Analysis of structures causing instabilities

Thomas Wilhelm*

Theoretical Systems Biology, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom

(Received 16 January 2007; revised manuscript received 2 May 2007; published 17 July 2007)

We present a simple new method to systematically identify all topological structures (e.g., positive feedback loops) potentially leading to locally unstable steady states: ICSA—The instability causing structure analysis. Systems without any instability causing structure (i.e., not fulfilling the necessary topological condition for instabilities) cannot have unstable steady states. It follows that common bistability or multistability and Hopf bifurcations are excluded and sustained oscillations and deterministic chaos are most unlikely. The ICSA leads to new insights into the topological organization of chemical and biochemical systems, such as metabolic, gene regulatory, and signal transduction networks.

DOI: 10.1103/PhysRevE.76.011911 PACS number(s): 82.39.−k, 82.40.Bj, 47.50.Gj

I. INTRODUCTION

Poincaré once said a law of nature is a differential equation (DE). Although much of our knowledge about nature is written in DE terms, there is no general procedure for solving them. Rather, most systems are nonintegrable and admit no exact solutions [1]. After that appreciation, methods for qualitative analysis of DEs have been established. However, detailed knowledge of kinetics is usually needed for it, but often not available. Typically a rather vague knowledge about systems exist: one only knows which variables influence which others, but often also if it is an activating or inhibiting influence. Well known examples are gene regulatory and signal transduction systems. It is therefore important to develop a deeper dynamical understanding of these underlying topological structures: sometimes already some topological knowledge yields useful information about systems dynamics. For (bio)chemical systems initial results have been presented already some decades ago [2–5]. Analyses of the stoichiometric matrix became especially popular during recent years [6–10]. Here we present a new approach for topological analyses of such and other systems, for which usually no stoichiometric matrix is analyzed, for instance, gene regulatory and signal transduction systems.

Mass balance equations describing the dynamics of spatially homogeneous (bio)chemical reaction systems can be written in the form

$$\frac{d\mathbf{x}}{dt} = \mathbf{S}\mathbf{v} = f(\mathbf{x}), \tag{1}$$

where $\mathbf{x}=(x_1,x_2,\ldots,x_n)^T$ denotes the concentration vector of all metabolites, $\mathbf{S}$ the stoichiometric matrix, $\mathbf{v}=(v_1,v_2,\ldots,v_m)^T$ the flux vector describing all reaction velocities, and $f(\mathbf{x})$ the phase flow in phase space [6,7]. Different methods restricted to analyses of the stoichiometric matrix $\mathbf{S}$ instead of the complete dynamical system (1) became popular during the last decade [8]. Many sequencing projects are already completed now, providing the basis for genome-scale reconstructions of metabolic networks and their stoichiometric matrices [8]. Different network reconstruction methods have been developed during recent years [11]. Analyses of the stoichiometric matrix are an important part of "constraint based modeling," which can be subdivided into "two fundamental types of constraints: balances and bounds" [8]. Linear balance equations of the metabolites are found by analyzing the left null space of $\mathbf{S}$ [6,7,12,13]. The right null space (kernel) of $\mathbf{S}$ corresponds to the set of all solutions ($\mathbf{v}$) of the steady state condition for system (1),

$$\mathbf{S}\cdot\mathbf{v} = \mathbf{0}. \tag{2}$$

Without additional restrictions of reaction velocities (e.g., $v_i \geq 0$) it describes the space of all possible steady state fluxes of system (1). The kernel is also the basis for different definitions of metabolic pathways, taking into account different linear inequalities. Best known are elementary modes comprising unique minimal sets of enzymes that could operate at steady state [9,14], and extreme pathways, unique sets of generating vectors spanning the convex steady state flux cone [7]. Because in the presence of reversible reactions there are often more elementary modes than are needed to span the flux cone, extreme pathways typically comprise a subset of all elementary modes [7]. Convex spaces, which are based on linear equality and inequality conditions, are also the basis for the well-known flux balance analysis (FBA), defining optimal solutions in the allowed flux space [15].

However, all these "balances and bounds" are linear constraints; nonlinear constraints are nearly not discussed so far [8]. By taking into account additional knowledge of reaction kinetics one can also identify nonlinear conservation relations and nonlinear constraints in the space of steady state fluxes. For instance, the mass-action kinetic system $dx/dt=(k_1S_1-k_2)x-2k_3x^2$, $dy/dt=k_2x-k_4xy$ (in the following, S_i and P_i denote constant outer reactants) has the nonlinear conservation relation $F(x,y)=\ln(k_2+2k_3x-k_1S_1)/(2k_3)-\ln(k_2-k_4y)/k_4=\text{const}$ [16]. More importantly, the demand for local stability of steady states leads to additional (nonlinear) constraints in the space of steady state fluxes (example in Sec. II). Local stability can be calculated using the well-known criterion of Routh-Hurwitz [17] (Sec. II). In Sec. III we present a general procedure for systematical identification

*thomas.wilhelm@bbsrc.ac.uk

of all potential sources of instabilities in a given chemical or biological reaction network (metabolic, signal transduction, or gene regulatory network), which is based on the Routh-Hurwitz criterion—the instability causing structure analysis (ICSA). If a system does not contain any instability source, it can be deduced that all its steady states are locally stable. Only in such a case the demand for local stability yields no additional constraints in the space of steady state fluxes. An example is a system without any feedback loop. A corresponding elementary proof is also presented. Importantly, this analysis needs no knowledge of kinetics. For corresponding analyses of chemical or metabolic networks, in the simplest case it is sufficient to know only the signs of the elements of the stoichiometric matrix. For signal transduction and/or gene regulatory networks the typical information of activator and inhibitor interactions is already sufficient for the ICSA.

