Stability Properties of Biochemical Cascades

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Abstract

In this work we review a number of results concerning the existence of stable steady states for systems of ordinary differential equations of the type usually termed as biochemical cascades. These are typically nonlinear systems involving interconnected activation-inhibitory feedback loops, and are known to play a basic role in the regulation of key physiological human functions. In particular, conditions will be recalled that ensure the existence of at most one stable steady state as opposed to multistability, i.e. the existence of several stable steady states. The latter is considered to be a key feature in biological processes mediated by biochemical cascades.

1 Introduction

Many physiological human functions are mediated by biochemical cascades, either at the cellular or at systemic level. Such cascades are complex systems of chemical reactions, where products of ongoing reactions are consumed in subsequent ones. In general, such networks are made up of positive and negative feedback loops whose interaction results in efficient operation. Typical examples are provided by the mitogen-activated protein kinase (MAPK) system ([1]), the blood coagulation cascade (BCC) (cf. [2, 3, 4, 5]) and the immunological response (IR) to pathogen invasion, which results in the production of cytotoxic substances to neutralize infectious agents (cf. [6, 7, 8]).

As it turns out, the presence of a highly wired set of activation-inhibition feedback loops seems to be essential for the full functionality of any of those systems. Such structure provides a scheme of checks and balances which yields efficient regulation. By this we mean that biochemical function is neither too small nor too large, but is kept instead at a level appropriate to the nature of the challenge to be met. For this reason, any disruption in the operation of such network may have serious consequences. For instance, lack of efficient coagulation response is associated to a number of bleeding diseases, of which those going under the name of haemophilia are most widely known ([9]). On the other hand, overactivation of the coagulatory system results in thrombotic disorders. Among them, disseminated intravascular coagulation (DIC) is receiving increased attention as a major threat in late-stage

tumour processes ([10]).

The importance of such chemical networks raises at once a number of questions. For instance, one may wonder what is the structural design of such biochemical systems, how their regulatory properties are achieved, and where their fragility points lie, that is where those chemicals reactions are located whose impairment might result in irreversible operational failure.

To address the previous issues, a useful approach consists in viewing the system under consideration as a circuit that can be modeled by means of a suitable set of differential equations, which govern the evolution in time of the concentrations of the chemicals that mediate the corresponding processes. Once the model has been established, one is led to identifying significant solution behaviours and to describe their relevance for the dynamics of the equations considered. For instance, of particular interest in this context is the possible existence of stable steady states (equilibria), the characterization of their basins of attraction, and the dependence of such equilibria with respect to changes in the parameters involved. A related question is that of detecting (and when possible, to prevent) sharp changes in the values of solutions components. If unchecked, such large variations may disrupt crucial feedback loops, thus compromising the functionality of the full system.

When it comes to analyzing the dynamics of the equations under consideration, a major obstacle to be overcome is the large number of uncertainties that are usually present in these models. For instance, little is known about the precise nature of many of the chemical reactions involved in actual biochemical cascades. To gain insight into the process at hand, a typical approach consists in focusing in a particular feature of the system, as for instance stability properties of a relevant (sub)network, and to select by means of guesses (whether educated or wild) a set of parameters for which significant information about the property being considered can first be obtained, and then be compared with experimental or clinical data. Then a sensitivity analysis is performed, to check whether the solution behaviours thus obtained are preserved when parameter values are subject to variations within a sufficiently large range. When this occurs, it is often said that the behaviour analyzed is a robust one. However, such procedure is far from being safe from criticism. For instance, it is well known that when systems with even a moderate number of unknown parameters are considered, one may obtain similar properties for very different parameter choices, each of them corresponding to quite different assumptions on the nature of the underlying kinetics (cf. [11] for an discussion on this situation).

Bearing these facts in mind, an alternative and suggestive approach may consist in exploring which type of properties (if any) can be ensured for systems for which a precise knowledge of the parameters therein involved is lacking .For instance, one may wonder what stability properties can be derived for a system where the general structure of the reactions involved is known, but the values of the corre-

sponding reaction rates are not. In this note we shall review some of the results obtained when such approach is applied to the issue of multistability in biochemical cascades. In particular, we shall be concerned with criteria to ensure (or to prevent) the existence of several stable steady states in arbitrarily large chemical networks as those used to describe biochemical cascades.

