# Multistationarity in biochemical networks: Results, analysis, and examples

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## 1 Introduction

Biochemical networks are complex objects, almost always containing nonlinear interactions among a usually large number of chemical species. The smallest activation or inhibition interactions involve two components (a gene and its protein, or an inhibitor and an enzyme), are well understood biologically and have simple stable long-term behavior that can be inferred straight from the interaction diagram ([1]; Figure 1**a**). But if the interaction involves a larger number of components, or if feedback loops are present, then the dynamics may become far more complicated, and understanding it requires strategies more subtle than chasing paths in the interaction diagram. Instabilities of different kinds are possible even for small biological structures and are associated with signaling events [2, 3, 4, 5]. One key class of instabilities are those leading to multiple positive steady states (Figure 1**b**), also known as *multistationarity*, and seen experimentally as irreversible switch-like behavior. There is significant theoretical evidence, backed by experiment, that important pathways may exhibit multistationarity as response to chemical signaling. This phenomenon is particularly relevant in crucial cell behaviors, including generating sustained oscillatory responses, remembering transitory stimuli, differentiation, or apoptosis [2, 4, 6, 7, 8, 9]. Multistationarity occurs in chemistry and chemical engineering as well, but is much less common. There is, in fact, a great deal of stable behavior in networks of chemical reactions, and (to a lesser degree) in biological networks. This can be explained in part by the fact that the possibility of exotic behavior places rather delicate constraints on the structure of an interaction network [10, 11, 12, 13, 14, 15, 16, 17, 18]. A seminal remark is due to Thomas [19] who conjectured that positive feedbacks in the logical structure of an interaction network are necessary for multistationarity. Much theoretical and simulation work followed, and proposed a series of increasingly refined design principles for pathways allowing multistationarity [1, 13, 20, 21, 22, 23]. Building on this effort, multistationarity has been demonstrated experimentally in bacterial synthetic genetic networks [24].

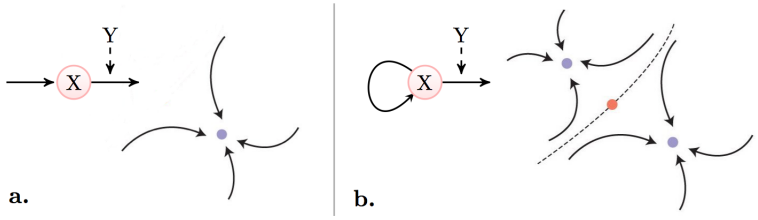



Figure 1: Even simple interactions may authorize complicated behaviors. **a.** In reactions of synthesis and degradation the steady state response X is unique for any value of signal Y [1]. **b.** In the presence of positive feedback, as is the case of autocatalysis, certain values of signal Y may generate multiple positive steady states for X [13].

The structure of reaction networks can be encoded by their reaction graph, Jacobian matrix sign pattern, various stoichiometric matrices, and so on. Recent notable work has established subtle connections between properties of these objects and multistationarity [11, 12, 14, 15, 16, 17, 25, 26, 27, 28, 29]. In particular, various features of the stoichiometric matrix may allow strong conclusions about the existence of multiple equilibria; this chapter is a review of results along this line. We note that a survey such as this will necessarily be not exhaustive, and we have omitted a number of multistationarity methods, including powerful algebraic tools well-suited for enzymatic networks [30, 31], as well as a series of results based on degree theory [32, 33]. We also refer the reader to the recent review [34].

The chapter is structured as follows. Section 2 is dedicated to terminology and notation; we follow a matrix-theoretical framework, much like in [27, 28]. The next two sections focus on necessary conditions for multistationarity of reaction networks. There is a wealth of results in this direction; we present theorems based on injectivity [26, 27, 35, 36, 37, 38, 39] (Section 3) and the DSR graph [37, 40] (Section 4). We then continue with two sections on sufficient conditions for multistationarity: recent work on inheritance of multistationarity [28, 29] from subnetworks is presented in Section 5, and the determinant optimization method [26] is reviewed in Section 6. Finally, methods for ruling out multistationarity based on deficiency theory [41, 42, 58] are collected in Section 7. Exercises are proposed at the end of each section. Some of them are important theoretical facts not included in the main text, while others are applications of the results contained in that section.

We restrict the presentation to mass action, where, informally, the rate of a chemical reaction is proportional to the number of molecular collisions, and therefore proportional to the concentration of each of the reactants. We note, however, that many of the results apply to more general kinds of kinetics. Our focus is on readability and a unifying presentation of the various results from the literature, rather than on giving the strongest statements possible. For these, the reader is encouraged to consult the references accompanying the theorems shown here.

## 2 Reaction network terminology and background

We proceed rather informally at first, using examples to introduce some basic terminology, which is later revisited in a general context. For ease of presentation, some of these examples are chosen somewhat artificially. Others are important systems from biochemistry; for example, throughout this chapter we will often consider versions of the *futile cycle*

$$E + S \rightleftharpoons ES \to E + P, \qquad F + P \rightleftharpoons FP \to F + S, \tag{1}$$

a well-studied structure that serves as building blocks in cellular signaling pathways [20, 22, 59, 60]. The name comes from the fact that, in may cases, the two opposite pathways (from $S$ to $P$ and from $P$ to $S$) run simultaneously and have no overall effect other than to dissipate energy. An important instance of a futile cycle is the *phosphorylation-dephosphorylation* network, where the substrate (often a protein) $S$ is converted into the product $P$ by adding a phosphoryl group. The process is triggered by an enzyme $E$, which binds to the substrate, forming an intermediate enzyme-substrate complex $ES$; this, in turn, dissociates to release $E$ and $P$, the phosphorylated form of $S$. The reverse process of dephosphorylation proceeds similarly, catalyzed by the enzyme $F$. Dynamical properties, and in particular multistationarity of systems of coupled futile cycles, have been studied extensively [2, 4, 50, 56, 61, 73, 74, 75]. The methods overviewed here can conclude whether or not many such enzymatic systems are multistationary.

### 2.1 Chemical Reaction Networks and their dynamics.

A *chemical reaction network* (CRN), also called *biochemical reaction network*, or simply *reaction network* throughout the text, is a list of *reactions* involving a finite list of *species*. The futile cycle above involves

six reactions among six species $E$, $S$, $ES$, $P$, $F$, $FP$. Likewise, the example in Figure 2 involves seven reactions among five species $A, B, C, D, E$, taking place in a defined reaction environment, such as a cell or a chemostat. Each arrow in the diagram represents a *reaction* and is interpreted in a natural way: for example, in $A + B \to 2C$, one molecule of species $A$ combines with one molecule of species $B$ to produce two molecules of $C$.

A reaction arrow connects formal linear combinations of species called *complexes*: it starts at a *source* complex and it ends at a *product* complex. Degradation, or discharge of species $E$ from the system is encoded as an *outflow reaction* $E \to 0$. The exterior of the reaction environment is represented in the CRN by the *zero complex* 0. Likewise, the *inflow reaction* $0 \to E$ encodes a constant supply of $E$ into the system. A CRN containing inflow and outflow reactions for all of its species is called *fully open*. Adding to a CRN the missing inflow and outflow reactions ($A \rightleftharpoons 0,\ B \rightleftharpoons 0,\ C \rightleftharpoons 0,\ D \rightleftharpoons 0$ for the example in Figure 2) yields its *fully open extension*.

A pair of reactions that switch their source and product complexes (for example, $B + C \rightleftharpoons 2A$) is called a *reversible reaction*. One can always view a reversible reaction as two separate, irreversible reactions, and most of our treatment follows this convention. We will clearly state when that is not the case. Species involved in the source complex of a certain reaction are called *reactants*. As Figure 2 illustrates, the CRN can be viewed as a directed graph with complexes as vertices; this is called the *reaction graph* of the CRN.

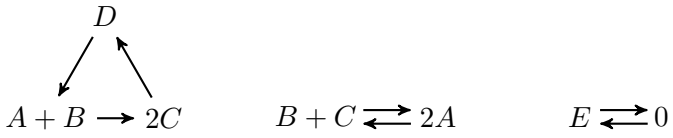



Figure 2: A reaction network with seven reactions involving five species $A, B, C, D, E$ and the complexes $A + D, 2C, D, B + C, 2A, E, 0$.

The molar *concentrations* $x_A, x_B, x_C, x_D, x_E$ of species $A, B, C, D, E$ are non-negative quantities varying with time according to a system of ordinary differential equations, as follows. The net gain of molecules for each species in a single occurrence of a reaction is encoded in a column vector called its *reaction vector*: setting species $A, B, C, D, E$ in this order, the reaction vector of $A + B \to 2C$ is $[-1, -1, 2, 0, 0]^t$. Each reaction "pushes" the vector field in the direction of its reaction vector, at a rate proportional to the product of its reactant concentrations (this way of constructing rates is called *mass action*): the *rate* of $A + B \to 2C$ is equal to $k_1 x_A x_B$, where $k_1 > 0$ is a reaction-dependent quantity called its *rate constant*. Sometimes the rate constant sits on top of the reaction arrow, for example $A + B \xrightarrow{k_1} 2C$. Inflow reactions (like $0 \to E$) have constant rates, equal to their rate constants. Aggregating the contributions of all reactions results in the following system of differential equations,

$$\begin{bmatrix} \dot{x}_A \\ \dot{x}_B \\ \dot{x}_C \\ \dot{x}_D \\ \dot{x}_E \end{bmatrix} = k_1 x_A x_B \begin{bmatrix} -1 \\ -1 \\ 2 \\ 0 \\ 0 \end{bmatrix} + k_2 x_C^2 \begin{bmatrix} 0 \\ 0 \\ -2 \\ 1 \\ 0 \end{bmatrix} + k_3 x_D \begin{bmatrix} 1 \\ 1 \\ 0 \\ -1 \\ 0 \end{bmatrix} + k_4 x_B x_C \begin{bmatrix} 2 \\ -1 \\ -1 \\ 0 \\ 0 \end{bmatrix} + k_5 x_A^2 \begin{bmatrix} -2 \\ 1 \\ 1 \\ 0 \\ 0 \end{bmatrix} + k_6 x_E \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ -1 \end{bmatrix} + k_7 \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 1 \end{bmatrix},$$

conveniently rewritten as the product of the *stoichiometric matrix* of the network and its *rate vector*

$$\begin{bmatrix}\dot{x}_A\\ \dot{x}_B\\ \dot{x}_C\\ \dot{x}_D\\ \dot{x}_E\end{bmatrix} = \begin{bmatrix}-1 & 0 & 1 & 2 & -2 & 0 & 0\\ -1 & 0 & 1 & -1 & 1 & 0 & 0\\ 2 & -2 & 0 & -1 & 1 & 0 & 0\\ 0 & 1 & -1 & 0 & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & -1 & 1\end{bmatrix} \begin{bmatrix}k_1 x_A x_B\\ k_2 x_C^2\\ k_3 x_D\\ k_4 x_B x_C\\ k_5 x_A^2\\ k_6 x_E\\ k_7\end{bmatrix}. \tag{2}$$

The stoichiometric matrix is made out of reaction vectors arranged as columns, and it is therefore dependent on the ordering of reactions (corresponding to its columns) and the ordering of species (corresponding to its rows). Clearly, this does not change the expression of the vector field (2), nor does it have any impact on the results presented here, which are independent of these orderings.

It is easy to see (Exercise 2) that the non-negative orthant $\mathbb{R}^5_{\geq 0}$ is *forward invariant* with respect to (2), i.e. solutions of (2) with non-negative initial conditions will stay non-negative. This important remark holds for any reaction network driven by mass action.

## 2.2 Some useful notation

Throughout this chapter we denote the stoichiometric matrix by $\Gamma$ and the rate vector by $v$. We note that every complex can be viewed as a (column) vector, for example $A + B$ corresponds to $[1, 1, 0, 0, 0]^t$. Source complexes can be arranged as columns in the *reactant matrix*, denoted by $\Gamma_l$. For example, the reactant matrix corresponding to Figure 2 is

$$\Gamma_l = \begin{bmatrix}1 & 0 & 0 & 0 & 2 & 0 & 0\\ 1 & 0 & 0 & 1 & 0 & 0 & 0\\ 0 & 2 & 0 & 1 & 0 & 0 & 0\\ 0 & 0 & 1 & 0 & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & 1 & 0\end{bmatrix}.$$

A useful notation in the context of mass action is vector exponentiation: for $u, v \in \mathbb{R}^n_{\geq 0}$, $u^v$ is defined as the product of componentwise powers: $u^v = \prod_{i=1}^n u_i^{v_i}$. Mass action rates have convenient expressions using this notation; for example if $x = [x_A, x_B, x_C, x_D, x_E]^t$ denotes the *concentration vector* for our network in Figure 2, then the reaction rate $k_1 x_A x_B$ of $A + B \to 2C$ is simply $k_1 x^{[1,1,0,0,0]^t}$; note that the exponent is the reactant vector of the reaction, and therefore it figures as a column in $\Gamma_l$. We introduce yet another abbreviation, that of exponentiation by a matrix, to write the vector of monomials

$$w(x)^t = [x_A x_B, x_C^2, x_D, x_B x_C, x_A^2, x_E, 1] = \begin{bmatrix}x_A\\ x_B\\ x_C\\ x_D\\ x_E\end{bmatrix}^{\begin{bmatrix}1 & 0 & 0 & 0 & 2 & 0 & 0\\ 1 & 0 & 0 & 1 & 0 & 0 & 0\\ 0 & 2 & 0 & 1 & 0 & 0 & 0\\ 0 & 0 & 1 & 0 & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & 1 & 0\end{bmatrix}} = x^{\Gamma_l}$$

so that the rate vector becomes

$$v(x) = D_k (x^{\Gamma_l})^t,$$

where $D_k$ denotes the diagonal matrix with rate constants $k_i$ on the diagonal.