II. ROUTH-HURWITZ STABILITY CRITERION

If system (1) is known completely, i.e., its stoichiometry **S** and the kinetics **v**, the local stability of its steady states can be determined: the considered steady state is locally stable if the real parts of all eigenvalues λ_i of the Jacobian matrix $\mathbf{J}=\{\partial f(\mathbf{x})/\partial \mathbf{x}|_{\text{st.st.}}\}$ are negative. Fortunately, a necessary and sufficient condition for local stability of steady states exists that works without actually calculating the λ_i. The characteristic polynomial of **J** is

$$\lambda^n + a_{n-1}\lambda^{n-1} + \cdots + a_1\lambda + a_0 = 0. \tag{3}$$

Hurwitz [17] has shown that all eigenvalues λ_i have negative real parts, i.e., the corresponding steady state is locally stable, if and only if

$$a_i > 0 (i = 0, \ldots, n-1) \quad \text{and} \quad H_j > 0 (j = 2, \ldots, n-1), \tag{4a}$$

where a_i are the coefficients of the characteristic polynomial and H_j denote the upper left subdeterminants (principal minors) of the Hurwitz determinant

$$H = \begin{vmatrix} a_{n-1} & a_{n-3} & a_{n-5} & a_{n-7} & \cdots \\ 1 & a_{n-2} & a_{n-4} & a_{n-6} & \cdots \\ 0 & a_{n-1} & a_{n-3} & a_{n-5} & \cdots \\ 0 & 1 & a_{n-2} & a_{n-4} & \cdots \\ \cdots & \cdots & \cdots & \cdots & \ddots \end{vmatrix}, \tag{4b}$$

for instance, $H_2 = a_{n-1}a_{n-2} - a_{n-3}$.

The first step in local stability analysis usually is the solution of the steady state equation (2), which is, unfortunately, in most cases impossible. However, additional constraints in the space of steady state fluxes can be found without solving Eq. (2). Consider, as an example, the smallest chemical reaction system with Hopf bifurcation[1] [18–20] as follows:

$$X + S_1 \xrightarrow{k_1} 2X,$$

$$X + Y \xrightarrow{k_2} Y + P_1,$$

$$Y \xrightarrow{k_3} P_2,$$

$$X \xrightarrow{k_4} Z,$$

$$Z \xrightarrow{k_5} Y, \tag{5a}$$

which can be described by the ordinary differential equations (ODEs) in the form of Eq. (1) as follows:

$$\begin{pmatrix} \dot{x} \\ \dot{y} \\ \dot{z} \end{pmatrix} = \begin{pmatrix} 1 & -1 & 0 & -1 & 0 \\ 0 & 0 & -1 & 0 & 1 \\ 0 & 0 & 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} v_1 = \tilde{k}_1 x \\ v_2 = k_2 xy \\ v_3 = k_3 y \\ v_4 = k_4 x \\ v_5 = k_5 z \end{pmatrix}. \tag{5b}$$

If the Jacobian is written in terms of reaction velocities[2]

$$J = \begin{pmatrix} \frac{v_1}{x} - \frac{v_4}{x} - \frac{v_2}{x} & -\frac{v_2}{y} & 0 \\ 0 & -\frac{v_3}{y} & \frac{v_5}{z} \\ \frac{v_4}{x} & 0 & -\frac{v_5}{z} \end{pmatrix},$$

one obtains the conditions

$$T = \frac{v_1}{x} - \frac{v_2}{x} - \frac{v_4}{x} - \frac{v_3}{y} - \frac{v_5}{z} < 0,$$

$$K = \frac{v_3}{y}\left(\frac{v_1}{x} - \frac{v_2}{x} - \frac{v_3}{x}\right) + \frac{v_5}{z}\left(\frac{v_1}{x} - \frac{v_2}{x} - \frac{v_4}{x} - \frac{v_3}{y}\right) < 0,$$

$$D = v_5(v_1v_3 - v_2v_3 - v_2v_4 - v_3v_4)/(xyz) < 0,$$

and $H_2 = TK + D > 0$. Because concentrations are always positive, $D < 0$ yields a nice nonlinear constraint in steady state flux space, which is independent of any steady state values.

However, for such analyses detailed knowledge of kinetics is necessary. We now show that, importantly, insight into destabilizing topological structures of dynamical systems can be found without knowing the kinetics.

[1] A corresponding proof that this system is the unique "smallest" one according to an also presented definition was given in [18]. Other "smallest" limit cycle systems with only two variables necessarily contain unrealistic trimolecular reactions. A comprehensive review of "smallest" oscillating two- and three-variable systems was published in 1997 in the doctoral thesis of the author and is available on request.

[2] Considering mass-action kinetic systems, inverse concentrations naturally enter the Jacobian if it is written in terms of reaction velocities. These inverse concentrations are the first n components (for n-dimensional systems) of Clarke's convex parameters used to discuss stability issues (instead of the usual kinetic parameters) [4].

