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Addition of flow reactions preserving multistationarity and bistability

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Abstract

We consider the question whether a chemical reaction network preserves the number and stability of its positive steady states upon inclusion of inflow and outflow reactions. Often a model of a reaction network is presented without inflows and outflows, while in fact some of the species might be degraded or leaked to the environment, or be synthesized or transported into the system. We provide a sufficient and easy-to-check criterion based on the stoichiometry of the reaction network alone and discuss examples from systems biology.

Keywords: multistationarity; open system; steady states; model reduction; reaction network

1 Introduction

Bistability and multistationarity in general are considered important biological mechanisms, providing explanations for co-existence of differentiable phenotypes and switch-like behaviour [13,17]. The question of whether bistability is present in a given system therefore arises naturally in many contexts [7,9,14–16]. However, it is not straightforward to decide a priori whether this is the case.

The objects of interest here are reaction networks describing the evolution of concentrations of chemical species over time, and modeled by means of systems of autonomous Ordinary Differential Equations (ODEs). Such an ODE system typically admits linear conservation laws, that is, linear first integrals, due to the reactions alone, independently of the kinetics. The first integrals restrict the dynamics of the ODE system to the so-called stoichiometric compatibility classes, and questions about the existence of multiple (stable) steady states are to be addressed relatively to each class. Furthermore, the ODE system depends on (potentially many) unknown parameters, which adds to the difficulty of the problem as the number and stability of the steady states must be investigated for general parameters. In particular, a reaction network is said to be multistationary, or bistable, if that is the case for some choice of parameter values.

A successful strategy to determine whether a network is multistationary or bistable is the following. First the number and stability of the steady states of a reduced reaction network is studied, and then these steady states (and their properties) are “lifted” to the original network. As the ODE system arises from a reaction network, a substantial amount of recent work has focused on determining modifications of the reactions that preserve properties at steady state upon lifting. Specifically, we consider two reaction networks \( F \) and \( G \), with respective kinetic network

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Perhaps, the first work in this direction is due to Craciun and Feinberg [5]. They show that multistationarity is preserved if a reaction of the form $X \rightleftharpoons 0$ is added to a network $F$ for all species $X$ in $F$. Subsequent work by Joshi and Shiu [11] consider the case where $G$ is obtained from $F$ by adding reactions in such a way that the stoichiometric compatibility classes are preserved. They also consider the case of embedded networks, where $G$ is obtained from $F$ by adding species in a specific way. Feliu and Wiuf [8] show that the number and properties of the steady states of a network are preserved upon the addition of intermediate species. Banaji and Pantea [1] introduce additional operations that preserve steady states properties.

Here we revisit the situation in which reactions of the form $0 \longrightarrow X, \quad X \longrightarrow 0$, called inflow and outflow reactions, respectively, and jointly flow reactions, are added to $F$, where $X$ is a species already in $F$. The addition of such reactions destroys the linear first integrals involving the concentration of $X$ and hence increases the actual dimension of the ODE system. Some recent methods to count the number of steady states and to determine their stability rely heavily on the existence of a parametrization of the steady state manifold, not restricted to a particular stoichiometric compatibility class [2–4, 18, 20]. Finding such parametrizations often requires sufficient freedom, which comes from the codimension of the stoichiometric compatibility classes. As a consequence, the direct determination of the number and stability of the steady states of a reaction network with flow reactions is much harder than for networks without them.

As mentioned above, if both flow reactions are added for all species of $F$, then statements of the desired type can be obtained (lifting of steady states appears first in [5]; stability in [11]). Hence, the network without flow reactions provides information about the network with all flow reactions. However, from a biochemical or metabolic point of view, the network with all flow reactions makes generally little sense. Here, $0 \longrightarrow X$ often represents synthesis of $X$ or transport of $X$ into a compartment, while $X \longrightarrow 0$ represents degradation or transport out of a compartment. Modeling, for example, the inflow of a protein complex, such as a kinase-substrate complex, is in general not meaningful. Indeed, in realistic models inflow and outflow reactions are only considered for selected species.

The discussion raises the following question:

What flow reactions can be added to a reaction network while preserving the number and stability of the steady states?

(in the sense discussed above). We should not expect that an arbitrary selection of flow reactions is allowed. As a simple illustration, consider the reaction network $X_1 \rightleftharpoons X_2$ with mass-action kinetics. It has one positive steady state in each stoichiometric compatibility class (for any choice of reaction rate constants). By adding the outflow reaction $X_2 \longrightarrow 0$, the network has no positive steady states for any choice of reaction rate constants.

