



## Preclusion of switch behavior in networks with mass-action kinetics

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### ABSTRACT

We study networks taken with mass-action kinetics and provide a Jacobian criterion that applies to an arbitrary network to preclude the existence of multiple positive steady states within any stoichiometric class for any choice of rate constants. We are concerned with the characterization of injective networks, that is, networks for which the species formation rate function is injective in the interior of the positive orthant within each stoichiometric class. We show that a network is injective if and only if the determinant of the Jacobian of a certain function does not vanish. The function consists of components of the species formation rate function and a maximal set of independent conservation laws. The determinant of the function is a polynomial in the species concentrations and the rate constants (linear in the latter) and its coefficients are fully determined. The criterion also precludes the existence of degenerate steady states. Further, we relate injectivity of a network to that of the network obtained by adding outflow, or degradation, reactions for all species.

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### 1. Introduction

Dynamical systems arising from the law of mass-action kinetics are commonly used to model phenomena described by networks of interacting species. The main examples are found in chemistry and molecular biology but instances are also found in ecology and epidemiology (such as the Lotka-Volterra and SIR models). Typical systems found in molecular biology are high-dimensional and contain many parameters that are unknown or poorly determined. It is therefore of interest to determine properties of these systems that are inherent of the network structure alone and independent of the specific choice of parameters.

Of particular relevance for applications in systems biology is the determination of network structures for which the corresponding dynamical systems have multiple positive steady states for some choice of parameters. Multistationarity in cellular systems provides a mechanism for switching between different cellular responses and can be crucial for cellular decision making. Even though different features, such as feedback loops, are known that facilitate multistationarity in systems, it is in general difficult to decide whether a particular system has the capacity to exhibit multiple steady states. Various criteria have therefore been developed to preclude the existence of multiple positive steady states. These criteria typically utilize the structure or qualitative features of the system [1–3] or properties of the class of kinetics that are allowed [4–6]. Additionally, algorithms to positively assert multistationarity have also been developed [7,8].

It is the aim of this paper to introduce a criterion for a network taken with mass-action kinetics to preclude the existence of multiple positive steady states within any stoichiometric class for any choice of rate constants (i.e., the parameters of the dynamical system). The criterion is based on the species formation rate function and characterizes when this function is injective for positive concentration vectors within each stoichiometric class. If this is the case then the network is said to be injective (Definition 5.1) and there cannot exist multiple positive steady states within a stoichiometric class.

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Specifically, we provide a Jacobian criterion that characterizes injectivity for any network. We consider a modified species formation rate function (Definition 4.4) obtained by replacing some components of the species formation rate function by equations for independent conservation laws. We show that a network is injective if and only if the determinant of the Jacobian of this function does not vanish (Corollary 5.4). If this is the case then any positive steady state is *non-degenerate* (Corollary 5.4). We show that the determinant of the Jacobian of the function is a polynomial, linear in the rate constants, and characterize its coefficients (Theorem 7.6). All together provides a criterion that depends only on the structure of the network and that is easy to implement using any computational algebra software.

Our work extends and embraces the previous Jacobian criterion by [4] on injectivity of networks in the context of a *continuous flow stirred tank reactor* (here called fully open networks). In a fully open network all species are assumed to flow out of the system or, alternatively, all species are being degraded. This implies that the system has no conservation laws, that is, the stoichiometric subspace has maximal dimension. Their criterion, however, is not informative for networks with conservation laws. To circumvent this, Craciun and Feinberg lay a route to preclude the existence of multiple positive steady states in arbitrary networks [9,5]: it consists of first deciding whether the associated fully open network is injective and then determining if degenerate steady states can occur. However, a network can be injective even if the associated fully open network is not.

The work presented here provides a direct path to preclude multiple steady states by avoiding the detour to fully open networks and enlarges the class of networks for which an injectivity criterion applies. We show that injectivity of an arbitrary network can be assessed regardless of the injectivity of the associated fully open network. Further, if the associated fully open network is injective, the occurrence of degenerate steady states in a network is completely characterized.

A different route to injectivity of a fully open network was taken by Banaji and co-workers [6]. A criterion is given that ensures that minus the Jacobian of the species formation rate function is a  $P$ -matrix (the definition is given in Section 10). It then follows from the results of [10] that the network is injective. Our results imply that, after changing the sign of certain rows, the Jacobian of the modified species formation rate function is a  $P$ -matrix and, hence, that the network is injective as well. Using this approach and the notion of strongly sign-determined matrices, Banaji and co-workers extend the injectivity results of Craciun and Feinberg for fully open networks taken with mass-action kinetics to kinetics satisfying some mild conditions [6], see also [3]. Our work is currently restricted to mass-action kinetics and the extension to general kinetics is under investigation.

The outline of the paper is as follows. In Section 2 we introduce some notation and the main definitions relating to networks and mass-action kinetics. In Section 3 we introduce the stoichiometric classes and the distinction between fully open and closed networks. We proceed in Section 4 to study degenerate steady states. Injectivity of networks is discussed in Section 5, where the definition of injectivity and the Jacobian criterion are introduced. In Section 6 and Section 7 we focus on open and closed networks, respectively. Section 8 provides a characterization of networks with only degenerate steady states. Finally, in Section 9 we relate injectivity of open networks to that of closed networks and in Section 10 the relationship between  $P$ -matrices and injectivity is discussed. We end with a few remarks including a discussion on the effective implementation of the criterion.

## 2. Networks with mass-action kinetics

### 2.1. Notation

Let  $\mathbb{R}_+$  denote the set of positive real numbers (without zero) and  $\overline{\mathbb{R}}_+$  the set of non-negative real numbers (with zero). Similarly, let  $\mathbb{N}$  be the set of non-negative integers. Given a finite set  $\mathcal{E}$ , let  $\overline{\mathbb{N}}^{\mathcal{E}}$  be the semi-ring of formal sums  $v = \sum_{E \in \mathcal{E}} \lambda_E E$ , with  $\lambda_E \in \mathbb{N}$ . If  $\lambda_E \in \mathbb{N}$  for all  $E \in \mathcal{E}$ , then we write  $v \in \mathbb{N}^{\mathcal{E}}$ . The semi-rings  $\overline{\mathbb{R}}_+^{\mathcal{E}}$  and  $\mathbb{R}_+^{\mathcal{E}}$  are defined analogously.

The ring of polynomials in  $\mathcal{E}$  is denoted  $\mathbb{R}[\mathcal{E}]$ . The total degree of a monomial  $\prod_{E \in \mathcal{E}} E^{n_E}$ , with  $n_E$  a non-negative integer for all  $E$ , is the sum of the degrees of the variables,  $\sum_{E \in \mathcal{E}} n_E$ . The degree of a polynomial is the maximum of the total degrees of its monomials.

If a polynomial  $p$  vanishes for all assignments  $a : \mathcal{E} \rightarrow \mathbb{R}_+$  then  $p = 0$  identically. Further, if  $p$  is a non-zero polynomial in  $\mathbb{R}[\mathcal{E}]$  such that the degree of each variable in each monomial is either 1 or zero, then all the coefficients of  $p$  are non-negative if and only if  $p(a(\mathcal{E})) > 0$  for any assignment  $a : \mathcal{E} \rightarrow \mathbb{R}_+$ .

### 2.2. Networks

We follow the approach to networks presented in Chemical Reaction Network Theory. See for instance [1,11] for background and extended discussions. The main concept is the definition of network, also called chemical reaction network or reaction network in the literature.

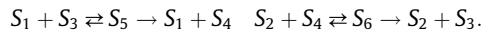
**Definition 2.1.** A network consists of three finite sets:

- (1) A set  $S$  of species.
- (2) A set  $C \subset \overline{\mathbb{N}}^S$  of complexes.
- (3) A set  $\mathcal{R} \subset C \times C$  of reactions, such that  $(y, y) \notin \mathcal{R}$  for all  $y \in C$ , and if  $y \in C$ , then there exists  $y' \in C$  such that either  $(y, y') \in \mathcal{R}$  or  $(y', y) \in \mathcal{R}$ .

Following the usual convention, an element  $r = (y, y') \in \mathcal{R}$  is denoted by  $r : y \rightarrow y'$ . The *reactant* and *product* (complexes) of a reaction  $r : y \rightarrow y'$  are  $y$  and  $y'$ , respectively. By definition, any complex is either the reactant or product of some reaction. The zero complex  $0 \in \mathcal{C}$  is allowed by definition. Reactions of the form  $y \rightarrow 0$  are called *outflow* reactions and reactions of the form  $0 \rightarrow y$  are called *inflow* reactions [12]. In particular, *species inflow* and *species outflow* reactions are reactions of the form  $0 \rightarrow S$  and  $S \rightarrow 0$ , respectively, for some  $S \in \mathcal{S}$ .

Let  $n$  be the cardinality of  $\mathcal{S}$ . We fix an order in  $\mathcal{S}$  so that  $\mathcal{S} = \{S_1, \dots, S_n\}$  and identify  $\overline{\mathbb{N}}^{\mathcal{S}}$  with  $\overline{\mathbb{N}}^n$ . The species  $S_i$  is identified with the  $i$ th canonical  $n$ -tuple of  $\overline{\mathbb{N}}^n$  with 1 in the  $i$ th position and zeroes elsewhere. Accordingly, a complex  $y \in \mathcal{C}$  is given as  $y = \sum_{i=1}^n y_i S_i$  or  $(y_1, \dots, y_n)$ . Although  $\overline{\mathbb{N}}^n$  is not a vector space,  $n$ -tuples  $v \in \overline{\mathbb{N}}^n$  will be called *vectors*.

**Example 2.2.** Consider the network with set of species  $\mathcal{S} = \{S_1, S_2, S_3, S_4, S_5, S_6\}$ , set of complexes  $\{S_1 + S_3, S_1 + S_4, S_2 + S_4, S_2 + S_3, S_3, S_5, S_6\}$  and reactions



That is, the reactions are  $r_1 : S_1 + S_3 \rightarrow S_5$ ,  $r_2 : S_5 \rightarrow S_1 + S_3$ ,  $r_3 : S_5 \rightarrow S_1 + S_4$ ,  $r_4 : S_2 + S_4 \rightarrow S_6$ ,  $r_5 : S_6 \rightarrow S_2 + S_4$  and  $r_6 : S_6 \rightarrow S_2 + S_3$ . This network is a main building block in protein modification systems and is known as the *futile cycle*. It assumes the Michaelis–Menten enzyme mechanism in which a substrate  $S_3$  is modified into a substrate  $S_4$  through the formation of an intermediate complex  $S_5$ . The reaction is catalyzed by an enzyme  $S_1$ . The modification can be reversed via a similar set of reactions with an intermediate complex  $S_6$  and an enzyme  $S_2$ . Each *reversible* reaction is written as two *irreversible* reactions, e.g. the reactions  $r_1$  and  $r_2$  are considered two distinct reactions and not one reversible reaction.

### 2.3. Mass-action kinetics

Let  $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a network. We denote the molar concentration of species  $S_i$  at time  $t$  by  $c_i = c_i(t)$  and associate with any complex  $y = (y_1, \dots, y_n)$  the monomial  $c^y = \prod_{i=1}^n c_i^{y_i} \in \mathbb{R}[c_1, \dots, c_n]$ . For example, if  $y = (2, 1, 0, 1) \in \overline{\mathbb{N}}^4$ , then the associated monomial is  $c^y = c_1^2 c_2 c_4$ .

A *rate vector* is an element  $\kappa = (k_{y \rightarrow y'})_{y \rightarrow y'} \in \mathbb{R}_+^{\mathcal{R}}$  given by the assignment of a positive *rate constant*  $k_{y \rightarrow y'} \in \mathbb{R}_+$  to each reaction  $r : y \rightarrow y' \in \mathcal{R}$ . The (mass-action) *species formation rate function* corresponding to the rate vector  $\kappa$  is defined by:

$$\mathbb{R}^n \xrightarrow{f_\kappa} \mathbb{R}^n, \quad c \mapsto \sum_{y \rightarrow y' \in \mathcal{R}} k_{y \rightarrow y'} c^y (y' - y).$$

Let  $f_{\kappa,i}(c)$  denote the  $i$ th entry of  $f_\kappa(c)$ , that is  $f_{\kappa,i}(c) = \sum_{y \rightarrow y' \in \mathcal{R}} k_{y \rightarrow y'} c^y (y'_i - y_i)$ .

The set of reactions together with a rate vector give rise to a polynomial system of ordinary differential equations (ODEs):

$$\dot{c} = f_\kappa(c).$$

These ODEs describe the dynamics of the concentrations  $c_i$  in time. The *steady states* of the network with rate vector  $\kappa$  are the solutions to the system of polynomial equations in  $c_1, \dots, c_n$  obtained by setting the derivatives of the concentrations to zero:

$$0 = f_\kappa(c).$$

This system of equations is referred to as the steady-state equations. We are interested in the positive steady states, that is, the solutions  $c$  to the steady-state equations such that all concentrations are positive,  $c \in \mathbb{R}_+^n$ .

**Example 2.3.** The ODEs system of the futile cycle taken with mass-action kinetics is:

$$\begin{aligned} \dot{c}_1 &= -k_1 c_1 c_3 + (k_2 + k_3) c_5 & \dot{c}_4 &= -k_4 c_2 c_4 + k_3 c_5 + k_6 c_6, \\ \dot{c}_2 &= -k_4 c_2 c_4 + (k_5 + k_6) c_6 & \dot{c}_5 &= k_1 c_1 c_3 - (k_2 + k_3) c_5, \\ \dot{c}_3 &= -k_1 c_1 c_3 + k_2 c_5 + k_6 c_6 & \dot{c}_6 &= k_4 c_2 c_4 - (k_5 + k_6) c_6, \end{aligned}$$

where the rate constant of reaction  $r_i$  is denoted by  $k_i$ .

**Remark 2.4.** If  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and/or  $c \in \mathbb{R}_+^n$  are not fixed then the function  $f_\kappa(c)$  can be seen as a polynomial function taking values in  $\mathbb{R}[c]$ ,  $\mathbb{R}[\kappa]$  or  $\mathbb{R}[c \cup \kappa]$ .

### 3. Stoichiometrically compatible steady states

The dynamics of a network might preserve quantities that remain constant over time. If this is the case, the dynamics takes place in a proper invariant subspace of  $\mathbb{R}^n$ , fixed by the initial concentrations  $c_i(0)$  of the system. Let  $v \cdot v'$  denote the Euclidian scalar product of two vectors  $v, v'$ . Let  $v'$  denote the transpose of a vector  $v$ .

**Definition 3.1.** The stoichiometric subspace of a network  $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  is the following vector subspace of  $\mathbb{R}^n$ :

$$\Gamma = \langle y' - y | y \rightarrow y' \in \mathcal{R} \rangle.$$

Two vectors  $c, c' \in \mathbb{R}^n$  are called *stoichiometrically compatible* if  $c - c' \in \Gamma$ , or equivalently, if  $\omega \cdot c = \omega \cdot c'$  for all  $\omega \in \Gamma^\perp$ .

We denote by  $s$  the dimension of  $\Gamma$ . Note that  $\Gamma$  is independent of the choice of rate constants and depends only on the structure of the reactions. Being stoichiometrically compatible is an equivalence relation which partitions  $\mathbb{R}_+^n$  into classes, called *stoichiometric classes*. In particular, the *stoichiometric class* of a concentration vector  $c \in \mathbb{R}_+^n$  is  $\{c + \Gamma\} \cap \mathbb{R}_+^n$ .