More precisely, the plan of this work is as follows. In Section 2 below we provide an introductory discussion on complexity and stability. The key fact therein recalled is a seminal result on conditions ensuring that the trivial solution of a class of linear systems of ordinary differential equations (ODEs) is stable, using only the signs (but not the precise values) of the systems coefficients. We then start to explore the subject of multistability for general autonomous systems in Section 3. Following the approach in reference ([12]), the focus is made on conditions ensuring the existence of at most one single equilibrium, a fact usually termed as injectivity. As will be explained there, the conditions thus derived heavily depend on the actual structure and parameters of the system under consideration, and turn out to be computationally unwieldy in most cases. Taking this into account, we then recall in Section 4 conditions yielding injectivity under large parameter uncertainty. Two results are provided there that concern respectively general autonomous systems and a particular class of them, involving only polynomial-type nonlinearities, which provide a typical framework for the biochemical cascades that motivate this work. As it turns out from the results in those Sections, conditions for the existence of a single equilibrium can be derived under rather general assumptions, whereas conditions sufficient for multistability remain more elusive to this day. A short discussion on this last issue is then provided in Section 5 as a complement for the contents of the previous Sections. In particular we shall observe that coupling through positive feedback of so-called monotone systems may lead to the onset of bistability in the resulting system. The paper then concludes with a final Section which summarizes the views previously presented and provides a short discussion on them.

2 Stability and Complexity

A key underlying issue in our approach is the relation between stability and complexity in population dynamics models. As a matter of fact, and arising from areas as diverse as Economics (cf. [13, 14]), Chemistry (cf. [15, 16, 17]) and Ecology ([18]), the following long-standing question has been raised: To determine stability conditions for the solutions of a (linear or nonlinear) differential system without a precise knowledge of its coefficients. More precisely, consider first the following linear system:

$$\frac{dx_i}{dt} = \sum_{j=1}^{n} a_{ij} x_j(t) \qquad i = 1, ..., n \tag{1}$$

where for $i, j = 1, ..., n$ the a_{ij} are real coefficients whose actual values are not known. Assume that $x^* = (x_1^*, ..., x_n^*)$ is a steady state (also called an equilibrium) of (1), so that:

$$\sum_{j=1}^{n} a_{ij} x_j^* = 0 \quad for \quad i = 1, ..., n \tag{2}$$

Notice that the origin: $x_i^* = 0$ for $1 \leq i \leq n$ is always a steady state of (1). As in ([18]) we may wonder whether the stability of x^* can be ascertained merely from the knowledge of the signs $(+, -, 0)$ of the $\{a_{ij}\}$. In an ecological context, parameters $\{a_{ij}\}$ represent the interaction coefficients describing the effect of species j upon species i, and the corresponding matrix $A = (a_{ij})$ can be thought of as a representation of the trophic web involving species $x_1, ..., x_n$. In particular, the effect of species j upon species i is positive, negative or neutral depending on the cases $a_{ij} > 0$, $a_{ij} < 0$ or $a_{ij} = 0$ respectively. It is well known that the steady state x^* is (asymptotically) stable if

$$\begin{array}{l} Re\lambda < 0 \ \ for\ any\ eigenvalue\ of\ A,\ i.e.\ for\ any\ solution\ of \\ det(A - \lambda I) = 0,\ I\ being\ the\ identity\ matrix. \end{array} \tag{3}$$

Our former question can then be recast as follows. Is it possible to ensure that condition (3) holds knowing only the signs of the coefficients $\{a_{ij}\}$ in A? As it turns out a positive, easy-to-check answer was obtained in ([14]), that can be stated as follows. Let $A = (a_{ij})$ be a given matrix. A is said to be qualitatively stable if every matrix $\overline{A} = (\overline{a}_{ij})$ whose coefficients $\overline{a}_{ij}$ have the same signs as the a_{ij}'s satisfies (3). Then the following result holds

Theorem 2.1 *([14])*

The following are necessary and sufficient conditions for $A = (a_{ij})$ to be qualitatively stable:

(i) $a_{ij} a_{ji} \leq 0$ for $i \neq j$.

(ii) $i_1 \neq i_2 \neq ... \neq i_m$, $a_{i_1 i_2} \neq 0$, $a_{i_2 i_3} \neq 0$, ..., $a_{i_{m-1} i_m} \neq 0$ implies $a_{i_m i_1} = 0$ for any $m > 2$.