In this paper we give a sufficient and easy-to-check criterion based on stoichiometry alone to decide on the question above. In particular, the criterion implies that the flow reactions must at least fulfill the following. If an outflow reaction is added for a species that is in a conservation law that cannot be written as the sum of two conservation laws involving each less variables, then outflow reactions must be added for all species in the conservation law. In contrast, if an inflow reaction is added for a species that is not also in an outflow reaction, then the species must not be conserved in the original network. Furthermore, we give various examples and illustrate, also by example, that our conditions are not necessary.

2 Reaction Networks and Steady States

Let $\mathbb{R}_{>0}$ and $\mathbb{R}_{\geq 0}$ denote the set of positive real numbers and the set of non-negative real numbers, respectively. Let $\mathbb{N}$ be the set of non-negative integers. $\langle v_1, \ldots, v_k \rangle$ denotes the linear span of the vectors $v_1, \ldots, v_k$ (in some vector space).
A reaction network $F = (\mathcal{C}, \mathcal{R})$ on a non-empty set $\mathcal{X} = \{X_1, \ldots, X_n\}$ is a directed graph without self-edges whose nodes are linear combinations in $\mathcal{X}$ with non-negative integer coefficients. The elements $y \in \mathcal{C}$ are complexes and of the form $y = \sum_{i=1}^{n} \lambda_i X_i$ with $\lambda_i \in \mathbb{N}$, $i = 1, \ldots, n$. The elements in $\mathcal{R}$ are reactions. It is assumed that there are no isolated nodes, that is, every complex is part of a reaction. We further consider the set of reactions to be ordered and let $m$ be its cardinality.

The zero complex $0 \in \mathcal{C}$ is allowed by definition. For $X \in \mathcal{X}$, recall from the introduction that the reactions

$$X \rightarrow 0 \quad \text{and} \quad 0 \rightarrow X$$

are outflow and inflow reactions, respectively, and jointly referred to as flow reactions. In this case $X$ is said to be a flow species.

We identify the species $X_i$ with the $i$-th canonical vector of $\mathbb{R}^n$ with 1 in the $i$-th position and zeroes elsewhere. Hence, each complex $y \in \mathcal{C}$ is a vector in $\mathbb{R}^n$. The stoichiometric matrix $N \in \mathbb{R}^{n \times m}$ of $F$ is the matrix whose $j$-th column is the vector $y^j - y$, where $y \rightarrow y'$ is the $j$-th reaction. In particular, the $(i, j)$-th entry of $N$ encodes the net production of species $X_i$ in the $j$-th reaction. The stoichiometric subspace $S \subseteq \mathbb{R}^n$ of $F$ is the span of the column vectors of $N$,

$$S := \text{im}(N) \subseteq \mathbb{R}^n.$$

We denote by $s$ the dimension of $S$ (that is, the rank of $N$) and by $d$ the dimension of the orthogonal complement subspace $S^\perp$ of $S$. Hence $s + d = n$.

The species concentrations change over time as a consequence of the reactions taking place. To describe the time evolution we introduce a kinetics and the species-formation rate function. A kinetics for $F$ is a $C^1$-function

$$K : \Omega \rightarrow \mathbb{R}^m_{\geq 0},$$

where $\mathbb{R}^m_{\geq 0} \subseteq \Omega \subseteq \mathbb{R}^n_{\geq 0}$ (differentiability is with respect to the open set $\mathbb{R}^n_{>0}$). The entry $K_j(x)$ is called the rate of the $j$-th reaction. A common choice of kinetics is mass-action kinetics, with $\Omega = \mathbb{R}^n_{\geq 0}$ and

$$K_j(x) = \kappa_j \prod_{i=1}^{n} x_i^{y_i}, \quad \text{if } y \rightarrow y' \text{ is the } j \text{-th reaction},$$

where $\kappa_j > 0$ is the reaction rate constant of the reaction. Under this kinetics, the reactions are usually labeled with the reaction rate constants.