For any rate vector  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$ , the image of  $f_\kappa$  is contained in  $\Gamma$ . Thus, for any choice of rate vector  $\kappa, c \in \mathbb{R}^n$ , and  $\omega \in \Gamma^\perp$ , we have that  $\omega \cdot f_\kappa(c) = 0$  and thus  $\omega \cdot \dot{c} = 0$ . If  $\omega = (\lambda_1, \dots, \lambda_n)$  then  $\sum_{i=1}^n \lambda_i c_i$  is independent of time and determined by the initial concentrations of the system. These conserved quantities are generally referred to as *total amounts*. Since  $a - b \in \Gamma$  if and only if  $\omega \cdot a = \omega \cdot b$  for all  $\omega \in \Gamma^\perp$ , total amounts are conserved within each stoichiometric class and characterize the class.

**Example 3.2.** The stoichiometric subspace of the futile cycle (Example 2.2) is

$$\Gamma = \langle S_1 + S_3 - S_5, S_5 - S_1 - S_4, S_2 + S_4 - S_6 \rangle$$

and has dimension  $s = 3$ . The dimension of the orthogonal space  $\Gamma^\perp$  is  $d = n - s = 3$  as well and a basis is  $\Gamma^\perp = \langle \omega^1, \omega^2, \omega^3 \rangle$  with

$$\omega^1 = S_1 + S_5, \quad \omega^2 = S_2 + S_6, \quad \omega^3 = S_3 + S_4 + S_5 + S_6. \tag{3.3}$$

Indeed, we have  $\dot{c}_1 + \dot{c}_5 = \dot{c}_2 + \dot{c}_6 = \dot{c}_3 + \dot{c}_4 + \dot{c}_5 + \dot{c}_6 = 0$ . In this example, three total amounts determine each stoichiometric class.

**Remark 3.4.** Questions like “How many steady states does a system possess?” refer to the number of steady states within each stoichiometric class. If this restriction is not imposed and  $s = \dim(\Gamma) < n$ , then the steady states describe an algebraic variety of dimension at least one over the complex numbers.

**Definition 3.5.** A network  $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  has the *capacity for multiple positive steady states* if there exists a rate vector  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and distinct vectors  $a, b \in \mathbb{R}_+^n$  such that  $a - b \in \Gamma$  and  $f_\kappa(a) = f_\kappa(b) = 0$ .

Note that in this work we focus mainly on the existence of multiple *positive* steady states. However, as we will show, the methods developed can preclude the existence of a specific type of multiple steady states on the boundary of  $\mathbb{R}_+^n$ .

If a basis  $\{\omega^1, \dots, \omega^d\}$  of  $\Gamma^\perp$  is chosen, then a network  $\mathcal{N}$  has the capacity for multiple positive steady states if there exists a rate vector  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and distinct  $a, b \in \mathbb{R}_+^n$  such that  $f_\kappa(a) = f_\kappa(b) = 0$  and  $\omega^i \cdot a = \omega^i \cdot b$  for all  $i = 1, \dots, d$ . In particular, if the map  $\bar{f}_\kappa : \mathbb{R}^n \rightarrow \mathbb{R}^{d+n}$  defined by  $\bar{f}_\kappa(c) = (\omega^1 \cdot c, \dots, \omega^d \cdot c, f_{\kappa,1}(c), \dots, f_{\kappa,n}(c))$  is injective, then the network does not have the capacity for multiple positive steady states. This function is the focus of study in this paper.

If species in- or outflow reactions exist for all species in a network then the stoichiometric subspace has maximal dimension  $n$ . Therefore, the requirement  $a - b \in \Gamma$  in the previous definition is superfluous. Specifically, if there is a reaction  $S_i \rightarrow 0$  or  $0 \rightarrow S_i$  for some species  $S$  then there are no vectors in  $\Gamma^\perp$  with non-zero  $i$ th entry.

**Lemma 3.6.** Let  $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a network. If  $S_i \rightarrow 0 \in \mathcal{R}$  or  $0 \rightarrow S_i \in \mathcal{R}$  for some  $S_i \in \mathcal{S}$ , then  $\lambda_i = 0$  for all  $\omega = (\lambda_1, \dots, \lambda_n) \in \Gamma^\perp$ .

**Proof.** Since  $\pm S_i \in \Gamma$ , we have  $0 = \omega \cdot (\pm S_i) = \pm \lambda_i$ .  $\square$

**Definition 3.7.** Let  $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a network. We say that

- (1)  $\mathcal{N}$  is *open* if  $\Gamma = \mathbb{R}^n$ .
- (2)  $\mathcal{N}$  is *fully open* if the outflow reaction  $S_i \rightarrow 0$  belongs to  $\mathcal{R}$  for all  $S_i \in \mathcal{S}$ .
- (3)  $\mathcal{N}$  is *closed* if  $\Gamma \neq \mathbb{R}^n$ .

If  $\mathcal{N}$  is any network then the *associated fully open network*,  $\mathcal{N}^o = (\mathcal{S}, \mathcal{C}^o, \mathcal{R}^o)$ , is the network with  $\mathcal{C}^o = \mathcal{C} \cup \mathcal{S} \cup \{0\}$  and  $\mathcal{R}^o = \mathcal{R} \cup \{S_i \rightarrow 0 | i = 1, \dots, n\}$ .

Lemma 3.6 ensures that a fully open network is also open. Fully open networks are considered by [4] in the context of continuous flow stirred tank reactors and their results extend to arbitrary open networks. A closed network is allowed to have outflow reactions as well, but not for all species since  $\Gamma \neq \mathbb{R}^n$ .

**Remark 3.8.** A closed network could also be made open by adding species inflow reactions to the set of reactions, but to be fully open requires species outflow reactions.

**Notation.** Let  $\mathcal{N} = (S, \mathcal{C}, \mathcal{R})$  be a network and  $\kappa$  a rate vector. At this point we have defined the species formation rate function  $f_\kappa$ , the stoichiometric subspace  $\Gamma$ , and used  $n$  for the number of species,  $s$  for the dimension of  $\Gamma$  and  $d = n - s$  for the dimension of  $\Gamma^\perp$ . None of these objects incorporate reference to the specific network in the notation. This notation is used without further mentioning throughout the paper. Additionally, to ease notation, in some examples species are called  $A, B, \dots$  and the respective concentrations  $c_A, c_B, \dots$ .

#### 4. Degenerate steady states

For any function  $f = (f_1, \dots, f_p) : \mathbb{R}^m \rightarrow \mathbb{R}^p$  let  $J_c(f)$  denote the Jacobian of  $f$  at  $c$ , that is, the  $p \times m$  matrix with entry  $(i, j)$  being  $\partial f_i(c) / \partial c_j$ .

**Definition 4.1.** Let  $\mathcal{N} = (S, \mathcal{C}, \mathcal{R})$  be a network and  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  a rate vector. A steady state  $c^* \in \mathbb{R}^n$  of  $\mathcal{N}$  is *degenerate* if  $\ker(J_{c^*}(f_\kappa)) \cap \Gamma \neq \{0\}$ .

**Remark 4.2.** A steady state  $x^*$  of a system of ODEs  $\dot{x} = \varphi(x)$  is often said to be degenerate if the Jacobian of  $\varphi$  at  $x^*$  is singular (for example as in [13]). With this definition, the existence of conserved amounts would make *all* steady states degenerate in the systems considered here (see Lemma 4.5 below). The reason is that, in this case, the dynamics is confined to a proper affine subspace of  $\mathbb{R}^n$  determined by the initial conditions. Consequently, the determinant of the Jacobian is zero and Jacobian-based approaches to the preclusion of multistationarity cannot be used. In fact, it is very likely that multiple steady states exist if  $\Gamma$  does not have maximal dimension. However, in biochemical applications the goal is to understand the dynamics within each stoichiometric class and the existence of multiple steady states in *each stoichiometric class* is the question of relevance. Therefore, our study restricts to stoichiometric classes and we adjust the notion of degeneracy to the singularities of the Jacobian in the stoichiometric space.

It is proven in [11, Section 5] that for any  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$ ,  $c \in \mathbb{R}_+^n$ , and  $\gamma \in \mathbb{R}^n$ ,

$$J_c(f_\kappa)(\gamma) = \sum_{y \rightarrow y' \in \mathcal{R}} k_{y \rightarrow y'} c^y (y'_* \gamma) (y' - y), \quad \text{where } v_* c w = \sum_{i=1}^n \frac{v_i w_i}{c_i}. \quad (4.3)$$

It is our aim to understand  $\ker(J_{c^*}(f_\kappa)) \cap \Gamma$ . We find a criterion to determine whether this intersection consists of the zero vector or is a proper subspace. The criterion does not require the computation of  $\ker(J_{c^*}(f_\kappa))$ . In particular, we find that the existence of degenerate steady states is linked to the function  $\bar{f}_\kappa$  being injective.

Since vectors of  $\Gamma$  are characterized by being orthogonal to all vectors in  $\Gamma^\perp$ , we obtain the following proposition (stated here for a general vector subspace  $F$ ).

**Proposition 4.4.** Let  $\mathcal{N}$  be a network and  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  a rate vector. Let  $F$  be a vector subspace of  $\mathbb{R}^n$ ,  $\{\omega^1, \dots, \omega^m\}$  a basis of  $F^\perp$  and define  $\bar{f}_\kappa : \mathbb{R}^n \rightarrow \mathbb{R}^{m+n}$  by

$$\bar{f}_\kappa(c) = (\omega^1 \cdot c, \dots, \omega^m \cdot c, f_{\kappa,1}(c), \dots, f_{\kappa,n}(c)).$$

Fix  $c^* \in \mathbb{R}^n$ . Then,  $\ker(J_{c^*}(f_\kappa)) \cap F = \{0\}$  if and only if the Jacobian  $J_{c^*}(\bar{f}_\kappa)$  of  $\bar{f}_\kappa$  at  $c^*$  has maximal rank  $n$ .

**Proof.** Let  $J_{c^*}(\bar{f}_\kappa)$  be the Jacobian of  $\bar{f}_\kappa(c)$  at  $c^*$ . It is an  $(m+n) \times n$  matrix. The rank of  $J_{c^*}(\bar{f}_\kappa)$  is maximal if and only if  $\ker(J_{c^*}(\bar{f}_\kappa)) = \{0\}$ . For  $i = 1, \dots, m$ , the  $i$ th row of  $J_{c^*}(\bar{f}_\kappa)$  equals the vector  $\omega^i$ . The lower  $n \times n$  matrix of  $J_{c^*}(\bar{f}_\kappa)$  (obtained by removing the first  $m$  rows) is equal to  $J_{c^*}(f_\kappa)$ . A vector  $v$  belongs to  $F$  if and only if  $\omega^i \cdot v = 0$  for all  $i$ . It follows that  $v \in \ker(J_{c^*}(\bar{f}_\kappa))$  if and only if  $v \in \ker(J_{c^*}(f_\kappa))$  and  $v \in F$ . Thus,  $\ker(J_{c^*}(f_\kappa)) \cap F = \{0\}$  if and only if  $\ker(J_{c^*}(\bar{f}_\kappa)) = \{0\}$ .  $\square$

By letting  $F = \Gamma$  in the previous lemma, we have shown that a steady state  $c^*$  is non-degenerate if and only if  $J_{c^*}(\bar{f}_\kappa)$  has maximal rank  $n$ . Since  $J_{c^*}(\bar{f}_\kappa)$  is a  $(n+d) \times n$  matrix,  $d$  rows of the matrix are linearly dependent of the remaining  $n$ . We describe now a procedure that is independent of  $c$  and  $\kappa$  to determine  $d$  rows with this property. Removal of these  $d$  rows from  $J_{c^*}(\bar{f}_\kappa)$  provides a square matrix whose rank agrees with the rank of  $J_{c^*}(\bar{f}_\kappa)$ .

**Lemma 4.5.** If  $\omega \in \Gamma^\perp$  then  $\omega^t J_c(f_\kappa) = 0$  for all  $c \in \mathbb{R}^n$  and all rate vectors  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$ .

**Proof.** If  $\omega \in \Gamma^\perp$  then  $\omega \cdot f_\kappa(c) = 0$  for all  $c$  and hence the scalar product vanishes as a polynomial in  $c$ . It follows that  $0 = \frac{\partial(\omega \cdot f_\kappa(c))}{\partial c_i} = \omega \cdot \frac{\partial f_\kappa(c)}{\partial c_i}$  for all  $i = 1, \dots, n$  and  $\omega^t J_c(f_\kappa) = 0$ .  $\square$

Hence, each  $\omega \in \Gamma^\perp$  provides a vanishing linear combination of the rows of  $J_c(f_\kappa)$ .

**Definition 4.6.** A basis  $\{\omega^1, \dots, \omega^d\}$  of  $\Gamma^\perp$  with  $\omega^i = (\lambda_1^i, \dots, \lambda_n^i)$  is said to be *reduced* if  $\lambda_i^i = 1$  for all  $i$  and  $\lambda_j^i = 0$  for all  $j = 1, \dots, \hat{j}, \dots, d$ . Given a rate vector  $\kappa$  and a reduced basis  $\{\omega^1, \dots, \omega^d\}$ , the associated extended rate function  $\tilde{f}_\kappa : \mathbb{R}^n \rightarrow \mathbb{R}^n$  is the function defined by

$$\tilde{f}_\kappa(c) = (\omega^1 \cdot c, \dots, \omega^d \cdot c, f_{\kappa, d+1}(c), \dots, f_{\kappa, n}(c)).$$

For the sake of simplicity, reference to the chosen reduced basis is not indicated in the notation of  $\tilde{f}_\kappa$ . After reordering the species, such a basis of  $\Gamma^\perp$  always exist (use for instance Gaussian elimination on any basis of  $\Gamma^\perp$ ). We assume from now on that a reduced basis is chosen, implying that the species are ordered accordingly.

**Example 4.7.** The basis  $\omega^1, \omega^2, \omega^3$  of  $\Gamma^\perp$  of the futile cycle given in (3.3) is reduced. The associated extended rate function is

$$\tilde{f}_\kappa(c) = (c_1 + c_5, c_2 + c_6, c_3 + c_4 + c_5 + c_6, -k_4 c_2 c_4 + k_3 c_5 + k_6 c_6, k_1 c_1 c_3 - (k_2 + k_3) c_5, k_4 c_2 c_4 - (k_5 + k_6) c_6).$$

Let  $J_{c,i}(f_\kappa)$  denote the  $i$ th row of  $J_c(f_\kappa)$ . If  $\{\omega^1, \dots, \omega^d\}$  is a reduced basis with  $\omega^i = (\lambda_1^i, \dots, \lambda_n^i)$ , then it follows from Lemma 4.5 that

$$0 = (\omega^i)^t J_c(f_\kappa) = J_{c,i}(f_\kappa) + \sum_{j=d+1}^n \lambda_{c,j}^i J_{c,j}(f_\kappa), \quad i = 1, \dots, d.$$

Thus, the rows  $1, \dots, d$  of  $J_c(f_\kappa)$  are linear combinations of the rows  $d + 1, \dots, n$ . It follows that the rank of  $J_{c^*}(\tilde{f}_\kappa)$  equals the rank of  $J_{c^*}(f_\kappa)$ . The latter is a square  $n \times n$  matrix and has maximal rank if and only if its determinant does not vanish.