III. CLASSIFICATION AND SYSTEMATIC IDENTIFICATION OF DESTABILIZING TOPOLOGICAL STRUCTURES: ICSA—INSTABILITY CAUSING STRUCTURE ANALYSIS

We define all different types of topological structures potentially causing local instability of steady states and present a method to systematically identify them. If a system does not contain any instability structure, it follows that all steady states are always (for all parameter values) locally stable. The simplest example for an instability causing structure is an autocatalytic reaction. We name such structures *type 1 instability* structures. Importantly, the only information that is needed for the ICSA is the topological structure of the (bio)chemical system (e.g., metabolic network or signal transduction system), knowledge of the kinetics is not required. Consider, for example, the Schilling-Letscher-Palsson (SLP) system [7]

$$\begin{array}{l} \overset{v_1}{A \to B} \\ \overset{v_2}{B \to C} \\ \overset{v_3}{C \to B} \\ \overset{v_4}{C \to D} \\ \overset{v_5}{D \to C} \\ \overset{v_6}{C \to E} \\ \overset{v_7}{A \to P_1} \\ \overset{v_8}{B \to P_2} \\ \overset{v_9}{D \to P_3} \\ \overset{v_{10}}{E \to P_4} \end{array} \quad \text{with} \quad \mathbf{S} \cdot \mathbf{v} = \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \end{pmatrix} \begin{pmatrix} v_1(A) \\ v_2(B) \\ v_3(C) \\ v_4(C) \\ v_5(D) \\ v_6(C) \\ b_1(A) \\ b_2(B) \\ b_3(D) \\ b_4(E) \end{pmatrix}. \tag{6}$$

A,B,C,D,E are internal and P_1,P_2,P_3,P_4 are constant external reactants. For (bio)chemical reaction networks, the minimum information needed for an ICSA are simply the signs of the nonzero elements in the stoichiometric matrix. Usually, these signs show the net effect of each reaction, for instance, the first column of **S** indicates that the first reaction consumes A and produces B. Knowing not only the signs, but the complete stoichiometric factors allows a somewhat more detailed ICSA. This information is already available for different large metabolic networks in different databases, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG [21]) and BRENDA [22]. It also results from new sequencing projects [8]. Although not necessary for a basic ICSA, additional information about catalyzing reactants [e.g., Y in reaction 2 of system (5a)], and for non-mass-action systems about activators and inhibitors of enzyme reactions [e.g., threonine inhibits the glycine hydroxymethyltransferase (EC 2.1.2.1) and isoleucine inhibits the threonine ammonia-lyase (EC 4.3.1.19)] can reveal additional instability causing structures. A lot of corresponding information can be found in BRENDA [22]. Signal transduction and gene regulatory networks typically contain activator and inhibitor interactions such as $A \to B$, i.e. A activates B, and $B \dashv C$, i.e., B inhibits C. This information is enough for a corresponding ICSA. Different signal transduction databases exist; two regularly updated examples are KEGG [21] and the dynamic signaling maps (http://www.hippron.com/hippron/index.html).

A. General definition and method to identify all instability types

We demonstrate the general method to identify all destabilizing structures of a given system by means of the example system (5a). As mentioned, the minimum information needed for the analysis of this chemical reaction system are the signs of the stoichiometric matrix elements (5b). However, knowing all substrates for all reactions, e.g., knowing also that Y catalyzes reaction 2 (which is not indicated by the stoichiometric matrix), allows a more detailed ICSA[3]. Multiplication of the sign matrix and the velocity substrates

[3]In mass-action kinetic systems higher substrate concentrations always enhance the reaction velocity. However, if general biochemical reactions are considered, it is useful to know for each substrate if it is activating or inhibiting the reaction [simply knowing that a substance E influences the reaction velocity $v(S,E)$ does not tell if $\partial v/\partial E > 0$ or <0].

vector[4] $[v_1(x),v_2(x,y),v_3(y),v_4(x),v_5(z)]^T$ and differentiation yields the corresponding general Jacobian of system (5a) and (5b) as follows:

$$J=\begin{pmatrix} v_{1,x}-v_{4,x}-v_{2,x} & -v_{2,y} & 0 \\ 0 & -v_{3,y} & v_{5,z} \\ v_{4,x} & 0 & -v_{5,z} \end{pmatrix}, \tag{7a}$$

where $v_{i,j}=\partial v_i/\partial x_j$ denotes the corresponding general partial derivatives.

Generally, we assume a constant sign of $\partial v_i/\partial x_j$ in the considered phase space. In (bio)chemical systems it is therefore important to consider each reversible reaction as two independent irreversible reactions: this ensures that the reaction velocity expression depends on the substrates in a monotonic manner [6][5]. Just for the sake of convenience: if $\partial v_i/\partial x_j<0$ (i.e., x_j is an inhibitor), the corresponding partial derivative term should be replaced by a modified expression explicitly showing the negative sign: $v_{i,j}=-\tilde{v}_{i,j}$. An example is the simple competitive inhibition kinetics $v(S,I)=v_m S/(K_m+S+I/K_I)$ [6]: $\partial v/\partial I=v_{.,I}=-\tilde{v}_{.,I}$. However, in mass-action kinetic systems $\partial v_i/\partial x_j>0\ \forall\ i,j$.