The species-formation rate function of $F$ with kinetics $K$ is the map $f : \Omega \rightarrow S \subseteq \mathbb{R}^n$ defined by

$$f(x) := NK(x).$$

The dynamics of the species concentrations of the network $F$ with kinetics $K$ is described by a set of ODEs given by the species-formation rate function:

$$\dot{x} = f(x), \quad x \in \Omega,$$

(1)

where $\dot{x} = \dot{x}(t)$ denotes the derivative of $x(t)$ with respect to time $t$.

If $K_j(x)$ vanishes whenever $x_i = 0$ for $i$ an index for which $N_{ij}$ is negative (as it is the case for mass-action kinetics), then both $\Omega$ and $\mathbb{R}^n_{\geq 0}$ are forward invariant by the solutions of $\dot{x} = f(x)$. Additionally, as $\text{im}(f) \subseteq S$, a solution of $\dot{x} = f(x)$ is confined to an invariant linear space of the form $x_0 + S$, where $x_0 \in \Omega$ is the initial point of the solution. The set $(x_0 + S) \cap \Omega$ is called a stoichiometric compatibility class. Given a matrix $W \in \mathbb{R}^{d \times n}$ whose rows form a basis of $S^\perp$, the stoichiometric compatibility classes might be parametrized by $T = (T_1, \ldots, T_d) \in W(\Omega) \subseteq \mathbb{R}^d$ as follows:

$$\mathcal{L}_{W,T} := \{x \in \Omega | Wx = T\}.$$  

(2)

Note that given $x_0 \in \Omega$, there exists a unique $T$ such that $x_0 \in \mathcal{L}_{W,T}$. The vector $T$ is commonly referred to as the vector of total amounts and any relation $\sum_{i=1}^{n} \omega_i x_i = T$, with $\omega \in S^\perp$, is called a conservation law. Consequently, we call a matrix $W$ whose rows form a basis of $S^\perp$ a matrix of conservation laws.
Definition 1. Given a vector \( u \) in \( \mathbb{R}^n \) we define its support to be the subset of species \( X_i \in \mathcal{X} \) where \( u_i \neq 0 \). We say a species \( X_i \) is non-conserved if it is not in the support of any vector in \( S^\perp \), that is, the \( i \)-th canonical vector of \( \mathbb{R}^n \) belongs to \( S \).

Note that \( X_i \) is non-conserved if and only if the \( i \)-th column of a matrix of conservation laws is zero.

Example 2. Consider the reaction network with mass-action kinetics, given by
\[
X_1 + X_4 \xrightarrow{\kappa_1 \kappa_2} X_2 \xrightarrow{\kappa_3 \kappa_4} X_3 + X_4
\]
where \( \kappa_1, \kappa_2, \kappa_3, \kappa_4 \) are positive constants. The dynamics of the species concentrations are described by the following ODE system:
\[
\begin{align*}
\dot{x}_1 &= -\kappa_1 x_1 x_4 + \kappa_2 x_2 \\
\dot{x}_2 &= \kappa_1 x_1 x_4 - (\kappa_2 + \kappa_3)x_2 + \kappa_4 x_3 x_4 \\
\dot{x}_3 &= \kappa_3 x_2 - \kappa_4 x_3 x_4 \\
\dot{x}_4 &= -\kappa_1 x_1 x_4 + (\kappa_2 + \kappa_3)x_2 - \kappa_4 x_3 x_4.
\end{align*}
\]
The stoichiometric matrix and the stoichiometric subspace are
\[
N = \begin{pmatrix}
-1 & 1 & 0 & 0 \\
1 & -1 & -1 & 1 \\
0 & 0 & 1 & -1 \\
-1 & 1 & 1 & -1
\end{pmatrix}, \quad S = \langle (-1, 1, 0, -1), (0, -1, 1, 1) \rangle.
\]
Therefore \( n = 4, m = 4, s = 2, \) and \( d = 2 \).

The steady states of a network \( F \) with kinetics \( K \) are the solutions to the system of equations
\[
\dot{x} = f(x) = NK(x) = 0, \quad x \in \Omega,
\]
(3) which we refer to as the steady state equations. If \( N' \in \mathbb{R}^{r \times m} \) is any matrix such that \( \ker(N) = \ker(N') \), then the steady states of the network are precisely the solutions to \( N'K(x) = 0 \). In particular, if the rank of \( N \) is \( s \), then there exists a matrix \( N' \in \mathbb{R}^{s \times m} \) of maximal rank such that \( \ker(N) = \ker(N') \). Therefore, the system of \( n \) equations can always be reduced to an equivalent system of \( s \) equations.