**Corollary 4.8.** Let  $\mathcal{N}$  be a network,  $\kappa \in \mathbb{R}_+^R$  a rate vector,  $\{\omega^1, \dots, \omega^d\}$  a reduced basis of  $\Gamma^\perp$  and  $\tilde{f}_\kappa(c)$  the associated extended rate function. If  $c^* \in \mathbb{R}^n$ , then  $\ker(J_{c^*}(f_\kappa)) \cap \Gamma = \{0\}$  if and only if  $\det(J_{c^*}(\tilde{f}_\kappa)) \neq 0$ . In particular, a steady state  $c^* \in \mathbb{R}^n$  of  $\mathcal{N}$  is degenerate if and only if  $\det(J_{c^*}(f_\kappa)) = 0$ .

**Example 4.9.** The Jacobian matrix  $J_c(\tilde{f}_\kappa)$  of the futile cycle associated with the reduced basis of  $\Gamma^\perp$  in (3.3) is

$$J_c(\tilde{f}_\kappa) = \begin{pmatrix} 1 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 1 & 1 & 1 & 1 \\ 0 & -k_4 c_4 & 0 & -k_4 c_2 & k_3 & k_6 \\ k_1 c_3 & 0 & k_1 c_1 & 0 & -k_2 - k_3 & 0 \\ 0 & k_4 c_4 & 0 & k_4 c_2 & 0 & -k_5 - k_6 \end{pmatrix}.$$

The determinant of  $J_c(\tilde{f}_\kappa)$  is

$$-\det(J_c(\tilde{f}_\kappa)) = (c_1 c_2 + c_1 c_4) k_1 k_3 k_4 + c_1 k_1 k_3 k_5 + (c_1 c_2 + c_2 c_3) k_1 k_4 k_5 + c_2 k_2 k_4 k_5 + c_2 k_3 k_4 k_5 + c_1 k_1 k_3 k_6. \tag{4.10}$$

All coefficients of  $-\det(J_c(\tilde{f}_\kappa))$  as a polynomial in  $c, \kappa$  are positive. Thus  $\det(J_c(\tilde{f}_\kappa))$  does not vanish for any  $c \in \mathbb{R}_+^n$  and  $\kappa \in \mathbb{R}_+^R$  and all positive steady states of the futile cycle are non-degenerate. In this example,  $\det(J_c(\tilde{f}_\kappa))$  is linear in the rate constants. If at least one coefficient in the polynomial was negative then we could find  $c, \kappa$  for which  $\det(J_c(\tilde{f}_\kappa)) = 0$ , implying that a degenerate steady state could occur. This observation holds for any network and will be discussed in the following sections.

**Remark 4.11.** The minimal space containing the image of  $f_\kappa$  is the *kinetic subspace*:

$$\Lambda_\kappa := \langle \text{im } f_\kappa \rangle \subseteq \Gamma. \tag{4.12}$$

In general, the two spaces  $\Lambda_\kappa$  and  $\Gamma$  might not agree for a fixed rate vector  $\kappa$ . If this is the case then  $\ker(J_c(f_\kappa)) \cap \Gamma \neq \{0\}$  for any  $c \in \mathbb{R}_+^n$ : as above, if  $\omega \in \Lambda_\kappa^\perp$  then  $0 = \omega \cdot f_\kappa = \omega \cdot \dot{c}$ . Similarly to the proof of Lemma 4.5 we have that  $\omega^t J_c(f_\kappa) = 0$  and hence  $\langle \text{im } J_c(f_\kappa) \rangle \subseteq \Lambda_\kappa$ . If  $\Lambda_\kappa \subsetneq \Gamma$  then  $\langle \text{im } J_c(f_\kappa) \rangle$  has at most dimension  $s - 1$  and it follows that the dimension of  $\ker(J_c(f_\kappa)) \cap \Gamma$  is at least 1. Thus, if the stoichiometric and the kinetic spaces do not agree for some  $\kappa$ , then all steady states corresponding to  $f_\kappa$  are degenerate.

**Example 4.13.** Consider the network with reactions  $r_1 : A \rightarrow B$  and  $r_2 : A \rightarrow C$ . The species formation rate function  $f_\kappa : \mathbb{R}^3 \rightarrow \mathbb{R}^3$  is

$$f_\kappa(c_A, c_B, c_C) = -(k_1 + k_2)c_A, k_1c_A, k_2c_A,$$

where  $k_i$  denotes the rate constant of reaction  $r_i$ . The stoichiometric subspace has dimension 2 and a basis of  $\Gamma^\perp$  is  $A + B + C$ . However,  $k_2B - k_1C \in \Lambda_\kappa^\perp \setminus \Gamma^\perp$  and thus for all  $\kappa$ , all steady states are degenerate.

**Example 4.14.** The stoichiometric and the kinetic spaces of the previous example never agree. For some networks, the two spaces do not agree only for certain rate vectors. Consider for example the network with reactions  $r_1 : A \rightarrow B, r_2 : A \rightarrow C$  and  $r_3 : B + C \rightarrow 2A$ . The species formation rate function  $f_\kappa : \mathbb{R}^3 \rightarrow \mathbb{R}^3$  is

$$f_\kappa(c_A, c_B, c_C) = -(k_1 + k_2)c_A + 2k_3c_Bc_C, k_1c_A - k_3c_Bc_C, k_2c_A - k_3c_Bc_C.$$

If we let  $\omega = (\lambda_1, \lambda_2, \lambda_3)$  then the equation  $\omega \cdot f_\kappa(c) = 0$  for all  $c$  gives  $2\lambda_1 = \lambda_2 + \lambda_3$  and  $(\lambda_2 - \lambda_3)(k_1 - k_2) = 0$ . If  $k_1 \neq k_2$ , then  $(1, 1, 1)$  is a basis of  $\Gamma^\perp$  as well as  $\Lambda_\kappa^\perp$  and the stoichiometric and the kinetic spaces agree. However, if  $k_1 = k_2$  then  $(1, 2, 0) \in \Lambda_\kappa^\perp \setminus \Gamma^\perp$  and the two spaces do not agree. Further, in this case all steady states are degenerate.

**Remark 4.15.** Note that Proposition 4.4, Lemma 4.5 and Corollary 4.8 do not depend on the kinetics being of mass-action type, but hold for general (differentiable) kinetics with  $f_\kappa$  and  $\tilde{f}_\kappa$  changed accordingly. In particular, Corollary 4.8 characterizes degenerate steady states in terms of the determinant of the Jacobian of  $\tilde{f}_\kappa$ .

### 5. Injective networks

Here we introduce the notion of injectivity: a network is injective if for all rate vectors  $\kappa$  the function  $\tilde{f}_\kappa$  is injective over  $\mathbb{R}_+^n$ . The definition is an extension of the definition of injectivity for fully open networks [4, Def. 2.8] to arbitrary networks and, together with Proposition 5.2 below, it is in line with the definition given in a recent paper on concordant networks [14]. We show that a network is injective if and only if the Jacobian of  $\tilde{f}_\kappa$  is non-singular for all positive concentrations  $c$  and for all  $\kappa$ .

In [5, Def. 6.1] a definition of injectivity is given for networks with  $\Gamma \subseteq \mathbb{R}^n$ . It relies on the Jacobian of the species formation rate function restricted to the stoichiometric subspace. We prove in Theorem 5.6 below that our definition agrees with their definition. The equivalence is also claimed in Remark 6.4 by [5] without a proof.

**Definition 5.1.** A network  $\mathcal{N}$  is said to be injective if for all rate vector  $\kappa$  and any distinct  $a, b \in \mathbb{R}_+^n$  such that  $a - b \in \Gamma$ , we have  $f_\kappa(a) \neq f_\kappa(b)$ .

The definition of injectivity is restricted to the interior of the positive orthant. However, we show below that being injective also precludes the existence of distinct  $a, b$  such that  $a - b \in \Gamma$  and  $f_\kappa(a) = f_\kappa(b)$ , provided either that  $a \in \overline{\mathbb{R}_+^n}, b \in \mathbb{R}_+^n$  or that  $a, b \in \overline{\mathbb{R}_+^n}$  are on the boundary of  $\mathbb{R}_+^n$  and fulfill a certain condition. For  $a \in \overline{\mathbb{R}_+^n}$ , let  $I_a = \{i | a_i = 0\}$  be the indices for which  $a_i$  is zero and let  $\mathcal{Y}_a = \{y | y \rightarrow y' \in \mathcal{R}, I_a \cap \text{supp}(y) \neq \emptyset\}$ , where  $\text{supp}(y) = \{i | y_i \neq 0\}$  is the support of  $y$ . That is,  $\mathcal{Y}_a$  is the set of reactant complexes involving at least one species  $S_i$  for which  $a_i = 0$ . It follows that  $y$  belongs to  $\mathcal{Y}_a$  if and only if  $a^y = 0$ .

**Proposition 5.2.** Let  $\mathcal{N}$  be a network. The following two statements are equivalent:

- (i)  $\mathcal{N}$  is injective.
- (ii) For any distinct  $a, b \in \overline{\mathbb{R}_+^n}$  such that  $\mathcal{Y}_a \cap \mathcal{Y}_b = \emptyset$  and  $a - b \in \Gamma, f_\kappa(a) \neq f_\kappa(b)$ .

In particular, if  $b \in \mathbb{R}_+^n$ , that is  $I_b = \emptyset$ , then  $\mathcal{Y}_a \cap \mathcal{Y}_b = \emptyset$  is fulfilled for any  $a \in \overline{\mathbb{R}_+^n}$ .

**Proof.** (ii) obviously implies (i). To prove the reverse we assume that there are  $a, b \in \overline{\mathbb{R}_+^n}$ , such that  $\mathcal{Y}_a \cap \mathcal{Y}_b = \emptyset, b - a \in \Gamma$  and  $f_\kappa(a) = f_\kappa(b)$  and show that  $\mathcal{N}$  cannot be injective. Let  $\gamma_i = b_i - a_i$  and for a set  $M \subseteq \{1, \dots, n\}$  and  $y \in \mathcal{C}$  define  $x^{yM} = \prod_{i \in M} x^{y_i}$ . Further, define  $I = I_a \cup I_b$  and  $J = I^c$ . If  $I = \emptyset$ , then clearly  $\mathcal{N}$  cannot be injective. Hence, assume that  $I \neq \emptyset$ . We seek to define a rate vector  $\tilde{\kappa} = (\tilde{k}_{y \rightarrow y'})_{y \rightarrow y'}$  and  $\tilde{a}, \tilde{b}$  such that  $\tilde{a}_i = a_i, \tilde{b}_i = b_i$  for  $i \notin I, \tilde{a}_i, \tilde{b}_i > 0$  and  $\tilde{b}_i - \tilde{a}_i = \gamma_i$  for  $i \in I$ , and

$$\sum_{y \rightarrow y' \in \mathcal{R}} \tilde{k}_{y \rightarrow y'} (\tilde{a}^y - \tilde{b}^y) (y' - y) = 0,$$

that is,  $f_{\tilde{\kappa}}(\tilde{a}) = f_{\tilde{\kappa}}(\tilde{b})$ . Then, since  $\tilde{a}, \tilde{b} \in \mathbb{R}_+^n$  and  $\tilde{b} - \tilde{a} \in \Gamma$ , it follows that  $\mathcal{N}$  is not injective. The equality  $f_\kappa(a) - f_\kappa(b) = 0$  can be rewritten as:

$$0 = \sum_{\substack{y \rightarrow y' \\ y \in \mathcal{C}_a^c \cap \mathcal{C}_b^c}} k_{y \rightarrow y'} (a^y - b^y) (y' - y) + \sum_{\substack{y \rightarrow y' \\ y \in \mathcal{C}_a \cap \mathcal{C}_b^c}} k_{y \rightarrow y'} (a^y - b^y) (y' - y) + \sum_{\substack{y \rightarrow y' \\ y \in \mathcal{C}_a^c \cap \mathcal{C}_b}} k_{y \rightarrow y'} (a^y - b^y) (y' - y),$$

Assume that we can find  $\tilde{a}_i, \tilde{b}_i > 0$  with  $\tilde{b}_i - \tilde{a}_i = \gamma_i$  for all  $i, \tilde{a}_i = a_i, \tilde{b}_i = b_i$  for  $i \notin I$ , and  $\alpha_y > 0$  such that

$$\frac{\alpha_y}{\alpha_y + 1} \tilde{b}^y = \tilde{a}^y, \quad \text{if } y \in \mathcal{Y}_a \cap \mathcal{Y}_b^c, \quad \text{and} \quad \frac{\alpha_y}{\alpha_y + 1} \tilde{a}^y = \tilde{b}^y, \quad \text{if } y \in \mathcal{Y}_a^c \cap \mathcal{Y}_b. \tag{5.3}$$

Then, let  $\tilde{k}_{y \rightarrow y'} = k_{y \rightarrow y'}$  for  $y \in \mathcal{Y}_a^c \cap \mathcal{Y}_b^c$ ,  $\tilde{k}_{y \rightarrow y'} = (\alpha_y + 1)k_{y \rightarrow y'} b^y / \tilde{b}^y$  for  $y \in \mathcal{Y}_a \cap \mathcal{Y}_b^c$  and  $\tilde{k}_{y \rightarrow y'} = (\alpha_y + 1)k_{y \rightarrow y'} a^y / \tilde{a}^y$  for  $y \in \mathcal{Y}_a^c \cap \mathcal{Y}_b$ . With these definitions, if  $y \in \mathcal{Y}_a \cap \mathcal{Y}_b^c$  since  $a^y = 0$  we have

$$\tilde{k}_{y \rightarrow y'} (\tilde{a}^y - \tilde{b}^y) = k_{y \rightarrow y'} (\alpha_y + 1) (\tilde{a}^y b^y / \tilde{b}^y - b^y) = k_{y \rightarrow y'} (\alpha_y b^y - (\alpha_y + 1) b^y) = k_{y \rightarrow y'} (-b^y) = k_{y \rightarrow y'} (a^y - b^y)$$

Analogously,  $\tilde{k}_{y \rightarrow y'} (\tilde{a}^y - \tilde{b}^y) = k_{y \rightarrow y'} (a^y - b^y)$  if  $y \in \mathcal{Y}_a^c \cap \mathcal{Y}_b$ , using  $b^y = 0$ . It follows that for  $\tilde{\kappa} = (\tilde{k}_{y \rightarrow y'})_{y \rightarrow y'}$ , we have  $f_{\tilde{\kappa}}(\tilde{a}) - f_{\tilde{\kappa}}(\tilde{b}) = f_{\tilde{\kappa}}(a) - f_{\tilde{\kappa}}(b) = 0$  as desired.

All that remains is to find  $\tilde{a}_i, \tilde{b}_i > 0$  for  $i \in I$  and  $\alpha_y > 0$  such that (5.3) is fulfilled. If  $y \notin \mathcal{Y}_b$ , then  $\tilde{b}^y, \tilde{a}^y$  are independent of the value of  $\tilde{a}_i, \tilde{b}_i$  for  $i \in I_b$ . Therefore, we define first  $\tilde{a}_i, \tilde{b}_i$  for  $i \in I_a$  and focus on fulfilling (5.3) for  $y \in \mathcal{Y}_a \cap \mathcal{Y}_b^c$ . Further, since we want  $\tilde{b}_i - \tilde{a}_i = \gamma_i$ , once  $\tilde{a}_i$  is defined, we have  $\tilde{b}_i = \tilde{a}_i + \gamma_i$ .

