Proceedings of the 11th International Symposium on
Computer Applications in Biotechnology (CAB 2010),
Leuven, Belgium, July 7-9, 2010
Julio R. Banga, Philippe Bogaerts, Jan Van Impe,
Denis Dochain, Ilse Smets (Eds.)

Parametric Condition for Multistationarity in Biochemical Reaction Networks ⋆

Irene Otero-Muras, Julio R. Banga, Antonio A. Alonso*

Process Engineering Group, IIM-CSIC, Spanish Council for Scientific Research. Eduardo Cabello 6, 36208 Vigo, Spain.

*Corresponding author: e-mail: antonio@iim.csic.es

Abstract: Chemical Reaction Network theory allows us to decide whether many classes of networks have the capacity for multiple positive equilibria, based on their structural properties. In this way, the deficiency zero theorem asserts that every weakly reversible network of zero deficiency has a unique equilibrium, for any choices of parameter values. We make use of CRNT, aiming not only to discriminate whether a (positive deficiency) network can exhibit multiple steady states, but also to characterize the whole space of the parameters regarding to their capability to produce multistationarity. In this work, we provide a condition, on the parameters of biochemical networks, for the appearance of multistationarity.

Keywords: CRNT, bistability, nonlinear analysis, bifurcations, biochemical reaction networks.

1. INTRODUCTION

The system-level understanding of a biological system requires insight into its structure and dynamics, identifying not only the interactions and mechanisms configuring the system network, but its behaviour over time under various conditions (Kitano, 2002). The time evolution of the concentrations $\mathbf{c}$ of the species involved in a biochemical reaction network can be described, under standard assumptions, by a system of nonlinear first order ordinary differential equations (ODEs) of the form:

$$\dot{\mathbf{c}} = f(\mathbf{c}, k) \tag{1}$$

where k refers to the kinetic parameters.

In the context of biochemical network modelling, quantitative knowledge is often very limited. To overcome this lack of information, formal methods are needed that link the network structure and the dynamics (Conradi et al., 2007), in order to unravel how the observed biological behaviour arises out of the network topology and the parameters (Lu et al., 2006).

Specifically, much effort has been devoted to explore the capacity for multistability in biochemical reaction networks. Multistationarity plays an important role in biological phenomena such as cell differentiation and memory. Angeli et al. (2004) developed a graphical method for deducing the stability behavior and bifurcation diagrams for a class of feedback systems of arbitrary order. In a recent work, Conradi et al. (2007) provide a method to decompose a biochemical network in subnetworks capable of being analyzed with the deficiency one algorithm (Feinberg, 1995). Furthermore, conditions are given under which, if multistability appears in a small subnetwork for some values of the parameters, a range of kinetic constants can be computed giving rise to multiplicities for the overall system. The deficiency one algorithm, the advanced deficiency theory, the deficiency zero and deficiency one theorems are part of the Chemical Reaction Network theory in which networks are classified by means of a nonnegative integer index called deficiency –a property of the graph of complexes of a network– and some structural conditions are evaluated to decide whether networks have the capacity for multiple positive equilibria. Chemical reaction network theory provides also results based on the inspection of other different graphs associated to the networks (Craciun et al., 2006; Craciun and Feinberg, 2005).

In this work, we make use of CRNT aiming not only to discriminate whether a network can exhibit multiple steady states, but also to characterize the whole space of parameters in terms of their capability to produce multiplicities. In a previous paper (Otero-Muras et al., 2009), we have exploited the structure of the graph of complexes of a biochemical network to obtain an expression of the locus of equilibria –the set of points $\mathbf{c}^*$ such that $f(\mathbf{c}^*, k) = 0$ in (1)– in terms of as many parameters as the deficiency of the network. For those networks that we have denoted as *proper*, the dimension of the equilibrium manifold coincides with the deficiency of the network. By continuation of the new variation parameters associated to the deficiency, we managed to divide the space of kinetic parameters in regions with different qualitative dynamic behaviour. Understanding the equilibrium points as intersections of the locus of equilibria with the so called reaction polyhedron (Otero-Muras et al., 2008), a geometric condition for multistability was checked. In the present work, we make use of this insight in order to provide a general condition for the existence of multiplicities, valid for all classes of networks (satisfying weak reversibility and mass action kinetics).

⋆ The authors acknowledge financial support received from Spanish MICINN project MultiSysBio DPI2008-06880-C03-02.

2. FUNDAMENTALS

Let us consider a generic biochemical network consisting of a set of m species $\mathcal{S} = \{\mathcal{S}_1, \ldots, \mathcal{S}_j, \ldots, \mathcal{S}_m\}$ interacting among themselves through a set of reactions with a given kinetics. Under standard assumptions (Otero-Muras et al., 2009) the network dynamics can be described by a set of ordinary differential equations of the form (1) where $\mathbf{c}$ is the vector of continuous positive real variables that represents the m species concentrations. The *species formation function* $f(\cdot, k) : \mathbb{R}^m \to \mathbb{R}^m$ depends on the kinetics, and k refers to the internal or external conditions held constant during the process.

Employing Feinberg's description (Feinberg, 1979), a reaction network is represented by an n-node directed graph, where the edges correspond to the irreversible reaction steps taking place and the nodes stand for the so-called complexes, that are the multisets of reactants or products that appear on the left and right hand sides of each reaction step.