## 2.3 The Jacobian matrix

Much of the theory to be presented here relies on Jacobian properties of the CRN vector field. The notation introduced so far allows for a useful expression of the Jacobian matrix, as follows: if $f = \Gamma v$ denotes the mass action vector field, then $Df = \Gamma Dv$, and $Dv$ has a useful factorization, illustrated here for the CRN in Figure 2:

$$
\begin{aligned}
(\partial v/\partial x) &= \begin{bmatrix} k_1x_B & k_1x_A & 0 & 0 & 0 \\ 0 & 0 & 2k_1x_C & 0 & 0 \\ 0 & 0 & 0 & k_3 & 0 \\ 0 & k_4x_C & k_4x_B & 0 & 0 \\ 2k_5x_A & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_6 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix} = \begin{bmatrix} k_1x_Ax_B & k_1x_Ax_B & 0 & 0 & 0 \\ 0 & 0 & 2k_1x_C^2 & 0 & 0 \\ 0 & 0 & 0 & k_3x_D & 0 \\ 0 & k_4x_Bx_C & k_4x_Bx_C & 0 & 0 \\ 2k_5x_A^2 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_6x_E \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} 1/x_A & 0 & 0 & 0 & 0 \\ 0 & 1/x_B & 0 & 0 & 0 \\ 0 & 0 & 1/x_C & 0 & 0 \\ 0 & 0 & 0 & 1/x_D & 0 \\ 0 & 0 & 0 & 0 & 1/x_E \end{bmatrix} \\
&= \begin{bmatrix} k_1x_Ax_B & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & k_1x_C^2 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & k_3x_D & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & k_4x_Bx_C & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_5x_A^2 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & k_6x_E & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & k_7 \end{bmatrix} \begin{bmatrix} 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 2 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 1 & 0 & 0 \\ 2 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} 1/x_A & 0 & 0 & 0 & 0 \\ 0 & 1/x_B & 0 & 0 & 0 \\ 0 & 0 & 1/x_C & 0 & 0 \\ 0 & 0 & 0 & 1/x_D & 0 \\ 0 & 0 & 0 & 0 & 1/x_E \end{bmatrix} \\
&= D_k D_w \Gamma_l^t D_{1/x}.
\end{aligned}
$$

Here $D_k$, $D_w$ and $D_{1/x}$ denote diagonal matrices with diagonal entries coming from the vector of rate constans $k$, monomials $w(x)$, and the vector $[1/x_A, 1/x_B, 1/x_C, 1/x_D]^t$ respectively. This factorization holds for any mass action CRN. Note that, although $Df$ is defined everywhere on $\mathbb{R}^5_{\geq 0}$, $D_{1/x}$ is not defined on the boundary of the $\mathbb{R}^5_{\geq 0}$. However, that is not important for the purposes of this chapter, as our Jacobian calculations are restricted to the positive orthant.

## 2.4 Stoichiometry classes

A simple derivation from (2) shows that $\dot{x}_A + \dot{x}_B + \dot{x}_C + 2\dot{x}_D = 0$, which means that $x_A + x_B + x_C + 2x_D$ stays constant along trajectories. This linear combinaton of concentrations is called a *conservation law*. A systematic way to find the conservation laws of a CRN is to look for vectors $c \in \ker \Gamma^t$ since then $c \cdot \dot{x} = 0$ (here "$\cdot$" denotes the usual dot product). In our example, $[1, 1, 1, 2, 0]^t$ forms a basis of $\ker \Gamma^t$, and therefore, while the phase space is $\mathbb{R}^5_{\geq 0}$, the trajectories are constrained to four dimensional affine spaces orthogonal to $[1, 1, 1, 2, 0]^t$, called *stoichiometry classes*. Note that since $(\ker \Gamma^t)^\perp = \operatorname{im} \Gamma$, the stoichiometry classes are affine spaces parallel to the *stoichimoetric subspace* $\operatorname{im} \Gamma$. (Throughout the chapter we let $\ker A$ and $\operatorname{im} A$ denote the nullspace and the column space of a matrix $A$ respectively).

The stoichiometry classes foliate the phase space; the simpler example

$$A \rightleftharpoons 2B \tag{3}$$

allows for a helpful picture – see Figure 3. Here the stoichiometric subspace is the span of $[-1, 2]^t$, and translating it by $p \in \mathbb{R}^2_{\geq 0}$ into $\mathbb{R}^2_{\geq 0}$ yields the stoichiometry classes $\{p + s \begin{bmatrix} -1 \\ 2 \end{bmatrix} \mid s \in \mathbb{R}\} \cap \mathbb{R}^2_{\geq 0}$. Each stoichiometry class corresponds to a choice of "total mass" $T$ and can also be defined as $\{[x_A, x_B]^t \in \mathbb{R}^2_{\geq 0} \mid 2x_A + x_B = T\}$. For this example, there is only one independent conservation law $[2, 1]^t$, and it spans $\ker \Gamma^t$.

As trajectories are confined to stoichiometry classes, these, rather than the whole phase space, are the relevant spaces where dynamical behaviors of CRNs (like existence or uniqueness of equilibria) are studied. We will come back to this in the next subsection.

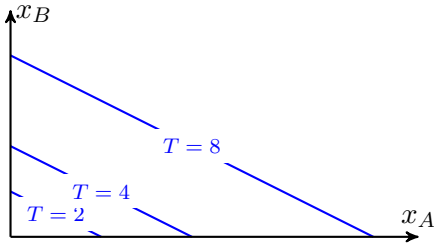



Figure 3: Stoichiometry classes for the network $A \rightleftharpoons 2B$. Although the phase space is two-dimensional, every trajectory is either stuck at the origin, or constrained to one of the one-dimensional stoichiometry classes.

### 2.5 Equilibria

An *equilibrium* (or *steady state*) of a reaction network is a point in the phase space where its vector field vanishes, i.e. a single-point trajectory of the CRN dynamics. In this chapter, we focus on the study of equilibria with positive coordinates (from now on called *positive equilibria*), and in particular, on their uniqueness. As described in the introduction, the existence of two or more positive equilibria is of central importance in the study of many important cellular processes.

Equilibrium points sit at the intersection of *nullclines*, which are manifolds defined by the vanishing of the derivative of one of the variables. In the case of mass action, these are algebraic varieties cut out by a single multivariate polynomial. For example, the network

$$2A \xrightarrow{1} B,\ B \xrightarrow{1} A,\ A \xrightarrow{1} A + B \tag{4}$$

with all rate constants chosen to be equal to 1, gives rise to the mass action system

$$\begin{bmatrix} \dot{x}_A \\ \dot{x}_B \end{bmatrix} = \begin{bmatrix} -2 & 1 & 0 \\ 1 & -1 & 1 \end{bmatrix} \begin{bmatrix} x_A^2 \\ x_B \\ x_A \end{bmatrix}$$

and therefore the equilibria sit at the intersection of

$$\dot{x}_A = -2x_A^2 + x_B = 0 \text{ and } \dot{x}_B = x_A^2 - x_B + x_A = 0$$

(see Figure 4**a.**). A quick calculation shows that there is only one positive equilibrium at $(1, 2)$. Note that the stoichiometric matrix here has rank 2, and so there are no conservation laws. When these are present, the discussion on equilibria becomes a little more subtle; for example, let's revisit network (3)

$$A \underset{1}{\overset{1}{\rightleftharpoons}} 2B \tag{5}$$

with both rate constants set to 1. The steady state equations read

$$\dot{x}_A = -x_A + x_B^2 = 0, \quad \dot{x}_B = 2x_A - 2x_B^2 = 0,$$

the two polynomials are multiple of each other, and therefore we really only have one equation in two variables. This produces a continuum of equilibria $x_A = x_B^2$ called the *steady state manifold* – see Figure 4**b**. However, now we have a conservation law $2x_A + x_B = const$. When speaking about uniqueness of positive equilibria in networks with conservation laws, the relevant question is whether steady states are

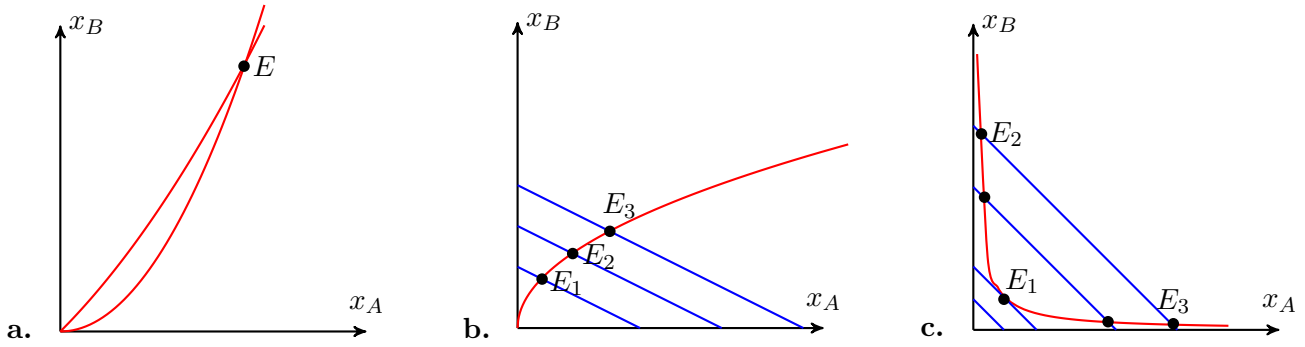



Figure 4: **a.** Nullclines for (4). The unique positive equilibrium is $(1, 2)$. **b.** The two nullclines for (5) coincide. There is a unique equilibrium in each positive stoichiometry class, and it is nondegenerate. **c.** The two nullclines for (6) coincide. Some stoichiometry classes contain two nondegenerate equilibria, some of them contain no equilibria, while the stoichiometry class $x_A + x_B = 2$ has a single degenerate equilibrium.

unique *within some (same) stoichiometry class*. It is easy to check that for any $T > 0$, the stoichiometry class $2x_A + x_B = T$ contains precisely one equilibrium at $(\frac{1+4T-\sqrt{1+8T}}{8}, \frac{-1+\sqrt{1+4T}}{4})$ (see also Figure 4**b**.

On the other hand, the network

$$2A + B \xrightarrow{1} 3A, \ A \xrightarrow{1} B \tag{6}$$

has two positive equilibria within some stoichiometry classes. Indeed, the mass action equations read

$$\begin{bmatrix} \dot{x}_A \\ \dot{x}_B \end{bmatrix} = \begin{bmatrix} 1 & -1 \\ -1 & 1 \end{bmatrix} \begin{bmatrix} x_A^2 x_B \\ x_A \end{bmatrix}$$

and the steady state equations are

$$\dot{x}_A = x_A^2 x_B - x_A = 0, \quad \dot{x}_B = -x_A^2 x_B + x_A = 0.$$

Note that the two equations are equivalent (linearly dependent), and in the positive quadrant they both reduce to $x_A x_B = 1$. The intersection of this curve with the stoichiometry class $x_A + x_B = T$, $T > 0$ yields two positive equilibria $(\frac{T+\sqrt{T^2-4}}{2}, \frac{T-\sqrt{T^2-4}}{2})$ and $(\frac{T-\sqrt{T^2-4}}{2}, \frac{T+\sqrt{T^2-4}}{2})$ if $T > 2$, a single equilibrium if $T = 2$, and no equilibria if $T < 2$ (see Figure 4**c**).

Since there exists at least one stoichiometry class that contains more than one steady state, we say that network (6) has multiple positive equilibria. Here we happened to fix the rate constants, but throughout this chapter that won't be the case. We say that a network has multiple positive equilibria if *for some choice of rate constants* there exists a stoichiometry class with two or more steady states.

Network (6) is a favorite example in the multistationarity literature [58, 28, 29, 43]: it is simple enough for calculations to be done by hand, but it is complex enough to have interesting features, like multistationarity and degenerate equilibria. We will often use versions of this network to illustrate various points throughout this chapter.