Let  $I_a = \{i_1, \dots, i_m\}, I_k = \{i_1, \dots, i_k\}$  ( $k \leq m$ ) with  $I_0 = \emptyset$  and  $J_k = J \cup I_k$ . Further, recursively define  $\mathcal{Y}_k = \{y \in \mathcal{Y}_a \mid I_a \cap \text{supp}(y) \subseteq I_k\} \setminus \mathcal{Y}_{k-1}$  with  $\mathcal{Y}_0 = \emptyset$ . It follows that  $\bigcup_{k=1}^m \mathcal{Y}_k = \mathcal{Y}$  such that any complex in  $\mathcal{Y}$  is in precisely one  $\mathcal{Y}_k$ . That is, if  $y \in \mathcal{Y}_k$  then  $i_k$  is the largest index in  $I_a$  in the support of  $y$ . We define  $\tilde{a}_{i_k}, \tilde{b}_{i_k}$  recursively. Note that  $\gamma_i > 0$  for all  $i \in I_a$ . Assume that  $\tilde{a}_{i_1}, \dots, \tilde{a}_{i_{k-1}}$  and  $\tilde{b}_{i_1}, \dots, \tilde{b}_{i_{k-1}}$  are defined and hence the products  $\tilde{b}^{y_{i_{k-1}}}, \tilde{a}^{y_{i_{k-1}}}$  are fixed. For  $i_k$  do the following: if  $\mathcal{Y}_k = \emptyset$ , let  $\tilde{a}_{i_k} = 1$  and  $\tilde{b}_{i_k} = 1 + \gamma_{i_k}$ . If  $\mathcal{Y}_k \neq \emptyset$ , observe that for  $y \in \mathcal{Y}_k$  (for which  $y_{i_k} \neq 0$ ), the equality (5.3) can be written as

$$\frac{\tilde{a}_{i_k}^{y_{i_k}}}{(\tilde{a}_{i_k} + \gamma_{i_k})^{y_{i_k}}} = \frac{\alpha_y}{\alpha_y + 1} \frac{\tilde{b}^{y_{i_{k-1}}}}{\tilde{a}^{y_{i_{k-1}}}}, \quad \text{that is,} \quad \alpha_y = \frac{r_{i_k}(\tilde{a}_{i_k}, y_{i_k})}{\frac{\tilde{b}^{y_{i_{k-1}}}}{\tilde{a}^{y_{i_{k-1}}}} - r_{i_k}(\tilde{a}_{i_k}, y_{i_k})}, \tag{5.4}$$

with  $r_{i_k}(\tilde{a}_{i_k}, y_{i_k}) := \tilde{a}_{i_k}^{y_{i_k}} / (\tilde{a}_{i_k} + \gamma_{i_k})^{y_{i_k}} > 0$ . The function  $r_{i_k}$  is increasing in  $a_{i_k}$  and  $r_{i_k}(0, y_{i_k}) = 0$  for all  $y_{i_k}$ . By defining  $\tilde{a}_{i_k} > 0$  arbitrarily such that

$$r_{i_k}(\tilde{a}_{i_k}, y_{i_k}) < \min \left( \frac{\tilde{b}^{y_{i_{k-1}}}}{\tilde{a}^{y_{i_{k-1}}}} \mid y \in \mathcal{Y}_k \right)$$

for all  $y \in \mathcal{Y}_k$  (which is a finite set), we obtain  $\alpha_y > 0$  fulfilling (5.4) as desired.

The same procedure is applied to define  $\tilde{a}_i, \tilde{b}_i$  for  $i \in I_b$ , with the roles of  $a$  and  $b$  reversed. In this case, however,  $b_i = 0$  implies that  $\gamma_i < 0$  and  $\tilde{a}_i = \tilde{b}_i - \gamma_i$  for  $i \in I_b$ . Therefore,  $r_{i_k}(\tilde{b}_{i_k}, y_{i_k})$  becomes  $\tilde{b}_{i_k}^{y_{i_k}} / (\tilde{b}_{i_k} - \gamma_{i_k})^{y_{i_k}}$ , which also is increasing.  $\square$

The assumption  $\mathcal{Y}_a \cap \mathcal{Y}_b = \emptyset$  in Proposition 5.2(ii) cannot be relaxed. Consider the network with  $\mathcal{S} = \{A, B\}, \mathcal{C} = \{A + B, A + 2B, 0\}$  and reactions  $A + B \rightarrow 0, A + 2B \rightarrow 0$ . This network is injective and open. If  $a = (1, 0)$  and  $b = (0, 1)$  then  $\mathcal{Y}_a \cap \mathcal{Y}_b = \{A + B, A + 2B\} \neq \emptyset$  and for any rate vector  $\kappa$  we have  $f_{\kappa}(a) = f_{\kappa}(b)$ .

**Remark 5.5.** In [14], a network  $\mathcal{N}$  with arbitrary kinetics is said to be injective if  $f_{\kappa}(a) \neq f_{\kappa}(b)$  for any pair of stoichiometrically compatible concentration vectors  $a, b$ , at least one of which is positive. The condition given in Proposition 5.2(ii) is slightly more general in that both  $a$  and  $b$  can be non-negative.

If  $\mathcal{N}$  is injective, then for any choice of rate vector  $\kappa$  at most one positive steady state can exist within each stoichiometric class, i.e., for every  $c_0 \in \mathbb{R}_+^n$  there exists at most one  $c \in \{c_0 + \Gamma\} \cap \mathbb{R}_+^n$  such that  $f_{\kappa}(c) = 0$ . In other words, if  $\mathcal{N}$  is injective

then  $\mathcal{N}$  does not have the capacity for multiple positive steady states. However, the reverse might not be true: non-injective networks exist that do not have the capacity for multiple positive steady states. An example is provided in Example 7.11.

The proof of the following theorem is adapted from the proof of [4, Th 3.1].

**Theorem 5.6.** Let  $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a network. Then,  $\mathcal{N}$  is injective if and only if  $\ker(J_c(f_{\kappa})) \cap \Gamma = \{0\}$  for all  $c \in \mathbb{R}_+^n$  and  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$ .

**Proof.**  $\mathcal{N}$  is not injective if and only if there exists  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and distinct  $a, b \in \mathbb{R}_+^n$  such that  $a - b \in \Gamma$  and  $f_{\kappa}(a) = f_{\kappa}(b)$ . Further,  $\ker(J_c(f_{\eta})) \cap \Gamma \neq \{0\}$  for some  $c \in \mathbb{R}_+^n$  and  $\eta \in \mathbb{R}_+^{\mathcal{R}}$  if and only if there exists  $\gamma \in \Gamma$  such that  $J_c(f_{\eta})(\gamma) = 0$ . By definition and using (4.3),

$$f_{\kappa}(a) - f_{\kappa}(b) = 0 \iff \sum_{y \rightarrow y' \in \mathcal{R}} k_{y \rightarrow y'} (a^y - b^y) (y' - y) = 0,$$

$$J_c(f_{\eta})(\gamma) = 0 \iff \sum_{y \rightarrow y' \in \mathcal{R}} \eta_{y \rightarrow y'} c^y (y * c \gamma) (y' - y) = 0.$$

We will show that given distinct  $a, b \in \mathbb{R}_+^n$  such that  $\gamma := a - b \in \Gamma$  and some rate vector  $\kappa$ , there exist  $c \in \mathbb{R}_+^n$  and  $\eta \in \mathbb{R}_+^{\mathcal{R}}$  such that

$$k_{y \rightarrow y'} (a^y - b^y) = \eta_{y \rightarrow y'} c^y (y * c \gamma) \tag{5.7}$$



and vice versa. Consider distinct  $a, b \in \mathbb{R}_+^n$  such that  $\gamma := a - b \in \Gamma, \gamma \neq 0$ . If  $a_i - b_i \neq 0$  define  $c_i := \frac{a_i - b_i}{\log a_i / b_i} > 0$  and let  $c_i = 1$  otherwise. Since  $\gamma_i = 0$  if  $a_i = b_i$ , we have  $a^y = b^y e^{y^* c \gamma}$ . Note that the signs of  $a^y - b^y = b^y (e^{y^* c \gamma} - 1)$  and  $y^* c \gamma$  agree. If  $y^* c \gamma = 0$ , let  $\eta_{y \rightarrow y'} = 1$ . Otherwise, we let  $\eta_{y \rightarrow y'} = \frac{k_{y \rightarrow y'} (a^y - b^y)}{\partial^y (y^* c \gamma)} > 0$  and (5.7) is fulfilled. Reciprocally, given  $\gamma \in \Gamma, \gamma \neq 0, c \in \mathbb{R}_+^n$  and  $\eta \in \mathbb{R}_+^{\mathcal{R}}$ , define  $a, b \in \mathbb{R}_+^n$  by  $b_i = a_i = 1$  if  $\gamma_i = 0$  and  $b_i = \gamma_i / (e^{\eta_i / c_i} - 1) > 0, a_i = b_i e^{\eta_i / c_i} > 0$  otherwise. Then  $a - b = \gamma \in \Gamma$  and  $a^y - b^y = b^y (e^{y^* c \gamma} - 1)$  has the same sign as  $y^* c \gamma$ . If  $a^y - b^y = 0$ , define  $k_{y \rightarrow y'} = 1$ . Otherwise, define  $k_{y \rightarrow y'} = \frac{\eta_{y \rightarrow y'} (y^* c \gamma)}{\partial^y (a^y - b^y)} > 0$  and equality (5.7) is fulfilled.  $\square$

**Remark 5.8.** The summand in  $f_\kappa$  corresponding to an inflow reaction  $0 \rightarrow y'$  in  $\mathcal{R}$  takes the form  $k_{0 \rightarrow y'}$  and thus is independent of the concentration vector  $c$ . It follows that  $J_c(f_\kappa)$  is independent of the presence or absence of inflow reactions in  $\mathcal{R}$  and so is the property of being injective.

We have thus obtained a characterization of injective networks in terms of the Jacobian associated with the species formation rate function. Together with Corollary 4.8 we obtain:

**Corollary 5.9.** Let  $\mathcal{N}$  be a network,  $\{\omega^1, \dots, \omega^d\}$  a reduced basis of  $\Gamma^\perp$  and  $\tilde{f}_\kappa(c)$  the associated extended rate function.  $\mathcal{N}$  is injective if and only if  $\det(J_c(\tilde{f}_\kappa)) \neq 0$  for all  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and  $c \in \mathbb{R}_+^n$ .

**Remark 5.10.** Statements similar to Theorem 5.6 and Corollary 5.9 cannot be stated for individual  $\kappa$ , since  $\kappa$  and  $\eta$  generally are different in the proof of Theorem 5.6. However, if the total degree of each of polynomials in the components in  $\tilde{f}_\kappa$  is at most two then  $\tilde{f}_\kappa$  is an injective function if and only if the Jacobian is non-singular [15].

Note that  $\mathcal{N}$  is injective if and only if the extended rate function  $\tilde{f}_\kappa$  associated with a reduced basis is injective over  $\mathbb{R}_+^n$ . Further, as is observed in (4.10) for the futile cycle,  $\det(J_c(\tilde{f}_\kappa))$  is a homogeneous polynomial in the entries of  $\kappa$  with total degree  $s$ , because the rows  $1, \dots, d$  of  $J_c(\tilde{f}_\kappa)$  are constant (that is, independent of  $c$  and  $\kappa$ ).

**Remark 5.11.** A general version of the corollary above has recently been formulated in [16] for a certain class of polynomial maps, without the restriction to species formation rate functions and conservation laws associated to networks.

From Definition 4.1 and Theorem 5.6 we obtain the following corollary.

**Corollary 5.12.** Let  $\mathcal{N}$  be a network. If  $\mathcal{N}$  is injective then there exist no degenerate positive steady states.

**Remark 5.13.** It follows from Theorem 5.6 and Remark 4.11 that if the two spaces  $\Gamma$  and  $\Lambda_\kappa$  are not identical for some rate vector  $\kappa$  then the network cannot be injective.

As noticed in Example 4.10, the determinant of the Jacobian of the extended species rate formation function of the futile cycle can never vanish. Thus, the futile cycle is injective and does not have the capacity for multiple positive steady states.

The coefficients of the determinant of the Jacobian of fully open networks are characterized by Craciun and Feinberg in [4] and this characterization easily generalizes to open networks. Thus, in order to characterize the coefficients of the determinant of the Jacobian of an arbitrary network  $\mathcal{N}$ , we consider the associated fully open network,  $\mathcal{N}^o$ , and “match” the terms of the respective determinants. This is done in Section 7 after we discuss some results about open networks in the next section.

### 6. Injective open networks

Recall that a network is open if its stoichiometric subspace is  $\mathbb{R}^n$ . If this is the case then  $\tilde{f}_\kappa = f_\kappa$ . By Theorem 5.6, an open network  $\mathcal{N}$  is injective if and only if the Jacobian  $J_c(f_\kappa)$  is non-singular, i.e.,  $\det(J_c(f_\kappa)) \neq 0$  for all rate vectors  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and all  $c \in \mathbb{R}_+^n$ . Hidden in the proof of Theorem 3.1 in [4] and Theorem 5.6 above we find a simplification of the characterization of injective open networks: for a network to be injective it suffices to fix any concentration vector  $c \in \mathbb{R}_+^n$  and show that  $\det(J_c(f_\kappa)) \neq 0$ , for all rate vectors  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$ .

**Proposition 6.1.** An open network  $\mathcal{N}$  is injective if and only if  $\det(J_c(f_\kappa)) \neq 0$  for all rate vectors  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and a fixed positive  $c \in \mathbb{R}_+^n$ .

**Proof.** By Theorem 5.6, it is enough to prove that  $\det(J_c(f_\kappa)) \neq 0$  for all rate vectors  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and all  $c \in \mathbb{R}_+^n$  if and only if the statement holds for a fixed  $c \in \mathbb{R}_+^n$ . The forward implication is obvious. To see the reverse, assume that  $\det(J_x(f_\kappa)) = 0$  for some rate vector  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and  $x \in \mathbb{R}_+^n$ , that is, there exists a non-zero vector  $\gamma \in \mathbb{R}^n$  such that  $0 = J_x(f_\kappa)(\gamma)$ . Define

$\eta = (\eta_{y \rightarrow y'})_{y \rightarrow y'} \in \mathbb{R}_+^{\mathcal{R}}$  with  $\eta_{y \rightarrow y'} = k_{y \rightarrow y'} \lambda^{y'} / c^{y'} > 0$ , and  $\delta = (\delta_1, \dots, \delta_n)$  with  $\delta_i = \gamma_i c_i / x_i$ . With these definitions, we have  $k_{y \rightarrow y'} \lambda^{y'} = \eta_{y \rightarrow y'} c^{y'}$  and  $y^* \lambda^y = y^* c^y \delta$ . Then, using (4.3), we have

$$J_c(f_\eta)(\delta) = \sum_{y \rightarrow y' \in \mathcal{R}} \eta_{y \rightarrow y'} c^{y'} (y^* c^y \delta)(y' - y) = J_x(f_\kappa)(\gamma) = 0,$$

which implies that  $\det(J_c(f_\eta)) = 0$  and we have reached a contradiction.  $\square$

The simplification presented here applies to any open network, independently of whether it contains outflow reactions or not. We have proved that injectivity of an open network can be checked using the Jacobian criterion with the concentration vector fixed to  $\mathbf{1} := (1, \dots, 1)$ . In this case the determinant of the Jacobian is a polynomial depending only on the rate constants and this reduces the number of variables substantially. Further, the polynomial is homogeneous of total degree  $n$ .