From Eq. (7a) one obtains the following coefficients of the characteristic polynomial and the Hurwitz determinant H_2:

$$a_2=v_{4,x}+v_{2,x}+v_{3,y}+v_{5,z}-v_{1,x},$$

$$a_1=(v_{2,x}+v_{4,x})(v_{3,y}+v_{5,z})+v_{3,y}v_{5,z}-v_{1,x}(v_{3,y}+v_{5,z}),$$

$$a_0=v_{2,x}v_{3,y}v_{5,z}+v_{2,y}v_{4,x}v_{5,z}+v_{3,y}v_{4,x}v_{5,z}-v_{1,x}v_{3,y}v_{5,z},$$

$$H_2=\text{positive terms}-v_{1,x}[2v_{3,y}(v_{2,x}+v_{4,x})+v_{3,y}^2+2v_{5,z}(v_{2,x}+v_{3,y})+2v_{4,x}v_{5,z}+v_{5,z}^2]-v_{2,y}v_{4,x}v_{5,z} \tag{7b}$$

As stated above in Eq. (4a), a steady state is locally stable if all $a_i>0$ and all corresponding $H_i>0$. Note that the trace a_{n-1} always contains summands with only *one* factor, a_{n-2} contains only *two* factors terms, and so on. Generally, all summands in a_{n-i} are products of i factors. The same holds for H_i: H_2 only contains $1+2=3$ factors terms, H_3 (for four- and higher-dimensional systems) contains $1+2+3=6$ factors terms, H_4 (for five- and higher-dimensional systems) contains $1+2+3+4=10$ factors terms. Generally, H_i contains $i(i+1)/2$ factors terms. Note that usually each factor belongs to another reaction. In other words, the higher i, the more complicated topological structures are involved, for both, the coefficients a_i and the Hurwitz determinants H_i.

Generally, the coefficients a_i (3) are related to the elements of the Jacobian b_{ij} [23] by

$$a_{n-1}=(-1)^1\sum_i b_{ii},$$

$$a_{n-2}=(-1)^2\left(\sum_{i,j} b_{ii}b_{jj}-\sum_{i,j} b_{ij}b_{ji}\right),$$

$$a_{n-3}=(-1)^3\left(\sum_{i,j,k} b_{ii}b_{jj}b_{kk}-\sum_{i,j,k} b_{ii}b_{jk}b_{kj}+\sum_{i,j,k} b_{ij}b_{jk}b_{ki}\right),$$

$$a_{n-4}=(-1)^4\left(\sum_{i,j,k,l} b_{ii}b_{jj}b_{kk}b_{ll}+\sum_{i,j,k,l} b_{ii}b_{jk}b_{kl}b_{lj}-\sum_{i,j,k,l} b_{ii}b_{jj}b_{kl}b_{lk}+\sum_{i,j,k,l} b_{ij}b_{ji}b_{kl}b_{lk}-\sum_{i,j,k,l} b_{ij}b_{jk}b_{kl}b_{li}\right),$$

$$\begin{aligned} a_{n-5}=(-1)^5\Bigg(&\sum_{i,j,k,l,m} b_{ii}b_{jj}b_{kk}b_{ll}b_{mm}-\sum_{i,j,k,l,m} b_{ii}b_{jk}b_{kl}b_{lm}b_{mj}\\ &+\sum_{i,j,k,l,m} b_{ii}b_{jj}b_{kl}b_{lm}b_{mk}-\sum_{i,j,k,l,m} b_{ii}b_{jj}b_{kk}b_{lm}b_{ml}\\ &+\sum_{i,j,k,l,m} b_{ii}b_{jk}b_{kj}b_{lm}b_{ml}-\sum_{i,j,k,l,m} b_{ij}b_{ji}b_{kl}b_{lm}b_{mk}\\ &+\sum_{i,j,k,l,m} b_{ij}b_{jk}b_{kl}b_{lm}b_{mi}\Bigg), \end{aligned}$$

$$\ldots,$$

$$a_0=(-1)^n\,\mathrm{Det}(\mathbf{J}), \tag{8}$$

Equation (8) shows that all terms in a_{n-1} correspond to cycles of length one: $v_{i,j}$ in row j and column j denotes an influence of reactant j onto itself (via reaction i). All terms in a_{n-2} contain only cycles of length one or two, but no other combination of factors. Similarly, a_{n-3} contains only terms with cycles of length one, one and two, or three, and so on.[6] Generally, a_{n-i} contains only terms corresponding to feedback cycles (up to a length i). It follows that also all terms in H_i only contain cycles [either positive (autocatalytic) or negative feedback cycles]. Therefore, treelike dynamical systems without any feedback cycle cannot have unstable steady states [4] (if $b_{ii}<0\ \forall\ i$). This also implies that any system with an arbitrary number of feed-forward loops (positive or negative), but no feedback cycle is always stable.

Moreover, we conjecture the following for all terms a_{n-i}: the summands containing an odd number of cycles have always a negative sign (e.g., $\Sigma_{i,j,k,l,m} b_{ii}b_{jj}b_{kl}b_{lm}b_{mk}$ in a_{n-5}: two cycles of length one and one cycle of length three), all summands containing an even number of cycles have always a positive sign (cf. $a_{n-1},a_{n-2},\ldots,a_{n-5}$). It follows that at least one of the cycles must be a positive feedback loop (only activating interactions or an even number of inhibitory inter-

[4]The velocity substrates vector contains information about all substrates for each reaction, and for more complicated biochemical velocity expressions also information about activators and inhibitors.

[5]This holds true for all common reaction velocity expressions in (bio)chemical systems [6]. However, also hypothetical complicated expressions violating this rule can be handled by corresponding decompositions of the velocity terms.