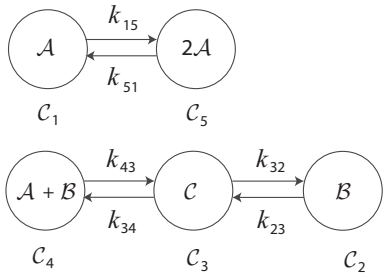


Fig. 1. Graph for the Edelstein network: the mechanism involves 3 species $\mathcal{A}$, $\mathcal{B}$ and $\mathcal{C}$, distributed in five complexes and two linkage classes.

Let $\mathcal{C} = \{\mathcal{C}_1, \ldots, \mathcal{C}_i, \ldots, \mathcal{C}_n\}$ be the set of complexes of the network. Each node or complex $\mathcal{C}_i$ is characterized by a set $\mathcal{I}_i$ of integer elements such that:

$$\mathcal{I}_i = \{j \in \{1, \ldots, n\} |\ \mathcal{C}_j \ \text{ is reachable from } \mathcal{C}_i\} \tag{2}$$

plus a pair of vectors $\{\mathbf{y}_i, \boldsymbol{\varepsilon}_i\}$.

Let $\mathbb{R}^m_+$ denote the positive orthant (i.e. $\mathbb{R}^m_+ = \{x \in \mathbb{R}^m | c_i > 0 \ \ \forall i = 1, \ldots, m\}$) and $\overline{\mathbb{R}}^m_+$ denote the nonnegative orthant (i.e. $\overline{\mathbb{R}}^m_+ = \{x \in \mathbb{R}^m | c_i \geq 0 \ \ \forall i = 1, \ldots, m\}$).

The vector $\mathbf{y}_i \in \overline{\mathbb{R}}^m_+$ indicates the stoichiometry associated with the complex i (the entries are the molecularities of each of the species in the complex i), while $\boldsymbol{\varepsilon}_i$ is a vector of the standard basis of $\mathbb{R}^n$ such that:

$$\varepsilon_{ji} = \begin{cases} 1 & \text{if } i = j \quad (i, j = 1, \ldots, n) \\ 0 & \text{otherwise.} \end{cases} \tag{3}$$

In addition, the complete set of edges in the graph is constructed by connecting $\mathcal{C}_i \to \mathcal{I}_i$ for all $i = 1, \ldots, n$. The reaction rates are incorporated in the graph description by associating to each node i a scalar function:

$$\psi_i : \overline{\mathbb{R}}^m_+ \to \overline{\mathbb{R}}_+ \tag{4}$$

and a set of positive parameters $k_{ij} > 0$ representing kinetic constants for every edge leaving $\mathcal{C}_i$ and entering $\mathcal{C}_j$. Keeping with the formalism described above, the dynamic evolution of concentrations can be encoded into the following set of ordinary differential equations (Feinberg, 1979):

$$\dot{\boldsymbol{c}} = Y \cdot A[\boldsymbol{\psi}(\mathbf{c})] \tag{5}$$

where $Y \in \overline{\mathbb{R}}^{m \times n}_+$ is the *molecularity* matrix, with columns being the vectors $\mathbf{y}_i$, and the vector $\boldsymbol{\psi}(\mathbf{c}) \in \overline{\mathbb{R}}^n_+$ contains the scalar function of the concentrations (4) corresponding to each complex. A maps from $\mathbb{R}^n$ to $\mathbb{R}^n$ and the expression for $A[\boldsymbol{\psi}(\mathbf{c})]$ reads:

$$A[\boldsymbol{\psi}(\mathbf{c})] = \sum_{i=1}^{n} \psi_i(\mathbf{c}) \sum_{j \in \mathcal{I}_i} k_{ij} \cdot (\boldsymbol{\varepsilon}_j - \boldsymbol{\varepsilon}_i) \tag{6}$$

where $\boldsymbol{\varepsilon}_i$ has been defined in (3). Assuming that the reaction rates obey the mass action law, the expression for $\psi_i(\mathbf{c})$ in (4) is of the form:

$$\psi_i(\mathbf{c}) = \prod_{j=1}^{m} c_j^{y_{ji}}. \tag{7}$$

The mass action law (7) leads to the following relationship, provided that $\mathbf{c} > 0$ (i.e. $\mathbf{c} \in \mathbb{R}^m_+$):

$$\ln \boldsymbol{\psi}(\mathbf{c}) = Y^T \ln \mathbf{c} \tag{8}$$

where the natural logarithm operator $\ln(\cdot)$ acts on any vector element-wise.

