



Multistationarity in Cyclic Sequestration-Transmutation Networks

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Abstract

We consider a natural class of reaction networks which consist of reactions where either two species can inactivate each other (i.e., sequestration), or some species can be transformed into another (i.e., transmutation), in a way that gives rise to a feedback cycle. We completely characterize the capacity of multistationarity of these networks. This is especially interesting because such networks provide simple examples of “atoms of multistationarity”, i.e., minimal networks that can give rise to multiple positive steady states.

Keywords Reaction networks · Mass-action kinetics · General kinetics · Multistationarity · Sequestration-transmutation networks · Atoms of multistationarity · VEGFR dimerization

1 Introduction

An important problem in the theory of reaction networks is to identify the networks that allow multiple (stoichiometrically compatible) positive equilibria (Banaji and Craciun 2009; Banaji et al. 2007; Banaji and Craciun 2010; Banaji and Pantea 2016;

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Conradi and Mincheva 2017; Craciun and Feinberg 2005, 2006; Joshi and Shiu 2012, 2013; Mincheva and Roussel 2007; Wiuf and Feliu 2013; Yu and Craciun 2018). This phenomenon, also referred to as *multistationarity*, underlies switching behavior in biochemistry (Angeli et al. 2004; Craciun et al. 2006; Ferrell 2002). In particular, multistationarity is necessary in order for a reaction system to be able to generate multiple outputs in response to different external signals or stimuli. One prominent approach for identifying multistationary networks, developed over the last decade, is that of *network inheritance*, which says that multistationarity in a large, complex network can be established via studying smaller component networks that are also multistationary. Precise conditions for inheritance of multistationarity have been established (Banaji and Pantea 2018; Joshi and Shiu 2013). This creates the possibility of “lifting” multistationarity from certain idealized network motifs to larger and more realistic networks. Classes of motifs that have been catalogued by their presence or absence of multistationarity include all open networks with one (reversible or irreversible) reaction with arbitrary stoichiometry (Joshi 2013), bimolecular open networks with two reactions (both reactions reversible or irreversible) (Joshi and Shiu 2013), fully open as well as isolated sequestration networks in arbitrary number of species and reactions (Joshi and Shiu 2015), and certain small non-open reaction networks, notably those with two species and two reactions (Joshi and Shiu 2017). In this paper, we add a new class of motifs to this catalog: cyclic sequestration-transmutation (CST) networks. We establish precise conditions for when this class of networks admits multistationarity. Our results contribute to the theoretical understanding of multistationarity in sparse networks, but they also have practical consequences. For example, in Sect. 8 we use inheritance of multistationarity and our results on CST networks to prove multistationarity of a well-known VEGFR dimerization model (Mac Gabhann and Popel 2007).

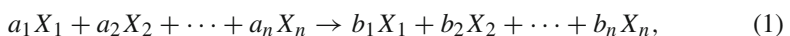
Furthermore, our analysis is not limited to the fully open case as in many previous studies, but extends to partially open and non-open or isolated versions of CST networks. Moreover, the kinetics that we consider are not only mass action kinetics, but includes a much broader class. These general kinetics must satisfy only mild requirements, such as: in order for a reaction to take place all reactants must have positive concentration, and the rate of reaction must increase if the concentration of a reactant increases.

2 Background and Notation

We introduce terminology and recall some important results that will be useful in our proofs below.

2.1 Reaction Networks and Kinetics

The general form of a *reaction* is



where $X_1, \dots, X_n$ is a list of *species*, and $a = [a_1, \dots, a_n]^T$ and $b = [b_1, \dots, b_n]^T$ are nonnegative integer-valued vectors whose entries are called *stoichiometric coefficients*. Formal linear combinations of $X_1, \dots, X_n$ are called *complexes*; in particular $a \cdot X := a_1 X_1 + a_2 X_2 + \dots + a_n X_n$ is called the *source complex* of (1). Species X_i for which $a_i > 0$ are called *reactant species*. Finally, a and $b - a$ are called the *source vector*, respectively *reaction vector* of (1).

Given a list of (distinct) species $X = (X_1, \dots, X_n)$, a *reaction network* is a finite list of reactions on $X_1, \dots, X_n$. It is customary to impose that the source and product complexes differ for each reaction, that every species participates in at least one reaction, and that no reaction is listed multiple times. However, none of these assumptions is needed in this paper.

For a reaction network with m reactions and a fixed ordering of the reactions, the *source matrix*, or *left stoichiometric matrix* $\Gamma_l \in \mathbb{R}^{n \times m}$ has the m source vectors as columns. Likewise the *stoichiometric matrix* Γ is the $n \times m$ matrix whose columns are the m reaction vectors of the reaction list. The image of the stoichiometric matrix is called the *stoichiometric subspace* of the network.

The vector of *concentrations* of $X_1, \dots, X_n$ is denoted by $x \in \mathbb{R}_{\geq 0}^n$.¹

We ascribe a rate to each reaction, an assignment that is referred to as *kinetics*. Under *mass action kinetics*, reaction rates are proportional to the product of the concentrations of reactants (taken with multiplicity). To be precise, the rate of reaction $a_1 X_1 + \dots + a_n X_n \xrightarrow{k} b_1 X_1 + \dots + b_n X_n$ is $kx^a = kx_1^{a_1} \dots x_n^{a_n}$. Here, k is a positive constant that depends on the reaction, called *reaction rate constant*. For mass-action kinetics it is customary to indicate the reaction rate on top of the reaction arrow.

Some of the results to follow hold under *general kinetics* (Banaji and Pantea 2016, Definition 4.5), a large class of reaction rates that includes mass-action kinetics as a special case. General kinetics places minimal physical requirements on reaction rates, like “concentrations do not become negative”, “reactions proceed if and only if all reactants are present”, and “reaction rates are nondecreasing with reaction concentration”. To be exact, v is a *general kinetics* rate vector if

1. v is defined and C^1 on $\mathbb{R}_{\geq 0}^n$;
2. $v_j \geq 0$; $v_j(x) = 0$ if and only if $x_i = 0$ for some reactant X_i of reaction j ;
3. $\partial v_j / \partial x_i = 0$ if X_i is not a reactant in reaction j ; $\partial v_j / \partial x_i$ is non-negative on $\mathbb{R}_{\geq 0}^n$ and strictly positive on the interior of the positive orthant if X_i is a reactant in reaction j .

In deterministic spatially homogeneous models, x varies with time according to the ODE system

$$\dot{x} = \Gamma v(x) \quad (2)$$

where $v(x) = (v_1(x), \dots, v_m(x))$ is the vector of reaction rates, or *rate vector* (see Example 1 on the next page).

A reaction of the form $X_i \rightarrow 0$ (by which X_i is depleted or degraded) is called an *outflow reaction*; its reaction vector $[0, \dots, 0, -1, 0, \dots, 0]^T$ has a single nonzero

¹ Throughout this article we use the convention that species names are in the upper case while their concentrations are the corresponding lower case letter. For example, the concentrations of species $X_1, \dots, X_n$, are denoted $x_1, \dots, x_n$.

entry at index i . With mass-action kinetics the outflow reaction $X_i \rightarrow 0$ has rate kx_i . Likewise the *inflow reaction* $0 \rightarrow X_i$ has reaction vector $[0, \dots, 0, 1, 0, \dots, 0]^T$, and constant rate under mass-action. Inflow and outflow reactions are referred to as *flow reactions*.

Definition 1 (*Open, fully open and closed networks*) A network which contains at least one flow reaction is called *open*. A network which contains inflow and outflow reactions $0 \rightarrow X_i$ and $X_i \rightarrow 0$ for each species X_i is called *fully open*. A network that is not open is called *closed*.