## 2.6 Nondegenerate equilibria

An equilibrium is called *nondegenerate* if the various manifolds at whose intersection it lies (linearly independent nullclines and conservation laws, when the latter exist) intersect transversally. This technicality is important in various places during our presentation: for example, we will be interested to see how equilibria survive under small perturbations of the vector field (and therefore of the nullclines), which leads naturally

to considering the notion of nondegeneracy. Figure 4 suggests that all equilibria of (4) and (5) are nondegenerate, whereas the steady state $E_1$ of (6) is degenerate. To make this precise, note that nondegeneracy is equivalent to saying that the normal vectors to the (linearly independent) hypersurfaces that cut out an equilibrium point are linearly independent. When there are no conservation laws, these normal vectors are the rows of the Jacobian matrix, and therefore nondegeneracy is equivalent to non-vanishing of the Jacobian at the equilibrium point. The Jacobian matrix of network (4) is

$$Df = \begin{bmatrix} -4x_A & 1 \\ 2x_A + 1 & -1 \end{bmatrix}$$

and the Jacobian is $\det(Df) = 2x_A - 1$. At equilibrium (1,2) this is positive, and nondegeneracy follows.

When conservation laws are present, linearly dependent nullclines are replaced by linearly independent conservation laws, and the Jacobian of the new algebraic system is then computed. For network (5), nondegeneracy is checked by computing the Jacobian of the algebraic system

$$-x_A + x_B^2 = 0, \quad 2x_A + x_B - T = 0,$$

i.e.

$$\det \begin{bmatrix} -1 & 2x_B \\ 2 & 1 \end{bmatrix} = -1 - 4x_B.$$

This is negative everywhere in the positive orthant, and therefore equilibria are nondegenerate. As for network (6), one computes the Jacobian of the system

$$x_A^2 x_B - x_A = 0, \quad x_A + x_B - T = 0$$

to get

$$\det \begin{bmatrix} 2x_A x_B - 1 & x_A^2 \\ 1 & 1 \end{bmatrix} = 2x_A x_B - 1 - x_A^2. \tag{7}$$

At a positive equilibrium point $(p_A, p_B)$ we have $p_A p_B = 1$, so the Jacobian is equal to $1 - p_A^2$, which shows that all equilibria are nondegenerate except for when $p_A = 1$, i.e. when the steady state is $E_1 = (1, 1)$.

### 2.7 The reduced Jacobian

Here we illustrate another way to think about nondegeneracy. Let's revisit network (6) and the nondegeneracy condition (7) for an equilibrium $p \in \mathbb{R}^2$. This condition means that any non-zero vector $v \in \mathbb{R}^2$ satisfying $[2p_A p_B - 1,\ p_A^2]^t v = 0$ cannot be orthogonal to $[1, 1]^t$, i.e. it cannot lie in the stoichiometric subspace $\mathrm{span}([-1, 1]^t)$. In other words, the Jacobian map of the network at $p$, $Df(p) : \mathbb{R}^2_{\geq 0} \to \mathrm{span}([-1, 1]^t)$ does not vanish on non-zero vectors of the stoichiometric subspace $\mathrm{im}\,\Gamma$, i.e. its restriction to $\mathrm{im}\,\Gamma$ is invertible. To compute this restriction, we use local coordinates on the stoichometric subspace as follows. For $y \in \mathrm{im}\,\Gamma$, let $z \in \mathbb{R}$ denote the coordinate of $y$ in our basis, $y = \begin{bmatrix} 1 \\ -1 \end{bmatrix} z$. Then, recalling that $v(x) = [x_A^2 x_B, x_A]^t$, we have

$$\begin{aligned} Df(p)y &= \Gamma Dv(p) \left( \begin{bmatrix} 1 \\ -1 \end{bmatrix} z \right) = \begin{bmatrix} -1 & 1 \\ 1 & -1 \end{bmatrix} \begin{bmatrix} 2p_A p_B & p_A^2 \\ 1 & 0 \end{bmatrix} \begin{bmatrix} 1 \\ -1 \end{bmatrix} z \\ &= \begin{bmatrix} 1 \\ -1 \end{bmatrix} [1 \ \ -1] \begin{bmatrix} 2p_A p_B & p_A^2 \\ 1 & 0 \end{bmatrix} \begin{bmatrix} 1 \\ -1 \end{bmatrix} z = \begin{bmatrix} 1 \\ -1 \end{bmatrix} (2p_A p_B - 1 - p_A^2) z \end{aligned} \tag{8}$$

and therefore $z \mapsto (2p_A p_B - 1 - p_A^2)z$ is the action of the Jacobian matrix $Df(p)$ using the basis $\{[1, -1]^t\}$ of the stoichiometric subspace. This is generally a linear map (it happens to be a scalar multiplication in

this example since $\operatorname{rank}\Gamma = 1$). Its determinant is called *the reduced Jacobian* of the system. Non-vanishing of the reduced Jacobian at $p$ is equivalent to $p$ being nondegenerate. Not coincidently, the reduced Jacobian computed here is the same as the determinant computed in (7). This is revisited in the next section, where the construction of the reduced determinant is presented in full generality.

### 2.8 General setup and preliminaries

Here we put the various terminology discussed so far into a general framework. Each notion introduced below is referred to a previous section where examples were discussed informally. We adopt a presentation of matrix-theoretical flavor along the lines of [27]. Throughout this section, we consider a CRN of $m$ reactions on $n$ species, and we fix an order of species and an order of reactions. We can then refer to "species $i$" or "reaction $j$". Reactions may be reversible, but we will view these as two separate irreversible reactions. For convenience, a reaction arrow proceeds from left to right: reactants sit on the left, and products on the right.

In what follows, $\mathbb{R}^n_{\geq 0}$ denotes be the nonnegative orthant in $\mathbb{R}^n$ with interior $\mathbb{R}^n_{>0}$. The image (column space) of a matrix $A \in \mathbb{R}^{n\times m}$, a linear subspace of $\mathbb{R}^n$, is denoted $\operatorname{im} A$, and the nullspace of $A$ is denoted by $\ker A$. The transpose of $A$ is denoted by $A^t$. If $x \in \mathbb{R}^n$, we denote by $D_x \in \mathbb{R}^{n\times n}$ the diagonal matrix with elements of $x$ on the diagonal, i.e. $(D_x)_{ii} = x_i$. We will use the following notation for submatrices and minors: for a matrix $A \in \mathbb{R}^{n\times m}$ and sets $\alpha, \beta \subseteq \{1, \ldots, n\}$, $A(\alpha|\beta)$ denotes the submatrix of $A$ with rows from $\alpha$ and columns from $\beta$. If $|\alpha| = |\beta|$, then $A[\alpha|\beta] \stackrel{\text{def}}{=} \det(A(\alpha|\beta))$ denotes the corresponding minor of $A$. When $\alpha = \beta$, these are simply denoted by $A(\alpha)$ and $A[\alpha]$.

**Definition 1** (Reactant matrix, product matrix, stoichiometric matrix – *Sections 2.1, 2.2*)**.** *The* reactant matrix $\Gamma_l \in \mathbb{Z}^{n\times m}$ *and the* product matrix $\Gamma_r \in \mathbb{Z}^{n\times m}$ *are defined as follows:* $(\Gamma_l)_{ij}$ *is the number of molecules of species $i$ occurring on the reactant side (left-hand side) of reaction $j$;* $(\Gamma_r)_{ij}$ *is the number of molecules of species $i$ occurring on the product side (right-hand side) of reaction $j$. The* stoichiometric matrix *of the network is defined as* $\Gamma = \Gamma_r - \Gamma_l$.

Note that $\Gamma_l$ and $\Gamma_r$ have non-negative entries. The $(i, j)$ entry of the stoichiometric matrix is the net gain of molecules of species $i$ in reaction $j$. The stoichiometric matrix is not uniquely defined, as it depends on the orderings on the species and reactions. However, these orderings do not impact any of the results we discuss here.

**Definition 2** (Complexes, inflow/outflow reactions, reaction graph – *Section 2.1*)**.** *If species are denoted $X_1, \ldots, X_n$, we define formally the vector $X = (X_1, \ldots, X_n)^t$. The formal dot products of columns of $\Gamma_l$ and $\Gamma_r$ with $X$ are termed* complexes *of the network [57]. Each reaction converts its* source complex *into its* product complex*. The zero vector (as a column in $\Gamma_l$ or $\Gamma_r$) is called the* zero complex*, and simply denoted by 0. Reactions of the form $0 \to C$ and $C \to 0$ (where $C$ is a non-zero complex) are called* inflow *and* outflow *reactions, respectively. Any reaction can be viewed as an edge in a directed graph whose vertices are the network complexes. This graph fully characterizes the CRN, and is termed the* reaction graph *of the network.*

The time evolution of the vector of species concentrations $x = x(t) \in \mathbb{R}^n_{\geq 0}$ is governed by the ODE system

$$\dot{x} = \Gamma v(x) =: f(x) \tag{9}$$

where $\dot{x}$ denotes $\frac{dx}{dt}$, $\Gamma \in \mathbb{R}^{n\times m}$ denotes the stoichiometric matrix and $v \in \mathbb{R}^{m\times 1}$ denotes the vector of reaction rates or *kinetics* of the system. While there are many types of kinetics that arise in practice, we restrict our attention to *mass action*, perhaps the most important one.

It is convenient to introduce the vector power notation: given $x = (x_1, \ldots, x_n)^t, y = (y_1, \ldots, y_n)^t \in \mathbb{R}^n_{\geq 0}$, we let $x^y = \prod_{i=1}^n x_i^{y_i}$. If $M \in \mathbb{R}^{m \times n}$ has columns $M_1, \ldots, M_m$ we use $x^M$ as a convenient abbreviation for the $1 \times m$ vector $(x^{M_1}, \ldots, x^{M_m})$.

**Definition 3** (Mass action kinetics, rate constants – *Sections 2.1, 2.2*)**.** *Let $\Gamma_l$ and $\Gamma \in \mathbb{Z}^{n \times m}$ denote the reactant and stoichiometric matrices, respectively. For $k \in \mathbb{R}^n_{>0}$, we say that (9) with $v(x) = D_k(x^{\Gamma_l})^t$ is a* CRN with mass action kinetics *and* rate constants $k$*. In mass action, the rate $v_j$ of reaction $j$ is proportional to the product of reactant concentrations, and the proportionality factor is $k_j$. A CRN with mass action is therefore governed by the ODE system*

$$\dot{x} = \Gamma D_k(x^{\Gamma_l})^t =: f(x). \tag{10}$$

Note that the rate of an inflow reaction $0 \xrightarrow{k} A$ is constant, and equal to $k$.

It is easy to show that the nonnegative orthant is forward invariant under the dynamics of a CRN with mass action. The proof of this fact is left to the reader (Exercise 2). For $x \in \mathbb{R}^n_{>0}$, we let $1/x = (1/x_1, \ldots, 1/x_n)$. The Jacobian matrix of (9) factors as

$$Df(x) = \Gamma D_{v(x)} \Gamma_l^t D_{1/x} \tag{11}$$

(this is an easy calculation; see Section 2.3 for an example).

**Definition 4** (Stoichiometric subspace, stoichiometry classes – *Section 2.4*)**.** *Given a CRN with stoichiometric matrix $\Gamma \in \mathbb{R}^{n \times m}$, $\operatorname{im}\Gamma \subseteq \mathbb{R}^n$ is called the* stoichiometric subspace *of the network. For $p \in \mathbb{R}^n_{\geq 0}$, the coset of $\operatorname{im}\Gamma$ containing $p$ and intersected with $\mathbb{R}^n_{\geq 0}$*

$$S_p = (p + \operatorname{im}\Gamma) \cap \mathbb{R}^n_{\geq 0} = \{y \in \mathbb{R}^n_{\geq 0} \mid \ y - p \in \operatorname{im}\Gamma\}$$

*is called the* stoichiometry class of $p$*. A stoichiometry class which intersects $\mathbb{R}^n_{>0}$ is called* nontrivial*. The intersection of a stoichiometry class with $\mathbb{R}^n_{>0}$ (if non-empty) is a* positive stoichiometry class*.*

Integrating (9) yields

$$x(t) = x(0) + \Gamma \int_0^t v(x(s))ds$$

which shows that the solution $\{x(t) \mid \ t \geq 0\}$ of (9) is constrained to the stoichiometry class of $x(0)$. Put another way, trajectories of CRN dynamics satisfy certain linear constraints, called conservation laws.

**Definition 5** (Conservation laws – *Section 2.4*)**.** *A non-zero element of $\ker\Gamma^t$ is called a* conservation law *of the CRN. A basis of $\ker\Gamma^t$ is called a* complete set of independent conservation laws*.*

If $v$ is a conservation law, then (9) yields $v \cdot x(t) = \textit{constant}$. Since $\ker\Gamma^t = (\operatorname{im}\Gamma)^\perp$, a set of constants for a complete set of conservation laws uniquely defines a coset of $\operatorname{im}\Gamma$, i.e. a stoichiometry class.