[6]The number of different terms in a_{n-i} is equivalent to the number of different possibilities to subdivide i stones. Five stones can be subdivided in seven different ways (as can be seen in a_{n-5}), six stones in 11 ways, seven stones in 15 ways, and so on. A corresponding general formula of this famous so-called partition problem was first presented by Hardy and Ramanujan [24].

actions [4,25]) in order to violate a stability condition $a_{n-i} > 0$. In other words, systems without autocatalytic cycles have always positive coefficients of the characteristic polynomial. Note also that in a_{n-i} only a term corresponding to a cycle of length i can violate the stability condition, if all terms in all lower order coefficients are stabilizing. A positive feedback loop of length i is always an instability causing structure if it appears in a_{n-i}. However, terms in the Hurwitz determinants H_i show no such simple pattern. System (10) shows that the same negative feedback cycle can be stabilizing in a_0 and destabilizing (instability causing) in H_2.

We propose the following simple classification scheme of ODE systems: the coefficient a_i or the Hurwitz determinant H_i containing the terms with the lowest number of factors, which contains at least one negative term defines the simplest contained type of instability. This simplest instability type is used for the corresponding system designation. For instance, the example system (5a) is a type 1 instability system, because already the simplest coefficient $a_{n-1}=a_2$ contains a negative term: $-v_{1,x}$ Eq. (7b). Additional independent instability structures are most simply found by deleting all terms ($v_{i,j}=0$) corresponding to the simpler type of instability in the higher-order expressions a_i and H_i and looking for remaining negative terms. For instance, setting $v_{1,x}=0$ reveals that a_0, a_1, and a_2 then only contain positive terms, Eq. (7b). There is only one remaining negative term in H_2 Eq. (7b): $-v_{2,y}v_{4,x}v_{5,z}$, indicating a second potential source of instability in system (5a). That means the type 1 instability system (5a) contains a total of two instability causing structures: a type 1 instability and an H_2 *instability*. Generally, the system designation is the following: if a_{n-i} is the smallest coefficient with at least one negative term, it is a *type i instability* system (or simply type i system). If, however, the smallest Hurwitz determinant H_i with a negative term consists of summands with smaller products than the terms of the smallest coefficient of the characteristic polynomial with at least one negative term, then the system is called an H_i *instability* system (or simply H_i system).

A more detailed analysis (taking into account the complete information on kinetics) shows that replacing the autocatalytic reaction in Eq. (5a) with a simple constant input reaction $S \rightarrow X$ (i.e., $v_{1,x}=0$), gives a totally stable system: the positive steady state is locally stable for all allowed parameter values (generally, our identified topological instability structures are necessary, but no sufficient conditions for instability). In Sec. III B. we present a similar system containing only an H_2 instability, i.e., an H_2 *instability* system, where the H_2 term $-v_{2,y}v_{4,x}v_{5,z}$ indicates the only instability causing structure.

B. Example systems

System (5a) with its autocatalytic reaction (which we also denote as an autocatalytic cycle of first order) is a simple type 1 instability system. Here we present three other systems with three different types of instabilities.

1. Type 2 instability system

The following system contains a type 2 instability, but no type 1 instability. It is therefore denoted as a type 2 instability system. Type 2 instabilities are always autocatalytic cycles of second order.

Some years ago we [26] presented the system

$$\begin{aligned} S &\xrightarrow{k_1} X, \\ 2Y &\xrightarrow{k_2} Z, \\ Z &\xrightarrow{k_3} 2Y, \\ X + Z &\xrightarrow{k_4} Y + Z, \\ Y &\xrightarrow{k_5} P, \end{aligned} \tag{9}$$

to demonstrate that autocatalytic reactions are not necessary for Hopf bifurcations. For our analysis of the contained instability types we do not need the detailed kinetics contained in the ODEs, but only the following information: $v_1=\text{const}$, $v_2=f(y)$ consumes Y and produces Z, $v_3=f(z)$ consumes Z and produces Y, $v_4=f(x,z)$ consumes X and produces Y, $v_5=f(y)$ consumes Y. Note that this is exactly the information contained in the signs of the stoichiometric matrix elements and the velocity substrates vector. The general Jacobian therefore reads

$$J = \begin{pmatrix} -v_{4,x} & 0 & -v_{4,z} \\ v_{4,x} & -v_{2,y} - v_{5,y} & v_{3,z} + v_{4,z} \\ 0 & v_{2,y} & -v_{3,z} \end{pmatrix}.$$

One obtains the following a_i and H_2:

$$a_2 = v_{2,y} + v_{3,z} + v_{4,x} + v_{5,y},$$

$$a_1 = v_{2,y}(v_{4,x} - v_{4,z}) + v_{3,z}(v_{4,x} + v_{5,y}) + v_{4,x}v_{5,y},$$

$$a_0 = v_{3,z}v_{4,x}v_{5,y},$$

$$H_2 = \text{positive terms} - v_{2,y}v_{4,z}(v_{2,y} + v_{3,z} + v_{4,x} + v_{5,y}).$$

All terms in a_2 are positive, the system contains no autocatalytic reaction. Obviously, the term $-v_{2,y}v_{4,z}$ in a_1 is the only source of instability (after setting $v_{2,y}v_{4,z}=0$, H_2 contains only positive terms). The system is a type 2 instability system, it contains an autocatalytic cycle of second order: if y is enhanced, z becomes larger what then enhances y again. Note that this positive feedback loop could also be realized with two negative terms $-v_{2,y}$ and $-v_{4,z}$ in $\mathbf{J}$: y inhibits z and z inhibits y. As we have shown [26], this type 2 instability system, described by the ODEs $\dot{x}=1-k_4xz$, $\dot{y}=-y-2k_2y^2+2k_3z+k_4xz$, $\dot{z}=k_2y^2-k_3z$ (dimensionless quantities), has one steady state $(\bar{x},\bar{y},\bar{z})=[k_3/(k_2k_4),1,k_2/k_3]$ and a supercritical Hopf bifurcation [e.g., for $k_2=k_3=1$, $k_4=(\sqrt{325}-17)/6$].