Remark 1 While “fully open network” is standard terminology in reaction networks, the meaning of “open” and “closed” network may vary in the literature. Our notion of “open network” is the same as that of (Craciun and Feinberg 2006). Reactions of our “closed” networks are sometime referred to as “true” reactions (Craciun and Feinberg 2005).

Remark 2 A reaction network in n species which contains R outflow reactions and r_2 inflow reactions has stoichiometric and reactant matrices written in block form

$$\bar{\Gamma} = [\Gamma | -K | L] \quad \text{and} \quad \bar{\Gamma}_I = [\Gamma_I | K | 0]$$

where $K \in \mathbb{R}^{n \times R}$ and $L \in \mathbb{R}^{n \times r_2}$ are submatrices of the identity I_n . If the network is fully open then $K = L = I_n$.

As above, throughout the paper we will denote by Γ and Γ_I the source and stoichiometric matrices of closed network, whereas $\bar{\Gamma}$ and $\bar{\Gamma}_I$ will be used for open networks.

2.2 Compatibility Classes and Multistationarity

Integrating (2) with respect to time yields

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

Under general kinetics, $x(t)$ is nonnegative for any $t \geq 0$, and so the solutions of (2) are constrained to *compatibility classes*, i.e. sets of the form $(x_0 + \text{im}(\Gamma)) \cap \mathbb{R}_{\geq 0}^n$, where $x_0 \in \mathbb{R}_{\geq 0}^n$.

Let $\mathcal{R}$ denote a reaction network with stoichiometric matrix Γ and fix a general kinetics v . A *positive steady state* of $\mathcal{R}$ is a point $x^* \in \mathbb{R}_{>0}^n$ such that

$$\Gamma v(x^*) = 0.$$

A steady state x^* is called *nondegenerate* if the *reduced Jacobian*, (i.e. the Jacobian of the vector field $\Gamma v(x)$ restricted to the compatibility class of x^*) is nonzero (Banaji and Pantea 2016). Equivalently, if $r = \text{rank } \Gamma$ then x^* is nondegenerate if the sum of the $r \times r$ principal minors of the Jacobian matrix ΓDv computed at x^* is nonzero. We note that if the stoichiometric matrix $\Gamma \in \mathbb{R}^{n \times m}$ of $\mathcal{R}$ has full column rank n then

the reduced Jacobian coincide with the Jacobian of the vector field. We remark that the Jacobian matrix of a mass-action reaction network vector field has the convenient form

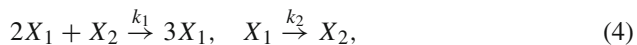
$$\left(\frac{\partial \Gamma v}{\partial x} \right) = \Gamma D_{v(x)} \Gamma_l^T D_{1/x}, \quad (3)$$

where $D_{v(x)}$ and $D_{1/x}$ are diagonal matrices with $(v_1(x), \dots, v_m(x))$ and $(1/x_1, \dots, 1/x_n)$ on the diagonals. Note that in this formula the inflow reactions can be excluded from Γ and Γ_l without changing the result.

Definition 2 (*Multistationarity, nondegenerate multistationarity*) Let $\mathcal{R}$ denote a reaction network with stoichiometric matrix Γ .

1. $\mathcal{R}$ is (*nondegenerately*) *multistationary under mass-action kinetics* if there exists a choice of mass action kinetics v (i.e. a choice of rate constants) such that (2) has two distinct (nondegenerate) positive steady states within the same compatibility class.
2. $\mathcal{R}$ is (*nondegenerately*) *multistationary under general kinetics* if there exists a choice of general kinetics v such that (2) has two (nondegenerate) distinct positive steady states within the same compatibility class.

Example 1 Consider for example the reaction network



one of the simplest networks with multistationarity (see also Joshi and Shiu 2013; Banaji and Pantea 2018).

The stoichiometric matrix and source matrix of the network are

$$\Gamma = \begin{bmatrix} 1 & -1 \\ -1 & 1 \end{bmatrix}, \quad \Gamma_l = \begin{bmatrix} 2 & 1 \\ 1 & 0 \end{bmatrix}$$

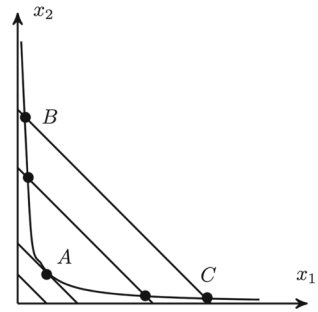
and the steady state manifold $k_1 x_1^2 x_2 - k_2 x_1 = 0$ intersects the positive quadrant along the curve $x_1 x_2 = k_2/k_1$. The positive compatibility classes are obtained by intersecting the positive quadrant with cosets of $\text{span}([1, -1]^T)$ i.e. lines of the form $x_1 + x_2 = T$. Compatibility classes may contain no positive steady states, a single degenerate steady state, or two nondegenerate steady states (see Fig. 1).

To be a little more precise, one computes the Jacobian matrix of the system as

$$\Gamma Dv = \begin{bmatrix} 1 & -1 \\ -1 & 1 \end{bmatrix} \begin{bmatrix} 2k_1 x_1 x_2 & k_1 x_1^2 \\ k_2 & 0 \end{bmatrix} = \begin{bmatrix} 2k_1 x_1 x_2 - k_2 & k_1 x_1^2 \\ -2k_1 x_1 x_2 + k_2 & -k_1 x_1^2 \end{bmatrix}$$

For simplicity set $k_1 = k_2 = 1$ (but the same calculation can be done for any choice of k_1 and k_2). Since $\text{rank } \Gamma = 1$, the reduced Jacobian at a steady state equals $\text{Tr}(\Gamma Dv) = 2x_1 x_2 - x_1^2 - 1 = 1 - x_1^2$. It follows that the only degenerate steady state is $(1, 1)$.

Fig. 1 Multistationarity of reaction network (4). Set $k_1 = k_2 = 1$. The compatibility class $x_1 + x_2 = T$ contains no positive steady states for $T < 2$, two nondegenerate steady states for $T > 2$, and a single degenerate equilibrium for $T = 2$



2.3 Ruling out Multistationarity: The Injectivity Property

A particularly successful approach in the study of multistationarity has been that of *injective* reaction networks, i.e. reaction networks for which the corresponding vector field is injective on each compatibility class, for any choice of kinetics.

Definition 3 (*Injective reaction networks*) Let $\mathcal{R}$ denote a reaction network with stoichiometric matrix Γ .

1. $\mathcal{R}$ is *injective under mass-action* if for any choice of mass-action kinetics $v(x)$, the restriction of the vector field $f(x) = \Gamma v(x)$ to any positive compatibility class is injective.
2. $\mathcal{R}$ is *injective under general kinetics* if for any choice of general kinetics $v(x)$, the restriction of the vector field $f(x) = \Gamma v(x)$ to any positive compatibility class is injective.

Since mass action kinetics are a subclass of general kinetics, it is clear that a network that is injective under general kinetics is injective under mass action kinetics. However, a network may fail to be injective under general kinetics while being injective under mass action kinetics (Banaji and Pantea 2016).

An injective reaction network cannot be multistationary, since requires that two different points in the same positive compatibility class be both mapped by f to zero. Note however that injectivity is not equivalent to the lack of capacity for multiple positive equilibria (Craciun and Feinberg 2005).

The study of injective reaction networks was started by Craciun and Feinberg for fully open networks (Craciun and Feinberg 2005) and has since been extended by work of many authors (Banaji and Pantea 2016; Müller et al. 2016; Joshi and Shiu 2012; Feliu and Wiuf 2013; Wiuf and Feliu 2013; Craciun and Feinberg 2006; Shinar and Feinberg 2012; Banaji et al. 2007). The characterization of injectivity we give in Theorem 1 is that of Banaji and Pantea (2018, Theorems 3, 5). For different versions of this result and for other related results the reader is referred to Joshi and Shiu (2012), Müller et al. (2016) and Shinar and Feinberg (2012).