**Definition 6** (Fully open systems, fully open extensions – *Section 2.1*)**.** *A CRN that contains inflow reactions $A \to 0$ and outflow reactions $0 \to A$ for all species $A$ is called a* fully open network*. A fully open network has stoichiometric subspace equal to $\mathbb{R}^n$, and no conservation laws. Adding all inflow and outflow reactions to a network defines its* fully open extension*.*

**Definition 7** (Equilibria, multiple positive equilibria – *Section 2.5*)**.** *A point $p \in \mathbb{R}^n_{\geq 0}$ is called an equilibrium of a CRN if $\Gamma v(p) = 0$. It is called a* positive equilibrium *if $p \in \mathbb{R}^n_{>0}$. A CRN has* the capacity for multiple positive equilibria (MPE) *[26] if there exist rate constants $k \in \mathbb{R}^n_{>0}$ for which (10) admits two distinct equilibria within the same stoichiometry class, i.e. there exist distinct $p_1, p_2 \in \mathbb{R}^n_{>0}$ such that $p_1 - p_2 \in \operatorname{im}\Gamma$ and*

$$\Gamma D_k(p_1^{\Gamma_l})^t = \Gamma D_k(p_2^{\Gamma_l})^t = 0.$$

Let $r > 0$ be the rank of the stoichiometric matrix $\Gamma$. Choose any basis for $\operatorname{im}\Gamma$ and arrange its vectors as columns of a matrix $\Gamma_0 \in \mathbb{R}^{n\times r}$. We can write $\Gamma = \Gamma_0 Q$ for some matrix $Q$. Letting $\Gamma'$ be a left inverse of $\Gamma_0$ we get $\Gamma'\Gamma = Q$, and therefore $\Gamma = \Gamma_0\Gamma'\Gamma$. We write $x \in \operatorname{im}\Gamma$ in coordinates corresponding to the basis $\Gamma_0$, i.e. $x = \Gamma_0 y$. The action of the Jacobian map $\Gamma Dv$ on $x \in \operatorname{im}\Gamma$ is as follows: $\Gamma Dv\Gamma_0 y = \Gamma_0\Gamma'\Gamma Dv\Gamma_0 y = \Gamma_0 z$ with $z = \Gamma'\Gamma Dv\Gamma_0 y$. Therefore the Jacobian matrix acts on local coordinates on $\operatorname{im}\Gamma$ according to $y \mapsto \Gamma'\Gamma Dv\Gamma_0 y$.

**Definition 8** (Reduced Jacobian [27] – *Section 2.7*)**.** *With the notations introduced above, a* reduced Jacobian matrix *of a CRN is defined as* $\Gamma'\Gamma Dv\Gamma_0$*; its determinant is called the* reduced Jacobian *and denoted by* $\det_\Gamma(\Gamma Dv)$.

Note that different choices of $\Gamma_0$ and $\Gamma'$ may result in different reduced Jacobian matrices, however, they are all similar matrices (see [27, Appendix A]). Therefore the reduced Jacobian does not depend on these choices and the notation $\det_\Gamma(\Gamma Dv)$ is unambiguous. In fact, one can show that the reduced Jacobian of a CRN is equal to the sum of $r \times r$ principal minors of the Jacobian:

**Proposition 1.** *[27]* $\det_\Gamma(\Gamma Dv) = \sum_{\substack{\alpha\subseteq\{1,\dots,n\}\\ |\alpha|=r}} (\Gamma Dv)[\alpha]$.

In particular, if $\operatorname{rank}\Gamma = n$ (for example if the network is fully open), then the reduced Jacobian and the Jacobian are equal.

**Definition 9** (Nondegenerate equilibria – *Sections 2.6, 2.7*)**.** *An equilibrium* $p \in \mathbb{R}^n_{\geq 0}$ *of a mass action CRN is called nondegenerate if* $\det_\Gamma(\Gamma Dv(p)) \neq 0$*. We say that a CRN has* the capacity for multiple positive nondegenerate equilibria (MPNE) *if there exist rate constants* $k \in \mathbb{R}^n_{>0}$ *for which (10) admits two distinct nondegenerate equilibria within the same positive stoichiometry class, i.e. there exist distinct* $p_1, p_2 \in \mathbb{R}^n_{>0}$ *such that* $p_1 - p_2 \in \operatorname{im}\Gamma$,

$$\Gamma D_k(p_1^{\Gamma_l})^t = \Gamma D_k(p_2^{\Gamma_l})^t = 0,$$

*and* $\det_\Gamma(\Gamma Dv(p_i)) \neq 0$ *for* $i = 1, 2$.

Alternatively, an equilibrium is nondegenerate if it sits at a *transversal* intersection of nullclines and hyperplanes defined by conservation laws. To see that this is equivalent to the previous definition, we use the following setup (see for example [35, 36]). First we reorder the species (i.e. the rows of $\Gamma$) so that a complete set of conservation laws can be arranged as the columns of the block matrix $\begin{bmatrix} W \\ I \end{bmatrix}$ with $I \in \mathbb{R}^{(n-r)\times(n-r)}$ denoting the identity matrix, and $W \in \mathbb{R}^{r\times(n-r)}$. In other words, the last $n-r$ concentrations can be linearly eliminated using conservation laws; also, their derivatives are linear combinations of the derivatives of the first $r$ concentrations. We form a new algebraic system characterizing the equilibria in some stoichiometric class by removing the equations corresponding to the last $n-r$ concentrations from the steady state system $\Gamma v(x) = 0$, and replacing them with the $n-r$ conservation laws which define the stoichiometric class $[W^t|I]x = T$ (here $T \in \mathbb{R}^{n-r}$ is the vector of conserved values). Nondegeneracy according to the new geometric interpretation amounts to the non-vanishing of the new algebraic system's Jacobian. It turns out that this is equivalent to the nondegeneracy in Definition 9. Indeed, if

$$\Gamma = \begin{bmatrix} \Gamma_1 \\ \Gamma_2 \end{bmatrix} \tag{12}$$

where $\Gamma_1 \in \mathbb{R}^{r\times m}$, then the new algebraic system reads

$$\Gamma_1 v(x) = 0, \quad [W^t|I]x - T = 0 \tag{13}$$

and we have the following result generalizing the calculations (7) and (8) in Sections 2.6 and 2.7.

**Proposition 2.** *The Jacobian of (13) is equal to the reduced Jacobian* $\det_\Gamma(\Gamma Dv)$.

*Proof.* Note that from (12) and the definition of $W$ we get $\Gamma_2 = -W^t\Gamma_1$. We rearrange reactions (columns of $\Gamma$) to get a nonzero principal minor $\Gamma(\{1,\dots,r\})$, and we write $\Gamma$ and $Dv$ in corresponding block form

$$\Gamma = \begin{bmatrix} \Gamma_{11} & \Gamma_{12} \\ -W^t\Gamma_{11} & -W^t\Gamma_{12} \end{bmatrix}, \quad Dv = \begin{bmatrix} V_{11} & V_{12} \\ V_{21} & V_{22} \end{bmatrix}$$

where $\Gamma_{11} \in \mathbb{R}^{r\times r}$ is nonsingular and $V_{11} \in \mathbb{R}^{r\times r}$. The Jacobian matrix of (13) is

$$\begin{bmatrix} \Gamma_{11}V_{11} + \Gamma_{12}V_{21} & \Gamma_{11}V_{12} + \Gamma_{12}V_{22} \\ W^t & I \end{bmatrix}. \tag{14}$$

To compute the reduced determinant, note that the columns of $\Gamma_0 = \begin{bmatrix} \Gamma_{11} \\ -W^t\Gamma_{11} \end{bmatrix}$ form a basis of $\operatorname{im}\Gamma$ and that $\Gamma' = [\Gamma_{11}^{-1}|0]$ is a left inverse of $\Gamma_0$. Then

$$\begin{aligned} \det_\Gamma(\Gamma Dv) &= \det(\Gamma'\Gamma Dv\Gamma_0) \\ &= \det\left([\Gamma_{11}^{-1}|0]\begin{bmatrix} \Gamma_{11} & \Gamma_{12} \\ -W^t\Gamma_{11} & -W^t\Gamma_{12} \end{bmatrix}\begin{bmatrix} V_{11} & V_{12} \\ V_{21} & V_{22} \end{bmatrix}\begin{bmatrix} \Gamma_{11} \\ -W^t\Gamma_{11} \end{bmatrix}\right) \\ &= \det\Gamma_{11}^{-1}\det\left([I|0]\begin{bmatrix} \Gamma_{11} & \Gamma_{12} \\ -W^t\Gamma_{11} & -W^t\Gamma_{12} \end{bmatrix}\begin{bmatrix} V_{11} & V_{12} \\ V_{21} & V_{22} \end{bmatrix}\begin{bmatrix} I \\ -W^t \end{bmatrix}\right)\det\Gamma_{11} \\ &= \det(\Gamma_{11}V_{11} - \Gamma_{11}V_{12}W^t + \Gamma_{12}V_{21} - \Gamma_{12}V_{22}W^t), \end{aligned}$$

which equals the determinant of (14) by the Schur determinant formula. □

Some of the results to follow regard not only the existence of multiple positive equilibria of a CRN, but also their linear stability in the following sense.

**Definition 10** (Linear stability, multiple positive linearly stable equilbria: MPSE)**.** *An equilibrium $p$ of (9) will be termed* linearly stable *if it is linearly stable w.r.t. its stoichiometry class, namely all eigenvalues of the reduced Jacobian matrix have negative real parts. A mass action CRN displays* multiple positive linearly stable equilibria *(MPSE) if, for some choice of its rate constants, it has two distinct positive equilibria in some same stoichiometry class, both linearly stable.*

Note that if a CRN has MPSE, then it also has MPNE. The network (6) shows that the converse is not true in general (Exercise 5).

## Exercises

1. *(Hungarian Lemma [44]).* Let $f_i(x) : \mathbb{R}^n \to \mathbb{R}$, $i \in \{1,\dots,n\}$ be polynomial functions and $f(x) = (f_1(x),\dots,f_n(x))$. Show that there exists a CRN whose mass action equations are $\dot{x} = f(x)$ if and only if any monomial with negative coefficient in $f_i$ contains $x_i$. (For example, this is not satisfied for $\dot{x}_1 = -2x_1x_2^2 + 1$, $\dot{x}_2 = -x_1^2 + 3x_2$, since the negative monomial $-x_1^2$ in the expression of $\dot{x}_2$ does not contain $x_2$.)

   *Remark. This shows that a large class of polynomial dynamical systems can be realized as mass action, and therefore results for mass action may have implications beyond reaction networks.*

2. *(Forward invariance under mass action).* Show that if $x(t)$, $t \geq 0$ is a solution of (10) with $x(0) \in \mathbb{R}^n_{\geq 0}$, then $x(t) \in \mathbb{R}^n_{\geq 0}$ for all $t \geq 0$.

3. *(The futile cycle).* Find a complete set of conservation laws for the following CRN (the *futile cycle (1)*, sometimes also called the *one-step phosphorylation-dephosphorylation network*), and compute its reduced Jacobian:

$$E + S \rightleftharpoons ES \to E + P$$
$$F + P \rightleftharpoons FP \to F + S.$$

4. *(Examples of small networks)* Analyze the capacity for MPE/MPNE/MPSE for the following mass action networks (see [27] for related examples, and [29] for results on two-species networks):
   a. $2A + B \to 3A, \quad A \to B, \quad A \to 0, \quad 0 \to B.$
   b. $2A + B \to 3A, \quad A \to B, \quad A \rightleftharpoons 0, \quad B \rightleftharpoons 0.$
   c. $2A + B \to 3A, \quad A \to B, \quad A + B \rightleftharpoons 0.$
   d. $A \to B, \ 2B \to 2A, \ 2A + 2B \to 3A + B.$
   e. $A + B \to 2B, \ 2A + B \to 2B, \ 3B \to A + 2B.$

5. *(MPNE does not imply MPSE).* Show that the CRN (6) does not have MPSE.

# 3 Necessary conditions for multistationarity I: injective CRNs

Much of the literature on multistationarity for CRNs has focused on studying necessary conditions for the existence of two or more positive equilibria. A particularly successful approach in this direction has been that of *injective* CRNs, i.e. CRNs for which the corresponding vector field $f = \Gamma v$ (10) is an injective function on positive stoichiometry classes for any choice of rate constants. A very simple, but perhaps illuminating example of a non-injective CRN is $2A \rightleftharpoons A$; for rate constants equal to one, the mass action vector field is equal to $-x_A^2 + x_A$, which is not injective on the unique stoichiometry class, namely $\mathbb{R}_{>0}$. Clearly, an injective CRN cannot have the capacity for multiple positive equilibria, since the latter requires that two points in the same positive stoichiometry class be both mapped by $f$ to 0. Note, however that injectivity is *not equivalent* to the lack of capacity for multiple positive equilibria (see Exercise 1 below).