(iii) $a_{ii} \leq 0$ for all i, $a_{kk} < 0$ for some k; $1 \leq k \leq n$.

(iv) $det(A) \neq 0$.

As a consequence of Theorem (2.1), the system (1) corresponding to a matrix A with the qualitative pattern described below is qualitatively stable provided that $det(A) \neq 0$, whereas that corresponding to matrix B is not.

$$A = \begin{pmatrix} - & + & + & + \\ - & - & 0 & 0 \\ - & 0 & - & 0 \\ - & 0 & 0 & - \end{pmatrix} \qquad B = \begin{pmatrix} - & + & + & + \\ - & - & + & + \\ - & - & + & - \\ - & - & + & - \end{pmatrix}$$

Concerning Theorem (2.1), a few remarks are in order. To begin with, there is a natural extension of that result to general autonomous systems of the type:

$$\frac{dx}{dt} = F(x) \tag{4}$$

where $x = (x_1, ..., x_n)$, $F = (f_1, ..., f_n)$ and for $1 \leq i \leq n$, $f_i \equiv f_i = f_i(x_1, ..., x_n)$ is a smooth (say continuously differentiable) real function. If (4) has a steady state $x^* = (x_1^*, ..., x_n^*)$ (so that $f_i(x_1^*, ..., x_n^*) = 0$ for $i = 1, ..., n$), on setting $x_i = x_i^* + \widetilde{x}_i$ with $|\widetilde{x}_i| << x_i^*$, $1 \leq i \leq n$, one readily sees that, to the lowest order, the small amplitude perturbations $\{\widetilde{x}_i\}$ satisfy a linear system (1) with coefficients:

$$a_{ij} = J_F(x^*) \tag{5}$$

J_F being the jacobian matrix $J_F = \left(\frac{\partial f_i}{\partial x_j}\right) \equiv (J_{ij})$, $1 \leq i, j \leq n$.

Therefore, Theorem (2.1) is still relevant to discuss linear stability of steady states of (5), although it provides no information concerning stability with respect to large perturbations thereof. Notice that a given system (1) (respectively (4)) may possess asymptotically stable (respectively, linearly asymptotically stable) equilibria when conditions in Theorem (2.1) are not satisfied. However, additional information on coefficients is then needed in order to further discuss stability. On the other hand, neutral stability (i.e. the case where $Re\lambda_j = 0$ for some eigenvalues λ_j with $1 \leq j \leq m \leq n$ with $Re\lambda_i < 0$ for $m < i \leq n$) falls out of the scope of such result.

A particularly interesting question in this context is that of determining the number of steady states of a given system (1), (4). We will address this issue in our following Sections.

3 Multistability in Nonlinear Autonomous Systems

In many cases of practical interest it is possible to ensure the existence of at least one steady state. For instance, consider a chemical network involving $S_1, S_2, ..., S_n$ chemical species and that m reactions take place among them. The j^{th}-reaction can thus schematically be represented as follows:

$$c_{j1}S_1 + c_{j2}S_2 + ... + c_{jn}S_n \rightarrow d_{j1}S_1 + d_{j2}S_2 + ... + d_{jn}S_n$$

c_{ji}, d_{ji} being nonnegative integers. When reactions are reversible, the sign $\rightarrow$ above is to be replaced by $\rightleftarrows$. Using mass action law, we may represent the overall process by means of the following system:

$$\frac{dx_i}{dt} = \sum_{j=1}^{m} a_{ij} x_1^{c_{j1}} \cdots x_n^{c_{jn}} \equiv \sum_{j=1}^{m} a_{ij} x^{c_j} \qquad i = 1, ..., n \tag{6}$$

where $a_{ij} = (d_{ji} - c_{ji})k_j$, k_j being the rate constant for the reaction being considered. For later purposes, we shall assume that $d_{ji} \neq c_{ji}$. System (6) is a particular case of (4), its main feature being that only polynomial nonlinearities are taken into account. Clearly, (6) always has a trivial steady state $x_0 = (0, ..., 0)$.