For a matrix $A \in \mathbb{R}^{n \times m}$ and two sets $\alpha \subseteq \{1, \dots, n\}$, $\beta \subseteq \{1, \dots, m\}$ of the same cardinality, we denote by $A[\alpha|\beta]$ the minor of A corresponding to rows α and columns β . We also let $\mathcal{Q}(A)$ denote the set of real $n \times m$ matrices whose entries have the same sign (+, − or 0) as the corresponding entry in A .

Theorem 1 Let $\mathcal{R}$ be a reaction network with stoichiometric matrix Γ and reactant matrix Γ_l . Let $r = \text{rank } \Gamma$.

1. $\mathcal{R}$ is injective under mass-action if and only if the products

$$\Gamma[\alpha|\beta]\Gamma_l[\alpha|\beta]$$

have the same sign for all choices of $\alpha \subseteq \{1, \dots, n\}$ and $\beta \subseteq \{1, \dots, m\}$ with $|\alpha| = |\beta| = r$, and at least one such product is nonzero.

2. $\mathcal{R}$ is injective under general kinetics if and only if for any $A \in \mathcal{Q}(\Gamma_l)$ the products

$$\Gamma[\alpha|\beta]A[\alpha|\beta]$$

have the same sign for all choices of $\alpha \subseteq \{1, \dots, n\}$, $\beta \subseteq \{1, \dots, m\}$ with $|\alpha| = |\beta| = r$, and at least one such product is nonzero.

2.4 The Jacobian Optimization Criterion

The following sufficient condition for multistationarity (Craciun and Feinberg 2005, Theorem 4.1) will be used in some of our proofs. Here we follow the formulation of this result given in Banaji and Pantea (2016, Theorem 5):

Theorem 2 Consider a fully open reaction network $\overline{\mathcal{R}}$ and let $\overline{\Gamma}$ and $\overline{\Gamma}_l$ denote the stoichiometric and source matrices corresponding to $\overline{\mathcal{R}}$ with all inflow reactions omitted. Suppose there exists a positive diagonal matrix D such that

$$(-1)^n \det \left(\overline{\Gamma} D \overline{\Gamma}_l^T \right) < 0 \text{ and } \overline{\Gamma} D \mathbf{1} \leq 0$$

where $\mathbf{1} \in \mathbb{R}^{2n \times 1}$ denotes the vector of 1's. Then the fully open CST admits multiple positive equilibria under mass action.

2.5 Inheritance of Multistationarity

The following useful result states that nondegenerate multistationarity of a mass-action network survives when we add all possible flow reactions (see Craciun and Feinberg 2006, Theorem 2; Joshi and Shiu 2013, Corollary 3.6; Banaji and Pantea 2018, Theorem 2).

Theorem 3 (Adding inflows and outflows of all species) Let $\mathcal{R}$ denote a mass-action reaction network with species $X_1, \dots, X_n$. Suppose we create $\mathcal{R}'$ from $\mathcal{R}$ by adding to $\mathcal{R}$ all the flow reactions $0 \rightleftharpoons X_1, \dots, 0 \rightleftharpoons X_n$. If $\mathcal{R}$ admits multiple positive nondegenerate steady states, then so does $\mathcal{R}'$.

1. (Adding a new species with inflow and outflow Joshi and Shiu 2013, Theorem 4.2; Banaji and Pantea 2018, Theorem 4). Suppose we create $\mathcal{R}'$ from $\mathcal{R}$, by adding into some reactions of $\mathcal{R}$ the new species Y in an arbitrary way, while also adding the new reaction $0 \rightleftharpoons Y$. If $\mathcal{R}$ is nondegenerately multistationary, then so is $\mathcal{R}'$.

2. (Adding a dependent reaction Joshi and Shiu 2013, Theorem 3.1; Banaji and Pantea 2018, Theorem 1). Suppose we create $\mathcal{R}'$ from $\mathcal{R}$, by adding to $\mathcal{R}$ a new irreversible reaction whose reaction vector lies in the stoichiometric subspace of $\mathcal{R}$. If $\mathcal{R}$ nondegenerately multistationary, then so is $\mathcal{R}'$. In particular, adding the reverse of any reaction in $\mathcal{R}$ preserves nondegenerate multistationarity.

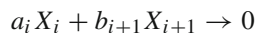
We note that the result in Theorem 3 is one of the simplest examples of modifications that preserve nondegenerate multistationarity. For a more complete list of such results the reader is referred to Banaji and Pantea (2018).

3 Cyclic Sequestration-Transmutation Networks

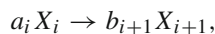
We are interested in two special reaction types, sequestration and transmutation. Each involves exactly two distinct species. In the first case both appear on the reactant side, while in the latter case one appears on the reactant end and the other on the product end. The central object of study in this paper is the set of cyclic sequestration-transmutation networks.

Definition 4 (CST networks)

1. A *sequestration reaction* is a reaction of the type $aX + bY \rightarrow 0$ for positive integers a, b and species X, Y .
2. A *transmutation reaction* is a reaction of the type $aX \rightarrow bY$ for positive integers a, b and distinct species X, Y .
3. A reaction network on species $X_1, \dots, X_n$ (with the convention $X_{n+1} = X_1$) containing reactions $R_1, \dots, R_n$, where for each $i \in \{1, \dots, n\}$ R_i is either a sequestration reaction

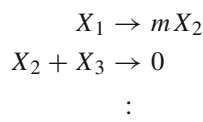


or a transmutation reaction



is called a *closed CST (cyclic sequestration-transmutation) network*. If in addition, the network contains at least one flow reaction, then it is called an *open CST network*. A *CST network* could mean either a closed or an open CST network. A *fully open CST network* contains inflow and outflows for all its species.

Remark 3 Subclasses of CST networks have been previously considered in the literature (Joshi and Shiu 2015). Notably, the class of open *sequestration networks*



$$\begin{aligned}
 X_{n-1} + X_n &\rightarrow 0 \\
 X_n + X_1 &\rightarrow 0 \\
 X_i &= 0, \quad 1 \leq i \leq n
 \end{aligned} \tag{5}$$

($n \geq 2$, $m \geq 1$ integers) has been shown to be multistationary if and only if $m > 1$ and n is odd (Joshi and Shiu 2015, Theorem 6.4). Note that Theorems 7 and 8 in this paper extend that result. Nondegeneracy of steady states in sequestration networks (5) has been shown for particular cases (including for network (5) with $n = 3$ in Félix et al. 2016 and in full generality in Tang and Wang 2021). Furthermore, the latter work shows *bistability* (existence of multiple stable steady states) of sequestration networks with $m > 1$ and n odd. We note that nondegeneracy holds for classes of CST networks that are not necessarily sequestration networks; this is ongoing work (Bai et al. in preparation).

3.1 Closed CST network

The stoichiometric matrix and the reactant matrix of a closed CST network can be written as

$$\Gamma = \begin{bmatrix} -a_1 & 0 & \dots & 0 & -b_1|b_1 \\ -b_2|b_2 & -a_2 & \dots & 0 & 0 \\ & \ddots & \ddots & \vdots & \vdots \\ & & \ddots & -a_{n-1} & 0 \\ & & & -b_n|b_n & -a_n \end{bmatrix} \quad \text{and} \quad \Gamma_l = \begin{bmatrix} a_1 & 0 & \dots & 0 & b_1|0 \\ b_2|0 & a_2 & \dots & 0 & 0 \\ & \ddots & \ddots & \vdots & \vdots \\ & & \ddots & a_{n-1} & 0 \\ & & & b_n|0 & a_n \end{bmatrix} \tag{6}$$

The convention here is that the entry $-b_i|b_i$ in Γ is equal to $-b_i$ if R_{i-1} is a sequestration reaction, and is equal to b_i if R_{i-1} is a transmutation reaction (R_0 is reaction R_n).