The study of injective CRNs was started by Craciun and Feinberg for fully open networks [26] and has since been extended by work of various authors [27, 35, 36, 37, 38, 39]. These papers have led to complete characterizations of injective CRNs, some of which are gathered in the theorem below. First, we introduce some terminology and notation.

**Definition 11** (Positive and negative matrices)**.** *For a real matrix (or vector) $A$, write $A \geq 0$ to denote the fact that all entries of $A$ are nonnegative, and $A > 0$ to denote the fact that $A \geq 0$ and $A \neq 0$.*

**Definition 12** (Qualitative class)**.** *Given $A \in \mathbb{R}^{n\times m}$, the* qualitative class $\mathcal{Q}(A) \subseteq \mathbb{R}^{n\times m}$ *of $A$ consists of all matrices with the same sign pattern as $A$, i.e., $B \in \mathcal{Q}(A)$ if and only if $(A_{ij} > 0) \Rightarrow (B_{ij} > 0)$; $(A_{ij} < 0) \Rightarrow (B_{ij} < 0)$; and $(A_{ij} = 0) \Rightarrow (B_{ij} = 0)$. If $\mathcal{A}$ is a set of matrices or vectors, we write $\mathcal{Q}(\mathcal{A})$ for $\cup_{A\in\mathcal{A}}\mathcal{Q}(A)$.*

Finally, if $A, B \in \mathbb{R}^{n\times m}$ we write $A \circ^r B > 0$ (respectively, $A \circ^r B < 0$) if the product of any pair of corresponding $r \times r$ minors of $A$ and $B$ is non-negative (non-positive), and at least one of these products is positive (respectively, negative). To be precise, $A \circ^r B > 0$ if for any $\alpha \subset \{1, \ldots, n\}, \ \beta \subset \{1, \ldots, m\}$ with $|\alpha| = |\beta| = r$ we have

$$A[\alpha|\beta]B[\alpha|\beta] \geq 0$$

and at least one such product is positive.

Consider a CRN with stoichiometric matrix $\Gamma$ and reactant matrix $\Gamma_l$, and let $r = \operatorname{rank}\Gamma$.

**Theorem 1.** *The following are equivalent:*

1. (Injectivity) *The mass action vector field* $\Gamma v(x) := \Gamma D_k(x^{\Gamma_l})^t$ *(as a function of* $x$*) is injective on any positive stoichiometry class, for any rate constants* $k \in \mathbb{R}^m_{>0}$.

2. (Nonvanishing of reduced Jacobian) *The reduced Jacobian* $\det_\Gamma(\Gamma Dv)$ *(as a polynomial in* $x$ *and* $k$*) is non-zero and all of its terms have the same sign.*

3. (Concordance) $\Gamma \circ^r \Gamma_l^t > 0$ *or* $\Gamma \circ^r \Gamma_l^t < 0$.

4. (Sign condition) $\Gamma_l^t(\mathcal{Q}(\operatorname{im}\Gamma)\backslash\{0\}) \cap \mathcal{Q}(\ker\Gamma) = \emptyset$.

For the proofs of these and other related results, the reader is referred to [27] and [35]. A network is called *concordant* if condition 3 in Theorem 1 above holds. Concordance was first defined in [38] in an equivalent way, and versions of the results above appear in that work. In many cases, the concordance condition may be easiest to check – note that its calculation does not involve the rate constants. On the other hand, the reduced Jacobian is also not hard to compute – recall from Proposition 1 that the reduced Jacobian is the sum of $r \times r$ principal minors of the Jacobian matrix $\Gamma Dv$.

Next, we discuss an example illustrating the applicability of Theorem 1. For more examples, the reader is referred to the exercises at the end of the section. Consider the following version of the futile cycle (1), where metabolite $S$ gets transformed into a product $P$ in a reaction catalyzed by enzyme $E$, while the reverse process does not require an enzymatic mechanism:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_3}{\to} E + P, \quad P \overset{k_4}{\to} S. \tag{15}$$

Setting species $E, S, ES, P$ in this order, the corresponding stoichiometric matrix $\Gamma$, reactant matrix $\Gamma_l$, and ODE system are

$$\Gamma = \begin{bmatrix} -1 & 1 & 1 & 0 \\ -1 & 1 & 0 & 1 \\ 1 & -1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix}, \quad \Gamma_l = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}, \quad \dot{x} = \begin{bmatrix} -1 & 1 & 1 & 0 \\ -1 & 1 & 0 & 1 \\ 1 & -1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix} \begin{bmatrix} k_1 x_1 x_2 \\ k_2 x_3 \\ k_3 x_3 \\ k_4 x_4 \end{bmatrix}.$$

The Jacobian matrix can be computed as

$$\Gamma Dv = \begin{bmatrix} -1 & 1 & 1 & 0 \\ -1 & 1 & 0 & 1 \\ 1 & -1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix} \begin{bmatrix} k_1 x_2 & k_1 x_1 & 0 & 0 \\ 0 & 0 & k_2 & 0 \\ 0 & 0 & k_3 & 0 \\ 0 & 0 & 0 & k_4 \end{bmatrix} = \begin{bmatrix} -k_1 x_2 & -k_1 x_1 & k_2 + k_3 & 0 \\ -k_1 x_2 & -k_1 x_1 & k_2 & k_4 \\ k_1 x_2 & k_1 x_1 & -k_2 - k_3 & 0 \\ 0 & 0 & k_3 & -k_4 \end{bmatrix},$$

and since $\operatorname{rank}\Gamma = 2$, the reduced Jacobian of the system is the sum of all $2 \times 2$ principal minors of $\Gamma Dv$, i.e.

$$\begin{aligned} \det_\Gamma(\Gamma Dv) &= \begin{vmatrix} -k_1 x_2 & 0 \\ 0 & -k_4 \end{vmatrix} + \begin{vmatrix} -k_1 x_1 & k_2 \\ k_1 x_1 & -k_2 - k_3 \end{vmatrix} + \begin{vmatrix} -k_1 x_1 & k_4 \\ 0 & -k_4 \end{vmatrix} + \begin{vmatrix} -k_2 - k_3 & 0 \\ k_3 & -k_4 \end{vmatrix} \\ &= k_1 k_3 x_1 + k_1 k_4 x_1 + k_1 k_4 x_2 + k_2 k_4 + k_3 k_4. \end{aligned}$$

This polynomial in $x_1, \ldots, x_4, k_1, \ldots, k_4$ has only positive coefficients, and so Theorem 1 implies that the vector field is injective on each positive stoichiometry class. Therefore, the mass action network (15) does not have the capacity for multiple positive equilibria.

One may reach the same conclusion by checking concordance of the network, i.e. by computing products of corresponding $2 \times 2$ minors of $\Gamma$ and $-\Gamma_l^t$. By inspection, the only non-zero products $\Gamma[\alpha|\beta]\Gamma_l[\alpha|\beta]$ occur

for $(\alpha|\beta) \in \{(\{1,4\}|\{1,4\}), (\{2,3\}|\{1,3\}), (\{2,4\}|\{1,4\}), (\{3,4\}|\{2,4\}), (\{3,4\}|\{3,4\})\}$. The corresponding products are

$$\begin{vmatrix} -1 & 0 \\ 0 & -1 \end{vmatrix} \begin{vmatrix} 1 & 0 \\ 0 & 1 \end{vmatrix}, \begin{vmatrix} -1 & 0 \\ 1 & -1 \end{vmatrix} \begin{vmatrix} 1 & 0 \\ 0 & 1 \end{vmatrix}, \begin{vmatrix} -1 & 1 \\ 0 & -1 \end{vmatrix} \begin{vmatrix} 1 & 0 \\ 0 & 1 \end{vmatrix}, \begin{vmatrix} -1 & 0 \\ 0 & -1 \end{vmatrix} \begin{vmatrix} 1 & 0 \\ 0 & 1 \end{vmatrix}, \begin{vmatrix} -1 & 0 \\ 1 & -1 \end{vmatrix} \begin{vmatrix} 1 & 0 \\ 0 & 1 \end{vmatrix},$$

and are all equal to one. Therefore the network is concordant, and injectivity follows. Note that the number of non-zero products of minors (five) equals the number of monomials in the reduced determinant computed above. This is not coincidental; it turns out that in general, the pairwise products of minors from the definition of concordance are precisely the coefficients of the polynomial $\det_\Gamma(\Gamma Dv)$ – see for example [27], and Exercise 2.

Finally, we illustrate how one checks the sign condition 4 in the theorem above. We note that our treatment here is rather rudimentary, and there is a great deal of subtlety that we miss in a short description like this one. For a detailed discussion of the sign condition and for insightful connections with the machinery of oriented matroids, the reader is referred to [45]. Informally, $\Gamma_l^t$ acts on sign vectors using the commutative addition rules $(+)+(+)=+,\ (-)+(-)=(-),\ (+)+(0)=+,\ (-)+(0)=-,\ (+)+(-)\in\{0,+,-\}$.

Choosing the second and fourth column of $\Gamma$ as basis, we can write $\operatorname{im}\Gamma = \{[x, x+y, -x, -y]^t \mid x, y \in \mathbb{R}\}$. The possible nonzero sign patterns of $\operatorname{im}\Gamma$ are obtained by listing all sign combinations of $x$ and $y$: $(0,+), (+,0), (+,+), (+,-), (0,-), (-,0), (-,-), (-,+)$. The first three sign combinations of $x$ and $y$ produce precisely one sign pattern and the fourth one, $(+,-)$ produces three more depending on the relative sizes of $|x|$ and $|y|$. The last four sign pairs give rise to the negative of the sign patterns we have so far. Therefore $\mathcal{Q}(\operatorname{im}\Gamma)\setminus\{0\}$ consists of twelve sign patterns, listed here:

$$\begin{bmatrix} 0 \\ + \\ 0 \\ - \end{bmatrix}, \begin{bmatrix} + \\ + \\ - \\ 0 \end{bmatrix}, \begin{bmatrix} + \\ + \\ - \\ - \end{bmatrix}, \begin{bmatrix} + \\ 0 \\ - \\ + \end{bmatrix}, \begin{bmatrix} + \\ + \\ - \\ + \end{bmatrix}, \begin{bmatrix} + \\ - \\ - \\ + \end{bmatrix}, \begin{bmatrix} 0 \\ - \\ 0 \\ + \end{bmatrix}, \begin{bmatrix} - \\ - \\ + \\ 0 \end{bmatrix}, \begin{bmatrix} - \\ - \\ + \\ + \end{bmatrix}, \begin{bmatrix} - \\ 0 \\ + \\ - \end{bmatrix}, \begin{bmatrix} - \\ - \\ + \\ - \end{bmatrix}, \begin{bmatrix} - \\ + \\ + \\ - \end{bmatrix}.$$

Applying $\Gamma_l^t$ to all sign vectors above, one obtains the sign patterns in $\Gamma_l^t(\mathcal{Q}(\operatorname{im}\Gamma)\setminus\{0\})$ (for simplicity, we only list the combinations with the first non-zero component equal to "+"; the remaining ones are simply the negative of these):

$$\begin{bmatrix} + \\ 0 \\ 0 \\ - \end{bmatrix}, \begin{bmatrix} + \\ - \\ - \\ 0 \end{bmatrix}, \begin{bmatrix} + \\ - \\ - \\ - \end{bmatrix}, \begin{bmatrix} + \\ - \\ - \\ + \end{bmatrix}, \begin{bmatrix} 0 \\ + \\ + \\ - \end{bmatrix}, \begin{bmatrix} + \\ + \\ + \\ - \end{bmatrix}. \tag{16}$$

On the other hand, $\ker\Gamma = \{[x+y, x, y, y]^t \mid x, y \in \mathbb{R}\}$ and therefore $\mathcal{Q}(\ker\Gamma)$ has the following non-zero sign patterns (once again, for shortness, we only list those whose first non-zero coordinate is "+"):

$$\begin{bmatrix} + \\ 0 \\ + \\ + \end{bmatrix}, \begin{bmatrix} + \\ + \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} + \\ + \\ + \\ + \end{bmatrix}, \begin{bmatrix} 0 \\ + \\ - \\ - \end{bmatrix}, \begin{bmatrix} + \\ + \\ - \\ - \end{bmatrix}, \begin{bmatrix} + \\ - \\ + \\ + \end{bmatrix}. \tag{17}$$

Since no sign pattern appears in both (16) and (17), the sign condition is satisfied and therefore the network is injective.

Checking the various injectivity conditions can be easily implemented computationally. For example, the software packages CoNtRol [46, 47] and CRNToolbox [48] include this functionality and many other CRN computation tools.

**Exercises**

1. *(1D injective CRNs).* Find all injective one-species mass action CRNs. Give an example of a non-injective CRN without capacity for MPE.