The graph associated to a reaction network is constituted by a number ℓ of "isolated" sub-graphs known in CRNT as *linkage classes*: $\{L_1, \ldots, L_k, \ldots, L_\ell\}$. Each linkage class L_k is accompanied by a vector $\boldsymbol{\Lambda}_k \in \overline{\mathbb{R}}^n_+$ with components being 1 at those places in the vector which correspond with the complexes present in the linkage class, and zero otherwise:

$$\Lambda_{jk} = \begin{cases} 1 & \text{if } \ \mathcal{C}_j \text{ in } L_k \\ 0 & \text{otherwise.} \end{cases} \tag{9}$$

A network is said to be *weakly reversible* if, provided a path from a complex $\mathcal{C}_i$ to another complex $\mathcal{C}_j$, there exists a path (in opposite direction) linking the complex $\mathcal{C}_j$ with the complex $\mathcal{C}_i$. We concentrate on weakly reversible reaction networks, for which, in addition, trajectories of (5) that start at any positive initial condition will not approach the boundary of the positive orthant (Angeli et al., 2007). For this class of networks, mass conservation constrains the evolution of the trajectories in the concentration space to a reduced convex region in the positive orthant known as the reaction polyhedron [1] (Otero-Muras et al., 2008). Let B be the matrix whose columns are an arbitrary basis of the null space of $Im(YA)$:

$$(YA)^T B = 0. \tag{10}$$

The *reaction polyhedron* can be defined with respect to a reference concentration vector $\mathbf{c}_0$ as:

$$\Omega(\mathbf{c}_0) = \{\mathbf{c} > 0 | B^T(\mathbf{c} - \mathbf{c}_0) = 0 \text{ with } (YA)^T B = 0\}. \tag{11}$$

For weakly reversible networks, the subspace spanned by YA is known in the CRNT as the *stoichiometric subspace* S. This subspace is defined here in terms of the matrix B in (11) as:

$$S = \{\mathbf{u} \in \mathbb{R}^m | B^T \mathbf{u} = 0\}. \tag{12}$$

Let us introduce now an important property concerning the *nature of the equilibrium points*. The equilibrium

[1] Also referred to in the literature as *stoichiometric compatibility class*.

points $\mathbf{c}^*$ of (5) must satisfy that $YA[\boldsymbol{\psi}(\mathbf{c}^*)] = 0$, then fulfilling at least one of the following conditions:

$$\boldsymbol{\psi}(\mathbf{c}^*) \in D_0 \equiv \ker A \tag{13}$$

$$A[\boldsymbol{\psi}(\mathbf{c}^*)] \in D_\delta \equiv \ker Y \cap \mathrm{ImA}. \tag{14}$$

The dimension of the subspace D_δ is the so called *deficiency*, computed for weakly reversible networks by the formula:

$$\delta = n - \ell - s \tag{15}$$

with ℓ being the number of linkage classes and s the dimension of the stoichiometric subspace S defined in (12). It can be deduced that all the reaction networks with deficiency equal to zero can only accept equilibrium solutions in D_0. On the other hand, a solution $\mathbf{c}^*$ such that $\boldsymbol{\psi}(\mathbf{c}^*) \in D_0$ is unique and stable (Feinberg, 1979). Therefore, as stated by the *deficiency zero theorem* (Feinberg, 1979), weakly reversible networks of zero deficiency have a unique and stable equilibrium point per stoichiometric compatibility class for any values taken by the reaction rate constants.

3. EQUILIBRIUM MANIFOLD

As it can be deduced from (14), the equilibrium points associated to the linear subspace D_δ are those $\mathbf{c}^*$ which image under $\boldsymbol{\psi}$ fulfills the following relation:

$$A[\boldsymbol{\psi}(\mathbf{c}^*)] = \sum_{i=1}^{\delta} \alpha_i \boldsymbol{w}_i \tag{16}$$

where α_i are real numbers and $\{\boldsymbol{w}_i\}_{i=1}^{\delta}$ is a basis for D_δ.

In (Otero-Muras et al., 2009), it has been proved that, for a given deficiency δ network, a basis for D_δ can be computed as follows:

$$\{\boldsymbol{w}_1, \ldots, \boldsymbol{w}_\delta\} = \ker\ [\Lambda \quad Y^T]^T \tag{17}$$

where Y is the molecularity matrix and Λ is a $n \times \ell$ matrix with columns being the vectors $\boldsymbol{\Lambda}_k$ defined by (9).

We will derive now an expression for the locus of equilibria or *equilibrium manifold*, denoted by $\mathcal{H}_s$, in terms of the new parameters α_i in (16). Our aim is to obtain a canonical expression for those $\mathbf{c}^*$ fulfilling $YA[\boldsymbol{\psi}(\mathbf{c}^*)] = 0$, which exploits the graph structure of the network. Here it is important to note that, a vector $\mathbf{c}^*$ belonging to the locus of equilibria (in the space of the species $\mathbb{R}_+^m$) must be such that its image under $\boldsymbol{\psi}$ simultaneously satisfies (16) and the mass action law (8). Solutions associated to D_0 will satisfy (16) with $\alpha_i = 0$ for $i = 1, \ldots, \delta$. Using $\boldsymbol{\psi}(\mathbf{c}^*)$ the equilibrium manifold can be described, in the space of the complexes $\mathbb{R}_+^n$, as the intersection between a linear variety coming from (16), that will be referred to as *the family of solutions* $\mathcal{F}$, and a nonlinear algebraic variety, corresponding to the mass action law condition, designated as *the mass action manifold* $\mathcal{M}$. Let us denote the locus of equilibria in the space of the complexes as $\mathcal{H}_c$. In order to obtain canonical expressions for both, the family of solutions $\mathcal{F}$ and the mass action manifold $\mathcal{M}$, the complexes of the network are numbered, without loss of generality, according to the following rules:

- r.1 the first ℓ nodes of the graph belong to different linkage classes,
- r.2 the first m rows of the matrix Y^T are linearly independent. Here we are assuming that the rank of Y is equal to the number of species. As it has been shown in (Otero-Muras et al., 2009), every network can be transformed in an equivalent network with as many pseudo species as the rank of the molecularity matrix.