In the same way the entry $b_i|0$ in Γ_l is equal to b_i if R_{i-1} is a sequestration and to 0 if R_{i-1} is a transmutation.

Under mass-action kinetics the rate of reaction R_i is either

$$v_i(x) = k_i x_i^{a_i} x_{i+1}^{b_{i+1}} \quad \text{or} \quad v_i(x) = k_i x_i^{a_i}$$

depending on whether R_i is a sequestration or transmutation reaction, respectively.

The Jacobian matrix $Dv(x)$ of $v(x)$ has only nonnegative entries for general kinetics. Furthermore, an entry of $Dv(x)$ is positive if and only if the corresponding entry of Γ_l is positive. In other words, $Dv(x)$ belongs to $\mathcal{Q}(\Gamma_l)$.

Remark 4 Unless we specify otherwise, we reserve Γ , Γ_l , and v for denoting the stoichiometric matrix, reactant matrix and the rate vector of a closed CST. For open CSTs the stoichiometric and reactant matrices and the rate vector will be denoted by $\bar{\Gamma}$, $\bar{\Gamma}_l$, and $\bar{v}$ respectively.

3.2 Minors of Γ and Γ_I for Closed CSTs

Both Γ and Γ_I corresponding to closed CSTs belong to the class $\mathcal{M} \subset \mathbb{R}^{n \times n}$ of matrices of the form

$$\begin{bmatrix} m_{1,1} & 0 & \dots & 0 & m_{1,n} \\ m_{2,1} & m_{2,2} & \dots & 0 & 0 \\ 0 & m_{3,2} & \dots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \dots & m_{n-1,n-1} & 0 \\ 0 & 0 & \dots & m_{n,n-1} & m_{n,n} \end{bmatrix} \quad (7)$$

Lemma 1 *Let $M \in \mathcal{M}$. Any minor of M of size less than n is a monomial in $m_{i,j}$. The determinant of M is equal to $\prod_{l=1}^n m_{l,l} + (-1)^{n+1} \prod_{l=1}^n m_{l,l-1}$, where by convention $m_{1,0} = m_{1,n}$.*

Proof Let $1 \leq i_1 < \dots < i_k \leq n$, $1 \leq j_1 < \dots < j_k \leq n$ and let $\alpha = \{i_1, \dots, i_k\}$, $\beta = \{j_1, \dots, j_k\}$. Then

$$M[\alpha|\beta] = \sum_{\sigma \in S_k} \epsilon(\sigma) \prod_{l=1}^k m_{i_l, j_{\sigma(l)}} \quad (8)$$

For any $\sigma \in S_k$ that produces a nonzero term in (8) we must have $j_{\sigma(l)} \in \{i_l - 1, i_l\}$ where the index 0 means n by convention. This convention does not affect the case $i_1 > 1$, where we have $j_{\sigma(1)} \leq i_1 \leq j_{\sigma(2)} \leq \dots \leq j_{\sigma(k)} \leq i_k$. In this case $j_{\sigma(1)} < \dots < j_{\sigma(k)}$, $j_{\sigma(l)} = j_l$ for all $l \in \{1, \dots, k\}$, and therefore there is only one permutation that produces a nonzero term in $M[\alpha|\beta]$ which is a monomial in $m_{i,j}$.

If $i_1 = 1$, then either $j_{\sigma(1)} = 1$ or $j_{\sigma(1)} = n$. If $j_{\sigma(1)} = 1$ the argument above yields the permutation $\sigma_1(l) = l$ for all l . If $j_{\sigma(1)} = n$ then $n \in \alpha$ and therefore $i_k = n$. We have

$$\begin{aligned} 1 = i_1 \leq i_2 - 1 \leq j_{\sigma(2)} \leq i_2 \leq i_3 - 1 \leq j_{\sigma(3)} \leq \dots \leq i_{k-1} \leq i_k - 1 \leq j_{\sigma(k)} \leq i_k \\ \leq j_{\sigma(1)} = n \end{aligned}$$

and we arrive at the permutation $\sigma_2(2) = 1, \sigma_2(3) = 2, \dots, \sigma_2(k) = k - 1$, and $\sigma_2(1) = k$. Therefore there are at most two permutations σ_1 and σ_2 that produce nonzero terms, and note that $\sigma_1(l) \neq \sigma_2(l)$ for all l . Since $j_{\sigma(l)} \in \{i_l - 1, i_l\}$, it follows that $i_l - 1 \in \alpha$ for all l . Therefore $n - 1 \in \alpha, n - 2 \in \alpha, \dots, 1 \in \alpha$. We have $\alpha = \beta = \{1, \dots, n\}$ and in this case $M[\alpha|\beta] = \det(M)$. $\square$

Lemma 2 *Let Γ and Γ_I denote the stoichiometric and source matrices of a closed CST network, and let $\alpha, \beta \subseteq \{1, \dots, n\}$ be such that $|\alpha| = |\beta| < n$. Then for any $M \in \mathcal{Q}(\Gamma_I)$ we have*

$$(-1)^{|\alpha|} \Gamma[\alpha|\beta] M[\alpha|\beta] \geq 0.$$

Proof Suppose $\Gamma[\alpha|\beta]M[\alpha|\beta] \neq 0$. By Lemma 1 the minors $\Gamma[\alpha|\beta]$ and $M[\alpha|\beta]$ each contain one term, corresponding to the same permutation σ in (8). The sign of $M[\alpha|\beta]$ is equal to $\epsilon(\sigma)$. If the monomial $\Gamma_I[\alpha|\beta]$ contains b_i as a factor, then R_i is a sequestration, and the entry $-b_i|b_i$ in Γ is equal to $-b_i$. Then $\Gamma[\alpha|\beta] = \epsilon(\sigma) \prod_{i \in I} (-a_i) \prod_{j \in J} (-b_j) = \epsilon(\sigma) (-1)^{|\alpha|} \prod_{i \in I} a_i \prod_{j \in J} b_j$, where I, J are disjoint subsets of $\{1, \dots, n\}$ with $|I \cup J| = |\alpha|$. It follows that $\text{sign}(\Gamma[\alpha|\beta]M[\alpha|\beta]) = (-1)^{|\alpha|} \epsilon(\sigma)^2$ and the conclusion follows. $\square$

4 Injectivity of CSTs

4.1 Closed CST Networks

We show that closed CST networks with mass-action are always injective. In fact, even under general kinetics, with the exception of one case, closed CST networks are injective. For the exception, which occurs when the number of species is even and transmutation reactions are absent, we can find a non-mass action kinetics that makes the closed CST network fail injectivity (Banaji and Pantea 2016).

Theorem 4 1. A closed CST network is not injective under general kinetics if and only if $s = n$, n is even and $\prod_{i=1}^n a_i \neq \prod_{i=1}^n b_i$.
2. Any closed CST network is injective under mass-action.

Proof 1. We apply Theorem 1 part 1 distinguishing the cases $\text{rank } \Gamma = n$ and $\text{rank } \Gamma \neq n$. Note that the second case is equivalent to $\text{rank } \Gamma = n - 1$ since the minor $\Gamma[\{1, \dots, n-1\}|\{1, \dots, n-1\}]$ equals $(-1)^{n-1} \prod_{i=1}^{n-1} a_i \neq 0$. Let $M = Dv(x) \in \mathcal{Q}(\Gamma_I)$; M is of the form (7) with non-negative entries, and strictly positive diagonal entries.