2. Type 3 instability system

The following is a simple example for a type 3 instability system, i.e., it contains an autocatalytic cycle of third order,

but no autocatalytic cycle of first (autocatalytic reaction) or second order:

$$S_0 \xrightarrow{k_0} X,$$

$$S_1 + Y \xrightarrow{k_1} X + Y,$$

$$S_2 + Z \xrightarrow{k_2} Y + Z,$$

$$S_3 + X \xrightarrow{k_3} Z + X,$$

$$X \xrightarrow{k_4} P_1,$$

$$Y \xrightarrow{k_5} P_2,$$

$$Z \xrightarrow{k_6} P_3.$$

The corresponding general Jacobian, a_i and H_2 are

$$J = \begin{pmatrix} -v_{4,x} & v_{1,y} & 0 \\ 0 & -v_{5,y} & v_{2,z} \\ v_{3,x} & 0 & -v_{6,z} \end{pmatrix},$$

$$a_2 = v_{4,x} + v_{5,y} + v_{6,z},$$

$$a_1 = v_{4,x}v_{5,y} + v_{4,x}v_{6,z} + v_{5,y}v_{6,z},$$

$$a_0 = v_{4,x}v_{5,y}v_{6,z} - v_{1,y}v_{2,z}v_{3,x},$$

$$H_2 = \text{only positive terms}.$$

The only source of instability is the term $-v_{1,y}v_{2,z}v_{3,x}$ in a_0, the system has the corresponding autocatalytic cycle of third order. A more detailed analysis based on the corresponding ODEs $\dot{x}=\tilde{k}_0+\tilde{k}_1 y-k_4 x$, $\dot{y}=\tilde{k}_2 z-k_5 y$, $\dot{z}=\tilde{k}_3 x-k_6 z$ shows that the necessary and sufficient condition for local (and global) stability of the only steady state $(\bar{x},\bar{y},\bar{z}) = (\tilde{k}_5\tilde{k}_6,\tilde{k}_2\tilde{k}_3,\tilde{k}_3\tilde{k}_5)\tilde{k}_0/D$ with $D=k_4k_5k_6-\tilde{k}_1\tilde{k}_2\tilde{k}_3$ is $D>0$ (this linear system has, of course, also a simple analytical solution).

3. H_2 instability system

The following system contains an H_2 instability as the only instability causing structure, i.e. it contains no autocatalytic cycle at all. As mentioned above, a modified system (5a) where the autocatalytic reaction is replaced with a simple constant input reaction, gives a totally stable system. However, a similar system with a constant input instead of the autocatalytic reaction and two reactions of higher molecularity is an H_2 system:

$$S_1 \xrightarrow{k_1} X,$$

$$X + Y \xrightarrow{k_2} Y + P,$$

$$Y \xrightarrow{k_3} P_2,$$

$$S_2 + 3X \xrightarrow{k_4} 3X + Z,$$

$$S_3 + 3Z \xrightarrow{k_5} 3Z + Y,$$

$$Z \xrightarrow{k_6} P_3. \tag{10}$$

The general Jacobian and the coefficients a_i and H_2 read

$$J = \begin{pmatrix} -v_{2,x} & -v_{2,y} & 0 \\ 0 & -v_{3,y} & v_{5,z} \\ v_{4,x} & 0 & -v_{6,z} \end{pmatrix},$$

$$a_2 = v_{2,x} + v_{3,y} + v_{6,z},$$

$$a_1 = v_{2,x}(v_{3,y} + v_{6,z}) + v_{3,y}v_{6,z},$$

$$a_0 = v_{2,x}v_{3,y}v_{6,z} + v_{2,y}v_{4,x}v_{5,z},$$

$$H_2 = \text{positive terms} - v_{2,y}v_{4,x}v_{5,z}.$$

Note the similarity to the expressions in Eqs. (7a) and (7b) with $v_{1,x}=0$. Obviously, the negative term $-v_{2,y}v_{4,x}v_{5,z}$ in H_2 indicates the only instability causing structure, which is not an autocatalytic cycle (i.e. positive feedback loop), but, interestingly, a negative feedback loop: X promotes the production of $Z(v_{4,x})$, Z promotes the production of $Y(v_{5,z})$, but Y does not promote the production of X, but inhibits it $(-v_{2,y})$.

A local stability analysis based on the corresponding ODEs $\dot{x}=\tilde{k}_1-k_2xy$, $\dot{y}=\tilde{k}_5z^3-k_3y$, $\dot{z}=\tilde{k}_4x^3-k_6z$ shows that the only positive steady state $(\bar{x},\bar{y},\bar{z}) = ([k_6^3k_3\tilde{k}_1/(\tilde{k}_4^3\tilde{k}_5k_2)]^{0.1}, \tilde{k}_1/(k_2\bar{x}), (k_3\bar{y}/\tilde{k}_5)^{1/3})$ undergoes a supercritical Hopf bifurcation at $H_2=k_3^2(k_6+k_2\bar{y})+k_3(k_5+k_2\bar{y})^2 + k_2[k_6\bar{y}(k_6+k_2\bar{y})-9\tilde{k}_4\tilde{k}_5\bar{x}^3\bar{z}^2]=0$.