2. *(Coefficients of the reduced determinant).* Show that the coefficients of the reduced Jacobian of a CRN (as a polynomial in $x$ and $k$) are precisely the products $\Gamma[\alpha|\beta]\Gamma_l[\beta|\alpha]$, with $\alpha \subseteq \{1,\dots,n\}$, $\beta \subseteq \{1,\dots,m\}$, $|\alpha| = |\beta| = \operatorname{rank}\Gamma$ (as usual, $\Gamma$ denotes the stoichiometric matrix of the CRN).

   *Hint. Use the Cauchy-Binet formula: Given $A \in \mathbb{R}^{n\times m}$, $B \in \mathbb{R}^{m\times n}$, and any $\alpha,\beta \subseteq \{1,\dots,n\}$, $|\alpha| = |\beta| = r > 0$, we have*

$$(AB)[\alpha|\beta] = \sum_{\substack{\gamma\subseteq\{1,\dots,m\}\\ |\gamma|=r}} A[\alpha|\gamma]B[\gamma|\beta].$$

3. *Phoshporylation networks.* Are the following networks injective? Use CoNtRol to double check your answer.

   a. *(futile cycle)*

$$E + S \rightleftharpoons ES \to E + P$$
$$F + P \rightleftharpoons FP \to F + S.$$

   b. *(distributive double phosphorylation)*

$$E + S_1 \rightleftharpoons ES_1 \to E + S_2 \rightleftharpoons ES_2 \to E + S_3$$
$$F + S_3 \rightleftharpoons FS_3 \to F + S_2 \rightleftharpoons FS_2 \to F + S_1.$$

   c. *(distributive double phosphorylation without shared enzymes)*

$$E_1 + S_1 \rightleftharpoons E_1S_1 \to E_1 + S_2, \quad E_2 + S_2 \rightleftharpoons E_2S_2 \to E_2 + S_3$$
$$F_1 + S_3 \rightleftharpoons F_1S_3 \to F_1 + S_2, \quad F_2 + S_2 \rightleftharpoons F_2S_2 \to F_2 + S_1.$$

   d. *(processive double phosphorylation)*

$$E + S_1 \rightleftharpoons ES_1 \to ES_2 \to E + S_3$$
$$F + S_3 \rightleftharpoons FS_3 \to FS_2 \to F + S_1.$$

   *Remark. Injectivity is just one example of how dynamics changes as an effect of enzyme sharing, or by switching from distributive to processive mechanisms. See [49, 50, 51] for further discussion.*

# 4 Necessary conditions for multistationarity II: the DSR graph

There is a great deal of stable behavior in networks of chemical reactions, and, to a lesser degree, in biological networks. This can be explained in part by the fact that the possibility of exotic behavior (such as multistability) places rather delicate constraints on the structure of an interaction network; a seminal remark is due to R. Thomas, who noticed that positive feedback in the logical structure of a CRN are necessary for multistationarity [19]. Subsequent theoretical work proved this claim [11]; here we discuss the DSR graph condition, a far-reaching refinement of Thomas' observation. The DSR (*directed species-reaction*) graph,

introduced by Banaji and Craciun [40] is based on earlier work by Craciun and Feinberg [14], and it provides an elegant sufficient condition for injectivity of CRNs. We note that this condition is not also necessary, so that the methods of Section 3 are more powerful than the results that follow here. However, the DSR graph is closely related to the typical diagram depicting a biological network, and it offers unique insight into the connection between its structure and its capacity for multiple equilibria.

Throughout this section we consider *nonautocatalytic networks*, i.e. networks for which no species occurs on both sides of the same reaction. We note that the DSR theory doesn't need this restriction. However, the exposition is significantly simpler for nonautocatalytic networks, and moreover, most networks in practice are nonautocatalytic. In what follows, we regard each reversible reactions as one reaction, as opposed to splitting them in two irreversible reactions, and we (arbitrarily) choose a left side and a right side of a reversible reaction. This way, every species that enters a reversible reaction is either a *left reactant* or a *right reactant*. We also recall that species involved in an irreversible reactions are either *reactant species* (inputs), *product species* (outputs), or possibly both.

**Definition 13** (DSR graph, [40])**.** *The DSR graph of a CRN is a labeled bipartite directed multigraph, with nodes corresponding to* species *and* reactions*. The labels are all positive, but the graph will contain* positive *and* negative *edges. Moreover, given a species node $S$ and a reaction node $R$, two edges $S \to R$ and $R \to S$ of the same sign are by convention merged into one undirected edge $S - -R$ of the same sign. The DSR is defined in the following way:*

1. *For every* irreversible reaction *$R$ and every one of its* reactant species *$S$, we draw an undirected negative edge (depicted as a dashed line) $S - -R$. The edge is labeled with the* stoichiometric coefficient *of $S$ in $R$, i.e. the number of molecules of $S$ consumed in reaction $R$.*

2. *For every* irreversible reaction *$R$ and every one of its* product species *$S$, we draw a directed positive edge (depicted as a solid arrow) $R \to S$. The edge is labeled with the* stoichiometric coefficient *of $S$ in $R$, i.e. the number of molecules of $S$ produced in reaction $R$.*

3. *For every* reversible reaction *$R$ and every one of its* left reactant species *$S$, we draw an undirected negative edge $S - -R$. The edge is labeled with the* stoichiometric coefficient *of $S$ in $R$, i.e. the number of molecules of $S$ that enter reaction $R$.*

4. *For every* reversible reaction *$R$ and every one of its* right reactant species *$S$, we draw an undirected positive edge $S - R$. The edge is labeled with the* stoichiometric coefficient *of $S$ in $R$, i.e. the number of molecules of $S$ that enter reaction $R$.*

Figure 5 is perhaps illuminating; it illustrates two examples of DSR graphs, one of which corresponds to CRN (15). By convention, edge labels equal to 1 are omitted from the figure. As we will see below, the way various cycles intersect in the DSR graph may allow conclusions about the lack of multiple equilibria of the CRN's fully open extension. We carry on with a little more terminology.

Recall that a cycle in a directed graph is a path from some vertex to itself which repeats no other vertices, and which respects the orientation of any edges traversed. The unoriented edges in the DSR graph can be viewed as having two orientations, and can be traversed either way. Let $|C|$ denote the length of a cycle in the DSR graph, i.e. the number of vertices (or edges) it contains. For an edge $e$, let $l(e)$ denote its positive label as defined above. Recall that $e$ is also assigned a sign, $+1$ (solid) or $-1$ (dashed). Also note that since DSR is bipartite, each cycle has even length.

**Definition 14** (Sign of cycles, e-cycles, o-cycles, s-cycles, odd intersections, [14, 40])**.** *Let $C$ be a cycle in a DSR graph.*

1. *The* sign *of $C$, denoted* $\operatorname{sign}(C)$*, is the product of the signs of its edges. In other words, a cycle is positive (has sign +1) if it contains an even number of negative edges.*

2. *$C$ is called an* e-cycle *if* $(-1)^{|C|/2}\text{sign}(C) = 1$ *and is called an* o-cycle *if* $(-1)^{|C|/2}\text{sign}(C) = -1$. *In other words, a cycle $C$ is an e-cycle if the number of its negative (equivalently, the number of its positive) edges has the same parity as $|C|/2$.*

3. *Let $\{e_1, \ldots, e_{2r}\}$ denote the edges of $C$ traversed in order. $C$ is called an* s-cycle *if*

$$\prod_{i=1}^{r} l(e_{2i-1}) = \prod_{i=1}^{r} l(e_{2i}).$$

*Note that this product does not depend on the vertex of $C$ where we start enumerating its edges.*

4. *Two cycles in the DSR graph are* compatibly oriented *if their orientations coincide on each undirected edge in their intersection. Two cycles of the DSR graph have* odd intersection *if they are compatibly oriented and each component of their intersection contains an odd number of edges.*

To illustrate, we refer to Figure 5**a**. The DSR has four species nodes, three reaction nodes, and two cycles: $C_1: \ 1 \to ES \to 2 \to E \to 1$ and $C_2: \ 1 \to ES \to 2 \to P \to 3 \to S \to 1$. Both are s-cycles, and e-cycles: for example, $C_2$ has 3 negative edges, the same as half of its length. Moreover, $C_1$ and $C_2$ are compatible oriented, and do not have odd intersection; their intersection is the path $1 \to ES \to 2$.

Likewise, Figure 5**b.** depicts the DSR graph of the network

$$2A + B \to C \to 3A, \quad A \rightleftharpoons B. \tag{18}$$

Here we have four cycles; $C_1: \ 1 \to C \to 2 \to A \to 1$, $C_2: \ 1 \to B \to 3 \to A \to 1$, $C_3: \ 1 \to A \to 3 \to B \to 1$, and $C_4: \ 1 \to C \to 2 \to A \to 3 \to B \to 1$. Note that $C_2$ and $C_3$ have the same edges, traversed in opposite directions. $C_1$ and $C_4$ are e-cycles, and $C_2$ and $C_3$ are o-cycles: for example, half of the length of $C_2$ is even (two), whereas the number of its negative edges is odd (one). None of the cycles are s-cycles: for example, the two products of alternating labels for $C_1$ are $1 \cdot 3 \neq 1 \cdot 2$. Cycles $C_1$ and $C_2$ have odd intersection, as do $C_1$ and $C_4$, and $C_3$ and $C_4$. For example, the latter pair intersect along the path of length three $A \to 3 \to B \to 1$.


Figure 5: Examples of DSR graphs: **a.** $E + S \rightleftharpoons ES \to E + P, \ P \to S$. **b.** $2A + B \to C \to 3A, \ A \rightleftharpoons B$.

**Theorem 2.** *(The DSR graph Theorem, [40]) Suppose $\mathcal{R}$ is a mass action CRN whose DSR graph satisfies the following property: all its e-cycles are s-cycles, and no two e-cycles have odd intersection. Then the fully open extension of $\mathcal{R}$ is injective, and therefore it doesn't have the capacity for MPE.*

Most networks found in applications only involve stoichiometric coefficients equal to one, and in that case all cycles are e-cycles; therefore the first condition in Theorem 2 is very often satisfied in practice. DSR e-cycles are related to feedback loops; the DSR theorem implies not only that positive feedback is needed for MPE (as in the conjecture of Thomas), but that they satisfy additional conditions. Indeed, the DSR theorem is a quite a bit more powerful result [52].

All cycles are s-cycles in (Figure 5**a.**), and since the two cycles do not have odd intersection, one quickly rules out the capacity for multiple positive equilibria of the fully open extension of network (15). On the other hand, Theorem 2 stays silent for the open extension of network (18). Here both conditions of the theorem fail: $C_1$ is an e-cycle, but not an s-cycle, and the e-cycles $C_1$ and $C_4$ have odd intersection. In fact, one can show by methods of Section 5 that the open extension of (18) does have the capacity for MPE. We emphasize that in general, however, failure of the hypotheses in Theorem 2 is merely a necessary condition for non-injectivity (see Exercise 1).

The DSR graph theorem has been implemented in CoNtRol [46], which also includes a useful tool for drawing DSR graphs.

### Exercices

1. *(Injective fully open CRN for which the DSR Theorem does not apply).* Construct the DSR graph of the following network (the futile cycle with enzyme sharing):

$$\begin{aligned} E + S &\rightleftharpoons ES \to E + P \\ E + P &\rightleftharpoons EP \to E + S \\ E \rightleftharpoons 0,\ S \rightleftharpoons 0,\ P \rightleftharpoons 0,\ ES \rightleftharpoons 0,\ EP &\rightleftharpoons 0. \end{aligned}$$

   What conclusions does the DSR theorem allow? Show that the network is injective.

2. *(DSR of some enzymatic networks).* Study the injectivity of the fully open extensions of CRNs in Exercise 3 of Section 3. When the DSR theorem stays silent, use the methods of Theorem 1.

3. *(The sequestration network [34, 53]).* Show that if $m = 1$ or $n \geq 1$ is even, then the fully open extension of the following mass action network is injective:

$$\begin{aligned} X_1 + X_2 &\to 0 \\ X_2 + X_3 &\to 0 \\ &\vdots \\ X_{n-1} + X_n &\to 0 \\ X_1 &\to mX_n. \end{aligned} \tag{19}$$

   *Remark. One can show that all remaining possibilities for $m, n$ lead to networks with capacity for MPE [34]. It is however an open question whether these equilibria are nondegenerate [53].*

## 5 Sufficient conditions for multistationarity: inheritance of multiple equilibria

There has been much recent interest in studying "network motifs" in biological systems, namely small, frequently occurring subnetworks from which dynamical behaviors of the whole network can be inferred [54]. The results collected in this section fall broadly in this research direction; they study how multistationarity of a network is inherited from smaller structures, or "motifs". Specifically, we list a number of situations where MPNE and MPSE (Definitions 9 and 10) persist as we build up a network from smaller subnetworks. Here we focus on results that are shown by analytic methods, e.g. the implicit function theorem [28, 29], although approaches of algebraic nature also exist in recent literature; for example the results in [30] stem from an algebraic technique for linear elimination of species.

