Exploring Multiplicity Conditions in Enzymatic Reaction Networks

Irene Otero-Muras, Julio R. Banga, and Antonio A. Alonso
Process Engineering Group, IIM-CSIC, Spanish Council for Scientific Research, Eduardo Cabello 6, 36208 Vigo, Spain
Correspondence concerning this article should be addressed to A. A. Alonso at antonio@iim.csic.es.

In this work, a novel algorithmic approach to detect multiplicity of steady states in enzymatic reaction networks is presented. The method exploits the structural properties of networks derived from the Chemical Reaction Network Theory. In first instance, the space of parameters is divided in different regions according to the qualitative behavior induced by the parameters in the long term dynamics of the network. Once the regions are identified, a condition for the appearance of multiplicities is checked in the different regions by solving a given optimization problem. In this way, the method allows the characterization of the whole parameter space of biochemical networks in terms of the appearance or not of multistability. The approach is illustrated through a well-known case of enzymatic catalysis with substrate inhibition.

Keywords: multistability, biochemical reaction networks, nonlinear analysis, CRNT, biological complexity

Introduction

It has been shown that biochemical reaction networks catalyzed by enzymes can exhibit complex nonlinear behavior, such as multiple steady states or oscillations, for some ranges of the kinetic rates (Qiao et al., 2007). Understanding how a particular observed biological behavior arises out of the network topology and parameters (Lu et al., 2006) requires the use of mathematical models. Assuming negligible spatial distribution and isothermal conditions, the model describing the evolution of the concentrations $c$ in a
reaction network consists on a system of nonlinear first order ordinary differential equations (ODEs) of the form:

\[ \dot{c} = f(c, k) \]  

(1)

where the kinetic rates constants included in \( k \) are the parameters of the model.

The Chemical Reaction Network Theory (CRNT) revealed some interesting structural features behind (1). One of the main achievements of the CRNT was to discriminate whether or not a given mechanism can support multiple steady states by analysis of the network topology. More precisely, based on properties of the graphs associated to a reaction network, one can assert whether the network will have a unique steady state or not, independently of the values taken by the parameters (Craciun et al., 2006).

However, for those enzymatic networks that cannot be proved to have a unique equilibrium in spite of the values of the parameters, a key question remains: which ranges of the parameters, or what regions in the parameter space will be associated to a particular long term dynamic behavior? (for example, what region in the parameter space will be associated to the appearance of multiple steady states?), in other words, which is the mapping from the parameter space to the space of model behavior?

Classical bifurcation analysis can be used to investigate this relationship in the whole parameter space provided that the number of variation parameters is small. Alternatively, a method based on the theory of monotone systems has been proposed by Angeli et al. (2004) to detect the appearance of multistability in biological positive-feedback networks, as well as the range of feedback strengths giving rise to it. The method exploits the property of monotonicity and can be applied for different kind of kinetics, such as Michaelis Menten or Hill type (i.e., reduced-order models).

In this work, we describe a methodology to detect multiplicities in biochemical reaction networks of the mass action type, based on the structural properties revealed by CRNT (Feinberg, 1979). The mass action networks correspond to full order models, capable of reproducing the behavior of the system in variable conditions, and are normally endowed with a high number of parameters (corresponding to the kinetic reaction rates of each reaction step). The proposed method allows us to partition the space of
parameters in regions according to the different qualitative features exhibited by the system solution set. On these regions, some conditions are examined to find possible multiplicities. In that aim, we first derive, from the underlying network structure, a canonical representation of the manifold of equilibria. Analyzing this canonical form, the regions with different qualitative behavior in the space of parameters arise by the variation of as few parameters as the deficiency of the network. The deficiency is the dimension of a subspace characteristic of reaction systems (Feinberg, 1979) and it is generally a low number, orders of magnitude lower than the total number of kinetic parameters. Such regions will be afterward explored for possible multiplicities by checking a given condition somehow related to the curvature of the solution manifold. The search for multiple equilibria will be carried out on an optimization framework.

It is important to highlight here the implications of the method proposed in robust control of enzymatic reaction systems. Once the space of parameters is divided in different regions in terms of the qualitative features of the system solution set, the desired behavior of the system can be attained just maintaining the whole set of parameters of the closed loop system within the appropriate range, where the system can be driven by manipulating, for example, enzyme concentrations (Otero-Muras et al., 2007).

The article is structured as follows: In the following section a standard enzymatic reaction system will be employed to discuss and to illustrate the basic ingredients of CRNT that will be needed in the sequel. ‘‘The Manifold of Equilibrium Solutions’’ section will be devoted to provide a formal description of the manifold of equilibrium solutions for reaction networks. Such canonical representation will serve as the basis to propose a classification of reaction networks in terms of the dimension of the manifold. Finally, the approach employed to detect possible multiplicities in the parameter space, including the algorithmic aspects of the method, will be presented in ‘‘An Algorithmic Approach for the Detection of Multiplicities’’ section. The article ends with some conclusions and guidelines for further work.

Essentials of CRNT
In this section, the basic ingredients of the CRNT needed in the sequel will be briefly described. In particular, we will focus on those aspects from CRNT regarding (i) the implications of the mass action law, (ii) the existence of reaction invariants and (iii) the nature of the equilibrium points. Special attention will be paid to the notion of deficiency of a given reaction network (Feinberg, 1979) as it defines the dimension of a particular subspace where multiple equilibrium solutions for the network may appear. The end of the section will be devoted to explain how open networks (those networks exchanging material with the environment by means of input and output flows through the boundary of the system), can be incorporated into the CRNT description. Although a more formal description of the CRNT is out of the scope of this work, details for the interested reader can be found in Feinberg’s lectures (Feinberg, 1979).

In order to motivate the main aspects of the theory and to prepare the reader for the algorithm for multiplicity detection to be discussed in the subsequent sections, the description will be developed on the basis of an example consisting of a given enzymatic reaction presented in Figure 1 in its graph form. The example consists of an enzymatic catalysis with substrate inhibition as depicted in Figure 1. In this mechanism, substrate \( S \) is converted into \( P \) by the enzyme \( E \), and the intermediate complex \( ES \) reacts also with the substrate to form a dead-end complex \( ESS \). It must be pointed out that under appropriate conditions, a reduced order dynamic description for this mechanism can be obtained (Tzafirir and Edelman, 2007). For example, conducting the enzymatic reaction under an excess of substrate, bound enzyme forms \( (ES, ESS) \) can be maintained in quasi-steady-state conditions, thus resulting into a reduced order model with states \([S]\) and \([P]\), which coincides with the standard Michaelis-Menten rate law with substrate inhibition*:

\[
\frac{d[S]}{dt} = -\frac{k_{21}E_T[S]}{K_M + [S] + [S]^2 / K_I}
\]

in this expression, \( E_T \) corresponds with the concentration of the total amount of enzyme (in bounded and unbounded form) while \( K_M \) and \( K_I \) coincide with the Michaelis–Menten and the inhibition constants, respectively. Both parameters are related to the constants of the original mechanism by the following equivalences:

\[
K_M = \frac{k_{21} + k_{23}}{k_{12}} \quad K_I = \frac{k_{54}}{k_{45}}
\]
However, such simplified versions of the dynamics can be misleading for certain intracellular conditions or even industrial applications thus calling for a ‘‘full order’’ mechanistic interpretation in terms of the mass action law. In addition, the reduced order dynamics might in some instances obscure the understanding of the observed behaviour when the assumptions are not correctly handled. In this way, the framework offered by a reaction network theory based on the mass action law seems to be fundamental in order to keep clarity and physical insight. However, such description presents a number of difficulties related with the assessment of complex dynamic behavior such as multiplicities of equilibrium states or oscillations. This is mainly due to a much larger number of kinetic rate constants which translates into a higher dimensional parameter space much more difficult to explore for possible multiplicities through classical bifurcation analysis. Fortunately, mass action law networks conceal a strong underlying structure that can be exploited once accommodated into the CRNT framework. Any enzymatic reaction system can be accommodated into the chemical reaction network framework under standard assumptions, namely that reactions take place in isothermal conditions, and that the spatial distribution of chemicals—including biocatalysts (Berendsen et al., 2006)—can be neglected.