IV. DISCUSSION

We presented the ICSA, the first definition and systematic method for the identification of all topological structures potentially causing instabilities of steady states. It fits into the general research field relating the dynamic behavior of a system to its topological structure, which was also the basis of our former work [18–20,26]. For (bio)chemical systems this goes back to earlier works of Higgins [2] and Clarke [3–5]. Clarke also considered the problem of steady state stability, but in contrast to our simple and general approach his much more complicated analysis was constricted to mass-action kinetic systems [3,4]. Instead Clarke derived more detailed results also on global stabilities (Lyapunov functions [19,25]), oscillations, and multistabilities [4,5]. However, in contrast to our simple method, his detailed analyses cannot

be carried out for large networks [5]. Some more recent results stand in the tradition of Clarke, all dealing with mass-action kinetic systems. Feinberg *et al.* [27] studied necessary conditions for bistability, culminating in a recently published new theorem defining a class of mass-action kinetic systems possessing a single steady state only [28]. Others focused on oscillations. Eiswirth *et al.* [29] proposed a classification of Hopf bifurcation oscillators. Related to this approach is the work of Goldstein *et al.* [23]. The authors studied the so-called "oscillophoretic" condition for oscillations in mass-action kinetic systems with monomolecular and bimolecular reactions. The coefficients of the characteristic polynomial (3) should fulfill the conditions $a_0>0$ and $a_i<0$ for some $i<n$ [30]. In contrast to our analysis the authors did not consider Hurwitz determinants. However, system (10) shows that this is not a necessary condition: this H_2 system has stable limit cycles although $a_i>0\ \forall\ i<n$. It was argued that also enzyme kinetics may be handled within this framework, because the elementary reactions are only monomolecular and bimolecular (for a general derivation of enzyme kinetic rate laws from elementary reactions see [31]), but in contrast to our ICSA, general activation and inhibition relations of signal transduction and gene regulatory networks are not in the focus of the Goldstein *et al.* approach.

Of course, the relationship of dynamic behavior and topological structures of systems has fascinated many other authors. A well-known result is Thomas' conjecture that at least one positive feedback loop is necessary for the existence of multiple steady states [32]. This was proven later by different authors, e.g., [33]. It was also shown that another ingredient is essential for bistability: there must be some mechanism to filter out small stimuli of the positive feedback loop [34], for instance zero-order, inhibitor, or cooperative ultrasensitivity [35]. Negative feedback loops, in contrast, can create oscillations [36]. Indeed, all three oscillating systems presented here contain negative feedback cycles: $x\rightarrow z\rightarrow y\dashv x$ in Eqs. (5a) and (10) and $x\rightarrow y\rightarrow z\dashv x$ in Eq. (9). It was found that bistable switches, coupled to slower negative feedback loops produce oscillations [34]. Systems (5a), (9), and (10) show that one negative feedback loop alone is already sufficient. It is tempting to speculate that a negative feedback loop is indeed a necessary condition for oscillations. Note that not all cycles eventually appear in a_i or H_i: the negative feedback cycle $-v_{4,z}v_{4,x}v_{2,y}$ in Eq. (9) can be seen in the Jacobian (in the elements $b_{13}b_{21}b_{32}$), but not in its determinant a_0 (and therefore also not in H_2), because it cancels out with the corresponding terms of $b_{11}b_{23}b_{32}$.

Recently, other approaches to bridge the gap between structural analyses [7,9,14] and explicit kinetic modeling have been proposed: Angeli *et al.* [25] presented a method for analyzing positive-feedback systems with respect to bistability and multistability and corresponding bifurcations, and Steuer *et al.* [37] did a statistical analysis of the allowed parameter space to determine the possible dynamic behavior of a metabolic system with given topology.

Our ICSA method works for all dynamical systems for which the required topological information can be provided. Importantly, if a system contains no instability causing topological structure, for instance an autocatalytic cycle, it follows that all steady states are always locally stable. This has additional important implications: because all known (bio)chemical systems with sustained oscillations also give rise to Hopf bifurcations, i.e., locally unstable steady states (a homoclinic bifurcation causing $p53$ oscillations might be an exception [38]), limit cycles, and chaotic behavior is highly improbable for all systems without instability causing topological structures [18]. An example is the five-dimensional SLP system [7]. From Eq. (6) the corresponding general Jacobian follows:

$$\begin{pmatrix} -v_{1,A}-b_{1,A} & 0 & 0 & 0 & 0 \\ v_{1,A} & -v_{2,B}-b_{2,B} & v_{3,C} & 0 & 0 \\ 0 & v_{2,B} & -v_{3,C}-v_{4,C}-v_{6,C} & v_{5,D} & 0 \\ 0 & 0 & v_{4,C} & -v_{5,D}-b_{3,D} & 0 \\ 0 & 0 & v_{6,C} & 0 & -b_{4,E} \end{pmatrix}.$$

An ICSA shows that the SLP system has only locally stable steady states: all a_i as well as H_2, H_3 and H_4 contain only positive terms. However, if a system contains instability causing structures (ICS), i.e., feedback cycles (one ICS can contain different feedback cycles, but without feedback cycles no ICS), the type of the contained cycles yields additional important information: without positive feedbacks bistability or multistability can be excluded, and negative feedbacks are (probably) necessary for sustained oscillations.[7]

[7]In our terminology, an instability causing structure (ICS) corresponds to at least one negative term in some a_i or H_i. Therefore, the negative feedback loop of system (9) is no ICS.