Using something like the implicit function theorem is quite natural: certain modifications of the network result in small perturbations of the vector field which allow for local continuations of each nondegenerate positive equilibrium. For example, adding new reactions to a network without changing the stoichiometric subspace, and assigning them small rate constants results in new nullcline manifolds that are merely perturbations of the original ones. In this case the steady state configuration will (locally) stay the same – imagine a small perturbation of the steady state curve in Figure 4**c.**. On the other hand, if the network is modified in a way that the stoichiometric subspace changes, then it is possible for equilibria to vanish, or become degenerate; see Exercise 1.

Throughout this section, we consider a mass action CRN $\mathcal{R}$ which the capacity for MPNE, and order its species in a vector $X = (X_1, \ldots, X_n)$. Recall that complexes are formal linear combinations of species, with non-negative integer coefficients. If $a = (a_1, \ldots, a_n) \in \mathbb{N}^n$, we use the convenient notation $a \cdot X$ to denote the complex $a_1X_1 + \cdots + a_nX_n$. The zero complex $0x_1 + \cdots + 0x_n$ will be denoted 0. The theorem below collects a series of results about the preservation of the capacity for MPNE when $\mathcal{R}$ is being "enlarged" into a new network $\mathcal{R}'$.

**Theorem 3.** *Let $\mathcal{R}$ be a CRN that admits MPNE. If $\mathcal{R}'$ is a CRN obtained by modifying $\mathcal{R}$ in any of the following ways, then $\mathcal{R}'$ admits MPNE. If additionally $\mathcal{R}$ admits MPSE, then $\mathcal{R}'$ does as well.*

1. (Adding a dependent reaction [28, 29]) *$\mathcal{R}'$ is obtained by adding a new irreversible reaction with reaction vector in the stoichiometric subspace of $\mathcal{R}$.*

2. (Adding a trivial species [28]) *$\mathcal{R}'$ is obtained by adding into the reactions of $\mathcal{R}$ a new species $Y$ which occurs with the same stoichiometry on both sides of each reaction in which it participates.*

3. (Adding inflows and outflows for all species [28, 29, 37, 40]) *$\mathcal{R}'$ is obtained by adding to $\mathcal{R}$ the reactions $0 \rightleftharpoons X_i$ for each $i \in \{1, \ldots, n\}$.*

4. (Adding a new species with inflow and outflow [28, 29]) *$\mathcal{R}'$ is obtained by adding into the reactions of $\mathcal{R}$ the new species $Y$ in an arbitrary way, while also adding the new reaction $0 \rightleftharpoons Y$.*

5. (Adding new reversible reactions involving new species [28]) *$\mathcal{R}'$ is obtained by adding $m \geq 1$ new reversible reactions involving $k$ new species such that the submatrix of the new stoichiometric matrix corresponding to the new species has rank $m$ (this forces $k \geq m$).*

6. (Adding intermediate complexes involving new species [28]) *$\mathcal{R}'$ is obtained by replacing each of the $m$ reactions:*

$$a_i \cdot X \to b_i \cdot X \text{ with a chain } \quad a_i \cdot X \to c_i \cdot X + \beta_i \cdot Y \to b_i \cdot X, \ (i = 1, \ldots, m)\,.$$

   *Here $Y$ is a list of $k$ new species whose coefficient matrix $\beta = (\beta_1|\beta_2|\cdots|\beta_m)$ has rank $m$ (this implies $k \geq m$), and $a_i$, $b_i$ and $c_i$ are arbitrary nonnegative vectors and any or all may coincide.*

**Remark.** The proof of Theorem 3 relies on a continuation of each nondegenerate equilibrium of $\mathcal{R}$ into an equilibrium of $\mathcal{R}'$. With that in mind, the theorem actually says more: $\mathcal{R}'$ has at least as many nondegenerate equilibria as $\mathcal{R}$. In particular, if $\mathcal{R}$ has a positive nondegenerate equilibrium, then so does $\mathcal{R}'$.

We illustrate the applicability of these results starting with our favorite example (6):

$$2A + B \to 3A, \ A \to B. \tag{20}$$

Recall from Sections 2.5 and 2.6 that this system has the capacity for MPNE. We now build on this network using modifications of the type shown in Theorem 3 to arrive at a significantly more complicated network, whose capacity for MPNE is otherwise not easy to study. For example, we can add $A \rightleftharpoons 0$, $B \rightleftharpoons 0$

(modification 2), and $B \to A$ (modification 1); note that one can always add the reverse of an existing reaction keeping the capacity for MPNE (or MPSE). Next, we add the reversible reactions $A + C \rightleftharpoons 2D$ and $C + D \rightleftharpoons 2B$; this is allowed by modification 5, since the submatrix of the stoichiometric matrix corresponding to the new species $C$ and $D$, i.e. $\begin{bmatrix} -1 & -1 \\ 2 & -1 \end{bmatrix}$ has rank 2. Next we can replace $A \to 0$ by $A + E \to E$ (modification 2), and then replace this reaction and the reaction $2A + B \to 3A$ by the two chains $A + E \to C + F \to E$ and $2A + B \to F + 2G \to 3A$; this is allowed by modification 6 since the matrix of coefficients $\begin{bmatrix} 1 & 1 \\ 0 & 2 \end{bmatrix}$ for the two new species $F$ and $G$ has rank 2. Finally, we can add $H \rightleftharpoons 0$ and add $H$ to the reactions $A \to B$ and $0 \to B$ by $A + 2H \to B$ and $2H \to B$ respectively. We conclude that the resulting network

$$\begin{aligned} 2A + B \to F + 2G \to 3A \\ A + 2H \to B \to A \rightleftharpoons 0 \\ H \rightleftharpoons 0 \to B \leftarrow 2H \\ A + C \rightleftharpoons 2D \\ C + D \rightleftharpoons 2B \\ A + E \to C + F \to E \end{aligned}$$

inherits the capacity for MPSE from network (20). This example may be overtly made up, but it illustrates how the simple modifications listed in Theorem 3 allow one to draw conclusions that are otherwise very difficult. We note that here we started with a simple network with capacity for MPNE and concluded that the (much more) complicated one keeps this property. In practice however, one starts with a large network, and the inverse process is needed, namely finding a MPNE "subnetwork" and a series of modifications that transforms it into the large network. This is a daunting task in general, although for relatively small networks, familiarity with MPNE motifs like (20) and some trial and error will work in many cases. Such an example is presented in Exercise 3, which discusses an important biological example.

## Exercises

1. *(Variations of network (20)).* a. Show that the fully open extension of network (18):

   $$2A + B \to C \to 3A,\ A \rightleftharpoons B,\ A \rightleftharpoons 0 \rightleftharpoons B$$

   admits MPNE.

   b. Show that the network

   $$2A + B \to 3A,\ A \to B,\ 0 \leftrightharpoons B$$

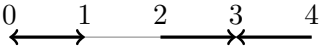
   does not have the capacity for MPE.

   c. Show that the network

   $$2A + B \to 3A,\ A \to B,\ 0 \leftrightharpoons A + B$$

   has the capacity for MPE, but not the capacity for MPNE.

   *Remark. CRNs b. and c. show that adding reactions may destroy the capacity for MPE/MPNE [28]).*

2. *(One-species networks [55]).* A network involving a single species can be naturally represented as a sequence of arrows on the real line. For example, $0 \leftrightharpoons A, 2A \to 3A, 4A \to 3A$ corresponds to the diagram 

a. Show that a one-species reaction network has the capacity for MPNE if and only if it contains an arrow subsequence of the form $\rightarrow \leftarrow \rightarrow$ or $\leftarrow \rightarrow \leftarrow$ . (Here the length of the arrow is not important, only its direction; the condition says that there are three reactions with distinct reactant complexes, arranged in order of their stoichiometric coefficient, that proceed left, right, left, or right, left, right.)

b. Characterize the one-species networks with capacity for MPE.

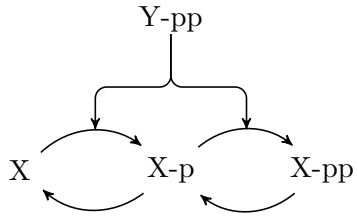
3. *(Adding enzymatic mechanisms [28]).* Let $\mathcal{R}$ be a CRN with capacity for MPNE and vector of species $X$ as in Theorem 3, whose notation we adopt.

a. Suppose $\mathcal{R}'$ is obtained by replacing reaction $a\cdot X \rightarrow b\cdot X$ with the chain $cE+a\cdot X \rightleftharpoons I \rightarrow cE+b\cdot X$, where $E$ and $I$ are new species and $c \geq 0$. Show that $\mathcal{R}'$ has the capacity for MPNE.

b. Suppose that we add the chain in a., while also keeping the reaction $a \cdot X \rightarrow b \cdot X$. Show that this new network has the capacity for MPNE.

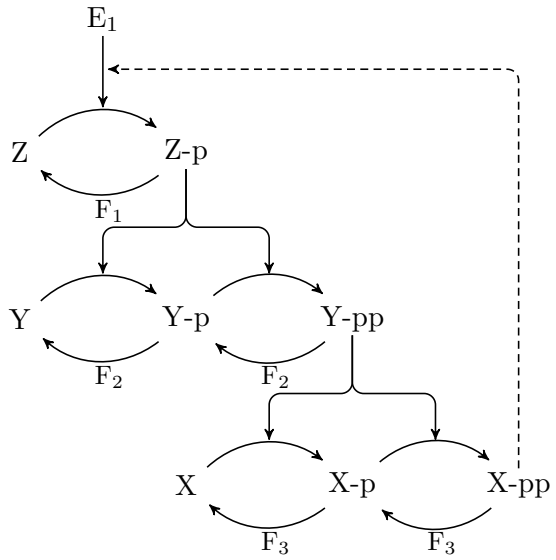
4. *(Multistationarity of the Huang-Ferrell-Kholodenko MAPK cascade with negative feedback [56]).*

a. Show that the following version of mass action double phosphorylation has the capacity for MPSE [28]. In the diagram, reactions $X \stackrel{E}{\rightarrow} Y$ are shortened representations of an enzymatic chain $E + X \rightleftharpoons EX \rightarrow E + Y$. The list of all reactions is also included.




Y-pp+X $\rightleftharpoons$ Y-pp–X $\rightarrow$ Y-pp+X-p $\rightarrow$ Y-pp+X-pp
X-pp$\rightarrow$X-p$\rightarrow$X

b. Show that the following network has the capacity for MPSE [28].




$E_1$+Z $\rightleftharpoons$ $E_1$–Z $\rightarrow$ $E_1$+Z-p,
$F_1$+Z-p $\rightleftharpoons$ $F_1$–Z-p $\rightarrow$ $F_1$+Z
Z-p+Y $\rightleftharpoons$ Z-p–Y $\rightarrow$ Z-p+Y-p $\rightleftharpoons$ Z-p–Y-p $\rightarrow$ Z-p+Y-pp
$F_2$+Y-pp $\rightleftharpoons$ $F_2$–Y-pp $\rightarrow$ $F_2$+Y-p $\rightleftharpoons$ $F_2$–Y-p $\rightarrow$ $F_2$+Y
Y-pp+X $\rightleftharpoons$ Y-pp–X $\rightarrow$ Y-pp+X-p $\rightleftharpoons$ Y-pp–X-p $\rightarrow$ Y-pp+X-pp
$F_3$+X-pp $\rightleftharpoons$ $F_3$–X-pp $\rightarrow$ $F_3$+X-p $\rightleftharpoons$ $F_3$–X-p $\rightarrow$ $F_3$+X
$E_1$ + X-pp $\rightleftharpoons$ $E_1$–X-pp
$E_1$–X-pp+Z $\rightleftharpoons$ $E_1$–X-pp–Z
$E_1$–X-pp–Z $\rightleftharpoons$ $E_1$–Z+X-pp.

# 6 Sufficient conditions for multistationarity II: the determinant optimization method

As we have seen (Section 3, Exercise 1), non-injectivity of a CRN does not imply its capacity for MPE. The following result shows that non-injectivity together with an additional condition on the stoichiometric

matrix insures the capacity for MPE for fully open systems. The result is essentially Theorem 4.1 in [26], but we present it here using the matrix-theoretic framework developed in [27]. We also refer the reader to [53] for related results.