As the dynamics of the system are confined to the stoichiometric compatibility classes, questions about the number, stability or other properties of the steady states will be addressed relatively to a given stoichiometric compatibility class. Specifically, the steady states of a network with kinetics \( K \) in a stoichiometric compatibility class \( \mathcal{L}_{W,T} \) are the solutions to the \( n \) equations
\[
N'K(x) = 0 \quad \text{and} \quad Wx = T, \quad x \in \Omega,
\]
where \( N' \in \mathbb{R}^{s \times m} \) is such that \( \ker(N) = \ker(N') \). We define accordingly
\[
\Phi(x) := \langle N'K(x), Wx - T \rangle \in \mathbb{R}^s \times \mathbb{R}^s \equiv \mathbb{R}^n, \quad x \in \Omega,
\]
(4) such that the steady states in \( \mathcal{L}_{W,T} \) are the solutions to \( \Phi(x) = 0 \). We say a network with kinetics \( K \) is multistationary if there exists a stoichiometric compatibility class containing at least two positive steady states.

Recall that the Jacobian \( J_f(x^*) \) of a \( C^1 \)-map \( f : U \subseteq \mathbb{R}^n \to \mathbb{R}^n \) evaluated at \( x^* \in \text{int}(U) \) is the \( n \times n \) matrix such that the \((i,j)\)-th entry is \( \frac{\partial f_j}{\partial x_i}(x^*) \). We say that a steady state \( x^* \) is non-degenerate if \( J_f(x^*) \) is non-singular on \( S \), i.e. \( \ker(J_f(x^*)) \cap S = \{0\} \). The following lemma is proved in \cite{22}.

Lemma 3. A steady state \( x^* \in \Omega \) in the stoichiometric compatibility class \( \mathcal{L}_{W,T} \) is non-degenerate if and only if the Jacobian \( J_{\Phi}(x^*) \) of \( \Phi \) defined in (3) evaluated at \( x^* \) is non-singular on \( \mathbb{R}^n \), that is, if and only if \( \det(J_{\Phi}(x^*)) \neq 0 \).
Given a steady state \( x^* \), the eigenvalues of \( J_f(x^*) \) convey information on the local stability of \( x^* \) relative to the stoichiometric compatibility class it belongs to. As the rank of \( J_f(x^*) \) is at most \( s \), 0 is an eigenvalue of \( J_f(x^*) \) with multiplicity at least \( d \), and the multiplicity is exactly \( d \) if the steady state is non-degenerate. If \( J_f(x^*) \) has \( s \) eigenvalues with negative real part (counted with multiplicity), then \( x^* \) is said to be exponentially stable, and is in particular asymptotically stable relative to the stoichiometric compatibility class. If at least one of the eigenvalues of \( J_f(x^*) \) has positive real part, then \( x^* \) is said to be exponentially unstable (this is not a standard term in the literature but used here for convenience), and is in particular unstable. If \( J_f(x^*) \) has \( s \) eigenvalues with non-vanishing real part, then the steady state is said to be hyperbolic.

3 Partially open extensions and lifting steady states

In this section we compare two reaction networks \( F \) and \( G \) with respective kinetics, such that \( G \) is obtained from \( F \) by adding some inflow and outflow reactions. In what follows the objects defined in the previous section, namely \( N, S, R, W, K \), are indexed by subscripts \( F \) and \( G \) indicating the network they are associated with. The following definition is inspired by [5,6].

**Definition 4.** Consider a reaction network \( F \) with kinetics \( K_F \) and species set \( \mathcal{X} \) of cardinality \( n \).

- We say that a reaction network \( G \) with kinetics \( K_G \) is a partially open extension of \( F \) with respect to the inflow set \( \mathcal{X}^i \subseteq \mathcal{X} \) and the outflow set \( \mathcal{X}^o \subseteq \mathcal{X} \), if the species set of \( G \) is \( \mathcal{X} \), the set \( \mathcal{R}_G \) decomposes as a disjoint union
  \[
  \mathcal{R}_G = \mathcal{R}_F \sqcup \{ 0 \rightarrow X \}_{X \in \mathcal{X}^i} \sqcup \{ X \rightarrow 0 \}_{X \in \mathcal{X}^o},
  \]
  and \( K_G \) agrees with \( K_F \) for the reactions in \( \mathcal{R}_F \) and is of mass-action type otherwise.