3.1 The family of solutions.

We call *family of solutions* to a linear variety in the space of complexes, denoted by $\mathcal{F}$, such that:

$$A\mathcal{F} = \sum_{j=1}^{\delta} \alpha_j \boldsymbol{w}_j \tag{18}$$

where the set of vectors $\{\boldsymbol{w}_j\}_{j=1}^{\delta}$ defines a basis for the deficiency subspace D_δ and α_j are real parameters. It can be proved that the solution $\mathcal{F}$ can be written as:

$$\mathcal{F} = \sum_{k=1}^{\ell} \mathbf{x}_k \psi_k + \sum_{j=1}^{\delta} \alpha_j \mathbf{f}_j \tag{19}$$

where the vectors $\mathbf{x}_k$, $\mathbf{f}_j \in \overline{\mathbb{R}}_+^n$ are solutions of the following equations:

$$A[\mathbf{x}_k] = 0 \quad k = 1, \ldots, \ell, \tag{20}$$

$$A[\mathbf{f}_j] = \boldsymbol{w}_j \quad j = 1, \ldots, \delta. \tag{21}$$

Vectors $\mathbf{x}_k \in \overline{\mathbb{R}}_+^n$ for $k = 1, \ldots, \ell$ constitute a basis for the kernel of A. In fact, the number of elements of the basis coincides with the number of linkage classes. The structure of each vector $\mathbf{x}_k$ associated to a linkage class L_k is of the form:

$$x_{ik} = \begin{cases} 1 & i = k, \\ \rho_{ik} > 0 & i \neq k, \quad \mathcal{C}_i \text{ in } L_k \\ 0 & i \neq k, \quad \mathcal{C}_i \text{ not in } L_k \end{cases} \tag{22}$$

where the parameter $\rho_{ik} > 0$, corresponding to the complex i within the linkage class L_k, is a function of the kinetic constants in the given linkage class. Similarly, the parameter $\mathrm{f_{ij}}$, contains combinations of the original parameters of the network. Equations (20) and (21) lead to the relationships between the canonical parameters (ρ_{ik}, $\mathrm{f_{ij}}$), and the original rate constants.

3.2 The mass action manifold.

Let us denote by Y_1 the matrix constituted by the first m columns of the molecularity matrix Y. Taking into account that Y_1 is invertible by construction (according to r.2), we define a new matrix Q by:

$$Q^T = Y_1^{-1} \cdot Y. \tag{23}$$

The mass action manifold $\mathcal{M}$ in the space of the complexes is defined in terms of the columns of the matrix Q, denoted by $\mathbf{q}_1, \ldots, \mathbf{q}_m$, as follows:

$$\ln \mathcal{M} = \sum_{j=1}^{m} \mathbf{q}_j \ln \psi_j \tag{24}$$

Note that from (24), it follows that each element $\mathcal{M}_i$ can be written as:

$$\mathcal{M}_i = \prod_{j=1}^{m} \psi_j^{q_{ij}}. \tag{25}$$

3.3 The equilibrium manifold and its dimension

The intersection $\mathcal{H}_c$ of the *family of solutions* and the *mass action manifold* in the space of complexes $\mathbb{R}_+^n$ is the algebraic variety defined by:

$$\mathcal{H}_c(\boldsymbol{\psi}_m, \alpha) := \mathcal{F} - \mathcal{M} = 0 \tag{26}$$

and represents the locus of equilibria in the space of complexes. In this expression, $\boldsymbol{\psi}_m$ is the vector containing the first m components of $\boldsymbol{\psi} \in \mathbb{R}^n_+$. At this point it is important to note that the matrix Y_1, previously introduced, defines a bijective mapping between $\boldsymbol{\psi}_m$ and $\mathbf{c}$ of the form:

$$\ln \boldsymbol{\psi}_m = Y_1^T \ln \mathbf{c} \tag{27}$$

that allows us to transform $\mathcal{H}_c(\boldsymbol{\psi}_m, \alpha)$ into the equilibrium manifold $\mathcal{H}_s$:

$$\mathcal{H}_c(\boldsymbol{\psi}_m, \alpha) \rightarrow \mathcal{H}_s(\mathbf{c}, \alpha). \tag{28}$$

As shown in (Otero-Muras et al., 2009) the dimension of this manifold, either in the complex space or in the species space, is:

$$\lambda = m - s. \tag{29}$$

This characteristic dimension is used in (Otero-Muras et al., 2009) to classify the networks in *proper networks* ($\lambda = \delta$), *under-dimensioned networks* ($\lambda > \delta$), and *over-dimensioned networks* ($\lambda < \delta$). According to the expressions (15) and (29), the number of species m results to be equal, greater or lower than $n - \ell$, for proper, under, and over dimensioned networks, respectively.