The rest of this section will be dedicated to outline the main features of the dynamic structure, taking the reaction network depicted in Figure 1 as a working example. To that purpose, let us interpret the kinetic mechanism as a graph with nodes $C_1, \ldots, C_n$ corresponding to the complexes of the network (i.e. reactants or products that appear on the left and right hand sides of each reaction step), and connected through edges indicating the reaction direction and positive rate constants $k_{ij}$ for $i, j = 1, \ldots, n$. The dynamic evolution of the species concentration will be described by a set of ordinary differential equations with the following structure:

$$\frac{dc}{dt} = Y \cdot A_k \cdot \psi(c)$$

where $c \in \mathbb{R}^r$ is a vector collecting the concentrations for the $r$ species, which in our case correspond with $E, S, ES, ESS$, and $P$.

Matrix $Y \in \mathbb{N}^{rxn}$, also known as the molecularity matrix, contains as elements the stoichiometric coefficients of the species (rows) in each complex (columns). $A_k \in \mathbb{R}^{nxn}$
is a linear matrix closely related to the adjacency matrix of the graph that contains the kinetic constants of the network. For the case considered, both matrices take the form:

\[
Y = \begin{bmatrix}
1 & 0 & 1 & 0 & 0 \\
1 & 0 & 0 & 1 & 0 \\
0 & 1 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 1 \\
0 & 0 & 1 & 0 & 0 \\
\end{bmatrix}
\]

(5)

\[
A_k = \begin{bmatrix}
-k_{i2} & k_{21} & 0 & 0 & 0 \\
k_{12} & -(k_{21} + k_{23}) & k_{32} & 0 & 0 \\
0 & k_{23} & -k_{32} & 0 & 0 \\
0 & 0 & 0 & -k_{45} & k_{54} \\
0 & 0 & 0 & k_{45} & -k_{54} \\
\end{bmatrix}
\]

Finally, the vector \( \psi(c) \in \mathbb{R}^n \) contains as elements the mass action law monomials associated to each complex \( j \) (node in the graph) so that:

\[
\psi_j(c) = c_1^{y_{1j}} c_2^{y_{2j}} \cdots c_n^{y_{nj}}
\]

(6)

where the exponents \( y_{ij} \) are elements of the molecularity matrix \( Y \). For the example in Figure 1, vector \( \psi(c) \) reads:

\[
\psi(c) = (c_1 c_2 \ c_3 \ c_1 c_3 \ c_2 c_3 \ c_4)^T
\]

(7)

Provided that \( c > 0 \), the mass action law (6) for all complexes can be alternatively written in the following more compact form, to be used later on:

\[
\ln \psi(c) = Y^T \ln c
\]

(8)

where the function \( \ln(-) \) operates on the vectors \( \psi \) and \( c \) element-wise.

In CRNT standard terminology, each of the “isolated” subgraphs that constitute the graph of a reaction network is known as a linkage class (Feinberg, 1979). The network represented in Figure 1 is composed of two linkage classes, one containing the complexes \( C_1, C_2 \) and \( C_3 \) while the other consists of complexes \( C_4 \) and \( C_5 \). A linkage class is said to be weakly reversible when every complex in the linkage class can be connected to the rest by sequences of direct paths. A set of weakly reversible linkage
classes constitutes a weakly reversible reaction network. The graph in Figure 1 is an example of weakly reversible reaction network. In this article we concentrate on weakly reversible reaction networks for which trajectories of (4) that start at any positive initial condition \( c_0 > 0 \) will remain in the positive orthant. For this class of networks, mass conservation constrains the evolution of the trajectories—solutions of (4)—in the concentration space to a reduced convex region in the positive orthant known as the reaction polyhedron. This region is constructed as the intersection of the reaction invariants of the network, being of the form \( C_T = B^T c \), with the positive orthant. By definition, reaction invariants satisfy:

\[
C_T = B^T Y \cdot A_k \cdot (c) = 0 \tag{9}
\]

Condition (9), obtained by computing the time derivative of \( C_T \) along (4) is employed to get matrix \( B \) and to formally define the reaction polyhedron as:

\[
\Omega(c_0) = \{ c > 0 / B^T (c - c_0) = 0 \text{ with } (Y \cdot A_k)^T B = 0 \} \tag{10}
\]

where \( c_0 \) is a given concentration reference so that \( C_T = B^T c_0 \). For the example in Figure 1, there exist two reaction invariants of the form:

\[
ET = c_1 + c_3 + c_4 \\
ST = c_2 + c_3 + 2c_4 + c_5 \tag{11}
\]

with \( E_T \) and \( S_T \) being the initial enzyme and substrate concentrations, respectively. The explicit form for matrix \( B \) then becomes:

\[
B = \begin{pmatrix} 1 & 0 & 1 & 1 & 0 \\ 0 & 1 & 1 & 2 & 1 \end{pmatrix}^T \tag{12}
\]

Geometrically, the reaction polyhedron associated to the example consists of a linear three-dimensional region in the space of the species determined by the intersection of (11) with the positive concentration orthant.

For a given initial condition \( c_0 \), an equilibrium \( c^* \) of (4)—namely one positive vector \( c^* \) such that \( c^* = 0 \)—corresponds with the intersection between the curve of solutions, that is, the set of points \( c \) satisfying \( Y \cdot A_k \cdot \psi (c) = 0 \) and the reaction polyhedron defined at the reference \( c_0 \) (10). A graphical interpretation is provided in Figure 2 for a three species network. A given point \( c^* \) is said to belong to the curve of equilibrium solutions
for (4) if the corresponding vector \( \psi(c^*) \) either lies on the kernel of \( A_k \), or its image through \( A_k \) lies on the kernel of the molecularity matrix \( Y \). Both the kernel of \( A_k \) and the intersection of the image of \( A_k \) with the kernel of \( Y \) define two subspaces we will refer to as \( D_0 \) and \( D_\delta \) respectively, where equilibrium solutions belong. This can be formally stated by saying that the set of equilibrium solutions \( c^* \) are those which satisfy at least one of the two following conditions:

\[
\begin{align*}
C.1. & \psi(c^*) \in D_0 = \ker A_k \\
C.2. & A_k \cdot \psi(c^*) \in D_\delta = \ker Y \cap \text{Im}(A_k)
\end{align*}
\]

Remarkably, CRNT shows that the intersection of the curve of solutions associated with \( D_0 \) (C.1) with any reaction polyhedron is unique and stable for any combination of positive reaction rate constants (Feinberg, 1979). Thus multiple equilibrium solutions, if there exist, must be found in the intersection between the reaction polyhedron and solutions satisfying C.2. The dimension of subspace \( D_\delta \) is known as the deficiency of the network and can be computed from the graph by the formula (Feinberg, 1979):

\[
\delta = n - l - s
\]

where \( n \) and \( l \) are the number of complexes and linkage classes of the network, respectively, and \( s \) is the dimension of the stoichiometric subspace associated to the reaction polyhedron.† In the sequel we will refer to subspace \( D_\delta \) as the deficiency subspace. Whenever \( \delta = 0 \) (i.e. the dimension of subspace \( D_\delta \) is zero) the curve of equilibrium solutions can only be associated to \( D_0 \). Consequently, its intersection with any reaction polyhedron (10) would be unique and stable despite the values taken by the set of reaction rate constants. This constitutes the essence of the well celebrated zero deficiency theorem (Feinberg and Horn, 1974).