**Theorem 4.** *[26, 27] Let $\mathcal{R}$ be a fully open mass action CRN and let $\Gamma$ and $\Gamma_l$ denote the stoichiometric matrix and reactant matrix of the CRN obtained from $\mathcal{R}$ by removing all inflow reactions $0 \to X_i$. Suppose there exists a diagonal matrix $D$ such that $\det(-\Gamma D \Gamma_l^t) < 0$ and $\Gamma D \mathbf{1} \leq 0$, where $\mathbf{1}$ denotes the column vector with all entries equal to 1. Then $\mathcal{R}$ has the capacity for MPE.*

Condition $\det(-\Gamma D \Gamma_l^t) < 0$ simply states that the the CRN is not injective (Exercise 1). Verifying the hypothesis of the theorem amounts to finding a solution for a nonlinear system of inequalities in the entries of $D$, which can be easily implemented computationally (the webserver CoNtRol [46] includes this calculation). On the other hand, for small CRNs this can be done by hand, as we illustrate for the following example. Consider the mass action network

$$2A + B \rightleftharpoons 3A,\ A \to B,\ A \rightleftharpoons 0,\quad B \rightleftharpoons 0.$$

It is already clear that this CRN has the capacity for MPE, as easily shown by using tools from previous sections. Here we apply Theorem 4, and search for positive $d_1, d_2, d_3, d_4$ such that

$$\det\left(\begin{bmatrix} -1 & 1 & 1 & 0 \\ 1 & -1 & 0 & 1 \end{bmatrix} \begin{bmatrix} d_1 & 0 & 0 & 0 \\ 0 & d_2 & 0 & 0 \\ 0 & 0 & d_3 & 0 \\ 0 & 0 & 0 & d_4 \end{bmatrix} \begin{bmatrix} 2 & 1 \\ 1 & 0 \\ 1 & 0 \\ 0 & 1 \end{bmatrix}\right) < 0,$$

and

$$\begin{bmatrix} 1 & -1 & -1 & 0 \\ -1 & 1 & 0 & -1 \end{bmatrix} \begin{bmatrix} d_1 \\ d_2 \\ d_3 \\ d_4 \end{bmatrix} \leq 0,$$

or equivalently,

$$d_1 d_3 - 2 d_1 d_4 + d_2 d_4 + d_3 d_4 \leq 0, \quad d_1 \leq d_2 + d_3, \quad d_2 \leq d_1 + d_4.$$

The first inequality can be rewritten as $-d_1 d_4 + d_1 d_3 + d_4(d_2 + d_3 - d_1)$, and one may notice that these inequalities are satisfied by choosing $d_1$ close to $d_2 + d_3$ and $d_4$ large enough; for example, $d_1 = 9,\ d_2 = d_3 = 5,\ d_4 = 6$.

## Exercises

1. With the notations of Theorem 4, show that there exists a positive diagonal matrix $D$ such that $\det(-\Gamma D \Gamma_l^t) < 0$ if and only if $\mathcal{R}$ is not injective.

2. Show that the following sequestration network has the capacity for MPE [53]:

$$X_1 + X_2 \to 0,\quad X_2 + X_3 \to 0,\quad X_3 \to 2X_1,$$
$$X_1 \rightleftharpoons 0,\ X_2 \rightleftharpoons 0,\ X_3 \rightleftharpoons 0.$$

3. Use the determinant optimization method to show that the following network [26] has the capacity for MPE:

$$2A + B \rightleftharpoons 3A,\ A \rightleftharpoons 0 \rightleftharpoons B.$$

# 7 Results based on deficiency theory

Network deficiency was introduced by Horn, Jackson, and Feinberg in the 1970s in a series of seminal papers [57, 41, 42]. The name 'deficiency' refers to a nonnegative integer that connects the structure of a reaction network and the existence of (multiple) equilibria for the corresponding system of ODEs.

The reaction network structure enters in the definition of the deficiency via the rank of the stoichiometric matrix (denoted by $s$), the number of complexes (denoted by $N$), and the number of connected components (denoted by $\ell$). For example, the network given in Figure 6 consists of $N = 6$ complexes and $\ell = 2$ connected components of the reaction graph.

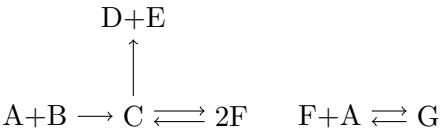



Figure 6: A reaction network with $N = 6$ complexes (nodes) and $\ell = 2$ connected components.

**Definition 15** (Network deficiency)**.** *The* deficiency $\delta$ *of a CRN is defined as*

$$\delta = N - \ell - s. \tag{21}$$

It turns out that the deficiency of a CRN is always a non-negative integer [42]. Quite a lot is known about CRNs of deficiency zero or one. We present below results pertaining to existence and uniqueness of positive equilibria, but we note that for deficiency zero CRNs, a great deal is known about the dynamics as well [41, 42, 58]. For now, we carry on with a few deficiency computations.

It is easy to see that the CRN in Figure 6 has stoichiometric subspace of dimension 4, so that its deficiency is $\delta = 6 - 4 - 2 = 0$. The network in Figure 2 has $N = 7$ complexes, $\ell = 3$ connected components, and its stoichiometric subspace has dimension $s = 4$. The deficiency of the CRN is $7 - 3 - 4 = 0$.

The following version of (6) also has deficiency zero:

$$2A + B \rightleftharpoons 3A. \tag{22}$$

Indeed, it is clearly the case that $s = 1$, and so $\delta = 2 - 1 - 1 = 0$.

For a biologically relevant example, consider again the futile cycle (1) and its stoichiometric matrix:

$$\begin{aligned} &E + S \rightleftharpoons ES \to E + P \\ &F + P \rightleftharpoons FP \to F + S \end{aligned} \qquad \Gamma = \begin{bmatrix} -1 & 1 & 1 & 0 & 0 & 0 \\ -1 & 1 & 0 & 0 & 0 & 1 \\ 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 1 & 0 \\ 0 & 0 & 0 & -1 & 1 & 1 \\ 0 & 0 & 0 & 1 & -1 & -1 \end{bmatrix}.$$

The species ordering here was chosen $(E, S, ES, P, F, FP)$. A simple calculation shows that $s = \operatorname{rank} \Gamma = 3$, and since the futile cycle involves $N = 6$ complexes and $\ell = 2$ connected components, its deficiency is $\delta = 6 - 3 - 2 = 1$.

## 7.1 The Deficiency Zero and Deficiency One Theorems

We present two theorems exhibiting settings where a CRN cannot have the capacity for MPE. The first result is a weak version of the Deficiency Zero Theorem [58]. Although our focus is on existence and uniqueness of equilibria, the real power of the Deficiency Zero Theorem resides in its strong conclusions about dynamical

properties of certain CRNs. While we omit these altogether, the reader is encouraged to survey the vast recent literature on global stability of equilibria in CRNs satisfying the hypotheses of the Deficiency Zero Theorem [62, 63, 64, 65, 66, 67, 68]. We start by setting up some terminology.

**Definition 16** (Strongly connected components, terminal strongly connected components, weakly reversible CRNs)**.** *Two complexes $C_1$ and $C_2$ are called* strongly connected *if $C_1 = C_2$ or if there exist reaction paths from $C_1$ to $C_2$, and from $C_2$ to $C_1$. A* strongly connected component *of the reaction graph is a maximal subset of nodes that are pairwise strongly connected. A strongly connected component $\mathcal{C}$ is called* terminal *if there is no reaction from a complex in $\mathcal{C}$ to a complex outside $\mathcal{C}$. A CRN is called* weakly reversible *if all connected components in its reaction graph are strongly connected. In other words, a CRN is weakly reversible if whenever there is a path following reaction arrows from a complex $C_1$ to a complex $C_2$, there is also a path from $C_2$ to $C_1$.*

For example, in Figure 6, $C$ and $2F$ are strongly connected while $A + B$ and $C$ are not, and neither are $C$ and $G$. The strongly connected components of the CRN in Figure 6 are $\{A + B\}$, $\{C, 2F\}$, $\{D + E\}$, $\{F + A, G\}$; out of these $\{D + E\}$ and $\{F + A, G\}$ are terminal. The network in Figure 1 is weakly reversible, and so is the CRN (22) – more generally, a CRN that contains only reversible reactions is clearly weakly reversible. On the other hand, the CRN in Figure 6 is not weakly reversible, and neither is the futile cycle: for instance, there is a path from $ES$ to $E + P$, but not the other way around.

**Theorem 5** (Equilibria in deficiency zero CRNs [58, Theorem 4.1])**.** *Consider a mass action CRN with zero deficiency.*

1. *If the network is not weakly reversible, then for any choice of rate constants, the CRN has no positive equilibria.*
2. *If the network is weakly reversible, then for any choice of rate constants, there exists exactly one equilibrium in each positive stoichiometry class.*

A quick application of this theorem shows, for example, that the CRN in Figure 1 has one equilibrium in each positive stoichiometry class, regardless of the choice of rate constants. The same is true for CRN (22). Notice that this network is related to our favorite multistationary example (6); in fact, adding inflows and outflows to (22) results in a CRN with capacity for MPE (Exercise 3 in Section 6). This is yet another illustration of the subtle connection between the structure of a network and its capacity for multistationarity. Finally, the CRN in Figure 6 has no positive equilibria, no matter what rate constants it is being assigned.

Deficiency theory can be applied to draw conclusions about the existence and uniqueness of positive equilibria even if the deficiency is strictly positive. In particular, a useful result is the Deficiency One Theorem which we present next.

**Theorem 6** (Deficiency One Theorem [58])**.** *Consider a CRN with mass action kinetics that satisfies the following conditions:*

1. *The deficiency of every connected component is either zero or one.*
2. *The deficiency of the overall network is the sum of the deficiencies of the connected components.*
3. *Every connected component contains exactly one terminal strongly connected component.*

*If for some values of rate constants the CRN has a positive equilibrium, then (for the same rate constants) the CRN has precisely one equilibrium in each positive stoichiometry class. Moreover, if the network is weakly reversible, then for any values of rate constants, the CRN has precisely one equilibrium in each positive stoichiometry class.*

Note that "Deficiency One" in the name of Theorem 6 refers to the deficiencies of the connected components of the network, and not to the deficiency of the network itself, which can be much higher.

## 7.2 The deficiency one algorithm and the advanced deficiency algorithm

The Deficiency Zero and Deficiency One theorems are remarkable results, not only due to the powerful conclusions they allow, but also because of their elegant statements. While unfortunately, many biologically relevant CRNs do not fall under the scope of these results, the theorems are the basis for widely applicable algorithms that have been developed by M. Feinberg and his research group [69, 70]. A precise discussion of these requires machinery beyond the scope of this chapter. We merely note that the *Deficiency One Algorithm* (applicable to networks of deficiency one) and the *Advanced Deficiency Algorithm* (for CRNs with arbitrary deficiency) translate the steady state equations into a collection of (potentially many) linear inequality systems. If at least one of these linear systems is feasible, then *multistationarity is possible* and every solution defines a pair of steady states and corresponding rate constants. If none of the systems are feasible, then *capacity for MPE is ruled out*. Both algorithms are implemented in the Chemical Reaction Network Toolbox [48]. We also refer the reader to a strand of very interesting results related to the Deficiency One Algorithm [76, 77].

The Deficiency One and Advanced Deficiency algorithms work well for many examples of biologically relevant networks. In particular, they can be used in a systems biology context to discriminate between different reaction mechanisms [71], or to study multistable signaling motifs [72], or to analyze whole families of enzymatic networks [50].

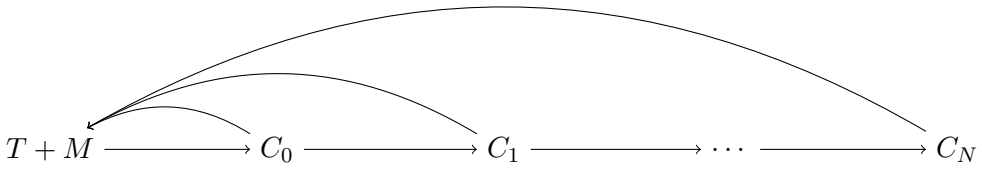
### Exercises

1. a. Show that the futile cycle with enzyme sharing has exactly one equilibrium in each positive stoichiometry class, for any values of the rate constants (see also Exercise 1 of Section 4):

$$E + S \rightleftharpoons ES \to E + P, \quad E + P \rightleftharpoons EP \to E + S.$$

   b. Show that adding reversible inflow-outflow reactions for any one species or for any combination of two species results in CRNs that have exactly one equilibrium in each positive stoichiometry class.

2. Let $N \in \mathbb{N}$. Show that the following mass action CRN (the McKeithan network [78]) has exactly one equilibrium in each stoichiometry class, for any choice of rate constants:




3. Consider the mass action CRN

$$2X \to Y + Z, \quad 2Y \to Z + X, \quad 2Z \to X + Y.$$

   a. Set up and solve the corresponding mass action algebraic system to show that the CRN has precisely one equilibrium in each positive stoichiometry class.

   b. Use deficiency theory to show the conclusion of a. *(Hint. Find a weakly reversible, deficiency zero reaction network with the same ODEs (dynamically equivalent, [79]) as the one in the exercise. An algorithm for finding weakly reversible networks, dynamically equivalent to a given CRN, is the object of [80].*

## Acknowledgement.

C. Pantea was supported by NSF-DMS Grant 1517577 *Multistationarity and Oscillations in Biochemical Reaction Networks*.

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