A particularly relevant question for equations (6) is under which assumptions such system has at least two stable equilibria. Since in many situations of practical interest x_0 turns out to be stable, the question can be reformulated in that case as when, and how, a new stable equilibria appears, a fact often referred to as bistability. More generally, one may wonder under which conditions does (6) possess several stable equilibria, a property termed as multistability. The latter feature is in particular associated to key switch-like processes in cell development, as well as to the onset of functional thresholds in the operation of biochemical cascades ([19, 20]). As a matter of fact, the question of multistability can be formulated for more general systems as (4), although specific results for systems (6) are particularly relevant.

As it turns out, it is often easier to exclude multistability then to derive it. In the sequel we shall elaborate on this statement, and for that purpose we shall closely follow the excellent revision provided in ([12]).

Let us consider first the general autonomous system (4). We say that F (and also (4)) is injective if $F(x_1) \neq F(x_2)$ whenever $x_1 \neq x_2$. Clearly, an injective system

cannot possess more than one steady state, and thus injectivity and multistability are mutually excludent. At first glance, injectivity conditions may seem easy to derive. Indeed, for one-dimensional, differentiable real functions $f : \Re \to \Re$ the condition $f'(x) \neq 0$ for all x implies injectivity. It thus seems natural to expect that in the higher-dimensional case (4) the condition $det(J_F) \neq 0$ will play a similar role. However, this is not the case. In particular, it has been shown in ([21]) that the map $F : \Re^2 \to \Re^2$ given by

$$F(x, y) = (e^{2x} - y^2 + 3, 4e^{2x}y - y^3) \tag{7}$$

is such that $det(J_F) > 0$ for any $(x, y) \in \Re^2$, but $F(0, 2) = F(0, -2) = 0$. Notice that (7) is not of the form (6). This result provided a counterexample to a conjecture raised in ([13]) according to which injectivity would be satisfied if all leading principal minors in J_F do not vanish. However, the authors of ([21]) were able to obtain a positive injectivity result by modifying the condition proposed in ([13]) in a suitable manner. More precisely, one says that M is a P-matrix if all of its principal minors are strictly positive, and that M is a weak P-matrix if $det(M) > 0$ and all other principal minors are nonnegative. Then one has:

Theorem 3.1 *([21])*

Let D be a rectangular region of $\Re^n$ $(n > 1)$ and let $F : D \to \Re^n$ be differentiable. Then if J_F is a P-matrix for all $x \in D$, F is injective. The same result holds true if J_F is a weak P-matrix and D is open.

The previous Theorem applies to general autonomous systems (4), and one may wonder if weaker injectivity conditions could be obtained for polynomial nonlinearities as those in (6). For instance, a natural question to consider is whether requiring $det(J_F) \neq 0$ for all $x \in \Re^n$ would suffice for that purpose. However, this is not in general the case. As a matter of fact, a polynomial function $F \equiv F(x, y) : \Re^2 \to \Re^2$ with $degree(x) = 10$ and $degree(y) = 35$ exists for which $det(J_F) \neq 0$ in $\Re^2$ but F is not injective ([22]). This fact notwithstanding, condition $det(J_F) \neq 0$ turns out to be sufficient to ensure injectivity in the case of quadratic nonlinearities, and the injectivity conjecture advanced in ([13]) has been proved for polynomial functions (6). We shall omit further details on this type of results, and refer instead to ([12]) for additional information.

4 Injectivity Under Parameter Uncertainty

Checking the assumption made to obtain injectivity in our previous Section needs a detailed knowledge about functions and parameters in equations (4) or (6).

However, for many reactions involved in biochemical cascades such information (for instance, the actual values of reactions rates) is beyond current experimental reach. This makes particularly relevant to discuss injectivity (or in general to decide about multistability) just based on information about the topology and general structure of the chemical network under consideration, without full knowledge of the parameters involved.