- If additionally \( G \) has an inflow and outflow reaction for all species in \( \mathcal{X} \), then \( G \) is called a fully open extension of \( F \).

- We let \( \mathcal{X}^f = \mathcal{X}^i \cup \mathcal{X}^o \) denote the set of flow species of \( G \) that are not flow species of \( F \).

Note that \( F \) might have flow reactions for species not in \( \mathcal{X}^f \). Our goal is to study for what sets \( \mathcal{X}^i, \mathcal{X}^o \), the number and stability of the positive steady states of \( F \) extend to \( G \) after appropriately choosing reaction rate constants for the added flow reactions. By [5], if \( F \) has multiple positive non-degenerate steady states, then so does the fully open extension, provided the reaction rate constants of the flow reactions of \( G \) that are not in \( F \) are chosen small enough. By [1], the maximal number of exponentially stable positive steady states of \( F \) within a stoichiometric compatibility class is also a lower bound of the maximal number of exponentially stable positive steady states \( G \) admits for arbitrary reaction rate constants of the flow reactions. Here we relax the condition that all missing inflow and outflow reactions must be added to preserve these characteristics.

A key ingredient of the main theorem below is to understand the image of the positive orthant by a matrix of conservation laws \( W \), which is a polyhedral cone. Thus, in preparation for the main theorem, we discuss a well-known property of polyhedral cones in \( \mathbb{R}^n \). Given a matrix \( M \in \mathbb{R}^{r \times n} \) with \( r \leq n \) and of rank \( r \), let \( \mathcal{C}(M) \) be the polyhedral cone generated by the columns of \( M \): if \( m^{(i)} \) denotes the \( i \)-th column of \( M \), then

\[
\mathcal{C}(M) = \left\{ \sum_{i=1}^{n} \lambda_i m^{(i)} \in \mathbb{R}^r \mid \lambda_1, \ldots, \lambda_n \geq 0 \right\} = \left\{ M\lambda \mid \lambda \in \mathbb{R}^n_{\geq 0} \right\}.
\]

We let \( \mathcal{C}^o(M) \) denote the corresponding open cone, obtained by imposing all \( \lambda_i \) to be positive.

For a subset \( I \subseteq \{1, \ldots, n\} \), we let \( M^I \) denote the submatrix of \( M \) given by the columns with index in \( I \). We say that \( M^I \) generates \( \mathcal{C}^o(M) \) if \( \mathcal{C}^o(M) = \mathcal{C}^o(M^I) \).
Lemma 5. Let \( M, M^* \in \mathbb{R}^{r \times n} \) be of rank \( r \leq n \) and such that \( M^* = PM \) with \( P \in \mathbb{R}^{r \times r} \) invertible. Given \( I \subseteq \{1, \ldots, n\} \), \( M^I \) generates \( C^o(M) \) if and only if \( M^{I^c} \) generates \( C^o(M^*) \).

Proof. Assume \( M^I \) generates \( C^o(M) \), such that the images of \( \mathbb{R}^n_{>0} \) by \( M \) and by \( M^I \) agree. Now we have

\[
C^o(M^*) = \{ M^* \lambda \mid \lambda \in \mathbb{R}^n_{>0} \} = \{ P(M \lambda) \mid \lambda \in \mathbb{R}^n_{>0} \} = \{ P(M^I \lambda) \mid \lambda \in \mathbb{R}^{|I|}_{>0} \}
\]

where \(|I|\) denotes the cardinality of \( I \). By symmetry of the argument, this concludes the proof. \( \Box \)

Under the hypothesis of Lemma 5, the rows of \( M \) and \( M^* \) generate the same vector subspace of \( \mathbb{R}^n \). In view of Lemma 5, the following definition is consistent and independent of the choice of \( W \).

Definition 6. Consider a reaction network \( F \) with species set \( \mathcal{X} \).

- For any subset \( \mathcal{X}_1 \subseteq \mathcal{X} \), the vector subspace of conservation laws with support in \( \mathcal{X}_1 \) is defined as
  \[
  S_{F, \mathcal{X}_1}^\perp = S_F^\perp \cap \langle e_i \mid X_i \in \mathcal{X}_1 \rangle.
  \]

- Given subsets \( \mathcal{X}_1 \subseteq \mathcal{X}_2 \subseteq \mathcal{X} \), let \( I \) be the index set of the species in \( \mathcal{X}_1 \), and \( W_2 \) be a matrix whose rows form a basis of the conservation laws with support in \( \mathcal{X}_2 \), \( S_{F, \mathcal{X}_2}^\perp \). Then \( \mathcal{X}_1 \) is said to generate \( S_{F, \mathcal{X}_2}^\perp \) if \( W_2^\perp \) generates \( C^o(W_2) \).