Suppose $\text{rank } \Gamma = n$. We have

$$\det \Gamma = (-1)^n \prod a_i + (-1)^{n+s+1} \prod b_i \neq 0,$$

i.e. s is odd, or $\prod_{i=1}^n a_i \neq \prod_{i=1}^n b_i$. In this case injectivity is equivalent to $\det M \neq 0$. If $s < n$ then $\det M$ is the product of its diagonal entries, and is therefore strictly positive. If $s = n$ then

$$\det M = \prod_{i=1}^n m_{i,i} + (-1)^{n+1} \prod_{i=1}^n m_{i,i-1}$$

by Lemma 1 and we have injectivity if and only if this expression is nonzero for any choice of positive $m_{i,j}$, i.e. if and only if n is odd. Therefore if $\text{rank } \Gamma = n$ the network is injective in all cases except when $s = n$ and n is even. Note that in this case $\text{rank } \Gamma = n$ is equivalent to $\prod_{i=1}^n a_i \neq \prod_{i=1}^n b_i$.

In the remaining case where $\text{rank } \Gamma = n - 1$ (i.e. s is even and $\prod a_i = \prod b_i$), Lemma 2 implies that if $|\alpha| = |\beta| = n - 1$ then the sign of $\Gamma[\alpha|\beta]M[\alpha|\beta]$ is equal to $(-1)^{n-1}$. We also note that if $\alpha = \beta = \{1, \dots, n-1\}$ this product is nonzero. This

completes the verification of the hypothesis in Theorem 1 part 2, and the network is injective.

2. We only need to discuss the case that fails injectivity under general kinetics, i.e. $s = n$ are even and $\prod_{i=1}^n a_i \neq \prod_{i=1}^n b_i$. In this case $\det \Gamma = \det \Gamma_l = \prod_{i=1}^n a_i - \prod_{i=1}^n b_i$ and so $\det \Gamma \det \Gamma_l > 0$, which completes the proof using Theorem 1 part 1. $\square$

A note on stability of equilibrium points While stability and convergence to equilibria for CST networks is not the focus of this paper, we briefly note that monotone systems (Angeli et al. 2010; see also Craciun et al. 2011) and deficiency theory (Horn and Jackson 1972; Horn 1972; Feinberg 2019; Craciun et al. 2009; Pantea 2012; Craciun 2019) are promising avenues for this type of question. In particular, the following proposition on closed CST networks is easy to prove. Not to distract from the main focus of the paper, we will assume familiarity with the statement of Deficiency Zero Theorem and related terminology; see Feinberg (2019).

Proposition 5 *Consider a closed CST network denoted as in Definition 4 such that s is odd or $\prod_{i=1}^n a_i \neq \prod_{i=1}^n b_i$ (i.e. Γ has rank n), and let $\mathcal{R}$ be obtained from this closed CST network by making all reactions reversible. Then $\mathcal{R}$ equipped with mass-action has a unique positive equilibrium, which is locally asymptotically stable.*

Proof The claim follows from the Deficiency Zero Theorem (Feinberg 2019, Section 7.1). There is a linkage class which is a graph-theoretical star that contains all sequestrations and no other reactions. Transmutation reactions form k linkage classes which are graph-theoretical paths (if one of these formed a cycle, then we would obtain linear dependencies between reaction vectors, and $\text{rank}(\Gamma) < n$). Suppose there are k linkage classes of transmutations. The number of complexes in transmutation reactions is $t + k$, and we compute the deficiency of the CST network as follows: $(s + 1 + t + k) - (1 + k) - n = 0$. $\square$

Remark 5 If $\prod_{i=1}^n a_i = \prod_{i=1}^n b_i$ then the fully open CST (without reverse reactions) has a unique positive steady state, which is linearly stable. This follows since the fully open CST is *delay stable*, i.e. when modeled as a mass-action system with delays, the steady state is linearly stable for any choice of the delay parameters (Craciun et al. 2021, Example 5.10).

4.2 Open CSTs without Outflows

The proof of Theorem 4 carries over without additional effort if we add inflows for some arbitrary subset of species (but no outflows). Indeed, if the stoichiometric matrix and left stoichiometric matrix of the CST (without inflows) are denoted Γ and Γ_l , then $\bar{\Gamma} = [\Gamma | L]$ and $\bar{\Gamma}_l = [\Gamma_l | 0]$ are the stoichiometric and left stoichiometric matrices of the open CST with inflows (see Remark 2). Then $\text{rank } \bar{\Gamma} = n$, and the only non-zero product $\bar{\Gamma}[\alpha | \beta] \bar{\Gamma}_l[\alpha | \beta]$ corresponds to $\alpha = \beta = \{1, \dots, n\}$. It follows from Theorem 1 part 2 that the CST network with inflows is injective. We have the following

Theorem 6 1. *An open CST network without outflows is not injective under general kinetics if and only if $s = n$, n is even and $\prod_{i=1}^n a_i \neq \prod_{i=1}^n b_i$.*

2. Any open CST network without outflows is injective under mass-action.

4.3 Open CSTs with Outflows

Theorem 7 An open CST with s sequestration reactions which contains at least one outflow reaction is injective under mass action if and only if $n = s$, or s is odd, or $\prod_{i=1}^n a_i \geq \prod_{i=1}^n b_i$.

Proof The stoichiometric and left stoichiometric matrices are in this case $\bar{\Gamma} = [\Gamma | -K | L]$ and $\bar{\Gamma}_l = [\Gamma_l | K | 0]$, where $K \in \mathbb{R}^{n \times k}$ is a submatrix of the identity matrix. We note that $\bar{\Gamma}$ has rank n . To apply Theorem 1b, we first show that at least one product of minors

$$\bar{\Gamma}[\{1, \dots, n\}|\beta] \bar{\Gamma}_l[\{1, \dots, n\}|\beta] \quad (9)$$

is nonzero for some index set β with $|\beta| = n$. Indeed, if species X_j is in the outflow, then consider the minor of $\bar{\Gamma}$ that corresponds to the column $[0, \dots, 0, -1, 0, \dots, 0]^T$ (-1 at position j) and all columns of Γ except column j . Consider also the corresponding minor of $\bar{\Gamma}_l$. The product of the two minors is equal to

$$\begin{vmatrix} -a_1 & & & & & & & & & & -b_1|b_1 \\ -b_2|b_2 & -a_2 & & & & & & & & & \\ & \ddots & \ddots & & & & & & & & \\ & & \ddots & -1 & & & & & & & \\ & & & 0 & \ddots & & & & & & \\ & & & & \ddots & -a_{n-1} & & & & & \\ & & & & & -b_n|b_n & -a_n & & & & \end{vmatrix} \begin{vmatrix} a_1 & & & & & & & & & & b_1|0 \\ b_2|0 & a_2 & & & & & & & & & \\ & \ddots & \ddots & & & & & & & & \\ & & \ddots & 1 & & & & & & & \\ & & & 0 & \ddots & & & & & & \\ & & & & \ddots & a_{n-1} & & & & & \\ & & & & & b_n|0 & a_n & & & & \end{vmatrix} \\ = (-1)^n \prod_{i \neq j} a_i^2 \neq 0.$$

We now consider possible cases for nonzero products of minors (9). Note that $\bar{\Gamma}_l[\{1, \dots, n\}|\{1, \dots, n\}]$ equals

$$\prod_{i=1}^n a_i + (-1)^{n+1} \prod_{i=1}^n b_i = (-1)^n \det \Gamma$$

if the CST contains only sequestration reactions (i.e. $s = n$), and $\prod_{i=1}^n a_i$ otherwise. Therefore the sign of $\bar{\Gamma}[\{1, \dots, n\}|\{1, \dots, n\}] \bar{\Gamma}_l[\{1, \dots, n\}|\{1, \dots, n\}]$ equals $(-1)^n$ if $s = n$ and equals the sign of $\det \Gamma = (-1)^n \prod_{i=1}^n a_i - (-1)^{n+s} \prod_{i=1}^n b_i = (-1)^n [\prod_{i=1}^n a_i - (-1)^s \prod_{i=1}^n b_i]$ otherwise.