The network considered in Figure 1 consists of 5 complexes, 2 linkage classes and a stoichiometric subspace of dimension \( s = 3 \). Thus according to (13) the deficiency of the network results to be \( \delta = 5 - 2 - 3 = 0 \). Consequently, and according to the zero deficiency theorem it only accepts a unique and stable equilibrium solution for any given reaction polyhedron.
The approach we discuss in this article exploits these facts to set up a systematic methodology to search for possible multiplicities in the deficiency subspace $D_δ$. This will be described in the next section.

We end up this section with some comments on how to accommodate open networks exchanging material with the environment into the CRNT formulation. Assuming complete mixing on a domain with volume $V$ and constant input-output fluxes, the species mole balances become:

$$\frac{dc}{dt} = \frac{1}{V} F(c_0 - c) + R(c) \quad (14)$$

where the first term in the right hand side describes the flow exchanged between the domain and the environment, and the second term coincides with the reaction rates taking place in the domain. $F \in \mathbb{R}^{nxr}$ is a diagonal matrix of nonnegative elements, being zero for those species which are not exchanged with the environment and positive otherwise. For instance, let us consider that the kinetic mechanism in Figure 1 is taking place on a well-mixed domain exchanging substrate and product with the environment. This can be the case, for example, when an immobilized enzyme remains in a stirred reactor with a constant flow of substrate and product. Equation (14) can then be encoded in the structure (4) by modeling the component inlet and outlet fluxes as pseudo reactions of zero and first order, respectively. The environment then becomes an extra node, represented in the network as a zero node. As a result, the new graph incorporates a new linkage class containing the zero node which represents the environment plus two nodes standing for the substrate and product, respectively. Such graph describing the open network is depicted in Figure 3. Assuming a constant inlet flow carrying substrate and product where $\phi$ is the inverse of the residence time and $S_0$ and $P_0$ their input concentrations, reactions leaving complex $C_7$ are zero order and have as rate constants $k_{76} = \phi S_0$ and $k_{78} = \phi P_0$. Reactions going to $C_7$ are first order with rate constants $k_{67} = k_{87} = \phi$.

For this example Eq. 14 in the form (4) now reads:
\[
\frac{dc}{dt} = \begin{bmatrix}
1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
1 & 0 & 0 & 1 & 0 & 1 & 0 & 0 \\
0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \\
\end{bmatrix}
\begin{bmatrix}
-k_{12} & k_{21} & 0 & 0 & 0 & 0 & 0 & 0 \\
k_{12} & -(k_{21} + k_{23}) & k_{32} & 0 & 0 & 0 & 0 & 0 \\
0 & k_{23} & -k_{32} & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -k_{45} & k_{54} & 0 & 0 & 0 \\
0 & 0 & 0 & k_{45} & -k_{54} & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & -k_{67} & k_{76} & 0 \\
0 & 0 & 0 & 0 & 0 & k_{67} & -(k_{76} + k_{78}) & k_{87} \\
0 & 0 & 0 & 0 & 0 & 0 & k_{78} & -k_{87} \\
\end{bmatrix}
\begin{bmatrix}
c_1c_2 \\
c_3 \\
c_1c_5 \\
c_2c_3 \\
c_4 \\
c_2 \\
c_5 \\
\end{bmatrix}
\]

(15)
This network accepts one reaction invariant corresponding to the conservation of $E_T$ in (11). Thus $B$ in (10) takes now the form:

$$B = \begin{pmatrix} 1 & 0 & 1 & 0 \end{pmatrix}^T$$

(16)

The deficiency of the resulting network now becomes $\delta = 8 - 3 - 4 = 1$. Consequently, the conditions of the zero deficiency theorem do not hold any longer, thus leaving room for multiplicity, at least for some reaction polyhedrons. Such solutions if they exist must somehow be related to the deficiency subspace $D_\delta$. The method we discuss in the following sections aims at exploring such subspace in search for possible multiplicities.

The Manifold of Equilibrium Solutions

In this section, a formal structure will be given for the set of equilibrium solutions $c^*$ of (4). Essentially we look at those concentrations which satisfy the equilibrium condition:

$$0 = c^* A_k \cdot \psi(c^*)$$

with $\psi(c^*)$ being the vector of mass action monomials discussed in the earlier section. To that purpose, we take advantage of the results by Feinberg on deficiency zero theory. In particular, use will be made of the subspaces $D_0$ and $D_\delta$ which as described in the earlier section (Conditions C.1. and C.2.) are related to all possible equilibrium solutions. Because conditions are stated in terms of complexes $\psi$, the set of solutions will be defined in the $n$-dimensional space of complexes rather than in the concentration space. In the complex space, the manifold of equilibrium solutions will emerge from the intersection of two distinct manifolds: a linear variety representing the vectors that satisfy either C.1. or C.2., which we will call the family of solutions, and a nonlinear manifold (the mass action manifold) which defines vectors compatible with the mass action law (8). The first part of this section will be devoted to describe the family of solutions and the mass action law, both in the space of complexes, as well as their intersection. In the second part of the section the dimensions of the resulting solution manifold will be discussed. On the basis of that, a network classification is proposed.

Canonical form of the solution manifold
Although the order of the complexes in the network (i.e. the assignment of a cardinal to each complex so to form the sequence $C_1, C_2, ..., C_n$) is arbitrary, for convenience we propose a set of ordering rules which will simplify the construction of a canonical representation for the solution manifold. The objective of such ordering criteria is twofold: Firstly, it defines a systematic way of constructing the manifold that can be easily automated. Secondly, it exploits the connections between the graph and the manifold structures.