In [4, Rk. 2.9] a different simplification is performed where the rate constants of all species outflow reactions are fixed to 1. We state this result as a proposition below and give the proof for completeness.

**Proposition 6.2.** *Let  $\mathcal{N}$  be an open network and fix a subset  $\mathcal{O} \subseteq \{1, \dots, n\}$ . The following statements are equivalent:*

- (1)  $\mathcal{N}$  is injective.
- (2)  $\det(J_c(f_\kappa)) \neq 0$  for all  $c \in \mathbb{R}_+^n$  and all rate vectors  $\kappa$  satisfying  $k_{S_i \rightarrow 0} = 1$  for all  $i \in \mathcal{O}$ .
- (3) For any distinct  $a, b \in \mathbb{R}_+^n$ ,  $f_\kappa(a) \neq f_\kappa(b)$  for all rate vectors  $\kappa$  satisfying  $k_{S_i \rightarrow 0} = 1$  for all  $i \in \mathcal{O}$ .

**Proof.** (i) implies (ii) and (iii) by Definition 5.1 and Theorem 5.6. We now prove that (ii) and (iii) separately imply (i). For any  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and  $z \in \mathbb{R}^n$  define  $\tilde{\kappa} = (k_{y \rightarrow y'})_{y \rightarrow y'} \in \mathbb{R}_+^{\mathcal{R}}$  and  $\tilde{z} \in \mathbb{R}^n$  by:

$$\tilde{z}_i = \begin{cases} z_i k_{S_i \rightarrow 0}, & i \in \mathcal{O} \\ z_i & i \notin \mathcal{O} \end{cases} \quad \tilde{k}_{y \rightarrow y'} = \frac{k_{y \rightarrow y'}}{\prod_{i \in \mathcal{O}} k_{S_i \rightarrow 0}^{y_i}}.$$

Clearly  $\tilde{k}_{S_i \rightarrow 0} = 1$  for all  $i \in \mathcal{O}$ ,  $\tilde{k}_{y \rightarrow y'}(\tilde{a})^y = k_{y \rightarrow y'} a^y$ , and further  $y^* \tilde{a}^y = y^* \tilde{\gamma}^y$  for any  $\gamma \in \mathbb{R}^n$ . It follows that  $f_{\tilde{\kappa}}(\tilde{a}) = f_\kappa(a)$ , and, similarly, for any  $\gamma \in \mathbb{R}^n$ ,  $J_{\tilde{a}}(f_{\tilde{\kappa}})(\tilde{\gamma}) = J_a(f_\kappa)(\gamma)$ . Therefore, if  $\mathcal{N}$  is not injective, then (1) there exists a rate vector  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and distinct  $a, b \in \mathbb{R}_+^n$  such that  $f_\kappa(a) = f_\kappa(b)$  and the construction of  $\tilde{\kappa} \in \mathbb{R}_+^{\mathcal{R}}$  and  $\tilde{a}, \tilde{b} \in \mathbb{R}_+^n$  as above implies that (iii) does not hold, and (2) there exist a rate vector  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$ ,  $a \in \mathbb{R}_+^n$  and  $\gamma \in \mathbb{R}^n$ ,  $\gamma \neq 0$ , such that  $J_a(f_\kappa)(\gamma) = 0$  and thus a rate vector  $\tilde{\kappa} \in \mathbb{R}_+^{\mathcal{R}}$ ,  $\tilde{a} \in \mathbb{R}_+^n$  and  $\tilde{\gamma} \in \mathbb{R}^n$  contradicting (ii). It follows that (ii) and (iii) both imply (i) and the proof is completed.  $\square$

The simplifications in Propositions 6.1 and 6.2 cannot be performed at the same time because it would constrain the choice of the free variables  $\kappa$  and  $c$  too much.

The next proposition is an extension of [4, Eq. (3.15)] where the statement is made for a specific class of open networks. However, the proof works line by line for the class of all open networks. In fact it does not depend on whether the network is open or not, but all terms in the proposition are zero unless the network is open. Notice however that our statement differs from the statement by [4] in the sign  $(-1)^n$ , because they establish the Jacobian criterion on the Jacobian of  $-f_\kappa$ .

Recall that  $n$  is the number of species and note that  $\det(J_c(f_\kappa))$  is a homogeneous polynomial of degree  $n$  in the rate constants. To simplify the notation we introduce the following: for any set of  $m$  reactions,  $R = \{y^1 \rightarrow y'^1, \dots, y^m \rightarrow y'^m\}$ , let

- $\mathcal{Y}(R)$  be the  $n \times m$  matrix whose  $i$ th column is  $y^i$ .
- $\Gamma(R)$  be the  $n \times m$  matrix whose  $i$ th column is  $y^i - y'^i$ .
- $\sigma(R) = (-1)^n \det(\mathcal{Y}(R)) \det(\Gamma(R))$  if  $m = n$ .

**Proposition 6.3** [4]. *Let  $\mathcal{N}$  be an open network and let  $R = \{y^1 \rightarrow y'^1, \dots, y^n \rightarrow y'^n\}$  be a set of  $n$  reactions. Viewed as a polynomial in the rate vector  $\kappa$ , the coefficient of the monomial  $\prod_{i=1}^n k_{y^i \rightarrow y'^i}$  in  $\det(J_c(f_\kappa))$  with  $c \in \mathbb{R}_+^n$  is*

$$\alpha(R) := \sigma(R) c^{-1 + \sum_{i=1}^n y^i}.$$

*In particular, the coefficient of the monomial  $\prod_{i=1}^n k_{y^i \rightarrow y'^i}$  in  $\det(J_1(f_\kappa))$  is  $\sigma(R)$ .*

**Remark 6.4.** The term  $\alpha(R)$  is a monomial in  $c$ : if  $\sum_{i=1}^n y_j^i = 0$ , then  $S_j$  has zero coefficient in  $y^i$  for all  $i$  and thus  $0 = \det(y^1, \dots, y^n) = \sigma(R)$ . If a reaction  $y^i \rightarrow y'^i$  appears twice in a set  $R$  then  $\sigma(R) = 0$ . Therefore the degree of each  $k_{y \rightarrow y'}$  in the polynomial  $\det(J_c(f_\kappa))$  is either zero or one.

**Remark 6.5.** Proposition 6.3 is proven by [4] following a direct approach. It also follows from the Cauchy-Binet formula. Given an  $n \times m$  matrix  $A$  and an  $m \times n$  matrix  $B$  with  $m \geq n$ , the formula expresses the determinant of the product of the matrices,  $\det(AB)$ , as the sum of the products of the corresponding  $n \times n$  minors of  $A$  and  $B$ . Assume that  $\mathcal{R}$  is ordered, so that  $\mathcal{R} = \{y^1 \rightarrow y'^1, \dots, y^m \rightarrow y'^m\}$ . The species formation rate function can be written in matrix form as  $f_\kappa(c) = -\Gamma(R)V$ , where  $V$  is the  $m \times 1$  matrix (i.e., a vector) whose  $j$ th entry is  $k_{y^j \rightarrow y'^j} c^{y^j}$ . It then follows that  $J_c(f_\kappa)$  decomposes as the product of two matrices:

$$J_c(f_\kappa) = -\Gamma(\mathcal{R})J_c(V).$$

The  $n \times n$  minors of  $\Gamma(\mathcal{R})$  are obtained by the choice of  $n$  columns, that is,  $n$  reactions  $R$  in  $\mathcal{R}$ , and hence they are the terms  $\det(-\Gamma(R)) = (-1)^n \det(\Gamma(R))$  in Proposition 6.3. The  $n \times n$  minors of  $J_c(V)$  correspond to the choice of  $n$  rows and are precisely given by

$$c^{-1 + \sum_{i=1}^n y^i} \left( \prod_{i=1}^n k_{y^i \rightarrow y^i} \right) \det(\mathcal{Y}(R)^t).$$

**Proposition 6.6.** *An open network  $\mathcal{N}$  is injective if and only if the non-zero coefficients  $\sigma(R)$  have the same sign for all sets  $R$  of  $n$  reactions and there exists at least one set for which  $\sigma(R) \neq 0$ .*

**Proof.** The reverse implication follows from Corollary 5.9. For the forward implication, assume that  $\mathcal{N}$  is injective. Clearly,  $\det(J_1(f_\kappa)) \neq 0$  and thus there exists at least one set  $R$  for which  $\sigma(R) \neq 0$ . Note that  $\det(J_1(f_\kappa))$  is a polynomial in  $\kappa$  with total degree  $n$  and degree at most one in each variable  $k_{y \rightarrow y'}$ . Assume that there exist two coefficients  $R_1, R_2$  satisfying  $\sigma(R_1) > 0$  and  $\sigma(R_2) < 0$ . Set  $k_{y \rightarrow y'} = 1$  if  $y \rightarrow y' \notin R_1$  and  $k_{y \rightarrow y'} = k$  if  $y \rightarrow y' \in R_1$ , where  $k$  is a positive parameter. After this transformation, the monomials corresponding to sets of reactions  $R \neq R_1$  have degree in  $k$  strictly lower than  $n$ . Then  $\det(J_1(f_\kappa))$  is a polynomial of degree  $n$  in  $k$ , with positive leading coefficient. It follows that if  $k$  tends to  $+\infty$ , then  $\det(J_1(f_\kappa)) > 0$ . Symmetrically, using  $R_2$  we can find rate constants for which  $\det(J_1(f_\kappa)) < 0$ . Since  $\det(J_1(f_\kappa))$  is continuous in a connected domain, there exists a rate vector for which  $\det(J_1(f_\kappa)) = 0$ , contradicting Proposition 6.1.  $\square$

The criterion in Proposition 6.6 is independent of the rate vector  $\kappa$ . The requirement that there exists at least one set  $R$  for which  $\sigma(R) \neq 0$  cannot be removed. Consider for example the network  $\mathcal{N}$  with set of reactions  $\{A \rightarrow B, A \rightarrow 0\}$ . The stoichiometric subspace has dimension 2 and thus  $\mathcal{N}$  is open. However,  $\det(J_c(f_\kappa)) = 0$  for all  $\kappa, c$ , since  $c_B$  is not a variable of  $f_\kappa$ . This requirement is not imposed in [4, Th. 3.3] because it holds automatically for fully open networks: in fact, the set of reactions  $\{S_1 \rightarrow 0, \dots, S_n \rightarrow 0\}$  provides the non-zero coefficient  $(-1)^n$  in the determinant expansion of the Jacobian. It is mentioned in [4, Rk 3.5] that the requirement is necessary if the network is not fully open.

### 7. Injective closed networks

We would like to have a characterization of the coefficients of the polynomial  $\det(J_c(\tilde{f}_\kappa))$  for closed networks  $\mathcal{N}$  similar to that of Proposition 6.3 and a characterization of injectivity similar to that of Proposition 6.6. To this end, we relate the terms of the polynomial  $\det(J_c(\tilde{f}_\kappa))$  to the terms of the determinant of the Jacobian of a function  $g_\kappa$ . This function is the species formation rate function of the fully open network, with rate constants of added outflow reactions set to 1.

Before getting into technicalities we illustrate the idea with the futile cycle. Define the function  $g_\kappa : \mathbb{R}^6 \rightarrow \mathbb{R}^6$  by  $g_\kappa = f_\kappa - id$ , that is

$$g_\kappa(c) = (-k_1 c_1 c_3 + (k_2 + k_3) c_5 - c_1, -k_4 c_2 c_4 + (k_5 + k_6) c_6 - c_2, -k_1 c_1 c_3 + k_2 c_5 + k_6 c_6 - c_3, -k_4 c_2 c_4 + k_3 c_5 + k_6 c_6 - c_4, k_1 c_1 c_3 - (k_2 + k_3) c_5 - c_5, k_4 c_2 c_4 - (k_5 + k_6) c_6 - c_6).$$

The Jacobian  $J_c(g_\kappa)$  of  $g_\kappa$  is

$$\begin{pmatrix} -k_1 c_3 - 1 & 0 & -k_1 c_1 & 0 & k_2 + k_3 & 0 \\ 0 & -k_4 c_4 - 1 & 0 & -k_4 c_2 & 0 & k_5 + k_6 \\ -k_1 c_3 & 0 & -k_1 c_1 - 1 & 0 & k_2 & k_6 \\ 0 & -k_4 c_4 & 0 & -k_4 c_2 - 1 & k_3 & k_6 \\ k_1 c_3 & 0 & k_1 c_1 & 0 & -k_2 - k_3 - 1 & 0 \\ 0 & k_4 c_4 & 0 & k_4 c_2 & 0 & -k_5 - k_6 - 1 \end{pmatrix}.$$

The determinant of  $J_c(g_\kappa)$  does not change if the fifth row is added to the first, the sixth row to the second, and the fourth, fifth and sixth to the third. Thus,

$$\det(J_c(g_\kappa)) = - \begin{vmatrix} 1 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 1 & 1 & 1 & 1 \\ 0 & -k_4 c_4 & 0 & -k_4 c_2 - 1 & k_3 & k_6 \\ k_1 c_3 & 0 & k_1 c_1 & 0 & -k_2 - k_3 - 1 & 0 \\ 0 & k_4 c_4 & 0 & k_4 c_2 & 0 & -k_5 - k_6 - 1 \end{vmatrix}$$

where the sign  $-$  in front corresponds to changing the sign of the first three rows. This determinant is almost equal to the determinant of  $J_c(\tilde{f}_\kappa)$  as one can see from Example 4.9. The difference between the two determinants arises from the  $-1$  in the diagonal entries of the matrix for the rows 4, 5, 6. Therefore, by splitting column 4 using  $(0, 0, 1, -k_4c_2 - 1, 0, k_4c_2) = (0, 0, 1, -k_4c_2, 0, k_4c_2) + (0, 0, 0, -1, 0, 0)$ , and similarly for columns 5, 6, we have

$$\det(J_c(g_\kappa)) = -\det(J_c(\tilde{f}_\kappa)) + \text{monomials of total degree at most 2 in } k_1, \dots, k_6. \tag{4.8}$$

The determinant  $\det(J_c(\tilde{f}_\kappa))$  is a homogeneous polynomial in  $k_1, \dots, k_6$  of degree  $s = 3$ . Thus, it agrees with the terms in  $-\det(J_c(g_\kappa))$  of total degree 3. As we will show, the terms of  $\det(J_c(g_\kappa))$  have already been characterized by Proposition 6.3. Thus, using (4.8) we can derive a description of the coefficients of the determinant expansion for closed networks.