4. CONDITION FOR MULTIPLE EQUILIBRIUM SOLUTIONS

In this section we introduce the main result of the paper: a condition on the parameters of a reaction network leading to multiple steady states.

Let us first define an augmented space setting the $m + \delta$ variables: $\psi_1, \dots \psi_m, \alpha_1, \dots, \alpha_\delta$. Let us denote by $D_{\boldsymbol{\psi}_m}\mathcal{H}_c \in \mathbb{R}^{(n-\ell)\times m}$ the jacobian of $\mathcal{H}_c$ with respect to $\boldsymbol{\psi}_m$, and by $D_\alpha \mathcal{H}_c \in \mathbb{R}^{(n-\ell)\times\delta}$ the jacobian of $\mathcal{H}_c$ with respect to the parameters α. The implicit function theorem guarantees the existence of a smooth manifold of solutions parameterized by α, provided that the following matrix:

$$D\mathcal{H}_c = [D_{\boldsymbol{\psi}_m}\mathcal{H}_c \quad D_\alpha \mathcal{H}_c] \tag{30}$$

is of full rank.

Let Ω represent the manifold given by the moiety conservation, see (11):

$$\Omega = B^T(\mathbf{c} - \mathbf{c}_0). \tag{31}$$

Let σ be the $m \times (m - s)$ matrix defined by:

$$\sigma^T = B^T \cdot diag(\mathbf{c}) \cdot (Y_1)^{-1} \cdot diag^{-1}(\boldsymbol{\psi}) \tag{32}$$

with $diag(\mathbf{v})$ and $diag^{-1}(\mathbf{v})$ being the diagonal and inverse diagonal matrices operating over the vector $\mathbf{v}$, respectively. We compute now the derivatives of Ω with respect to $\boldsymbol{\psi}_m$:

$$D_{\boldsymbol{\psi}_m}\Omega = \sigma^T \tag{33}$$

and with respect to α:

$$D_\alpha \Omega = 0. \tag{34}$$

Let us now build the following matrix:

$$G = \begin{bmatrix} D_{\boldsymbol{\psi}_m}\mathcal{H}_c & D_\alpha \mathcal{H}_c \\ D_{\boldsymbol{\psi}_m}\Omega & D_\alpha \Omega \end{bmatrix} \tag{35}$$

which has $(n - \ell + m - s)$ rows and $(m + \delta)$ columns. Taking into account the expression for the deficiency in (15), it can be deduced that $n - \ell + m - s = m + \delta$, and the matrix G is square by construction. If the matrix G is not of full rank, a vector $\boldsymbol{\tau} \neq 0 \in \mathbb{R}^{m+\delta}$ exists such that:

$$G \cdot \boldsymbol{\tau} = 0 \tag{36}$$

and therefore, the following equalities hold:

$$[D_{\boldsymbol{\psi}_m}\mathcal{H}_c \quad D_\alpha \mathcal{H}_c] \cdot \boldsymbol{\tau} = 0,$$
$$[\sigma^T \quad 0] \cdot \boldsymbol{\tau} = 0.$$

From these two equalities it can be deduced that the vector $\boldsymbol{\tau}$ is tangent to both the equilibrium manifold $\mathcal{H}_c$ and the nonlinear reaction polyhedron in the augmented space $\mathbb{R}^{m+\delta}$. As it is illustrated in the scheme of Fig. 2, this geometric condition is necessary for the manifold $\mathcal{H}_c$ and the reaction polyhedron in the augmented space $\mathbb{R}^{m+\delta}$ to intersect in more than one point. The polyhedron position (fixed by the initial concentration vector) will determine the number of intersections, which can be multiple provided that the geometric condition is fulfilled.

Summarizing, Let k be a parameter set for a weakly reversible biochemical reaction network with the dynamics given by (5). Let δ be the deficiency of the network graph. If for the set of parameters k there exists $\boldsymbol{\psi} \in \mathbb{R}^n_+$ and $\alpha \in \mathbb{R}^\delta$ such that

$$\text{rank}[G(\boldsymbol{\psi}_m, \alpha)] < m + \delta \tag{37}$$

with $G(\boldsymbol{\psi}_m, \alpha)$ defined by (35), the network will exhibit multiple steady states for some initial concentrations.

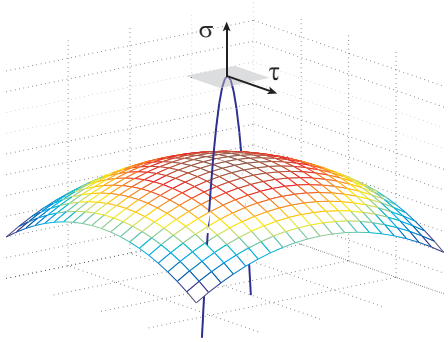


Fig. 2. Condition for multiplicities: the vector $\boldsymbol{\tau}$ is tangent to the equilibrium manifold (solid line) and orthogonal to the vector σ representing the reaction polyhedron (surface) in the augmented $m + \delta$ space

5. CASE STUDY: THE EDELSTEIN NETWORK

As a working example, we make use of the the so called Edelstein network (Feinberg, 1979), which is illustrated in Fig. 1. A number of variants of this network have been already introduced as case studies for the bifurcation analysis in the context of biochemical systems (Chickarmane et al., 2005). In Fig. 1 the complexes have been numbered according to ordering rules r.1 and r.2, such that the molecularity matrix (of rank 3) is:

$$Y = \begin{pmatrix} 1 & 0 & 0 & 1 & 2 \\ 0 & 1 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 \end{pmatrix}. \tag{38}$$