Let us consider a $\delta$-deficiency network with $n$ nodes, $\ell$ linkage classes and $r$ species. In addition, let us consider a a full rank molecularity matrix $Y$. We also assume that the number of species is smaller than the number of complexes‡ so that $\text{rank}(Y) = r$. Then complexes are ordered so that they satisfy the following two conditions:

1. The first complexes $C_1, ..., C_{\ell}$ are chosen one from each linkage class

2. complexes $C_1, ..., C_r$ are chosen so that the corresponding columns in matrix $Y$ are linearly independent

For example, applying these conditions to the open enzymatic mechanism in Figure 3 results into the equivalent representation depicted Figure 4. Columns of the molecularity matrix in Eq. 15 have being re-ordered accordingly so to get:

$$Y = \begin{pmatrix}
0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 \\
0 & 1 & 1 & 0 & 1 & 0 & 0 \\
1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 1
\end{pmatrix}$$ (18)

It is worth noting that by construction, the first $r = 5$ columns constitute an invertible $r \times r$ matrix. The corresponding $A_k$ and $\psi$ now read as:
\[
A_k = \begin{pmatrix}
-(k_{14} + k_{18}) & 0 & 0 & k_{41} & 0 & 0 & 0 & k_{81} \\
0 & -k_{26} & 0 & 0 & 0 & k_{62} & 0 & 0 \\
0 & 0 & -k_{37} & 0 & 0 & 0 & k_{73} & 0 \\
k_{14} & 0 & 0 & -k_{41} & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & -k_{57} & 0 & k_{75} & 0 \\
0 & k_{26} & 0 & 0 & 0 & -k_{62} & 0 & 0 \\
0 & 0 & k_{37} & 0 & k_{57} & 0 & -k_{75} + k_{73} & 0 \\
k_{18} & 0 & 0 & 0 & 0 & 0 & 0 & -k_{81}
\end{pmatrix}, \psi(c) = \begin{pmatrix} c_3 \\
\cdot \\
\cdot \\
\cdot \\
c_4 \\
c_2 \\
c_5 \\
c_2c_3 \\
c_1c_3 \\
1 \\
c_1c_4
\end{pmatrix}
\]
The Family of Solutions. We call family of solutions to a n-dimensional linear variety in
the space of complexes we denote by F and which corresponds with the solution of the
following linear equation:

\[ A_k F = \sum_{j=1}^{\delta} \alpha_j w_j \] (20)

where the set of vectors \( \{w_j\}_{j=1}^{\delta} \) defines a basis for the deficiency subspace \( D_\delta \), and \( \alpha_i \)
are real parameters. A method to compute a basis for \( D_\delta \) is presented in Appendix. The
explicit form of the solution \( F \) can be written as:

\[ F = \sum_{j=1}^{\ell} x_j(k) \psi_j + \sum_{j=1}^{\delta} \alpha_j f_j(k) \] (21)

where the vectors \( x_j, f_j \in \mathbb{R}^n \) are solutions of the following equations:

\[ A_k x_j = 0 \quad j = 1, \ldots, \ell \] (22)

\[ A_k f_j = w_j \quad j = 1, \ldots, \delta \] (23)

Vectors \( x_j \in \mathbb{R}^n \) for \( j = 1, \ldots, \ell \) constitute a basis for the kernel of \( A_k \). In fact, as described
in Proposition 4.1 of Feinberg’s lectures, the number of elements of the basis coincide
with the number of linkage classes. This proposition—we summarize in Appendix for
the sake of completeness—also describes the structure of each vector in the basis. In
this way, for each vector \( x_j \) associated to a linkage class \( L_j \), its elements are of the form:

\[ x_{ij} = \begin{cases} 1 & i = j, \\ \rho_{ij} & i \neq j, \ c_i \in L_j \\ 0 & i \neq j, \ c_i \notin L_j \end{cases} \] (24)

where the parameters \( \rho_{ij} > 0 \), corresponding to the complexes \( i \) within the linkage class
\( L_j \), are function of the kinetic constants in the given linkage class. Similarly, the
structural of of vectors \( f_j(k) \) contains combinations of the original kinetic parameters of
the network. For the open network depicted in Figure 4 the corresponding family of
solutions (21) takes the form:
Solution of (22) and (23) lead to the relationships between canonical parameters \( \rho_{ij} \) and \( f_{i1} \) and the original rate constants. For the open network, the relationships are presented in Table 1.

The Mass Action Manifold. Let us now obtain a canonical expression for the mass action manifold in the space of complexes. In this aim, a new matrix \( \mathbf{Q} \) is defined starting from the molecularity matrix \( \mathbf{Y} \) as follows:

1. We denote as \( \mathbf{Y}_1 \) the partition constituted by the first \( r \) columns of the molecularity matrix \( \mathbf{Y} \). As discussed previously this matrix is invertible by construction.

2. A new matrix \( \mathbf{Q} \) is then computed from \( \mathbf{Y}_1 \) and \( \mathbf{Y} \) as

\[
\mathbf{Q}^T = \mathbf{Y}_1^{-1} \cdot \mathbf{Y}
\]

(26)

The mass action manifold \( \mathbf{M} \) in the space of the complexes is defined in terms of the columns of the matrix \( \mathbf{Q} \), denoted as \( (q_1, \ldots, q_r) \):

\[
\ln \mathbf{M} = \sum_{j=1}^{r} q_j \ln \psi_j
\]

(27)

Note that from Eq. 27, it follows that each element \( M_i \) can be written as:

\[
M_i = \prod_{j=1}^{r} \psi_j^{q_j} \quad i = 1, \ldots, n
\]

(28)

This expression will be the basis to compute the elements of the jacobian associated to \( \mathbf{M} \). Following with the open Michaelis-Menten network example, matrix \( \mathbf{Y}_1 \)
corresponds with the first 5 columns of \( Y \) (18) which by construction is invertible. Thus for this case the manifold (27) takes the form:

\[
\begin{pmatrix}
1 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 1 \\
1 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 \\
0 & 0 & -1 & 1 & 1 \\
\end{pmatrix}
\begin{pmatrix}
\psi_1 \\
\psi_2 \\
\psi_3 \\
\psi_4 \\
\psi_5 \\
\end{pmatrix}
\]

\[
\text{In } M = \begin{pmatrix}
1 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 1 \\
1 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 \\
0 & 0 & -1 & 1 & 1 \\
\end{pmatrix}
\begin{pmatrix}
\psi_1 \\
\psi_2 \\
\psi_3 \\
\psi_4 \\
\psi_5 \\
\end{pmatrix}
\]

Note that the matrix in the above expression corresponds with \( Q \) in (26).

The Manifold of Equilibrium Solutions. The manifold of solutions in the space of complexes is given by the intersection of the linear \( \ell \)-dimensional family of solutions with the nonlinear \( r \)-dimensional mass action manifold. This is formally represented as follows:

\[
H_c(\psi_m, \rho, k, \alpha) := F - M = 0
\]

(30)

where \( w_m \) is the vector constituted by the first \( m = \sup(r, \ell) \) components of \( w \). For the example in Figure 3, the expression becomes:

\[
H_c(\psi_m, \rho, k, \alpha) :=
\begin{pmatrix}
\psi_1 - \psi_1 \\
\psi_2 - \psi_2 \\
\psi_2 - \psi_2 \\
\rho_{41}\psi_1 - \alpha f_{41}(k) - \psi_4 \\
\rho_{53}\psi_3 - \alpha f_{53}(k) - \psi_5 \\
\rho_{63}\psi_3 - \psi_4\psi_3 \\
\rho_{53}\psi_3 - \alpha f_{53}(k) - 1 \\
\rho_{81}\psi_1 + \alpha f_{81}(k) - \psi_4\psi_5\psi_3 \\
\end{pmatrix}
= 0
\]

(31)