To proceed in this direction, we borrow some notation from ([12]) and consider a general autonomous system (4) as represented by its interaction graph G. The latter consists of vertices S_i (one for each species whose concentration is denoted by x_i) and edges joining each pair of vertices (say S_{i1} and S_{i2}) with $J_{i1i2} \neq 0$, where $J_{ik} = \sum_{j=1}^{m} \frac{c_{jk}}{x_k} a_{ij} x^{c_j}$, m being the number of reactions involved, so that $J_F = (J_{ik})$. For $1 \leq j \leq m$, we shall denote by J_{ijk} the effect of S_k on S_i by means of the j^{th}-reaction, so that $J_{ik} = \sum_{j=1}^{m} J_{ijk}$. The sign of the edge joining S_{i1} and S_{i2} is that of J_{i1i2}. An important assumption to be retained in the sequel is that neither direct autocatalysis nor inhibition are allowed in the systems to be considered. In particular, self-edges as J_{ii} are excluded. Indirect autocatalysis (and autoinhibition) is however allowed by means of intermediate species, leading to the definition of cycles. A cycle in a graph is defined as an ordered subset $(i_1, i_2, ..., i_j)$ with $j \leq n$ such that there exist edges joining S_{i1} to S_{i2}, S_{i2} to S_{i3}, ... and S_{ij} to S_{i1}. A cycle is said to be positive (respectively negative) if the product of the signs of its edges is positive (respectively negative). Two cycles are disjoint if they have no vertices in common; otherwise we say that they interact.

The following example taken from ([12]) may be helpful to illustrate the previous concepts. Consider a hypothetical chemical network consisting of six reactions which involve eight species (to be labeled in alphabetical order) as follows:

1. $A + B + C \rightarrow X$
2. $A + B + D \rightarrow Y$
3. $C + E \rightarrow A$
4. $D + E \rightarrow B$
5. $A \rightarrow Z$
6. $Z \rightarrow D$

Such network can be translated into a system of eight differential equations (one for each species) in a straightforward manner. For instance, denoting by x_i the concentration of the i^{th}-species ($1 \leq i \leq 8$), one has that

$$\frac{dx_1}{dt} = -k_1 x_1 x_2 x_3 - k_2 x_1 x_2 x_4 + k_3 x_3 x_5 - k_5 x_1 \tag{8}$$

where the four terms in the right of (8) are obtained from equations 1, 2, 3 and 5 above, and constants k_1, k_2, k_3 and k_5 are the corresponding (positive) reaction rates. One of the several cycles that can be considered in that network is that denoted by S, which can be represented by the diagram: $C \Rightarrow A \Rightarrow Z \Rightarrow D \Rightarrow B \Rightarrow C$. On writing the full differential system associated to this network, and upon recalling that $J_{ik} = \frac{\partial}{\partial x_k}\left(\frac{dx_i}{dt}\right)$, we readily check that:

$$\begin{array}{lll} J_{13} = -k_1 x_1 x_2 + k_3 x_5, & J_{81} = k_5, & J_{48} = k_6 \\ J_{24} = -k_2 x_1 x_2 + k_4 x_5, & J_{32} = -k_1 x_1 x_3 & \end{array} \tag{9}$$

In particular, if $k_1 = k_2$ and $k_3 = k_4$, the product of these terms is negative and so is the sign of the cycle S.

We are now ready to state a first injectivity result for a general autonomous system (4). This reads as follows.

Theorem 4.1 *([23])*

Let D be an open rectangular region of $\Re^n$ $(n > 1)$ and let $F = (f_1, ..., f_n) : D \to \Re^n$ be a differentiable function. If the interaction graph of F has no positive cycles for any $x \in D$, then F is injective.

At this juncture, one may wonder whether sharper results could be obtained for the case of polynomial systems (6), of which the previous example is a particular case. As a matter of fact, the answer to that guess is a subtle one. We shall next see that injectivity can be proved irrespective of the reaction rates involved, provided that (6) satisfies some strict structure conditions. To describe these in detail, we need some additional notation taken again from ([12]). For any given cycle S, consider any of its edges, say that going from S_{i1} to S_{i2}. For any reaction (labeled as j) that contributes a term (denoted as J_{i2ji1}) to J_{i2i1}, we define the sub-sign of that edge as the sign of J_{i2ji1}. For a given set of N reactions, the sub-sign of a cycle is the product of the sub-signs of its edges with respect to the reactions involved. This product is called sub-positive (respectively sub-negative) if there is an even number (respectively an odd number) of sub-negative edges. Note that the sub-sign of an edge in general depends on the choice of the reaction j. Edges such that they are sub-positive for some j and sub-negative for other j are termed as reaction-ambiguous. Finally, given two cycles S^1, S^2 and choices of reactions j for each of their edges, S^1 and S^2 are said to strongly intersect if they share at least one common vertex S_i so that the reaction j chosen for the outgoing edge from S_i is the same in both cycles. Otherwise S^1 and S^2 are termed

as weakly disjoint. For example, consider cycle S in our previous example and assume that $k_1 = k_2$, $k_3 = k_4$ as before. On selecting the reactions corresponding to the terms J_{133}, J_{851}, J_{468}, J_{224} and J_{312}, we easily check that:

$$J_{133} = k_3 x_5, \quad J_{851} = k_5, \quad J_{468} = k_6$$
$$J_{224} = -k_2 x_1 x_2, \quad J_{312} = -k_1 x_1 x_3$$

Thus with respect to that choice S is sub-positive. However the sign of S corresponds to that of the product of the quantities in (9), which is always negative under our current assumptions.