It is expected that important biological insight will be gained by future systematic analyses of all known large-scale metabolic, gene regulatory, and signal transduction systems concerning topological structures causing instabilities.

ACKNOWLEDGMENTS

I thank an anonymous referee for many valuable comments. This work has been supported by the Federal Ministry of Education and Research, Germany (Grant No. 0312704E).

[1] I. Ekeland, *The Best of All Possible Worlds: Mathematics and Destiny* (Chicago University Press, 2006).
[2] J. Higgins, Ind. Eng. Chem. **59**, 18 (1967).
[3] B. L. Clarke, J. Chem. Phys. **60**, 1481 (1974).
[4] B. L. Clarke, Adv. Chem. Phys. **43**, 1 (1980).
[5] B. L. Clarke, Cell Biophys. **12**, 237 (1988).
[6] R. Heinrich and S. Schuster, *The Regulation of Cellular Systems* (Chapman & Hall, New York, 1996).
[7] C. H. Schilling, D. Letscher, and B. Ø. Palsson, J. Theor. Biol. **203**, 229 (2000).
[8] N. D. Price, J. L. Reed, and B. Ø. Palsson, Nat. Rev. Microbiol. **2**, 886 (2004).
[9] S. Schuster, D. A. Fell, and T. A. Dandekar, Nat. Biotechnol. **18**, 326 (2000).
[10] C. Wagner and R. Urbanczik, Biophys. J. **89**, 3837 (2005).
[11] R. A. Notebaart, F. H. J. Van Enckevort, C. Francke, R. J. Siezen, and B. Teusink, BMC Bioinf. **7**, 296 (2006).
[12] I. Famili and B. Ø. Palsson, Biophys. J. **85**, 16 (2003).
[13] M. Imielinski, C. Belta, H. Rubin, and A. Halasz, Biophys. J. **90**, 2659 (2006).
[14] S. Schuster and C. Hilgetag, J. Biol. Syst. **2**, 165 (1994).
[15] C. H. Schilling, J. S. Edwards, and B. Ø. Palsson, Biotechnol. Prog. **15**, 288 (1999).
[16] K. Schneider and T. Wilhelm, J. Math. Biol. **40**, 443 (2000).
[17] A. Hurwitz, Math. Ann. **46**, 273 (1895).
[18] T. Wilhelm and R. Heinrich, J. Math. Chem. **17**, 1 (1995).
[19] T. Wilhelm and R. Heinrich, J. Math. Chem. **19**, 111 (1996).
[20] T. Wilhelm, S. Schuster, and R. Heinrich, Nonlinear World **4**, 295 (1997).
[21] M. Kanehisa, S. Goto, M. Hattori, K. F. Aoki-Kinoshita, M. Itoh, S. Kawashima, T. Katayama, M. Araki, and M. Hirakawa, Nucleic Acids Res. **34**, D354 (2006) (Database issue).
[22] I. Schomburg, A. Chang, C. Ebeling, M. Gremse, C. Heldt, G. Huhn, and D. Schomburg, Nucleic Acids Res. **32**, D431 (2004) (Database issue).
[23] B. N. Goldstein, G. Ermakov, J. J. Centelles, H. V. Westerhoff, and M. Cascante, Eur. J. Biochem. **271**, 3877 (2004).
[24] M. du Sautoy, *The Music of the Primes: Searching to Solve the Greatest Mystery in Mathematics* (Harper Collins, New York, 2003).
[25] D. Angeli, J. E. Ferrell, Jr., and E. D. Sontag, Proc. Natl. Acad. Sci. U.S.A. **101**, 1822 (2004).
[26] T. Wilhelm and P. Hänggi, J. Chem. Phys. **110**, 6128 (1999).
[27] M. Feinberg, Arch. Ration. Mech. Anal. **132**, 311 (1995).
[28] G. Craciun, Y. Tang, and M. Feinberg, Proc. Natl. Acad. Sci. U.S.A. **103**, 8697 (2006).
[29] M. Eiswirth, A. Freund, and J. Ross, Adv. Chem. Phys. **80**, 127 (1991).
[30] A. N. Ivanova, Kinet. Katal. **20**, 1019 (1979).
[31] T. Wilhelm, E. Hoffmann-Klipp, and R. Heinrich, Bull. Math. Biol. **56**, 65 (1994).
[32] R. Thomas, in *Quantum Noise*, Springer Series in Synergetics 9, edited by C. W. Gardiner (Springer, Berlin, 1981), pp. 180–193.
[33] O. Cinquin and J. Demongeot, J. Theor. Biol. **216**, 229 (2002).
[34] J. E. Ferrell, Jr. and W. Xiong, Chaos **11**, 227 (2001).
[35] T. Eissing, S. Waldherr, F. Allgöwer, P. Scheurich, and E. Bullinger, Biosystems (to be published).
[36] J. J. Tyson, K. C. Chen, and B. Novak, Curr. Opin. Cell Biol. **15**, 221 (2003).
[37] R. Steuer, T. Gross, J. Selbig, and B. Blasius, Proc. Natl. Acad. Sci. U.S.A. **103**, 11868 (2006).
[38] J. J. Tyson, Mol. Syst. Biol. **2**, 32 (2006).