With the notation in Definition 6 let \( n_1, n_2 \) be the cardinality of \( \mathcal{X}_1, \mathcal{X}_2 \), respectively and \( W' \) the submatrix of \( W_2 \) given by the columns corresponding to the species in \( \mathcal{X}_2 \). Note that the other columns of \( W_2 \) are zero. Then Definition 6 is simply saying that the image of the positive orthant \( \mathbb{R}^n_{>0} \) by \( W' \) agrees with the image of the possibly lower dimensional orthant \( \{0\}^{n_2-n_1} \times \mathbb{R}^{n_1}_{>0} \) by \( W' \) (after appropriate reordering of the variables). Note that \( \mathcal{X}_1 \) needs to have at least as many species as rows of \( W_2 \). Furthermore, recall that the image of the positive orthant by a matrix of conservation laws determines the possible values of the vector of total amounts for positive (steady) states of the system.

Lemma 7. Consider a reaction network \( F \) with kinetics \( K_F \) and species set \( \mathcal{X} \) of cardinality \( n \). Let \( G \) be a partially open extension of \( F \) with \( \mathcal{X}' \) the set of added flow species as in Definition 4. Then

\[
S_G^\perp = S_{F, \mathcal{X}\setminus \mathcal{X}'}^\perp = S_F^\perp \cap \langle e_i \mid X_i \in \mathcal{X} \setminus \mathcal{X}' \rangle,
\]

that is, \( S_G^\perp \) is the vector subspace of \( S_F^\perp \) of vectors with support included in \( \mathcal{X} \setminus \mathcal{X}' \).

Proof. Recall that \( S_G, S_F \) are vector subspaces of \( \mathbb{R}^n \). By definition we have

\[
S_G = S_F + \langle e_i \mid X_i \in \mathcal{X}' \rangle,
\]

where \( e_i \) denotes the \( i \)-th canonical vector of \( \mathbb{R}^n \). Hence

\[
S_G^\perp = S_F^\perp \cap \langle e_i \mid X_i \in \mathcal{X}' \rangle^\perp = S_F^\perp \cap \langle e_i \mid X_i \in \mathcal{X} \setminus \mathcal{X}' \rangle.
\]

\( \Box \)

Theorem 8. Consider a reaction network \( F \) with kinetics \( K_F \) and species set \( \mathcal{X} \) of cardinality \( n \). Let \( G \) be a partially open extension of \( F \) with respect to the inflow set \( \mathcal{X}' \subseteq \mathcal{X} \) and the outflow set \( \mathcal{X}^0 \subseteq \mathcal{X} \), and with kinetics \( K_G \). Let \( d_1 \) be the dimension of \( S_{F, \mathcal{X}\setminus \mathcal{X}'}^\perp \). Assume that

(a) There is a direct sum decomposition

\[
S_F^\perp = S_{F, \mathcal{X}' \setminus \mathcal{X}'}^\perp \oplus S_{F, \mathcal{X}\setminus \mathcal{X}'}^\perp.
\]
\((b)\) \(\mathcal{X}^f \setminus \mathcal{X}^o \subseteq \mathcal{X}^c\) consists of non-conserved species of \(F\).

\((c)\) \(\mathcal{X}^c\) generates \(S_{F,\mathcal{X}^f}^+\).

Then the following statements hold:

\((i)\) If \(F\) has at least \(\ell\) positive non-degenerate steady states \(c_1, \ldots, c_\ell\) in one stoichiometric compatibility class \((x_0 + S_F) \cap \Omega\), then there exists a choice of reaction rate constants for the flow reactions of \(G\) not in \(F\) such that \(G\) with this kinetics has at least \(\ell\) positive non-degenerate steady states \(c'_1, \ldots, c'_\ell\) in the stoichiometric compatibility class \((x_0 + S_G) \cap \Omega\).

\((ii)\) With the appropriate numbering of steady states in \((i)\), if \(J_{F,c}(c_j)\) has at least \(r_1\) eigenvalues with positive real part and \(r_2\) eigenvalues with negative real part, then \(J_{F,c}(c'_j)\) has \(r_1\) eigenvalues with positive real part and \(r_2 + d_1\) eigenvalues with negative real part.