In the example, the row modifications done prior to the computation of the determinant were based on the fact that the futile cycle has conservation laws. This principle holds generally for any closed network. Let

$$\mathcal{O}(\mathcal{N}) := \{i \mid S_i \rightarrow 0 \notin \mathcal{R}\}$$

be the set of indices for which species outflow reactions do not belong to  $\mathcal{R}$ . For example, if  $\mathcal{N}$  is the futile cycle then  $\mathcal{O}(\mathcal{N}) = \{1, 2, 3, 4, 5, 6\}$ . If  $\{\omega^1, \dots, \omega^d\}$  is a reduced basis of  $\Gamma^\perp$  then by Lemma 3.6 we have that  $i \in \mathcal{O}(\mathcal{N})$  for  $i = 1, \dots, d$ . Thus, the cardinality of  $\mathcal{O}(\mathcal{N})$  is at least  $d$ .

Let  $\delta_i = 1$  if  $i \in \mathcal{O}(\mathcal{N})$  and  $\delta_i = 0$  otherwise, and let  $E_m^n$  be the  $n \times n$  matrix with zeroes everywhere but  $\delta_i$  in the diagonal entries  $(i, i)$  for  $i = m, \dots, n$ . We define  $g_\kappa(c)$  as

$$g_\kappa(c) := f_\kappa(c) - (\delta_1 c_1, \dots, \delta_n c_n). \tag{4.9}$$

**Proposition 7.3.** *Let  $\mathcal{N}$  be a closed network,  $\{\omega^1, \dots, \omega^d\}$  a reduced basis of  $\Gamma^\perp$  and  $\tilde{f}_\kappa(c)$  the associated extended rate function. For a rate vector  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$ , let  $g_\kappa(c), E_m^n$  and  $\delta_i$  be defined as above. Then,*

$$\det(J_c(g_\kappa)) = (-1)^d \det(J_c(\tilde{f}_\kappa) - E_{d+1}^n).$$

**Proof.** Since  $g_\kappa(c) = f_\kappa(c) - (\delta_1 c_1, \dots, \delta_n c_n)$ , we have that  $J_c(g_\kappa) = J_c(f_\kappa) - E_1^n$ . We let  $\omega^i = (\lambda_1^i, \dots, \lambda_n^i)$ . If  $\delta_j = 0$ , then  $S_j \rightarrow 0 \in \mathcal{R}$  and it follows from Lemma 3.6 that  $\lambda_j^i = 0$  for all  $i$ . Thus,  $(\omega^i)^t E_1^n = (\delta_1 \lambda_1^i, \dots, \delta_n \lambda_n^i) = \omega^i$  and from Lemma 4.5

$$(\omega^i)^t J_c(g_\kappa) = (\omega^i)^t J_c(f_\kappa) - (\omega^i)^t E_1^n = -\omega^i, \quad \text{for } i = 1, \dots, d.$$

Let  $P$  be the  $n \times n$  matrix whose  $i$ th row is  $-\omega^i$  for  $i = 1, \dots, d$  and the  $i$ th canonical vector  $e_i$  for  $i = d + 1, \dots, n$ . By the choice of  $\omega^i$ ,  $\det(P) = (-1)^d$ . Further,  $PJ_c(g_\kappa) = J_c(\tilde{f}_\kappa) - E_{d+1}^n$  and hence,

$$(-1)^d \det(J_c(g_\kappa)) = \det(PJ_c(g_\kappa)) = \det(J_c(\tilde{f}_\kappa) - E_{d+1}^n). \quad \square$$

**Corollary 7.4.** *The determinant expansion of  $J_c(\tilde{f}_\kappa)$  as a polynomial in  $\kappa$  agrees with the terms in the determinant expansion of  $(-1)^d \det(J_c(g_\kappa))$  of total degree  $s$ .*

**Proof.** For any non-empty set  $I \subseteq \{d + 1, \dots, n\}$ , let  $J_c^I(\tilde{f}_\kappa)$  be the matrix whose  $i$ th column equals that of  $J_c(\tilde{f}_\kappa)$  for  $i \notin I$  and is the vector  $-\delta_i e_i$  for  $i \in I$ . Then, by the column multilinear expansion of the determinant, we have

$$(-1)^d \det(J_c(g_\kappa)) = \det(J_c(\tilde{f}_\kappa)) + \sum_{\emptyset \neq I \subseteq \{d+1, \dots, n\}} \det(J_c^I(\tilde{f}_\kappa)).$$

If  $c$  is fixed then  $\det(J_c(\tilde{f}_\kappa))$  is a homogeneous polynomial in  $\kappa$  of total degree  $s$ , while the terms  $\det(J_c^I(\tilde{f}_\kappa))$  are polynomials in  $\kappa$  of total degree strictly lower than  $s$ .  $\square$

**Remark 7.5.** Let  $\mathcal{N}^\circ$  be the fully open network associated with  $\mathcal{N}$  such that

$$\mathcal{R}^\circ = \mathcal{R} \cup \{S_i \rightarrow 0 \mid i \in \mathcal{O}(\mathcal{N})\}.$$

Define the rate vector  $\kappa^\circ = (k_{y \rightarrow y'}^o)_{y \rightarrow y'} \in \mathbb{R}_+^{\mathcal{R}^\circ}$  by setting  $k_{S_i \rightarrow 0}^o = 1$  if  $i \in \mathcal{O}(\mathcal{N})$  and  $k_{y \rightarrow y'}^o = k_{y \rightarrow y'}$  for  $y \rightarrow y' \in \mathcal{R}$ . Then, the function  $g_\kappa(c)$  is exactly the species formation rate function of  $\mathcal{N}^\circ$  with rate vector  $\kappa^\circ$ .

Let  $\mathcal{N}$  be a network. Let  $\mathcal{R}_s$  be the set of all sets of  $n$  reactions formed by the union of a set  $R$  containing  $s$  reactions in  $\mathcal{R}$  and  $d = n - s$  outflow reactions  $S_i \rightarrow 0$  with  $i \in \mathcal{O}(\mathcal{N})$ . That is,

$$\mathcal{R}_s = \{R \cup \{S_{i_1} \rightarrow 0, \dots, S_{i_{n-s}} \rightarrow 0\} \mid R = \{r_1, \dots, r_s\} \subseteq \mathcal{R}, \text{ and } i_j \in \mathcal{O}(\mathcal{N})\}.$$

Since the cardinality of  $\mathcal{O}(\mathcal{N})$  is at least  $d$ ,  $\mathcal{R}_s \neq \emptyset$ . Let  $R' \in \mathcal{R}_s$  and let  $i_j, j = 1, \dots, d$ , be the indices for which outflow reactions  $S_{i_j} \rightarrow 0$  belong to  $R'$ . Then  $\det(\mathcal{Y}(R'))$  and  $\det(\Gamma(R'))$  are simply the minors of  $\mathcal{Y}(R')$  and  $\Gamma(R')$  with the  $i_j$ th rows and columns

removed. Equivalently,  $\det(\mathcal{Y}(R'))$  and  $\det(\Gamma(R'))$  are the minors obtained by removing the  $i_j$ th rows from  $\mathcal{Y}(R)$  and  $\Gamma(R)$ , respectively, for  $R = R' \cap \mathcal{R}$ . The matrices  $\mathcal{Y}(R)$  and  $\Gamma(R)$  are introduced above.

Let  $\mathcal{O}_d(\mathcal{N})$  be the set of subsets of  $\mathcal{O}(\mathcal{N})$  of cardinality  $d$ . If  $M$  is any  $n \times s$  matrix and  $I \in \mathcal{O}_d(\mathcal{N})$ , let  $M_I$  denote the  $s \times s$  submatrix of  $M$  obtained by removing the  $j$ th row for all  $j \in I$ .

The following theorem relies on Proposition 6.3 and the fact that  $g_\kappa$  is the species formation rate function of the fully open network  $\mathcal{N}^o$  associated with  $\mathcal{N}$  for rate vectors of a specific form.

**Theorem 7.6.** *Let  $\mathcal{N}$  be a network and  $s$  be the dimension of the stoichiometric subspace. The terms in the expansion of the determinant  $\det(J_c(\tilde{f}_\kappa))$  are monomials in  $\kappa$  of total degree  $s$  and linear in each rate constant. Further, let  $R = \{y^1 \rightarrow y^{s_1}, \dots, y^s \rightarrow y^{s_s}\}$  be a set of  $s$  reactions from  $\mathcal{N}$ . The coefficient of the monomial  $\prod_{i=1}^s k_{y^i \rightarrow y^{s_i}}$  in  $\det(J_c(\tilde{f}_\kappa))$  for  $c \in \mathbb{R}_+^n$  is*

$$(-1)^s c^{-1+\sum_{i=1}^s y^{s_i}} \sum_{I \in \mathcal{O}_d(\mathcal{N})} \det(\mathcal{Y}(R)_I) \det(\Gamma(R)_I) \prod_{i \in I} c_i.$$

Alternatively, the coefficient of  $\prod_{i=1}^s k_{y^i \rightarrow y^{s_i}}$  can be written as

$$(-1)^s c^{-1+\sum_{i=1}^s y^{s_i}} \sum_{R' \in \mathcal{R}_s, R=R' \cap \mathcal{R}} \sigma(R') \prod_{S_i \rightarrow 0 \in R' \setminus R} c_i.$$

**Proof.** Define the rate vector  $\kappa^o = (k_{y \rightarrow y'}^o)_{y \rightarrow y'} \in \mathbb{R}_+^{\mathcal{R}^o}$  by setting  $k_{S_i \rightarrow 0}^o = 1$  if  $i \in \mathcal{O}(\mathcal{N})$  and  $k_{y \rightarrow y'}^o = k_{y \rightarrow y'}$  for  $y \rightarrow y' \in \mathcal{R}$ . As pointed in Remark 7.5, the function  $g_\kappa(c)$  is exactly the species formation rate function of  $\mathcal{N}^o$  with rate vector  $\kappa^o$ . If  $\kappa$  is kept as a variable, by Corollary 7.4, the terms in the expansion of  $\det(J_c(\tilde{f}_\kappa))$  correspond to the terms in the expansion of  $\det(J_c(g_\kappa))$  of total degree  $s$  in  $\kappa$ . These terms are obtained from the determinant expansion of the species formation rate function of  $\mathcal{N}^o$  after setting the rate constant of each added outflow reaction to 1. Hence, by Proposition 6.3 and Remark 6.4,  $\det(J_c(\tilde{f}_\kappa))$  is linear in each rate constant. Every set  $R'$  obtained as the union of  $R$  and  $d$  outflow reactions in  $\mathcal{O}(\mathcal{N})$  such that  $\sigma(R') \neq 0$  provides a summand that is multiple of  $\prod_{i=1}^s k_{y^i \rightarrow y^{s_i}}$  in the polynomial  $\det(J_c(g_\kappa))$ . The form of the coefficients follow from Proposition 6.3 together with the discussion above the statement of this theorem.  $\square$

Observe that the vector  $\sum_{i=1}^s y^{s_i}$  is simply the row sum of the matrix  $\mathcal{Y}(R)$ . The corollary reduces to Proposition 6.3 if  $\mathcal{N}$  is open.

**Example 7.7.** Consider the futile cycle and (4.10). The coefficient of  $k_1 k_3 k_4$  in  $-\det(J_c(\tilde{f}_\kappa))$  is  $c_1 c_2 + c_1 c_4$ . It corresponds to the reactions  $R = \{r_1, r_3, r_4\}$  and the matrices  $\mathcal{Y}(R)$  and  $\Gamma(R)$  are:

$$\mathcal{Y}(R) = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{pmatrix}, \quad \Gamma(R) = \begin{pmatrix} 1 & -1 & 0 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & -1 & 1 \\ -1 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}.$$

The only sets of indices  $I \in \mathcal{O}_3(\mathcal{N})$  for which the product  $\det(\mathcal{Y}(R)_I) \det(\Gamma(R)_I)$  is non-zero are  $I = \{1, 2, 6\}$  and  $I = \{1, 4, 6\}$ . These sets give the coefficient  $c_1 c_2 + c_1 c_4$ . Since the last row of  $\mathcal{Y}(R)$  is zero, the index 6 belongs to all index sets  $I$  for which  $\det(\mathcal{Y}(R)_I) \det(\Gamma(R)_I) \neq 0$ .

**Remark 7.8.** The results above actually show that if the rank of  $J_c(f_\kappa)$  is  $s$ , then the product of the non-zero eigenvalues of  $J_c(f_\kappa)$  is exactly  $\det(J_c(\tilde{f}_\kappa))$ . Since the rank of  $J_c(f_\kappa)$  is at most  $s$ , then the characteristic polynomial of  $J_c(f_\kappa)$  is of the form

$$p(x) = (-1)^n x^n + a_{n-1} x^{n-1} + \dots + a_d x^d.$$

The constant  $a_d$  is non-zero only if zero has multiplicity  $d$  as an eigenvalue. In this case,  $a_d$  is exactly  $(-1)^d$  times the product of the non-zero eigenvalues of  $J_c(f_\kappa)$ . By the form of  $J_c(f_\kappa)$ , each non-zero coefficient  $a_i$  of  $p(x)$  is a homogeneous polynomial of degree  $n - i$  in  $\kappa$ . Therefore,  $a_i$  coincides with the sum of the terms of  $p(1)$  of degree  $n - i$  in  $\kappa$ . We claim that  $(-1)^d a_d$  is precisely  $\det(J_c(\tilde{f}_\kappa))$ . To see this, it suffices to show that  $a_d$  agrees with the terms in the determinant expansion of  $\det(J_c(g_\kappa))$  of total degree  $s$  in  $\kappa$  (Corollary 7.4). If the network has no outflows, this is straightforward because  $\det(J_c(g_\kappa)) = p(1)$ . If the network has some outflow reactions, we argue in the following way. The coefficient  $a_d$  is  $(-1)^d$  times the sum of the minors of size  $s$  of  $J_c(f_\kappa)$ . The difference between the terms of degree  $s$  in  $\kappa$  of  $\det(J_c(g_\kappa))$  and of  $p(1)$  is the following: the determinant  $\det(J_c(g_\kappa))$  ignores the minors of size  $s$  of  $J_c(f_\kappa)$  where some row and column indices in  $\{1, \dots, n\} \setminus \mathcal{O}(\mathcal{N})$  are removed, that is, the indices for which outflow reactions exist. However, these minors are always zero. Indeed, if there were a non-zero minor of  $J_c(f_\kappa)$  not involving an index  $i$ , and  $S_i \rightarrow 0$  is an outflow reaction of the network, then there would be a non-zero minor of size  $s$  in  $\Gamma$  not involving the reaction  $S_i \rightarrow 0$  (and hence not involving the  $i$ th row). The reactions corresponding to the  $s$

columns of this minor together with  $S_i \rightarrow 0$  provide  $s + 1$  independent reactions, contradicting that the rank of  $\Gamma$  is  $s$ . Therefore, the terms of degree  $s$  in  $\kappa$  of  $\det(J_c(\tilde{f}_\kappa))$  and of  $p(1)$  agree and the claim is proven.

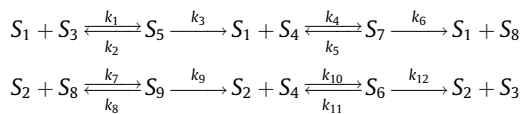
The same argument shows that the coefficients stated in Theorem 7.6 can be simply written as sums over all subsets  $I \subseteq \{1, \dots, n\}$  of cardinality  $d$ , because if  $I \notin \mathcal{O}_d(\mathcal{N})$  then the corresponding summand is zero.