If, on the other hand, $\beta \neq \{1, \dots, n\}$ then $\bar{\Gamma}[\{1, \dots, n\}|\beta] = \det(\Gamma(\{1, \dots, n\}|\beta_1) | (-I)(\{1, \dots, n\}|\beta_2))$, where $|\beta_1| + |\beta_2| = n$, and β_2 is nonempty. In the Laplace expansion of the determinant $\bar{\Gamma}[\{1, \dots, n\}|\beta]$ along its last $|\beta_2|$ columns, we note that there is only one nonzero minor of $(-I)(\{1, \dots, n\}|\beta_2)$, namely $(-I)[\beta_2|\beta_2]$. Then,

with β'_2 denoting the complement of β_2 in $\{1, \dots, n\}$ we have

$$\bar{\Gamma}[\{1, \dots, n\}|\beta] = \epsilon \Gamma[\beta'_2|\beta_1](-I)[\beta_2|\beta_2] = \epsilon(-1)^{|\beta_2|} \Gamma[\beta'_2|\beta_1],$$

where ϵ is the signature of the permutation corresponding to the pair (α_1, β_2) . With the same calculation for $\bar{\Gamma}_I[\beta'_2|\beta_1]$ we obtain

$$\bar{\Gamma}[\{1, \dots, n\}|\beta] \bar{\Gamma}_I[\{1, \dots, n\}|\beta] = (-1)^{|\beta_2|} \Gamma[\beta'_2|\beta_1] \Gamma_I[\beta'_2|\beta_1]. \quad (10)$$

By Lemma 2 the sign of any nonzero product (10) is $(-1)^{|\beta_2|+|\beta_1|} = (-1)^n$ and we conclude that the possible nonzero signs of the product of minors (9) are $(-1)^n$ and $\text{sign}(-1)^n (\prod_{i=1}^n a_i - (-1)^s \prod_{i=1}^n b_i)$, and that at least one such product is nonzero. It follows from Theorem 1 part 2 that the open CST with outflows is injective if and only if $n = s$ or $\prod_{i=1}^n a_i - (-1)^s \prod_{i=1}^n b_i \geq 0$, i.e. if either $n = s$, s is odd, or if $\prod_{i=1}^n a_i \geq \prod_{i=1}^n b_i$. $\square$

5 Fully Open Mass Action CSTs

In this section we focus on mass-action kinetics. Suppose the CST has s sequestration reactions. Theorem 7 implies that if $n = s$, s is odd, or if $\prod_{i=1}^n a_i \geq \prod_{i=1}^n b_i$, then the fully open CST is injective and has at most one positive equilibrium. In general, non-injectivity of a reaction network does not imply the existence of multiple positive equilibria. However, we now show that non-injective fully open CSTs are in fact multistationary, except for the special case of linear dynamics. Consider a non-injective fully open CST, i.e. suppose $s < n$, s is even and $\prod_{i=1}^n a_i < \prod_{i=1}^n b_i$. Let Γ and Γ_I denote the stoichiometric and source matrices of the CST without inflow and outflow reactions, and $\bar{\Gamma} = [\Gamma| -I]$ and $\bar{\Gamma}_I = [\Gamma_I| I]$ denote the stoichiometric and source matrices of the open CST with all outflow reactions added (see Remark 2).

Together with Theorem 7, the following result completes the characterization of multistationarity in fully open CST networks with mass action kinetics.

Theorem 8 *Suppose $s < n$, s is even, and $\prod_{i=1}^n a_i < \prod_{i=1}^n b_i$, i.e. the fully open CST network is not injective.*

1. *If $s > 0$, then the fully open CST network admits multiple positive equilibria under mass action kinetics.*
2. *If $s = 0$ and $1 < \prod_{i=1}^n a_i$, then the fully open CST network admits multiple nondegenerate positive equilibria under mass action kinetics.*
3. *If $s = 0$ and $a_i = 1$ for all i , then the fully open CST network does not admit multiple positive equilibria under mass action kinetics.*

Proof We prove part 1. Without loss of generality, assume that the last reaction is a sequestration, $a_n X_n + b_1 X_1 \rightarrow 0$. Let $\epsilon > 0$ and let $d_i = \frac{b_1 \dots b_i}{a_1 \dots a_i}$ for $1 \leq i \leq n$. Let $D_1 = \text{diag}(d_1, \dots, d_n)$ and

$$D = \begin{bmatrix} D_1 & 0 \\ 0 & \epsilon I \end{bmatrix}.$$

We have

$$\begin{aligned}\bar{\Gamma} D \mathbf{1} &= [\Gamma D_1] - \epsilon I \mathbf{1} < \Gamma D_1 \mathbf{1} \\ &\leq [-d_1 a_1 - d_n b_1, -d_2 a_2 + d_1 b_2, -d_3 a_3 + d_2 b_3, \dots, -d_n a_n + d_{n-1} b_n] \leq 0\end{aligned}$$

(all components except the first one are equal to zero).

Moreover, since $s < n$, $\det \Gamma_l$ contains only one positive monomial. We have $(-1)^n \det(\bar{\Gamma} D \bar{\Gamma}_l^T) = (-1)^n \det(\Gamma D_1 \Gamma_l^T - \epsilon I)$, and we see that $\lim_{\epsilon \rightarrow 0} (-1)^n \det(\bar{\Gamma} D \bar{\Gamma}_l^T) = (-1)^n \det \Gamma \det D_1 \det \Gamma_l = (\prod_{i=1}^n a_i - (-1)^s \prod_{i=1}^n b_i) \det D_1 \det \Gamma_l = (\prod_{i=1}^n a_i - \prod_{i=1}^n b_i) \det D_1 \det \Gamma_l < 0$. We can therefore pick $\epsilon > 0$ small so that the hypotheses of Theorem 2 are satisfied, and the conclusion follows.

For part 2 assume without loss of generality that $a_1 > 1$. We show that the CST with only the X_1 inflow and outflow reactions added (and not the ones for $X_2, \dots, X_n$) has nondegenerate multiple equilibria. Once this is done, it follows that the fully open CST multiple nondegenerate steady states as well by Theorem 3.

Consider then the CST system with inflow/outflow added for X_1 only. At steady state, $\dot{x}_i = k_{i-1} b_i x_{i-1}^{a_{i-1}} - k_i a_i x_i^{a_i} = 0$ for $i \geq 2$, which gives

$$x_n^{a_n} = \frac{k_{n-1}}{k_n} \cdot \frac{b_n}{a_n} x_{n-1}^{a_{n-1}} = \frac{k_{n-1} k_{n-2}}{k_n k_{n-1}} \cdot \frac{b_n b_{n-1}}{a_n a_{n-1}} x_{n-2}^{a_{n-2}} = \dots = \frac{k_1 b_2 \dots b_n}{k_n a_2 \dots a_n} x_1^{a_1} \quad (11)$$

Next, $\dot{x}_1 = 0$ yields

$$-k_1 a_1 x_1^{a_1} + k_n b_1 x_n^{a_n} - l_1 x_1 + f_1 = 0,$$

where l_1 and f_1 denote the rate constants of the outflow and inflow of X_1 . Using (11) we get

$$P(x_1) = k_1 a_1 \left(\frac{b_1 \dots b_n}{a_1 \dots a_n} - 1 \right) x_1^{a_1} - l_1 x_1 + f_1 = 0.$$