\((iii)\) If \(F\) has at least \(\ell_1\) exponentially stable and \(\ell_2\) exponentially unstable non-degenerate steady states in \((x_0 + S_F) \cap \mathbb{R}_+^n\), then so does \(G\) in \((x_0 + S_G) \cap \mathbb{R}_+^n\), for a choice of reaction rate constants for the flow reactions of \(G\) not in \(F\).

In particular, a choice of reaction rate constants of the flow reactions of \(G\) not in \(F\) such that \((i)-(iii)\) hold can be found with \(\theta\) small enough and

\[
X_j \xrightarrow{\theta} 0, \quad X_j \in \mathcal{X}^o, \quad 0 \xrightarrow{\theta x_j} X_j, \quad X_j \in \mathcal{X}^c,
\]

where \(\hat{x} \in \mathbb{R}_+^n\) has support \(\mathcal{X}^c\) and is such that \(W^f \hat{x} = W^f x_0\) for \(W^f\) a matrix whose rows form a basis of \(S_{F,\mathcal{X}^f}^+\).

The proof of Theorem 8 is given in Section 4 below. We remark that in the notation of Theorem 8, \(\ell_1 + \ell_2\) might not be \(\ell\), as not all \(\ell\) steady states need to be hyperbolic.

**Remark 9.** The assumptions of Theorem 8 can easily be verified by considering a matrix of conservation laws \(W_F\) of \(F\). In particular, assumption \((a)\) of Theorem 8 can be verified using Gauss reduction on \(W_F\). Indeed, assuming \(\mathcal{X}\) is ordered such that the species in \(\mathcal{X}^f\) are the first species, then \((a)\) holds if and only if there is a matrix of conservation laws of \(F\) of the form

\[
W_F = \begin{pmatrix} W_1 & 0 \\ 0 & W_2 \end{pmatrix},
\]

where the number of columns of \(W_1\) is the cardinality of \(\mathcal{X}^f\). It follows by Lemma 7 that \((0 \ W_2)\) is a matrix of conservation laws for \(G\), that is, its rows form a basis of \(S_G^+\). In particular \(S_G^+\) is the kernel of \((0 \ W_2)\). The blocks \(W_1\) and \(W_2\) might be empty.

Assumption \((b)\) in Theorem 8 says that for any species in \(\mathcal{X}^f\) for which the outflow reaction is not considered, then the corresponding column of \(W_1\) is identically zero. Hence all the species in the conservation laws of \(W_1\) must be outflow species (this species set is independent of \(W_1\)). Finally, assumption \((c)\) can be verified by determining the rays \(u_1, \ldots, u_k\) of the cone \(C(W_1)\). Then the columns of \(W_1\) corresponding to \(\mathcal{X}^c\) must contain scalar multiples of all the vectors \(u_1, \ldots, u_k\).

**Remark 10.** Assume a set of species \(\mathcal{X}^f \subseteq \mathcal{X}\) is given that satisfies assumption \((a)\) of Theorem 8. One might wonder how \(\mathcal{X}^f\) can be divided into inflow and outflow species such that \((b)\) and \((c)\) also hold. By choosing \(\mathcal{X}^c = \mathcal{X}^o = \mathcal{X}^f\), then \((b)\) and \((c)\) trivially hold.

As assumption \((b)\) stipulates that \((at least)\) the species in the conservation laws of \(W_1\) must be outflow species, \(\mathcal{X}^o\) is a set of outflow species for which \((b)\) holds if and only if it contains the conserved species of \(W_1\) and \(\mathcal{X}^o \subseteq \mathcal{X}^f\). Concerning the inflow species, assume \(\mathcal{X}^i_1, \ldots, \mathcal{X}^i_j \subseteq \mathcal{X}^f\) are the distinct smallest sets such that \((c)\) hold \((smallest\ in\ the\ sense\ that\ no\ proper\ subset\ of\ \mathcal{X}^i_j\ satisfies\ \((c)\)\). Hence \(\mathcal{X}^c\) is a set of inflow species for which \((c)\) is satisfied if and only if \(\mathcal{X}^i_j \subseteq \mathcal{X}^c \subseteq \mathcal{X}^f\) for some \(j = 1, \ldots, k\). If the rank of \(W_1\) is \(d_1\), then any set \(\mathcal{X}^i_j\) contains at least \(d_1\) elements, providing a lower bound on the size of the set.
As an easy consequence of Theorem \(8\), we recover two known cases, namely the case of fully open extensions as well as the case of \(S_G = S_F\). In the latter case, as \(F\) is a subnetwork of \(G\), the results on subnetworks in [11] apply.