**Corollary 7.9.** Let  $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a network. The following are equivalent:

- (i)  $\mathcal{N}$  is injective.
- (ii) The non-zero coefficients  $\sigma(R')$  have the same sign for all sets  $R' \in \mathcal{R}_s$ , and  $\sigma(R') \neq 0$  for at least one set  $R'$ .
- (iii) The non-zero products  $\det(\mathcal{N}(R)_I) \det(\Gamma(R)_I)$  have the same sign for all sets  $R$  of  $s$  reactions in  $\mathcal{R}$  and  $I \in \mathcal{O}_d(\mathcal{N})$ , and further  $\det(\mathcal{N}(R)_I) \det(\Gamma(R)_I) \neq 0$  for at least one set  $R$  and some  $I \in \mathcal{O}_d(\mathcal{N})$ .

**Proof.** The equivalence between (ii) and (iii) is a consequence of Theorem 7.6. If (ii) holds then (i) is a consequence of Theorem 7.6 and Corollary 5.9. To show that (i) implies (ii), we use the same argument as in the proof of Proposition 6.6. Using Theorem 7.6 and Corollary 5.9 it suffices to show that for any set  $R' \in \mathcal{R}_s$  we can find a concentration vector  $c$  and a rate vector  $\kappa$  for which the sign of  $\det(J_c(\tilde{f}_\kappa))$  agrees with the sign of  $(-1)^s \sigma(R')$ . Let  $k$  be a positive parameter and let  $m$  be an integer. Let  $R = R' \cap \mathcal{R}$ . Define  $k_{y \rightarrow y'} = k$  if  $y \rightarrow y' \in R$ , and  $k_{y \rightarrow y'} = 1/k^m$  if  $y \rightarrow y' \notin R$ . Define  $c_l = k$  if  $S_l \rightarrow 0 \in R$  and  $c_l = 1$  otherwise. Then the monomial corresponding to the set  $R' \in \mathcal{R}_s$  is the only monomial that tends to  $\pm\infty$  when  $k$  tends to infinity for  $m$  large enough.  $\square$

**Example 7.10.** We consider the extension of the futile cycle to incorporate two modification sites instead of one. The network consists of the reactions



with rate constants indicated next to each reaction. The enzyme  $S_1$  catalyzes the modification of  $S_3$  to  $S_4$  and subsequently to  $S_8$  via the formation of the intermediates  $S_5, S_7$ . Similarly, the enzyme  $S_2$  catalyzes the demodification of  $S_8$  to  $S_4$  and then to  $S_3$  via the intermediates  $S_9$  and  $S_6$ . A reduced basis of  $\Gamma^\perp$  of this network is given by the vectors  $S_1 + S_5 + S_7, S_2 + S_6 + S_9$ , and  $S_3 + S_4 + S_5 + S_6 + S_7 + S_8 + S_9$ . The extended rate function is obtained by substituting the components of  $f_\kappa$  with indices 1, 2, and 3 by  $c_1 + c_5 + c_7, c_2 + c_6 + c_9$ , and  $c_3 + c_4 + c_5 + c_6 + c_7 + c_8 + c_9$ , respectively.

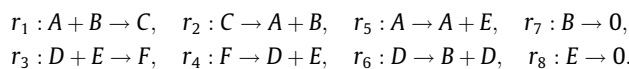
The determinant of the extended rate function has the monomials

$$k_1 k_3 k_4 k_7 k_9 k_{12} c_1 c_2 c_3 \quad \text{and} \quad -k_2 k_4 k_6 k_7 k_{10} k_{12} c_1 c_2 c_4.$$

The two terms have different signs. Therefore, this network is not injective. Note that the degree of the monomials in the rate constant is  $s = 6$ .

It is well known that this network can exhibit multistationarity for some choices of rate constants and total amounts [17]. More generally, Feliu and Wiuf [18] analyzed the occurrence of multistationarity in different small motifs accounting for enzyme sharing in protein modification, including the futile cycle and the two-site modification cycle. In their examples, all motifs that admit exactly one positive steady state for any total amounts are in fact injective. The motifs that can admit multiple positive steady states are obviously not injective.

**Example 7.11.** Being injective is not a necessary condition for the existence of at most one positive steady state within each stoichiometric class. Consider the network with reactions



For  $R_1 = \{r_2, r_4, r_7, r_8\}$ , we have  $\sigma(R_1) = 1$ , while for  $R_2 = \{r_1, r_3, r_5, r_6\}$ , we have  $\sigma(R_2) = -1$ . It follows that the network is not injective. However, by solving the steady-state equations together with the equations for the conservation laws, it is easily seen that there is exactly one positive steady state in each stoichiometric class.

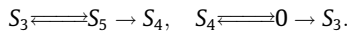
**8. Networks with all steady states degenerate**

If  $\det(J_c(\tilde{f}_\kappa))$  is not identically zero then there exist  $s$  linearly independent reaction vectors  $y^1 - y^{s^1}, \dots, y^s - y^{s^s}$  such that  $y^1, \dots, y^s$  are also linearly independent. Therefore, if the dimension of the vector space

$$\mathcal{Y} := \langle y \in \mathcal{C} | y \text{ is the reactant complex of some reaction } y \rightarrow y' \rangle \subseteq \mathbb{R}^n$$

is strictly smaller than  $s$  then  $\det(J_c(\tilde{f}_\kappa)) = 0$ . For example, if the reactant complexes of a network involve at most  $s - 1$  species, then all steady states of the network are degenerate. The network with reactions  $\{A \rightarrow B, A \rightarrow 0\}$  satisfies  $\dim \mathcal{Y} = 1 < 2$ , consistent with our computation that  $\det(J_c(\tilde{f}_\kappa)) = \det(J_c(f_\kappa)) = 0$ .

Let  $I \in \mathcal{O}_d(\mathcal{N})$  and let  $S_I = \{S_i \in \mathcal{S} | i \notin I\}$  be the set of species with indices not in  $I$ . Note that  $S_I$  has cardinality  $s$ . We consider the *projection* of the network  $\mathcal{N}$  to the set of species  $S_I, \mathcal{N}_I$ , induced by the projection  $\pi_I : \mathbb{R}^n \rightarrow \mathbb{R}^s$  on the coordinates not in  $I$ . For example, the projection of the futile cycle with  $I = \{1, 2, 6\}$  is the network with species  $S_I = \{S_3, S_4, S_5\}$  and reactions



If  $0 \rightarrow 0$  occurs then the reaction is discarded and like-wise redundant reactions are removed. The matrix  $\Gamma(R)_I$  varies over all sets of  $s$  reactions in  $\mathcal{N}_I$  as  $R$  varies. Similarly,  $\mathcal{Y}(R)_I$  varies over all sets of  $s$  reactant complexes in  $\mathcal{N}_I$  as  $R$  varies. Therefore, the requirement  $\det(\mathcal{Y}(R)_I) \det(\Gamma(R)_I) \neq 0$  for some  $R$  is equivalent to the existence of  $s$  independent reactions in  $\mathcal{N}_I$  such that the corresponding reactant vectors also are independent. Since  $\mathcal{N}_I$  has  $s$  species, a necessary condition is that  $\mathcal{N}_I$  is open, that is, the stoichiometric subspace has dimension  $s$ . In the example above, the set of reactions  $\{S_3 \rightarrow S_5, S_5 \rightarrow S_4, S_4 \rightarrow 0\}$  are independent and so are the reactant complexes. This implies (as also shown in Example 4.9) that the steady states of the futile cycle are not degenerate.

The following corollary is a consequence of Theorem 7.6, Corollary 4.8 and the preceding discussion.

**Corollary 8.1.** *Let  $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a network. The following statements are equivalent:*

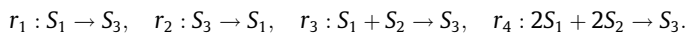
- (i)  $\ker(J_c(f_\kappa)) \cap \Gamma \neq \{0\}$  for all  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and  $c \in \mathbb{R}_+^{\mathcal{C}}$ .
- (ii)  $\det(\mathcal{Y}(R)_I) \det(\Gamma(R)_I) = 0$  for all sets  $R$  of  $s$  reactions in  $\mathcal{R}$  and  $I \in \mathcal{O}_d(\mathcal{N})$ .
- (iii) For all  $I \in \mathcal{O}_d(\mathcal{N})$  and for any set of  $s$  reactions  $y^1 \rightarrow y^1, \dots, y^s \rightarrow y^s$  in the projected network  $\mathcal{N}_I$ , if the vectors  $y^1 - y^1, \dots, y^s - y^s$  are linearly independent then the complexes  $y^1, \dots, y^s$  are linearly dependent.

If any of these hold then  $\mathcal{N}$  is not injective and any steady state is degenerate.

If  $\mathcal{N}$  is open, then  $d = 0$  and the only projection to consider is the identity. Therefore, condition (iii) reduces to the condition of the following corollary.

**Corollary 8.2.** *Let  $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be an open network. Then  $\ker(J_c(f_\kappa)) \cap \Gamma \neq \{0\}$  for all  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and  $c \in \mathbb{R}_+^{\mathcal{C}}$  if and only if for any set of  $n$  reactions  $y^1 \rightarrow y^1, \dots, y^n \rightarrow y^n$  such that the vectors  $y^1 - y^1, \dots, y^n - y^n$  are linearly independent, the complexes  $y^1, \dots, y^n$  are linearly dependent.*

If  $\mathcal{N}$  is fully open then the set of species outflow reactions provides a set of independent reaction vectors and independent reactant complexes. Therefore, fully open networks cannot fulfill that  $\ker(J_c(f_\kappa)) \cap \Gamma \neq \{0\}$  for all  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and  $c \in \mathbb{R}_+^{\mathcal{C}}$ . However, open networks that are not fully open might fulfill the condition. For example, consider the network with reactions



The dimension of  $\mathcal{Y}$  and  $\Gamma$  agree ( $s = 3$ ) and the network is open. The network has no outflow reactions. Reactions  $r_1, r_2$  are linearly dependent and thus any set of 3 independent reactions must contain  $r_3$  and  $r_4$ . In that case, however, the reactant complexes are linearly dependent. It follows that Corollary 8.2(ii) is fulfilled and hence all steady states of the network are degenerate.

**Remark 8.3.** If  $\mathcal{Y} \subsetneq \Gamma$ , in which case  $\dim(\mathcal{Y}) < s$  and Corollary 8.1(ii) is fulfilled, then all stoichiometric classes have either none or infinitely many positive steady states: let  $a \in \mathbb{R}_+^{\mathcal{C}}$  be a steady state. Then there is a vector  $\gamma \in \Gamma$  such that  $\gamma \cdot y = 0$  for all  $y \in \mathcal{Y}$ . Consequently, for all  $t \in \mathbb{R}$  and  $\mu \in \Gamma^\perp \subseteq \mathcal{Y}^\perp$  we have  $e^{y \cdot (t\gamma + \mu)} = 1$  and hence  $c_{t,\mu} = ae^{t\gamma + \mu}$  is a steady state. Further,  $c_{t,\mu} \in G_t = \{c \in \mathbb{R}_+^{\mathcal{C}} | \log(c) - \log(ae^{t\gamma}) \in \Gamma^\perp\}$  which intersects each stoichiometric class in exactly one point for each  $t$  [11]. It is easy to prove that  $G_t \cap G_{t'} = \emptyset$  if  $t \neq t'$  and hence there are infinitely many positive steady states in each stoichiometric class.  $\square$

**Remark 8.4.** In [5], a related determinant criterion is given to decide whether or not a closed network can admit degenerate steady states. Fix a determinant function  $\det_\Gamma$  on the stoichiometric subspace  $\Gamma$ . For each choice of scalar product  $*_c$  in Eq. (4.3) (that is, for each choice of  $c \in \mathbb{R}_+^{\mathcal{C}}$ ) non-singularity of  $J_c(f_\kappa)$  restricted to  $\Gamma$  is related to a polynomial expansion in  $\kappa$  of  $\det_\Gamma(J_c(f_\kappa))$ . Each term in the determinant expansion takes the form in Corollary 7.9 with ‘det’ replaced by  $\det_\Gamma$  and  $y \in \mathcal{Y}(R)_I$  replaced by  $\pi_c(y)$ , where  $\pi_c$  is the projection onto  $\Gamma$  as defined by the scalar product  $*_c$ . The criterion requires the coefficients of the terms in the determinant expansion to be of the same sign or zero for each choice of  $c \in \mathbb{R}_+^{\mathcal{C}}$  [5] [Prop. 10.3]. It is not obvious how to check whether this criterion is fulfilled using computational algebra software, in contrast to the criterion in Corollary 7.9.

**Remark 8.5.** For fully open networks, an algorithm is provided Joshi and Shiu in [19] to simplify the search for coefficients of the determinant of the Jacobian that have the “wrong” sign. The algorithm can be applied in the present setting, that is, to closed networks, to restrict the sets of  $s$  reactions to consider.

**Remark 8.6.** The projected networks are *embedded networks* as defined by Joshi and Shiu in [20]. We have shown that for an injective network all embedded networks obtained by selecting sets of  $s$  species as above are either injective or have all steady states degenerate. Consequently, if a network is injective then the embedded networks with  $s$  species do not have the capacity to admit multiple non-degenerate steady states.

**9. Open and closed networks and injectivity**

Craciun and Feinberg [9] preclude multistationarity in closed networks provided that (1) the fully open network is injective and (2) the closed network does not have degenerate steady states. In a later paper [5] [Th. 8.2], the authors provide a sufficient condition (namely that the *entrapped-species projection is a normal reaction network*) for (2) to hold provided (1) holds as well. All weakly reversible networks fulfill this condition.

Using the results of the previous sections we now relate injectivity of a network  $\mathcal{N}$  and injectivity of the associated fully open network  $\mathcal{N}^o$ . Recall that the function  $g_\kappa$  was defined in (4.9) and that it is the species formation rate function of  $\mathcal{N}^o$  for a specific choice of rate vector, namely,  $\kappa^o = (k_{y \rightarrow y'}^o)_{y \rightarrow y'} \in \mathbb{R}_+^{\mathcal{R}^o}$  defined by setting  $k_{S_i \rightarrow 0}^o = 1$  if  $i \in \mathcal{O}(\mathcal{N})$  and  $k_{y \rightarrow y'}^o = k_{y \rightarrow y'}$  for  $y \rightarrow y' \in \mathcal{R}$ .

**Theorem 9.1.** *Let  $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a closed network. If  $\mathcal{N}^o$  is injective then the following statements are equivalent:*

- (i)  $\mathcal{N}$  is injective.
- (ii) As a polynomial in  $\kappa$ ,  $\det(J_c(g_\kappa))$  has at least one monomial of total degree  $s$ .
- (iii)  $\ker(J_c(f_\kappa)) \cap \Gamma = \{0\}$  for some fixed  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and  $c \in \mathbb{R}_+^{\mathcal{N}}$ .
- (iv) There exists  $I \in \mathcal{O}_d(\mathcal{N})$  and a set of  $s$  reactions  $y^1 \rightarrow y^{1'}, \dots, y^s \rightarrow y^{s'}$  in the projected network  $\mathcal{N}_I$  such that the two sets of vectors  $y^1 - y^{1'}, \dots, y^s - y^{s'}$  and  $y^1, \dots, y^s$  are both linearly independent.