Applying (7), we obtain the vector of complexes $\boldsymbol{\psi}(\mathbf{c}) = (c_1, c_2, c_3, c_1c_2, c_1^2)^T$, and the expression for $A[\boldsymbol{\psi}]$ reads:

$$A[\boldsymbol{\psi}] = \begin{pmatrix} -k_{15} & 0 & 0 & 0 & k_{51} \\ 0 & -k_{23} & k_{32} & 0 & 0 \\ 0 & k_{23} & -(k_{32}+k_{34}) & k_{43} & 0 \\ 0 & 0 & k_{34} & -k_{43} & 0 \\ k_{15} & 0 & 0 & 0 & -k_{51} \end{pmatrix} \begin{pmatrix} \psi_1 \\ \psi_2 \\ \psi_3 \\ \psi_4 \\ \psi_5 \end{pmatrix}. \tag{39}$$

Table 1. Edelstein network parameter equivalences

ρ_{51}	ρ_{32}	ρ_{42}	f_3	f_4	f_5
$\frac{k_{15}}{k_{51}}$	$\frac{k_{23}}{k_{32}}$	$\frac{k_{23}k_{34}}{k_{32}k_{43}}$	$\frac{1}{k_{32}}$	$\frac{k_{32}+k_{34}}{k_{32}k_{43}}$	$\frac{1}{k_{51}}$

We construct a matrix Λ, with columns being the vectors $\mathbf{\Lambda}_k$ defined in (9):

$$\Lambda = \begin{pmatrix} 1 & 0 & 0 & 0 & 1 \\ 0 & 1 & 1 & 1 & 0 \end{pmatrix}^T. \tag{40}$$

The reaction polyhedron is defined by (11) with ($B^T = (0,\ 1,\ 1)$, that is, for the initial concentrations c_{1_0}, c_{2_0}, c_{3_0} the reaction polyhedron is the plane:

$$\Omega(\mathbf{c}_0): \quad c_2 + c_3 = c_{2_0} + c_{3_0}. \tag{41}$$

The stoichiometric subspace is two-dimensional, the network has five nodes and two linkage classes. Therefore, the deficiency according to the formula (15) is:

$$\delta = 5 - 2 - 2 = 1.$$

The basis for the subspace D_δ is computed using (17):

$$\boldsymbol{w} = (\ 1\ -1\ 0\ 1\ -1\ )^T. \tag{42}$$

The family of solutions $\mathcal{F}$ in (19) then reads:

$$\mathcal{F} = \begin{pmatrix} 1 \\ 0 \\ 0 \\ 0 \\ \rho_{51} \end{pmatrix} \psi_1 + \begin{pmatrix} 0 \\ 1 \\ \rho_{32} \\ \rho_{42} \\ 0 \end{pmatrix} \psi_2 + \alpha \begin{pmatrix} 0 \\ 0 \\ -\mathrm{f}_3 \\ -\mathrm{f}_4 \\ +\mathrm{f}_5 \end{pmatrix} \tag{43}$$

where the expressions for the parameters ρ_{ij} and f_{i1} (subindex 1 is omitted here), obtained by means of (20) and (21), are given in Table 1. To compute the mass action manifold we first obtain the matrix Y_1, constituted by the first m columns of the molecularity matrix:

$$Y_1 = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}. \tag{44}$$

Being Y_1 the identity matrix, the matrix Q^T (23) turns to be equal to the molecularity matrix Y (38). Then, the expression (24) for the mass action manifold reads:

$$\ln \mathcal{M} = \begin{pmatrix} 1 \\ 0 \\ 0 \\ 1 \\ 2 \end{pmatrix} \ln \psi_1 + \begin{pmatrix} 0 \\ 1 \\ 0 \\ 1 \\ 0 \end{pmatrix} \ln \psi_2 + \begin{pmatrix} 0 \\ 0 \\ 1 \\ 0 \\ 0 \end{pmatrix} \ln \psi_3. \tag{45}$$

The intersection of the family of solutions with the mass action manifold in the space of complexes, $\mathcal{H}_c(\boldsymbol{\psi}_m, \alpha)$ in (26), is given by the following equations:

$$\rho_{32}\psi_2 - \alpha \mathrm{f}_3 - \psi_3 = 0 \tag{46}$$
$$\rho_{42}\psi_2 - \alpha \mathrm{f}_4 - \psi_1\psi_2 = 0 \tag{47}$$
$$\rho_{51}\psi_1 + \alpha \mathrm{f}_5 - \psi_1^2 = 0. \tag{48}$$

Using the bijective mapping defined in (27), we have that:

$$\begin{pmatrix} \psi_1 \\ \psi_2 \\ \psi_3 \end{pmatrix} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} c_1 \\ c_2 \\ c_3 \end{pmatrix} \tag{49}$$

so that, the equilibrium manifold $\mathcal{H}_s(\mathbf{c}, \alpha)$ in (28) becomes:

$$\rho_{32}c_2 - \alpha \mathrm{f}_3 - c_3 = 0 \tag{50}$$
$$\rho_{42}c_2 - \alpha \mathrm{f}_4 - c_1c_2 = 0 \tag{51}$$
$$\rho_{51}c_1 + \alpha \mathrm{f}_5 - c_1^2 = 0. \tag{52}$$