Before stating our next result, some additional terminology is needed. Consider a cycle S given by vertices numbered as $(i_1, i_2, ..., i_k)$ and let $(j_1, j_2, ..., j_h)$ be reaction indexes so that $J_{i_{h+1} j_h i_h} \neq 0$ for $1 \leq h \leq k$. We then define the value V of that cycle with respect to the choice made of reaction indexes as follows:

$$V = \left| \frac{\prod_{h=1}^{k} J_{i_{h+1} j_h i_h}}{\prod_{h=1}^{k} J_{i_h j_h i_h}} \right|$$

Finally we slightly enlarge the class of polynomial nonlinearities considered in (6) by explicitly including inward and outward flows for each species. This amounts to replacing (6) by

$$\frac{dx_i}{dt} = f_i + \sum_{j=1}^{n} a_{ij} x^{c_j} - r_i x_i \qquad i = 1, ..., n \tag{10}$$

for some $f_i > 0$, $r_i > 0$. One then has:

Theorem 4.2 *([17], cf. also [12])*

Consider a system (10) such that any cycle that is sub-positive for some chosen reaction indexes has value $V = 1$. Then if two sub-positive cycles never strongly intersect, the network cannot have more than one steady state.

5 How can Multistability be Obtained

The results recalled in our previous Section display two significant features. First, they provide conditions sufficient to prevent multistability rather than to ensure

it. Second, they show that in many cases injectivity can be derived from information about the general structure of the chemical network being considered, without making use of any detailed knowledge of the actual system parameters. The overall conclusion is that multistability seems easier to exclude than to obtain.

However in many cases of practical interest, multistability (and in particular bistability) appears as a crucial property of biochemical networks. This is in particular the case when system operation makes use of switches, whereby different states can be achieved as a consequence of external inputs. As it turns out, bistability in that context is usually achieved only within a precise range of parameter values (cf. for instance [19]). This is a general situation, also observed in ecological problems ([24, 25]). It is worth mentioning in this context that, while in principle biochemical cascades can be derived by linking in many possible ways otherwise arbitrary elementary circuits, biological networks are prone to using a rather limited type of such building blocks, usually termed as motifs ([26]). Multistability thus requires of very precise conditions to be presented. This naturally leads to the question of characterizing at a theoretical level which classes of systems are able to sustain multistability, an important step towards understanding the origin and structure of actual multistable biochemical circuits.

While a detailed analysis of the previous question goes well beyond the scope of this work, we shall content ourselves with briefly remarking on a tool that has been shown to be relevant to that discussion. Specifically, we shall shortly describe the so-called monotone systems and their controllability, a subject which has been extensively developed in ([27, 28, 20]) and references therein.

More precisely, following ([27]) we define monotone systems as follows. Consider a general autonomous system (4) and its associated interaction graph G defined in our previous Section. A spin assignment $\sum$ for G consists in associating to any vertex S_i in G a number σ_i, where $\sigma_i = +1$ or $\sigma_i = -1$. For any edge joining vertex S_j to S_i, we say that such edge is consistent with the spin assignment if $J_{ij}\sigma_i\sigma_j = 1$. Finally, we say that $\sum$ is a consistent spin assignment for G if every edge in G is consistent with $\sum$. In particular, this means that if $J_{ij} > 0$ (respectively $J_{ij} < 0$) then S_i and S_j should have the same spin (respectively, they should have opposite spins). A system (4) is then said to be monotone if there exists at least one consistent spin assignment for its associated graph G. An important property of this class of systems is that when a positive perturbation is added to the concentration at a vertex S_i, the effect of such perturbation on the remaining vertices can be readily described. In particular, for vertices S_{ij} with $J_{ij} > 0$ (respectively $J_{ij} < 0$) the concentration at S_j will increase (respectively decrease). A symmetric result holds for the case of negative perturbations.