**Proof.** Let  $\{\omega^1, \dots, \omega^d\}$  be a reduced basis of  $\Gamma^\perp$  and  $\tilde{f}_\kappa$  the associated extended rate function for  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$ . Since  $\mathcal{N}^o$  is injective, then  $J_c(g_\kappa)$  is non-singular for all  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and for any  $c \in \mathbb{R}_+^{\mathcal{N}}$  (Proposition 6.2). Equivalently,  $\det(J_c(g_\kappa))$  is a polynomial in  $\kappa$ , with all non-zero coefficients having the same sign and thus all non-zero coefficients of  $\det(J_c(\tilde{f}_\kappa))$  have the same sign. It follows that either (1)  $\det(J_c(\tilde{f}_\kappa)) = 0$  as a polynomial in  $\kappa$  or (2)  $\det(J_c(\tilde{f}_\kappa)) \neq 0$  for all values of  $\kappa \in \mathbb{R}_+^{\mathcal{R}}$  and  $c \in \mathbb{R}_+^{\mathcal{N}}$ . The equivalence of the four statements follows from this observation, Theorem 5.6, Corollary 5.9 and Corollary 8.1.  $\square$

**Example 9.2.** Consider the open network  $\mathcal{N}$  with reactions  $r_1 : A + B \rightarrow D, r_2 : A \rightarrow C, r_3 : B \rightarrow D$ . The dimension of the stoichiometric subspace is 3. Let  $k_i$  denote the rate constant of reaction  $r_i$ . The associated fully open network is injective, since

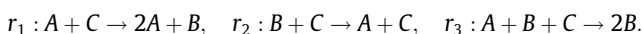
$$\det(J_c(g_\kappa)) = 1 + k_2 + k_3 + k_1 c_A + k_1 c_B + k_2 k_3 + k_1 k_2 c_A + k_1 k_3 c_B.$$

However, the determinant has no monomial involving  $k_1, k_2, k_3$ . It follows that  $\mathcal{N}$  is not injective and all steady states are degenerate. Alternatively, note that  $\mathcal{Y} = \langle A, B \rangle$  has dimension  $2 < s = 3$ .

**Corollary 9.3.** *Let  $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a closed network,  $\mathcal{N}^o = (\mathcal{S}, \mathcal{C}^o, \mathcal{R}^o)$  the associated fully open network, and  $f_\kappa^o$  the species formation rate function of  $\mathcal{N}^o$ . Assume that  $\mathcal{N}^o$  is injective. Then  $\mathcal{N}$  is injective if and only if as a polynomial in  $\kappa = (k_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}^o}$ ,  $\det(J_1(f_\kappa^o))$  has at least one monomial in  $k_{y^1 \rightarrow y^{1'}}, \dots, k_{y^s \rightarrow y^{s'}}$  with  $s$  reactions in  $\mathcal{R}$ . If this is the case, then all steady states of  $\mathcal{N}$  are non-degenerate.*

In view of these results, there might exist injective networks such that their open network counterparts are not injective. This can only occur if some monomials with less than  $s$  rate constants from the true reactions have the wrong sign. We provide two examples.

**Example 9.4.** Consider the network  $\mathcal{N}$  given by the reactions:



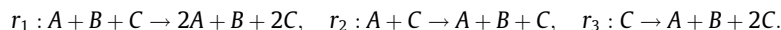
The stoichiometric subspace of  $\mathcal{N}$  has maximal dimension 3 and thus it is open. Let  $k_i$  denote the rate constant of reaction  $r_i$ , and  $k_A, k_B, k_C$  the rate constants of the outflow reactions. Then, for  $\kappa = (k_1, k_2, k_3, k_A, k_B, k_C)$ ,

$$\begin{aligned} \det(J_1(f_\kappa^o)) = & -2k_1 k_2 k_3 - k_1 k_2 k_A - k_1 k_2 k_B - k_2 k_3 k_B - k_1 k_A k_B - k_3 k_A k_B + 2k_1 k_2 k_C - 2k_1 k_3 k_C - k_2 k_A k_C + k_3 k_A k_C + k_1 k_B k_C \\ & - k_3 k_B k_C - k_A k_B k_C. \end{aligned}$$



Since the sign of the coefficients of  $\det(J_1(f_\kappa^0))$  as a polynomial in  $\kappa$  differ, the fully open network,  $\mathcal{N}^0$ , is not injective. However, there is one monomial only in  $k_1, k_2, k_3$ , which implies that  $\mathcal{N}$  is injective and has no degenerate steady states. This network, however, does not have positive steady states either.

**Example 9.5.** Consider the network  $\mathcal{N}$  given by the reactions:



The stoichiometric subspace of this network has dimension 2 and thus it is closed. Let  $k_i$  be the rate constant of reaction  $r_i$ . Then

$$\det(J_c(\mathbf{g}_\kappa)) = k_1 k_3 c_{AC} c_C + k_1 k_2 c_A^2 c_C + k_1 k_2 c_{AC} c_C^2 + k_3 + k_1 c_{AC} c_B + k_1 c_B c_C - 1.$$

We see that  $\mathcal{N}^0$  is not injective but since all monomials in  $k_1, k_2, k_3$  of total degree 2 have the same sign, it follows from Corollaries 7.4, 7.9, and Theorem 7.6 that  $\mathcal{N}$  is injective and there are no degenerate steady states.

**Remark 9.6.** The open network given in [9, Section 6] to illustrate that degenerate steady states can occur is not injective. Therefore the results of this work do not apply.

**Remark 9.7.** This remark is for the readers familiar with [5] and the terminology introduced in that paper. Let  $\mathcal{N}$  be a network such that  $\mathcal{N}^0$  is injective. We have provided a sufficient and necessary condition for  $\mathcal{N}$  to be injective as well, namely, that there exists  $I \in \mathcal{O}_d(\mathcal{N})$ , and a set of  $s$  reactions  $y^1 \rightarrow y^{1'}, \dots, y^s \rightarrow y^{s'}$  in the projected network  $\mathcal{N}_I$  such that the set of vectors  $y^1 - y^{1'}, \dots, y^s - y^{s'}$  and  $y^1, \dots, y^s$  are both linearly independent. Since it is a sufficient and necessary condition, any network fulfilling the condition in [5, Th. 8.2] for  $\mathcal{N}$  to be injective, that is, the entrapped-species projection is a normal reaction network, also fulfills our condition. In particular, weakly reversible networks are normal and hence they also fulfill our condition.

## 10. Weakly sign determined (WSD) networks

A square matrix is said to be a  $P$ -matrix if all principle minors are positive. If the principle minors are non-negative the matrix is said to be a  $P_0$ -matrix. [6] introduced a criterion (namely that the *stoichiometric matrix is WSD*) that relates to injectivity of a network  $\mathcal{N}$ . They restrict the class of networks to *non-autocatalytic networks* (NAC), which implies that the same species cannot both be a reactant and a product in the same reaction. We impose the same constraint in this section.

In our notation the criterion states that  $(*) \det(\mathcal{Y}(R)_I) \det(\Gamma(R)_I) \geq 0$  for any set  $I$  of  $n - k$  distinct indices in  $\{1, \dots, n\}$  and any set  $R = \{y^1 \rightarrow y^{1'}, \dots, y^k \rightarrow y^{k'}\}$  of  $k$  reactions from  $\mathcal{N}$  if and only if  $-J_c(f_\kappa)$  (minus the Jacobian) is a  $P_0$ -matrix [6] [Th. 4.1, Th. 4.3]. If  $\mathcal{N}$  is fully open then  $(*)$  (with inflow and outflow reactions excluded from the sets  $R$ ) is equivalent to  $-J_c(f_\kappa)$  being a  $P$ -matrix [6] [Cor. 4.2, Th. 4.4]. Using the results of [10], it follows that  $\mathcal{N}$  is injective.

If  $\mathcal{N}$  is closed and injective then  $J_c(f_\kappa)$  is not a  $P$ -matrix as the rank is less than  $n$ . Our criterion for injectivity states that if there exists  $\sigma \in \{0, 1\}$  such that  $(**) (-1)^\sigma \det(\mathcal{Y}(R)_I) \det(\Gamma(R)_I) \geq 0$  for any set  $I$  of  $n - s$  distinct indices in  $\{1, \dots, n\}$  and any set  $R = \{y^1 \rightarrow y^{1'}, \dots, y^s \rightarrow y^{s'}\}$  of  $s$  reactions from  $\mathcal{N}$ , and at least one of them is non-zero, then  $\det(J_c(\tilde{f}_\kappa))$  is non-zero and  $\mathcal{N}$  is injective. Assume that  $(**)$  holds. If all species in  $\mathcal{N}$  appear in some reactant complex then the NAC assumption guarantees that the diagonal entries of  $J_c(\tilde{f}_\kappa)$  in rows  $d + 1, \dots, n$  are non-zero and negative. Since the diagonal entries in rows  $1, \dots, d$  are all equal to one, it follows that the product of the diagonal entries is a term in the expansion of  $\det(J_c(\tilde{f}_\kappa))$  and has sign  $(-1)^s$ . Consider the matrix  $J_c^*(\tilde{f}_\kappa)$  obtained from  $J_c(\tilde{f}_\kappa)$  by multiplying the lower  $s$  rows by minus one. The matrix has full rank and is a  $P$ -matrix: if  $\mathcal{N}$  fulfills  $(**)$  then all non-zero terms in the determinant expansion of  $J_c^*(\tilde{f}_\kappa)$  have sign  $(-1)^s$ . Each principle minor can be obtained as a sum of terms in the determinant expansion of  $J_c^*(\tilde{f}_\kappa)$  divided by the (positive) diagonal entries of the rows not taken in the minor. Since this sum contains at least the non-zero diagonal product term, all principle minors are positive. Consequently,  $J_c^*(\tilde{f}_\kappa)$  is a  $P$ -matrix. Note that if  $\mathcal{N}$  has full rank (for example if it is fully open) then  $J_c^*(\tilde{f}_\kappa) = -J_c(f_\kappa)$ .

If  $S_i$  is a species that is only in product complexes of  $\mathcal{N}$  then the lower  $s$  rows of  $J_c(\tilde{f}_\kappa)$  are zero in the  $i$ th position. If  $S_i$  is not involved in any conservation law, then the  $i$ th column of  $J_c(\tilde{f}_\kappa)$  is zero,  $\det(J_c(\tilde{f}_\kappa)) = 0$ , and  $(**)$  does not hold. Generally, let  $S_{i_1}, \dots, S_{i_m}$  be the species of  $\mathcal{N}$  that are only in reactant complexes such that the lower  $s$  rows of  $J_c(\tilde{f}_\kappa)$  are zero in entries  $i_1, \dots, i_m$ . If  $(**)$  holds and  $\det(J_c(\tilde{f}_\kappa)) \neq 0$  then the columns  $i_1, \dots, i_m$  of  $J_c(\tilde{f}_\kappa)$  are linearly independent. Since the lower  $s \times m$  submatrix is identically zero, it follows that the upper  $d \times m$  matrix has rank  $m$  (and in particular  $m \leq d$ ). As a consequence, we can reorder the species in  $\mathcal{S}$  such that  $S_{i_1}, \dots, S_{i_m}$  are the first  $m$  species and we are guaranteed that there exists a reduced basis of  $\Gamma^\perp$  with that order. Because  $m \leq d$ , with this order the diagonal entries of  $J_c(\tilde{f}_\kappa)$  are non-zero and we can proceed as above.

To sum up, if  $(**)$  holds, then there exists an order of the species of  $\mathcal{N}$  such that the matrix obtained from  $J_c(\tilde{f}_\kappa)$  by changing the sign of the last  $s$  rows is a  $P$ -matrix. Using the results of [10] we conclude that  $\tilde{f}_\kappa$  is an injective function and hence that  $\mathcal{N}$  is injective.

## 11. Concluding remarks

In this paper we have provided a Jacobian criterion for the characterization of injective networks taken with mass-action kinetics. Injective networks have the important property that multiple positive steady states within any stoichiometric class cannot occur for any choice of rate constants. Further, the existence of multiple boundary solutions of a certain type is also precluded. Importantly, if an injective network has a positive steady state, then it cannot have any other non-negative steady state.

Since injective networks are characterized by a non-singular Jacobian of the species formation rate function (when restricted to the stoichiometric subspace), other interesting properties of this class of networks are expected. For instance, in [21] it is shown that their steady-state fluxes exhibit a certain degree of robustness against variation in rate constants.

The main novelty of this work is that injectivity of a network can be assessed directly. Previous work derived the non-existence of multiple steady states from the injectivity of the associated fully open network [4,9,5]. Since a network can be injective even if the associated fully open network is not, the method presented here can preclude multistationarity for a bigger class of networks. Further, the path to determine the existence of degenerate steady states in closed networks with injective associated fully open networks provided by [5] is also simplified.

The Jacobian criterion presented here can be effectively implemented using any symbolic computation software like Mathematica. Suggested steps for its implementation are the following (using pseudo-Mathematica commands):

- Definitions:
  - n**: number of species, **A**: stoichiometric matrix  $\Gamma$ ,
  - c** = **c**[1], ..., **c**[**n**]: Concentration vector, **v**: rate vector (with concentrations)  $(k_{y \rightarrow y'} C^y)_{y \rightarrow y' \in \mathcal{R}}$ ,
  - F** = **A.v**: species formation rate function  $f_{\mathcal{K}}$ .
- Conservation laws and associated extended rate function:
  - P** = **RowReduce**[**NullSpace**[**Transpose**[**A**]]]: find a reduced basis of  $\Gamma^\perp$ ,
  - ind**: vector of the indices of the first entry of each vector in **P**,
  - Ftilde**: remove entries **ind** of **F** and add the entries **P.c**.
- Compute the determinant of the Jacobian of **Ftilde**:
  - J** = **Table**[**D**[**Ftilde**[**[i]**],**c**[**j**]], {i,1,n},{j,1,n},
  - D** = **Det**[**J**].
- Determine the signs of the coefficients of **D**:
  - m** = **MonomialList**[**D**]: monomials of **D**,
  - coeffs** = **DeleteDuplicates**[**m**/.{**k**→1,**c**→1}]:
  - coefficient of each of the monomials (in the rate constants and **c**), and delete duplicates,
  - Pos** = **Select**[**coeffs**, **Positive**], **Neg** = **Select**[**coeffs**, **Negative**]: select the positive and the negative coefficients,
  - If **Length**[**Pos**]>0 and **Length**[**Neg**]=0:  $\mathcal{N}$  is injective,
  - If **Length**[**Pos**]=0 and **Length**[**Neg**]>0:  $\mathcal{N}$  is injective,
  - Otherwise:  $\mathcal{N}$  is not injective.

In our experience, this procedure works fast and reliably for not-so-big networks (at least up to around 15–20 species). For bigger networks, the computational cost in finding the determinant might be too high for a standard computer. In that case, one might construct the lists **Pos** and **Neg** above by computing one by one the signs of  $\det(\mathcal{J}(R)_I) \det(\Gamma(R)_I)$  for all sets  $R$  of  $s$  reactions and indices  $I \in \mathcal{O}_d(\mathcal{N})$ . If two conflicting (different) signs are found, the algorithm should stop and the network is not injective. In Mathematica, the command **Subsets**[**list**,{**s**}] provides the subsets of a list containing exactly  $s$  elements.

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