Table 2. Edelstein Network parameters

k_{15}	k_{51}	k_{23}	k_{32}	k_{34}	k_{43}
8.5	1	0.2	1	1	1

The dimension of the equilibrium manifold is $\lambda = \delta = 1$. The network is *proper* and $D_{\boldsymbol{\psi}_m}\mathcal{H}_c$ is square:

$$D_{\boldsymbol{\psi}_m}\mathcal{H}_c = \begin{pmatrix} 0 & \rho_{32} & -1 \\ -\psi_2 & \rho_{42} - \psi_1 & 0 \\ \rho_{51} - 2\psi_1 & 0 & 0 \end{pmatrix}. \tag{53}$$

Taking into account (49) we have, for the Edelstein network, $\sigma^T = B^T$, and the matrix G in (35) reads:

$$G = \begin{pmatrix} 0 & \rho_{32} & -1 & -\mathrm{f}_3 \\ -\psi_2 & \rho_{42} - \psi_1 & 0 & -\mathrm{f}_4 \\ \rho_{51} - 2\psi_1 & 0 & 0 & \mathrm{f}_5 \\ 0 & 1 & 1 & 0 \end{pmatrix}. \tag{54}$$

Computing the determinant of the matrix G:

$$\begin{aligned} |G| = &- \mathrm{f}_5\psi_2(1 + \rho_{32}) - ... \\ &... (2\psi_1 - \rho_{51})\left[\mathrm{f}_4(1 + \rho_{32}) + \mathrm{f}_3(\psi_1 - \rho_{42})\right] \end{aligned}$$

and setting $|G| = 0$, we arrive to:

$$\begin{aligned} 0 = &(k_{23} + k_{32})k_{43}\psi_2 + ... \\ &... (2k_{51}\psi_1 - k_{15})(k_{32} + k_{34} + k_{23} + k_{43}\psi_1). \end{aligned} \tag{55}$$

It is deduced from (55) that the determinant can only vanish provided that

$$\psi_1 = \frac{\beta\rho_{51}}{2} \quad \text{for some} \quad 0 < \beta < 1. \tag{56}$$

Note that expression (56) takes in account that ψ_1 must be positive for a feasible solution to exist. Substituting (56) into (48) we arrive to:

$$\alpha = \frac{\beta(\beta - 2)k_{15}^2}{4k_{51}} \tag{57}$$

that introduced in (47) gives us:

$$\psi_2 = \frac{\beta(\beta - 2)\rho_{51}^2 \mathrm{f}_4}{2\mathrm{f}_5(2\rho_{42} - \beta\rho_{51})} \tag{58}$$

since $0 < \beta < 1$, we have that $\beta - 2 < 0$, and so that ψ_2 is positive for

$$1 > \beta > 2\frac{\rho_{42}}{\rho_{51}} > 0. \tag{59}$$

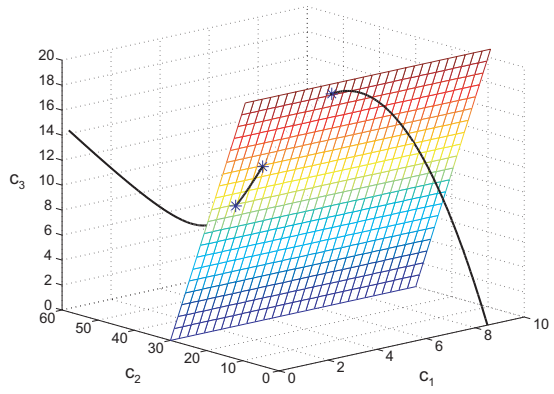
Substituting (57) and (58) in (46), it can be deduced that ψ_3 is positive provided (59). Summarizing, the Edelstein network will show multiplicities for those parameters fulfilling (55) with:

$$\psi_1 = \frac{\beta\rho_{51}}{2}, \quad \psi_2 = \frac{\beta(\beta - 2)\rho_{51}^2 \mathrm{f}_4}{2\mathrm{f}_5(2\rho_{42} - \beta\rho_{51})}$$

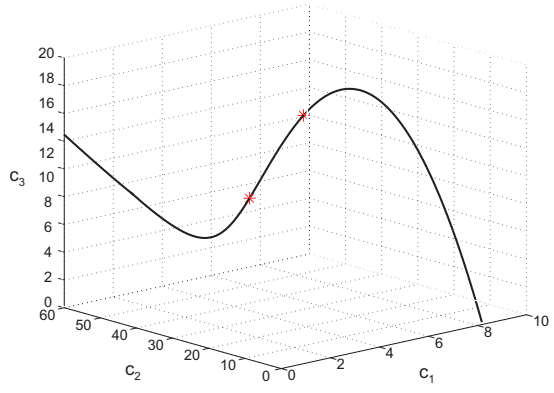
for β satisfying inequality (59). Let us consider the set of kinetic constants given in Table 2, they satisfy the condition (55) for $\beta = 0.6451$, and the resulting values for ψ_1 and ψ_2 are:

$$\psi_1 = 2.7416, \quad \psi_2 = 12.4230$$

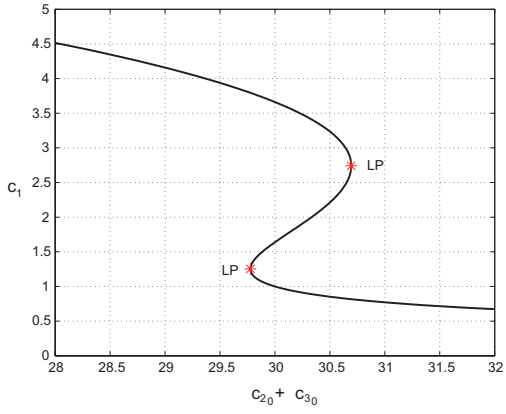
In Fig. 3(a), the equilibrium manifold for these values of the kinetic constants is depicted, together with the reaction polyhedron corresponding to $c_{2_0} + c_{3_0} = 30$. The manifold is one dimensional, and intersects the reaction polyhedron in three points, corresponding to three different equilibria. In Fig. 3(b), the equilibrium manifold is depicted showing the points fulfilling the rank deficiency condition, corresponding to $\alpha = -15.787$ and $\alpha = -9.079$. As it can be deduced from Fig. 3(a), three steady states



(a)



(b)



(c)

Fig. 3. (a) Equilibrium manifold for parameters in Table 2. Blue stars are steady states for $c_{2_0} + c_{3_0} = 30$. (b) Equilibrium manifold for parameters in Table 2. Red stars are points in which the rank deficiency condition is fulfilled. (c) Equilibrium curve for the Edelstein network, varying $c_{2_0} + c_{3_0}$. The kinetic parameters are kept fixed with the values shown in Table 2.

will exist for a range of the sum of initial concentrations $c_{2_0} + c_{3_0}$. In fact, performing a continuation of the curve of equilibria for the Edelstein network by varying the values of $c_{2_0} + c_{3_0}$, we obtain the curve shown in Fig. 3(c), where two limit points or saddle node bifurcations appear for $c_{2_0} + c_{3_0} = 29.7768$ and $c_{2_0} + c_{3_0} = 30.6949$. Within these values, corresponding to different positions of the reaction polyhedron, three steady states will exist.

6. CONCLUSIONS AND FUTURE WORK

In this work, we have provided a condition on the parameters of a biochemical network to have multiple steady states, extending previous results and concepts of CRNT. The applicability to large scale systems is limited by the computational capability of the symbolic solvers to obtain the matrix expressions in Section 4. In a future work, it will be shown how the condition can be systematically checked numerically through the parameter space by means of interval methods (Csendes and Pal, 2008).

REFERENCES

Angeli, D., de Leenheer, P., and Sontag, E. (2007). A petri nets approach to the study of persistence in chemical reaction networks. *Mathematical Biosciences*, 210, 598–618.

Angeli, D., Ferrell, J., and Sontag, E. (2004). Detection of multistability, bifurcations, and hysteresis in a large class of biological positive-feedback systems. *Proc Natl Acad Sci USA*, 101, 1822–1827.

Chickarmane, V., Paladugu, S., Bergmann, F., and Sauro, H. (2005). Bifurcation discovery tool. *Bioinformatics*, 21, 3688–3690.

Conradi, C., Flockerzi, D., Raisch, J., and Stelling, J. (2007). Subnetwork analysis reveals dynamic features of complex (bio)chemical networks. *Proc Natl Acad Sci USA*, 104, 19175–19180.

Craciun, G. and Feinberg, M. (2005). Multiple equilibria in complex chemical reaction networks: I. the injectivity property. *SIAM J Appl Math*, 65, 1526–1546.

Craciun, G., Tang, Y., and Feinberg, M. (2006). Understanding bistability in complex enzyme-driven reaction networks. *Proc Natl Acad Sci USA*, 103, 8697–8702.

Csendes, T. and Pal, L. (2008). A basic interval global optimization procedure for matlab/intlab. *Proceedings of the International Symposium on Nonlinear Theory and its Applications NOLTA2008, Budapest.*

Feinberg, M. (1979). Lectures on chemical reaction networks. *Notes of lectures given at the Mathematics Research Center, University of Wisconsin.*

Feinberg, M. (1995). Multiple steady states for chemical reaction networks of deficiency one. *Arch Rational Mech Anal*, 132, 371–406.

Kitano, H. (2002). Systems biology: A brief overview. *Science*, 295, 1662–1664.

Lu, J., Engl, H.W., and Schuster, P. (2006). Inverse bifurcation analysis: application to simple gene systems. *Algorithms for molecular biology*, 1, 1–11.

Otero-Muras, I., Banga, J.R., and Alonso, A.A. (2009). Exploring multiplicity conditions in enzymatic reaction networks. *Biotechnology Progress*, 25, 619–631.

Otero-Muras, I., Szederkenyi, G., Alonso, A.A., and Hangos, K.M. (2008). Local dissipative hamiltonian description of reversible reaction networks. *Systems & Control Letters*, 57, 554–560.