Monotone systems are known to provide important building blocks to produce multistable systems, even if any of the elementary monotone systems used for that purpose is not itself multistable. To achieve that goal, positive feedback among

building monotone systems is necessary. In order to make these ideas precise, we follow ([27, 28]) and consider the following input/output (I/O) system:

$$\frac{dx}{dt} = f(x,u)\ ; \qquad y = h(x) \tag{11}$$

where $x = (x_1, ..., x_n)$, $f = (f_1, ..., f_n)$, $u = (u_1, ..., u_k)$ and $y = (y_1, ..., y_j)$. Let us write $x \geq \overline{x}$ to denote that $x_i \geq \overline{x}_i$ for $1 \leq i \leq n$, and define $u \geq \overline{u}$ and $y \geq \overline{y}$ in a similar way. Then (11) is said to be a I/O monotone system if h is monotone (that is, $x \geq \overline{x}$ implies $y \geq \overline{y}$) and for any initial values x_0, $\overline{x}_0$ and any inputs u, $\overline{u}$ one has that, whenever $x_0 \geq \overline{x}_0$ and $u \geq \overline{u}$, then the output $y = h(x)$ satisfies $y \geq \overline{y}$.

The bistability of a system consisting of two interconnected, monostable monotone systems can be obtained as follows. Consider the systems:

$$\begin{array}{ll} \frac{dx_1}{dt} = f_1(x_1, u_1)\ ; & y_1 = h_1(u_1) \\ \frac{dx_2}{dt} = f_1(x_2, u_2)\ ; & y_2 = h_2(u_2) \end{array} \tag{12}$$

where $x_1 = (x_{11}, ..., x_{1n})$ and an obviously similar notation is being used for the remaining quantities in (12). Focusing on the input/output variables, we can represent the dependence of y_i on u_i $(i = 1, 2)$ in the form $y_1 = k_1(u_1)$, $y_2 = k_2(u_2)$ where k_1, k_2 are referred to as the corresponding characteristic functions. Clearly k_1, k_2 are monotone if both subsystems in (12) are monotone. Then, upon introducing positive feedback in (12) by setting:

$$u_2 = y_1\ , \qquad u_1 = y_2 \tag{13}$$

it can be shown that, under rather weak transversality assumptions on the characteristic functions of (12), the resulting coupled system (12), (13) is bistable. We refer to ([20]) for a detailed description of how these ideas can be used to obtain bistability for a system related to the MAPK cascade.

6 Concluding Remarks

In this note we have attempted at providing a short overview of results concerning multistability in biochemical cascades. More precisely, we have recalled conditions that enable us to ensure, or to discard, the existence of multiple stable steady

states for systems of nonlinear differential equations that model chemical networks known to be instrumental in a number of physiological functions. Actually, the availability of different stable equlibria is related to the switching properties of those networks, by means of which different biological responses can be elicited as a result of external inputs.

A picture that emerges from the facts recalled in the previous Sections is that a dichotomy can be observed between injectivity (the existence of at most one, possibly stable, steady state) and multistability. As it turns out, injectivity is shown to hold under loose conditions of coefficients for large classes of systems, provided that their internal structure meets some requirements. On the other hand, to obtain multistability (and in particular bistability, the case most often addressed in the literature) rather tight assumptions need to be met, both in terms of structure and parameter values. It is tempting to guess that, out of an extremely large set of possible chemical networks to play with, evolution seems to have selected those which operate according to particular internal structures, and remaining within precise parameter regions. We have just mentioned one such structure which is prone to sustaining bistability, that of monotone I/O systems. It would certainly be interesting to know which other types of circuits (if any) would also led to multistability when suitably interconnected. Needless to say, finding precise molecular mechanisms supporting the functioning of those models, remains always a key issue. The same can be said about procedures to estimate in detail parameter ranges at which multistability appears. The latter question represents a challenge for experimentalists and theoreticians alike. A further interesting question is that of externally tampering (for instance, by therapeutic means) with some of the circuits integrated in a given network, to create, shift or eliminate stability points. In this way one could produce, enhance or dispense with particularly relevant solution behaviors. Such issues are currently been dealt with, and are likely to receive increasing attention in the future.

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