Note that in the example, the intersection between \( F \) and \( M \) is trivially satisfied for the first three equations in (31). In general, the intersection will be trivially satisfied for the first \( j = \inf(r, \ell) \) equations in (30). Consequently, the manifold of equilibrium solutions can be described by the remaining \( n - k \) (5 equations in the above example). From now on, these will be the equations implied when referring to manifold \( H_c(\psi_m; \rho, k, \alpha) \).
Once the manifold of solutions is constructed in the space of complexes, one can derive its equivalent in the space of species. Using the matrix $Y_1$, we define the bijective mapping:

$$\ln \psi_m = Y_1^T \ln c$$  \hspace{1cm} (32)

This mapping allows us to transform the manifold $H_c(\psi_m; \rho, k, \alpha) \in \mathbb{R}^m$ into the ‘‘curve’’ of solutions $H_s \in \mathbb{R}^m$:

$$H_c(\psi_m, \rho, k, \alpha) \rightarrow H_s(c, \rho, k, \alpha)$$  \hspace{1cm} (33)

In this way, the manifold of solutions in the space of species corresponding to the example reads:

$$H_s(\psi_m, \rho, k, \alpha) := \begin{bmatrix}
\rho_{41}c_3 - \alpha f_{41}(k) - c_1c_2 \\
\rho_{53}c_2 - \alpha f_{51}(k) - c_5 \\
\rho_{62}c_4 - c_2c_3 \\
\rho_{73}c_2 - \alpha f_{71}(k) - 1 \\
\rho_{81}c_3 + \alpha f_{81}(k) - c_1c_5
\end{bmatrix} = 0$$  \hspace{1cm} (34)

Dimension of the manifold and classes of networks

The set of equilibrium solutions defined by expression (30) conforms a $\lambda$-dimensional manifold§ either in the space of complexes or—by the bijective mapping (32)—in the species space (33). This dimension $\lambda$, which at least partially depends on the deficiency of the network, conditions the number and nature of the possible equilibrium solutions attained when intersecting with the corresponding reaction polyhedron (10). In this way, it seems reasonable to explore the relationships between the manifold dimension $\lambda$ and the network deficiency $\delta$. This will be done in this section where in turn three different classes of reaction networks will be devised attending to a structural property we will refer to as the network layout $\theta$. This property is defined as the difference between the manifold dimension $\lambda$, and the deficiency of the network:

$$\theta = \lambda - \delta$$  \hspace{1cm} (35)

The term $\lambda$ can be computed as the number of degrees of freedom in (30). In other words, the difference between the number of variables, and the equations available.
From the discussion on the manifold of equilibrium solutions, it follows that for the general case the number of variables—including the elements of $\psi_m$ and the deficiency parameters $\alpha_i$—coincides with $\delta + \sup (r; \ell)$. On the other hand, and as pointed out also there, the number of (nontrivial) equations defined by $H_c(\psi_m; \rho, k, \alpha)$ is $n - \inf (r; \ell)$. Thus, the dimension $\lambda$ is then given by:

$$\lambda = \delta + \sup (r, \ell) - \left( n - \inf (r, \ell) \right) \equiv \delta - n + r + \ell$$

(36)

As expected, the expression (31) for the open Michaelis–Menten example is constituted by five independent equations and six unknowns ($\psi_1, \ldots, \psi_5, \alpha$). Equivalently, by using the definition of the deficiency (13), the expression above becomes of the form:

$$\lambda = r - s$$

(37)

which tells us that the dimension of the manifold of solutions coincides with the codimension of the reaction polyhedron in the species space (10). Depending on the sign taken by its layout, three different classes of networks will be devised, namely proper, under and over dimensioned networks.

Proper Networks. A network is considered proper if $\theta = 0$, that is, $\lambda = d$. The open Michaelis–Menten presented in Figure 4 is an example of proper network. According to (36), in these kind of networks, the number of species is equal to the number of nodes minus the number of linkage classes:

$$r = n - \ell$$

(38)

Because we are dealing with positive deficiency networks, proper networks always fulfill that $\lambda > 0$. This entails by observation of (37), that the trajectories evolve in a reduced linear submanifold of the species space (i.e. the reaction polyhedron is lower dimensional than the whole state space).

Under-Dimensioned Networks. A network is said to be under-dimensional if $\theta > 0$, that is, $\lambda > \delta$. In this case, according to (36), the number of species is greater than the number of nodes minus the number of linkage classes:

$$r > n - \ell$$

(39)
Hervagault and Canu (1987) investigate the dynamic behavior of a cyclic pathway with two intermediate metabolites S and P, that become the states of their reduced order model. The complete mechanism is derived here and its graph depicted in Figure 5. This mechanism is an example of an under-dimensioned network with $\lambda = 3$ and $\delta = 1$. Because $\lambda > 0$, as in the previous case, the reaction polyhedron is a linear submanifold of the species space.

Over-Dimensioned Networks. A network is said to be over-dimensioned if $\theta < 0$, that is, $\lambda < \delta$. In this case, according to (36), the number of species is lower than the number of nodes minus the number of linkage classes:

$$ r < n - \ell $$

(40)

We define an important subclass within the over-dimensioned networks, the so called fully open networks. A network is fully open if $\lambda = 0$. According to (37), the dimension of the reaction polyhedron and the space of species coincides, i.e. the reaction polyhedron occupies the whole concentration space. This means that none of the species is constrained by a conservation law. In addition, the reaction polyhedron does not depend on the initial conditions. The Brusselator network (Figure 6) is an example of fully open network which can exhibit oscillatory behavior.

An Algorithmic Approach for the Detection of Multiplicities

In this section, we will show how the concepts presented so far can be applied to determine, in a systematic way, those regions on the parameter space giving room for multiplicities. In that aim, we start with the manifold of solutions in the complex space (30), which for a given set of parameters $\lambda$ defines the set of equilibrium solutions (expressed in terms of the independent variables $\psi_m$) as a function of parameter $\alpha$.

From this point of view, and using classical bifurcation terminology, $\alpha$ becomes a sort of “continuation” parameter which we employ to check for continuity in the equilibrium solution manifold. Singularities, when they occur, divide (i.e. partition) the parameter space in regions where equilibrium solutions exhibit different qualitative behaviors. Such regions will be afterward explored for possible multiplicities by checking a given condition somehow related to the curvature of the solution manifold. The search for multiple equilibria will be carried out on an optimization framework.
This approach will be described in detail next in this section concentrating the discussion on deficiency one proper networks ($\theta = 0$). Finally and to keep the discussion as clear as possible, the open Michaelis-Menten network will be employed as an example to illustrate the different derivations and steps involved in the proposed method.

**Singularities on the manifold of solutions**

To detect singularities on the manifold of equilibrium solutions (30), we compute the derivative of $\psi_m$ with respect to $\alpha$ on the manifold $H_c = 0$, so that:

$$
\frac{dH_c}{d\alpha} \left( \frac{\partial F}{\partial \psi_m} - \frac{\partial M}{\partial \psi_m} \right) \frac{d\psi_m}{d\alpha} + \frac{\partial H_c}{\partial \alpha} = 0
$$

(41)

Note that because of the bijective mapping (32) between $\psi_m$ and $c$, we also have that:

$$
\frac{d\psi_m}{d\alpha} = D(\psi_m)Y^T \Gamma(c) \frac{dc}{d\alpha}
$$

(42)

where $D(v)$ and $\Gamma(v)$ denote diagonal operators acting on positive vectors $v$.