Letting

$$k_1 = a_1^{-1} \left(\frac{b_1 \dots b_n}{a_1 \dots a_n} - 1 \right)^{-1} > 0, \quad l_1 = 2^{a_1} - 1 > 0, \quad f_1 = 2^{a_1} - 2 > 0,$$

P has roots 1 and 2. Using (11) we compute two positive equilibria with $x_1 = 1$ and $x_1 = 2$, respectively. To see that these are nondegenerate equilibria note that the Jacobian matrix of the CST with flow reactions for X_1 can be written as [see (3)]

$$Df = \bar{\Gamma} D_{\bar{v}(x)} \bar{\Gamma}_l^T D_{1/x}$$

which is non-singular if and only if $\det(-\bar{\Gamma} D_{\bar{v}(x)} \bar{\Gamma}_l^T) \neq 0$, or equivalently

$$\det(-\Gamma D_{v(x)} \Gamma_l^T) + l_1 (-\Gamma D_{v(x)} \Gamma_l^T)[\{2 : n\}][\{2 : n\}] \neq 0 \quad (12)$$

We apply Cauchy–Binet to the second determinant:

$$(-\Gamma D_{v(x)} \Gamma_l^T)[\{2 : n\}|\{2 : n\}] = \sum_{\substack{\alpha \subseteq \{1, \dots, n\} \\ |\alpha| = n-1}} (-\Gamma[\{2 : n\}|\alpha]) D_{v(x)}[\alpha|\alpha] \Gamma_l[\{2 : n\}|\alpha]$$

and note that only one minor $\Gamma_l[\{2 : n\}|\alpha]$ is nonzero, namely when $\alpha = \{2 : n\}$. Therefore (12) implies that the condition for nondegeneracy of an equilibrium $x \in \mathbb{R}_{>0}^n$ is

$$\det(-\Gamma) \det D_{v(x)} \det(\Gamma_l) - l_1(-\Gamma[\{2 : n\}|\{2 : n\}] D_{v(x)}[\{2 : n\}|\{2 : n\}] \Gamma_l[\{2 : n\}|\{2 : n\}]) \neq 0,$$

or equivalently

$$\begin{aligned} & \left(\prod_{i=1}^n a_i - \prod_{i=1}^n b_i \right) \prod_{i=1}^n v_i(x) \prod_{i=1}^n a_i + l_1 \prod_{i=2}^n a_i^2 \prod_{i=2}^n v_i(x) \\ &= \prod_{i=2}^n a_i^2 \prod_{i=2}^n v_i(x) \left(\left(1 - \frac{b_1 \dots b_n}{a_1 \dots a_n} \right) a_1^2 v_1(x) + l_1 \right) \\ &= \prod_{i=2}^n a_i^2 \prod_{i=2}^n v_i(x) (-a_1 v_1(x)/k_1 + l_1) \neq 0. \end{aligned} \quad (13)$$

It remains to check (13) for our two equilibrium points. For the equilibrium with $x_1 = 1$ we get $v_1(x) = k_1$ and $-a_1 + l_1 = 2^{a_1} - a_1 - 1 > 0$ since $a_1 \geq 2$. For the equilibrium with $x_1 = 2$ we get $v_1(x) = k_1 2^{a_1}$ and $-a_1 2^{a_1} + l_1 = (1 - a_1) 2^{a_1} - 1 < 0$.

For part 3, suppose there are two distinct positive equilibria $x, y \in \mathbb{R}_{>0}^n$. Let k_i denote the reaction rate of $X_i \rightarrow b_{i+1} X_{i+1}$, let l_i denote the outflow rate of X_i , and let f_i denote the inflow rate of X_i . Set $D_1 = \text{diag}(k_1, \dots, k_n)$, $D_2 = \text{diag}(l_1, \dots, l_n)$, and $f = [f_1, \dots, f_n]$. We have $(\Gamma D_1 - D_2)x = (\Gamma D_1 - D_2)y = -f$. This implies that $\det(\Gamma D_1 - D_2) = 0$. Writing $x = D\mathbf{1}$, where $D = \text{diag}(x)$, we have $(\Gamma D_1 - D_2)D\mathbf{1} < 0$. With $\tilde{D}_1 = D_1 D = \text{diag}(\tilde{k}_i)$ and $\tilde{D}_2 = D_2 D = \text{diag}(\tilde{l}_i)$ we therefore have $(\Gamma \tilde{D}_1 - \tilde{D}_2)\mathbf{1} < 0$, or equivalently

$$\tilde{k}_i b_{i+1} < \tilde{k}_{i+1} + \tilde{l}_{i+1}, \quad i = 1, \dots, n. \quad (14)$$

However, $\det(\Gamma \tilde{D}_1 - \tilde{D}_2) = \det(\Gamma D_1 - D_2) \det D = 0$, so that (by Lemma 1)

$$\prod_{i=1}^n (\tilde{k}_i + \tilde{l}_i) = \prod_{i=1}^n \tilde{k}_i b_i,$$

which contradicts (14). $\square$

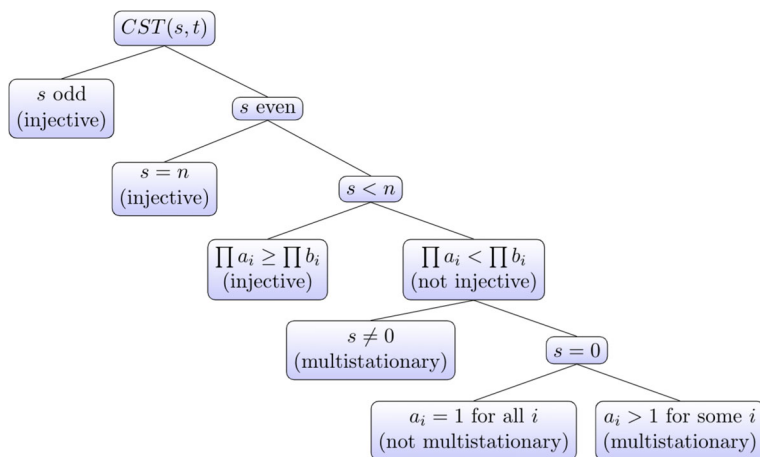


Fig. 2 Characterization of multistationarity for fully open CST networks. Here s and t represent the number of sequestration and transmutation reactions, respectively (color figure online)

6 A Simple Algorithm for Deciding the Capacity for Multistationarity in Fully Open CST Networks

We present here an algorithm for conclusively establishing the capacity for multistationarity (or lack thereof) for any fully open CST network. The algorithm takes the form of a flowchart (see Fig. 2) and also serves as a graphical summary of the main results in the previous section, i.e., Theorems 7 and 8. (Recall that an injective network does *not* have capacity for multistationarity.)

Remarkably, the input to the algorithm consists of only four integer parameters, two of which are counts of each type of reaction:

1. the number of sequestration reactions (s),
2. the number of transmutation reactions (t),

and the other two are simple functions of the stoichiometric coefficients:

3. $\text{sgn}(\prod a_i - \prod b_i)$,
4. $\text{sgn}(\prod a_i - 1)$.

Note, in particular, that the conditions above *do not depend on the order* in which the sequestration and transmutation reactions appear along the CST network cycle.

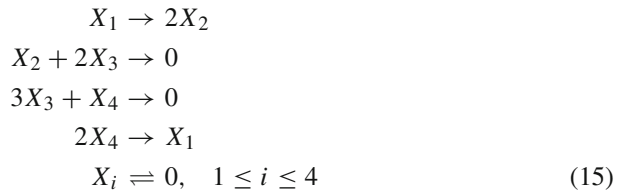
7 CST Atoms of Multistationarity

We conclude by noting that fully open CSTs described in Theorem 5.1 parts 1 and 2 are minimally multistationary (i.e., they are “atoms of multistationarity”) in the following sense.