Computing the derivatives appearing in (41) from the expressions given in Section 3 for the family of solutions (21) and the mass action manifold (27), and using (42), the Eq. 41 can be finally written as:

$$
(X^* - A^*) \cdot D(\psi_m) \cdot Y^T \Gamma(c) \frac{dc}{d\alpha} = -\left( f_{c,1}(k), \ldots, f_{c,n}(k) \right)^T
$$

(43)

The matrix $X^*$, associated to the jacobian of the family of solutions is of the form:

$$
X^*(\rho) = \begin{pmatrix}
x_{k,1,1}(\rho) & x_{k,1,2}(\rho) & \cdots & x_{k,1,l}(\rho) & 0 & \cdots & 0 \\
\vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\
x_{n,1}(\rho) & x_{n,2}(\rho) & \cdots & x_{n,l}(\rho) & 0 & \cdots & 0
\end{pmatrix}
$$

(44)

with $k = \inf (r, l)$ (as defined in Section 3.1) and $x_{ij}$ being the $i$th component of the vector $x_j$ as it appears in (24). Matrix $A^*$ in (43) is associated to the jacobian of the manifold of solutions and reads:

$$
A^* = \begin{pmatrix}
a_{k+1,1} & \cdots & a_{k+1,m} \\
\vdots & \ddots & \vdots \\
a_{n,1} & \cdots & a_{nm}
\end{pmatrix}
$$

(45)
where the elements $a_{ij}$ in $A^*$ represent the partial derivatives of the mass action manifold component $M_i$ with respect to the elements of $\psi_m$. Using expression (28), each element takes the form:

$$a_{ij} = \frac{\partial M_i}{\partial \psi_j} = \frac{q_{ij}}{\psi_j} M_i$$  \hspace{1cm} (46)$$

For the network depicted in Figure 4, matrices $X^*$ and $A^*$ become:

$$X^* = \begin{pmatrix}
\rho_{14} & 0 & 0 & 0 & 0 \\
0 & 0 & \rho_{35} & 0 & 0 \\
0 & \rho_{26} & 0 & 0 & 0 \\
0 & 0 & \rho_{37} & 0 & 0 \\
\rho_{18} & 0 & 0 & 0 & 0
\end{pmatrix}$$  \hspace{1cm} (47)$$

$$A^* = \begin{pmatrix}
0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 1 \\
\psi_{3} & 0 & \psi_{1} & 0 & 0 \\
0 & 0 & 0 & 0 & 0 \\
0 & 0 & -\psi_{4}\psi_{3} / 5\psi_{3}^2 & \psi_{5} / \psi_{3} & \psi_{4} / \psi_{3}
\end{pmatrix}$$

whereas the matrix $Y_1$ in (43), containing the first $m$ columns of the molecularity matrix $Y$ in (18) reads:

$$Y_1 = \begin{pmatrix}
0 & 0 & 0 & 1 & 0 \\
0 & 0 & 1 & 1 & 0 \\
1 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1
\end{pmatrix}$$  \hspace{1cm} (48)$$

Let us return to the general formulation, and re-write Eq. 43 in the following somehow more compact form as:

$$G(c, \rho, f, \alpha) \frac{dc}{d\alpha} = - \left( f_{r+1}(K), ..., f_n(k) \right)^T$$  \hspace{1cm} (49)$$

where matrix $G$ is of the form:

$$G(c, \rho, f, \alpha) = (X^* - A^*) \cdot D(\psi_m) Y_1^T \Gamma(c)$$  \hspace{1cm} (50)$$
A unique solution for \( c \) in \( H_\alpha(c, \rho, k, \alpha) \) (33) as a function of \( \rho, k, \) and \( \alpha \) can be found provided that the matrix \( G \) is invertible. Furthermore, the inverse matrix \( G^{-1} \) determines also the sign of the concentration derivatives \( \frac{dc}{d\alpha} \), conditioning also the qualitative behavior of the solution. In particular, multiple equilibrium solutions will appear—for some reaction polyhedrons—when the following condition holds:

\[
B^T \frac{dc}{d\alpha} = 0
\]

where matrix \( B \) has been introduced in Eqs. 9 and 10. Geometrically, condition (51) implies a folding of the manifold of solutions thus indicating more than one intersection with the reaction polyhedron. A pictorial description of the condition is presented in Figure 7. Condition (51) will be employed as the criterion for the assessment of multiplicities for certain regions in the parameter space, being their borders—as we will see next—related to the singularities of matrix \( G \) (50). Singularities will appear for those critical values \( \alpha^* \) for which the determinant of matrix (50) becomes zero. Moreover, because all terms in (50) except matrix \( (X^* - A^*) \) are invertible, critical parameters \( \alpha^* \) can be computed directly from the following determinant that we write as:

\[
|X^*(\rho) - A^*(c)| = \prod_{i=1}^{m} g_i(c, \rho, k)
\]

where factors \( g_i \) as they appear in (52) coincide with the eigenvalues of \( X^*(\rho) - A^*(c) \). Consequently, the critical values of the continuation parameter \( \alpha^* = \alpha(\rho, k) \) leading to a singularity in the equilibrium manifold are defined by the following condition:

\[
\lim_{\alpha \to \alpha^*} g_i[c(\rho, k, \alpha), \rho, k] = 0
\]

Solving (53) for \( i = 1, \ldots, m \), we obtain a set of \( l \) critical values \( \alpha^* \) which when different from zero refer to solutions in \( D_\delta \), the deficiency subspace (see conditions C.1 and C.2). In this way, because \( \alpha = 0 \) also defines a frontier in the parameter space, we consider \( \mu + 1 \) critical values of the continuation parameter \( \alpha \) and denote them by \( \tilde{\alpha}_i^* \). The following equalities:

\[
\tilde{\alpha}_i^*(\rho, k) = \alpha_{i^*}(\rho, k) \text{ for } i = 1, \ldots, \mu + 1
\]
divide the space of parameters in a number $n_R$ of regions with different qualitative behavior $R_1, R_2, \ldots, R_{n_R}$. To illustrate the ideas presented so far, we make use of the Michaelis–Menten open network and compute (52), using the matrices in (47) to get:

$$|X^*(\rho) - A^*(c)| = g_1(c, \rho, k) = c_2\rho_{s1} - c_3\rho_{41}$$  \hspace{1cm} (54)$$

For convenience, and to facilitate a graphical representation, let us employ the kinetic parameters $p_1, \ldots, p_2$ defined in Table 1 instead of the canonical ones solve condition (53), which for this case reduces to the following equation system:

$$c_2p_2 - c_3p_1 = 0$$
$$H_\alpha(c, \rho, k, \alpha) = 0$$

where $H_\alpha$ is expressed as in (34). Solving this system of equations we obtain one critical value of $\alpha$ as a function of the parameters:

$$\alpha^* = \frac{p_1p_5 - p_2p_4}{p_1 / k_{s7} + p_2 / k_{s7}}$$  \hspace{1cm} (55)$$