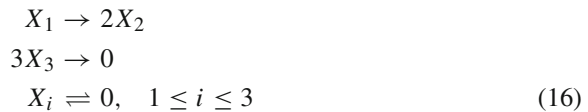
Theorem 9 *Let $\mathcal{R}$ be a fully open CST network. Suppose we obtain $\mathcal{R}'$ from $\mathcal{R}$ by removing (1) any number of species from all reactions in which they participate and*

(2) any number of non-flow reactions. If a trivial reaction (one in which the reactant and product complexes are the same) is obtained in $\mathcal{R}'$, then it is removed. Also removed are extra copies of repeated reactions in $\mathcal{R}'$. Then $\mathcal{R}'$ is injective.

Before we sketch the proof of the theorem, we make a few comments. The process of obtaining $\mathcal{R}'$ from $\mathcal{R}$ makes $\mathcal{R}'$ into an *embedded network* of $\mathcal{R}$. We will not present the detailed terminology here (the reader is referred to Joshi and Shiu 2013, Definition 2.2), but we briefly describe an example. Consider the fully open CST network $\mathcal{R}$



Removing the second reaction and species X_4 one obtains the network embedded in $\mathcal{R}$



Note that the fourth reaction of $\mathcal{R}$ becomes a duplicate of the inflow reaction $0 \rightarrow X_1$, which by convention we only list once.

Theorem 9 states that fully open CST networks are minimally multistationary with respect to the “embedded” relationship. In other words, they are a version of “atoms of multistationarity”, a notion introduced in Joshi and Shiu (2013). We remark that in that paper the authors require that the multiple steady states of an atom also be nondegenerate; the nondegeneracy of steady states for fully open CSTs is subject of future work.

Proof of Theorem 9 The proof uses the exact same argument from Theorem 6. We briefly sketch the argument in what follows. Let Γ and Γ_l denote the stoichiometric and reactant matrices of the non-flow part of the CST network $\mathcal{R}$ and let Γ' and Γ'_l denote the stoichiometric and reactant matrices of the non-flow part of the embedded network $\mathcal{R}'$. As usual then, $\bar{\Gamma}'$ and $\bar{\Gamma}'_l$ will denote the stoichiometric and reactant matrices of $\mathcal{R}'$. Minors of $\bar{\Gamma}'$ and $\bar{\Gamma}'_l$ are computed using Laplace expansion as in the proof of Theorem 7. Products of corresponding minors of $\bar{\Gamma}'$ and $\bar{\Gamma}'_l$ reduce to products of corresponding minors in Γ' and Γ'_l , see (10). On the other hand, minors of Γ' and Γ'_l are strict minors of Γ and Γ_l , which by Lemma 1 are monomials, or zero. Then, the argument after (10) follows through. $\square$

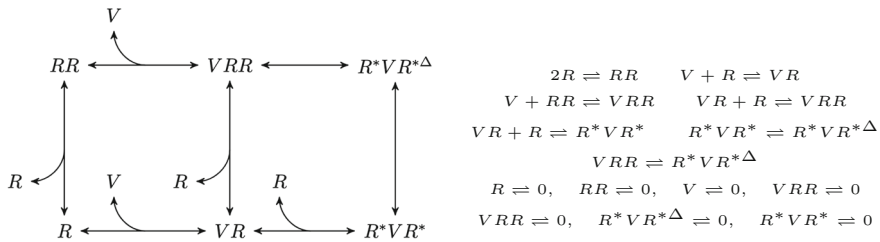


Fig. 3 Dimerization of VEGF receptors. R and V denote the VEGFR receptor monomer and VEGF, respectively. The naming convention of bound molecules reflect binding partners (for example VRR is VEGF bound to one VEGFR monomer of a VEGFR dimer, while RVR denotes VEGF bound to two VEGFR monomers. A phosphorylated receptor is marked with $*$, and Δ indicates that there is a bond between any two of the three components of the molecule. See Mac Gabhann and Popel (2007) for details

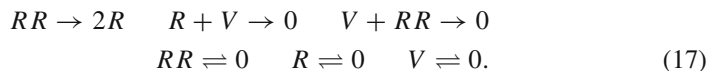
8 Application: Multistationarity in a Model of VEGFR Dimerization

Endothelial cells make up the lining of blood and lymphatic vessels, and plays important roles in many physiological mechanisms including regulation of vasomotor tone and blood fluidity, control of nutrients and leukocytes across the vascular wall, innate and acquired immunity, and angiogenesis (the growth of new blood vessels from existing vasculature). This diversity of roles of the endothelium is reflected in a remarkable structural and functional heterogeneity of endothelial cells, which can be related to the multistationarity of a pathway induced by VEGF (vascular endothelial growth factor), a key component of endothelial cell proliferation and angiogenesis (Regan and Aird 2012).

VEGF binds VEGF receptors (VEGFR) found on the surface of the cell via two binding sites, and the binding of a VEGF molecule to two VEGFR molecules induces signal transduction. A standard model for this dimerization, considered in Mac Gabhann and Popel (2007), is described in Fig. 3. We assume that each species has nonzero inflow and outflow rates, i.e. that the network is fully open. This is biologically relevant when, for example, there is an outside domain with high molecule concentration (Chen et al. 2013).

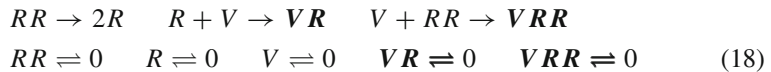
We use the results in this paper to show that the VEGFR dimerization network in Fig. 3 is multistationary. Namely, we exhibit a multistationary CST that can be built up to the VEGF network by way of modifications in Theorem 3. An automated way of searching for such a CST substructure in general networks is currently being implemented in CoNtRol (Donnell et al. 2014).

We start with the fully open CST network

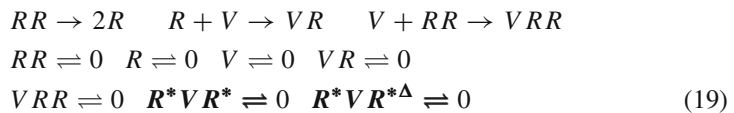


which is multistationary according to the first part of Theorem 8 (with $n = 3$, $s = 2$, $a_1a_2a_3 = 1$, $b_1b_2b_3 = 2$). This fully open three-species CST is in fact a sequestration network, and has nondegenerate multiple positive steady states (see Remark in Sect. 3).

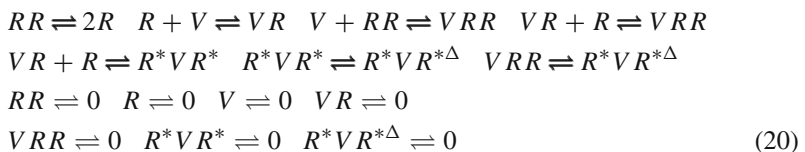
This allows us to apply Theorem 3 in the following way. At each step below, the network modifications are indicated in bold, and all modified networks have multiple positive nondegenerate steady states. First, we add species VR and VRR into the second and third reactions, together with inflow and outflow (Theorem 3 part 2):



Next, we add inflow/outflow reactions for the remaining species



Since all seven species have inflows and outflow reactions, the stoichiometric subspace of (19) is $\mathbb{R}^7$, and it contains any reaction among the species of (19). Theorem 3 part 3 implies that adding the remaining reactions [including the reverse of the first three reactions in (19)] preserves multistationarity:



Therefore, the VEGFR dimerization network (20) has multiple nondegenerate positive equilibria.

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