The parameter space is therefore divided by condition $\alpha^* = 0$ in two regions of different qualitative behavior, as depicted in Figure 8, separated by the following equality:

$$p_1p_5 - p_2p_4 = 0$$  \hspace{1cm} (56)$$

An optimization framework to detect multiplicities

Condition (51) which ensures multiple equilibrium solutions will be checked on the different regions of the parameter space with different qualitative behavior by solving a given optimization problem in each region $R_j (j = 1, \ldots, n_R)$. The objective function to be minimized over the set of representative kinetic parameters (we denote as a vector $k$) and $\alpha$ is of the form:

$$J(k, \alpha) = B^T \nabla_\alpha c(k, \alpha)[\nabla_\alpha c(k, \alpha)]^T B$$  \hspace{1cm} (57)$$

where $\nabla_\alpha c$ denotes the $\alpha$-derivative of $c$. The associated constrained optimization problem can be stated as follows:

Find $\min_{x \in \mathbb{R}^n} J(x)$ subject to: $Hc(k, \alpha) = 0$

$$p(k) \in R_j$$  \hspace{1cm} (58)$$
where \( x = [k, \alpha] \) is the vector of decision variables including the \( n_k \) kinetic rate constants and the continuation parameter \( \alpha \). Inequality constraints include those defining the lower and upper bounds (vectors \( x_l \) and \( x_u \), respectively) for the decision variables and strict positivity for the concentrations. The manifold \( H_c \) acts as a set of equality constraints defining the feasible regions where solutions are endowed with physical meaning. If a solution is found for any region \( R_j \) such that \( J(k, \alpha) = 0 \), then it can be concluded that in \( R_j \) multiplicities would appear for some reaction polyhedrons. On the other hand, for those regions where the minimum attained is positive (i.e. \( J(k, \alpha) > 0 \)) the possibility of multiple steady states can be ruled out.

For the open Michaelis-Menten example, where two regions with different qualitative behavior, \( R_1 \) and \( R_2 \), have been found as discussed in 4.1, one optimization problem of the form (58) is solved for each region. In this example, it has been assumed that the immobilized enzymes remain in a stirred tank reactor with a constant flow of substrate and product. Note that, under these conditions and by inspection of Figure 4 it can be deduced that \( k_{57} = k_{37} \).

We have used GLOBALm (Csendes, 2008), which is a multi-start clustering global optimization method for bounded constrained global optimization problems with black-box type objective functions. A particularly useful advantage of this method is that it reports all the optima (global and local) found during the search in the region of interest. Using this method, no feasible solutions were found in region \( R_2 \). On the contrary, GLOBALm has found multiple solutions belonging to \( R_1 \). In fact, it must be noted that the infinity of parameter combinations for which multiplicities arise cover continuous areas in the parameter space regions what is in agreement with the analytical approach given to the problem by Otero-Muras et al. (2007). Three representative solutions from those provided by GLOBALm are presented in Table 2. As illustrated in Figures 9 and 10, the form of the solution curve relative to the reaction polyhedron can give rise to multiple intersections which coincide with the number of equilibrium points of the dynamic system.** Finally, it must be pointed out that the location of the reaction polyhedron defined by the reference concentration \( c_0 \) in (10) is critical to induce multiple equilibrium solutions. In this example, the total amount of enzyme \( E_T \) is the
quantity which determines the location of the polyhedron in the concentration space and thus the number of equilibria points. As illustrated in Figure 10, the higher values of total enzyme correspond to a unique equilibrium at low concentration of $ESS (c_4)$; intermediate values of total enzyme give rise to three equilibria and, at lower values of total enzyme, a unique steady state appears at high concentration of $ESS$. The numeric values of the concentrations corresponding to all the steady states depicted in the figure are listed in Table 3.

Conclusions

In this work, we have explored the conditions under which multiplicities arise in enzymatic reaction networks. The method proposed exploits the underlying structure of biochemical networks to first divide the parameter space in regions leading to different dynamic behavior and then checking a condition for multiplicities within each of these regions. This procedure has been formulated as an optimization problem. The application of this approach has been illustrated through a well known case of enzymatic catalysis with substrate inhibition, where the parameter space resulted to be divided in two regions with different qualitative behavior. The optimization problem has been solved using GLOBALm and the results coincide with previous analytical studies indicating that multiplicities can arise only in one of the regions. In further works we aim to refine the method proposed using interval arithmetic techniques, in order to search within critical regions of the parameter space the exact areas where the multiplicity condition is always fulfilled.

Acknowledgments

The authors acknowledge financial support received from the Spanish Government (DPI2004 - 07444 - C04 - 03) and Xunta de Galicia (PGIDIT02 - PXIC40209PN).

Literature Cited

Appendix

A subspace orthogonal to the image of $A_k$, from (Feinberg, 1979)

For a given (bio)chemical network constituted by $n$ complexes $C_i (i = 1, \ldots, n)$ and $\ell$ linkage classes, $L_j (j = 1, \ldots, \ell)$ let us associate to each linkage class $L_j$ an $n$-dimensional vector $\Lambda_{ij}$ with entries constructed as follows:

$$
\Lambda_{ij} = \begin{cases} 
1 & C_i \in L_j \\
0 & C_i \not\in L_j 
\end{cases}
$$

(A1)
Vectors constructed in this way are known in Feinberg’s lectures (Feinberg, 1979) as the characteristic functions associated to the linkage classes (lecture 4, page 4-17) and constitute a basis for the subspace orthogonal to the one where the image of $A_k$ lies (Lemma 4.6 in Lecture 4, pp. 4–18). In fact if each linkage class of the network contains precisely one terminal strong linkage class (corollary 4.6 in Lecture 4, pp. 4–19) we have that the columns of the resulting matrix $\Lambda$ are a basis for the subspace orthogonal to the image of $A_k$. This we write it as follows:

$$\text{Im}(A_k) = \text{Im}(\Lambda) \quad \text{(A2)}$$

As an example, for the network depicted in Figure 4, the vectors associated to the linkage classes coincide with the columns of the following matrix:

$$\Lambda = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \end{pmatrix} \quad \text{(A3)}$$

Computing a basis for the deficiency subspace $D_6$

To compute a basis for the deficiency subspace $D_6 = \ker Y \cap \text{Im} A_k$ (defined in earlier section), let us first consider the following set of implications:

$$\left( \ker Y \cap \text{Im} A_k \right)^\perp = \left( \ker Y \right)^\perp \cup \left( \text{Im} A_k \right)^\perp \quad \text{(A4)}$$

$$\left( \ker Y \right)^\perp \cup \left( \text{Im} A_k \right)^\perp = \text{Im}(Y^T) \cup \text{Im}(\Lambda) \quad \text{(A5)}$$

where use has been made of relation (A2) in A1. From the above relations, it follows that each element $w \in D_6$ is orthogonal to $\text{Im}(Y^T) \cup \text{Im}(\Lambda)$ so that for any vector $v$ we have that:

$$w^T \left[ \Lambda \quad Y^T \right] v = v^T \left[ \Lambda \quad Y^T \right]^T w = 0 \quad \text{(A6)}$$

Consequently, a basis for $D_6$ for a deficiency d network can be computed from $Y$ and $\Lambda$ as:
\{w_1,\ldots, w_δ\} = \ker [A \ Y^T]^T \quad (A7)

For the example Figure 4 the resulting basis is:
\[ w = (0 \ 0 \ -1 \ 1 \ 1 \ 0 \ 0 \ -1)^T \quad (A8) \]

Finally, because it will be used in earlier section to construct the family of solutions, some results by Feinberg (Proposition 4.1, Remark 4.4 and Corollary 4.2, Lecture 4, pp. 4–10 to 4–12) describing the structure of the kernel of $A_k$ are summarized next in the following proposition:

Proposition 1. (Feinberg, 1979). For a weakly reversible network with linkage classes \{L_1, L_2; \ldots; L_ℓ\} the kernel of $A_k$ has a basis \{x_1, x_2,\ldots,x_ℓ\} where $x_i$ are nonnegative vectors in $\mathbb{R}^n$ such that:
\[ \text{supp } x_i = L_i \quad i = 1,\ldots, ℓ \quad (A9) \]

where supp denotes vector support.

On the rank of the molecularity matrix $Y$

The species participating on a reaction network can be numbered, without loss of generality, such that the first $m$ columns of $Y^T$ being linearly independent. If the molecularity matrix is rank deficient, the methodology is still applicable, although for an alternative space of species. To show this, let us order the columns in matrix $Y^T$ so that the first $m$ ($< r$) (m coincides with the rank of the matrix) are linearly independent. Then we have:
\[ Y^T = \left(y_1,\ldots,y_m \mid y_{m+1},\ldots,y_r\right) \quad \tilde{Y}^T = \left(y_1,\ldots,y_m\right) \quad (A10) \]

Because the last $r - m$ columns of $Y^T$ can be expressed as a linear combination of the first $m$ columns, we also have that:
\[ y_j = \sum_{i=1}^{m} \beta_{ji} \cdot y_i \quad \text{for } j = m + 1,\ldots,r \quad (A11) \]

where $\beta_{ji}$ are given scalars. Substituting (A11) in $Y^T$, mass action law (8) can be expressed as:
\[ \ln \psi = \sum_{i=1}^{n} y_i \cdot \left( \text{Inc}_i + \sum_{j=\infty+1}^{r} \beta_j \text{Inc}_j \right) \]  \hspace{1cm} (A12)

where the new \( \tilde{r} = m \) pseudo-species are of the form:

\[ \tilde{c}_i = c_i \cdot e^{\beta_j} \]  \hspace{1cm} (A13)

In this way, an equivalent formulation can be found for every network (with \( \tilde{r} \) pseudo-species and full rank molecularity matrix \( \tilde{Y} \)).
*Note that the assumption also requires that $k_{23} >> k_{32}$.

†This dimension coincides with the codimension of the subspace spanned by the columns of $B$ in the space of the species, i.e., $s = r - \text{rank}(B)$.

†If this is not the case the methodology will still be valid, although for a given combination of species as described in the Appendix.

§The scalar $\lambda$ gives the number of degrees of freedom that characterizes the dimension of the equilibrium manifold (30) for a given network. In this way, $\lambda = 1$ will correspond to a line, $\lambda = 2$ with a two dimensional surface, etc.

¶$D$ and $\Gamma$ act on a vector $v$ to produce diagonal matrices with diagonal elements being the components of $v$ in the case of $D$ or their inverses in the case of $\Gamma$.

**Note that these steady states might be stable or unstable.
Figure 1. Graph corresponding to a mechanism for enzymatic catalysis with substrate inhibition. The mechanism involves five different complexes represented by the five nodes of the graph: C₁, ..., C₅.

Figure 2. The equilibrium solution point as the intersection of the curve of solutions and the reaction polyhedron for a given network.

Figure 3. Graph for the open Michaelis-Menten mechanism with substrate inhibition, showing substrate and product flow through the system.

Figure 4. Canonically ordered graph for the open Michaelis-Menten mechanism with substrate inhibition, equivalent to the one depicted in Figure 3.

Figure 5. Graph for the binary substrate cycle.

Figure 6. Graph representing the Brusselator network.

Figure 7. Geometric interpretation of condition (51) and its relation with the appearance of multiplicities.

Figure 8. Regions of different qualitative behavior in Michaelis network.

Figure 9. Solution manifold and reaction polyhedron for different parameter sets in R₁ fulfilling (51) with (A) $\alpha = -0.138$, (B) $\alpha = -51.311$.

Figure 10. Intersections between the solution manifold and the reaction polyhedron for (A) $E_T = 1.746$, (B) $E_T = 1.296$, (C) $E_T = 1.046$, and (D) $E_T = 0.796$. 
Table 1. Relationships Between the Kinetic Parameters Employed and the Original Rate Constants for the Open Michaelis-Menten Network

Example

$$\rho_{41} = \frac{k_{14}}{k_{41}} \quad \rho_{81} = \frac{k_{18}}{k_{81}} \quad \rho_{62} = \frac{k_{26}}{k_{62}} \quad \rho_{73} = \frac{k_{27}}{k_{73}} \quad \rho_{53} = \frac{k_{53}}{k_{73}}$$

$$f_{41}(k) = \frac{1}{k_{41}} \quad f_{51}(k) = \frac{k_{25} + k_{73}}{k_{67}k_{73}} \quad f_{61}(k) = 0 \quad f_{71}(k) = \frac{1}{k_{73}} \quad f_{81}(k) = \frac{1}{k_{81}}$$

$$p_1 = \rho_{41} \quad p_2 = \rho_{81} \quad p_3 = \rho_{62} \quad p_4 = \frac{1}{\rho_{73}} \quad p_5 = \frac{\rho_{53}}{\rho_{73}}$$

Table 2. Parameter Sets in R1 Fulfilling the Multiplicity Condition

<table>
<thead>
<tr>
<th>Fig</th>
<th>(\alpha)</th>
<th>(k_{14})</th>
<th>(k_{41})</th>
<th>(k_{18})</th>
<th>(k_{81})</th>
<th>(k_{26})</th>
<th>(k_{62})</th>
<th>(k_{37})</th>
<th>(k_{73})</th>
<th>(k_{75})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.A</td>
<td>-0.138</td>
<td>1.000</td>
<td>100.000</td>
<td>101.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.017</td>
<td>0.251</td>
<td>0.00017</td>
</tr>
<tr>
<td>2</td>
<td>-20.955</td>
<td>15.742</td>
<td>77.182</td>
<td>98.757</td>
<td>0.058</td>
<td>99.394</td>
<td>32.918</td>
<td>0.883</td>
<td>38.676</td>
<td>78.249</td>
</tr>
</tbody>
</table>

Table 3. Equilibrium Points Corresponding to the Figure 10

<table>
<thead>
<tr>
<th>Fig</th>
<th>(E^t)</th>
<th>(\alpha)</th>
<th>(C_1)</th>
<th>(C_2)</th>
<th>(C_3)</th>
<th>(C_4)</th>
<th>(C_5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.A</td>
<td>1.746</td>
<td>-38.235</td>
<td>1.118</td>
<td>0.499</td>
<td>0.479</td>
<td>0.079</td>
<td>131.909</td>
</tr>
<tr>
<td>---</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.296</td>
<td>1.046</td>
<td>0.796</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-37.980</td>
<td>-34.249</td>
<td>-5.880</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.739</td>
<td>0.103</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.788</td>
<td>5.013</td>
<td>37.139</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.442</td>
<td>0.354</td>
<td>0.059</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.115</td>
<td>0.589</td>
<td>0.734</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>131.621</td>
<td>127.395</td>
<td>95.270</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>123.556</td>
<td>131.162</td>
<td>123.556</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>101.371</td>
<td>131.162</td>
<td>123.556</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>97.990</td>
<td>127.395</td>
<td>123.556</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>