**Corollary 11.** Consider a reaction network \(F\) with kinetics \(K_F\) and species set \(\mathcal{X}\) of cardinality \(n\), and the following two cases:

1. \(G\) is the fully open extension of \(F\).
2. \(G\) is a partially open extension of \(F\) with \(\mathcal{X}^i\) and \(\mathcal{X}^o\) consisting only of non-conserved species of \(F\), hence \(S_F = S_G\).

For both cases, conclusions (i), (ii) and (iii) of Theorem \(8\) hold.

**Proof.** We verify that assumption (a), (b) and (c) of Theorem \(8\) hold in the two cases.

1. We have \(S_{F,\mathcal{X}\setminus\mathcal{X}^i} = \emptyset\) and (a) holds. (b) holds trivially as \(\mathcal{X}^i \setminus \mathcal{X}^o = \emptyset\). (c) follows from \(\mathcal{X}^o = \mathcal{X}^f\).
2. Assumption (a) holds as \(S_{F,\mathcal{X}^i} = \emptyset\). (b) and (c) hold trivially.

Before giving the proof of Theorem \(8\) we illustrate it with several examples. Examples [12] and [13] illustrate further that the conditions of Theorem \(8\) cannot easily be relaxed.

**Example 12.** Consider the reaction network in Example \(2\), equipped with mass-action kinetics

\[
F: \quad X_1 + X_4 \rightleftharpoons X_2 \rightleftharpoons X_3 + X_4,
\]

and the following partially open extension of \(F\):

\[
G: \quad X_1 + X_4 \rightleftharpoons X_2 \rightleftharpoons X_3 + X_4
\]

\[
X_1 \rightleftharpoons 0 \quad X_2 \rightarrow 0 \quad X_3 \rightarrow 0 \quad X_4 \rightleftharpoons 0.
\]

Here \(\mathcal{X}^o = \mathcal{X} = \{X_1, X_4\}\). Let us verify that \(F,G\) satisfy the assumptions of Theorem \(8\). A matrix of conservation laws of \(F\) is

\[
W_F = \begin{pmatrix}
1 & 1 & 1 & 0 \\
0 & 1 & 0 & 1
\end{pmatrix}.
\]

We write \(W_F\) in the form \(5\) by choosing \(W_1 = W_F\) and \(W_2\) empty, and check the conditions of Theorem \(8\). As \(\mathcal{X}^f = \mathcal{X}^o = \mathcal{X}\), assumptions (a) and (b) hold. Finally, the cone \(C^o(W_F)\) is generated by the first and last columns of \(W_F\). Hence, the assumptions of Theorem \(8\) hold, and the conclusions (i)-(iii) regarding lifting properties of the steady states of \(F\) to \(G\) apply.

If \(F\) models a reversible enzymatic reaction, then the extension \(G\) includes degradation of every species, and assimilation of the substrate \(X_1\) and the enzyme \(X_4\) from the external environment.

Observe that as \(C^o(W_F)\) also is generated by the third and fourth columns of \(W_F\), Theorem \(8\) also applies to the partially open extension with inflow set \(\mathcal{X}^i\).

**Example 13.** (Hybrid histidine kinase) We consider a well-studied simplified model of a hybrid histidine kinase HK with two phosphorylated sites and transference of the phosphate group to a histidine phosphotransferase Hpt [12]. The reaction network \(F\) is

\[
F: \quad \text{HK}_{00} \rightarrow \text{HK}_{p0} \rightarrow \text{HK}_{pp} \quad \text{Hpt}_p \rightarrow \text{Hpt}_0
\]

\[
\text{HK}_{pp} + \text{Hpt}_0 \rightarrow \text{HK}_{p0} + \text{Hpt}_p \quad \text{HK}_{0p} + \text{Hpt}_0 \rightarrow \text{HK}_{00} + \text{Hpt}_p.
\]

Assuming mass-action kinetics, this network can have one or three positive non-degenerate steady states, depending on the choice of reaction rate constants and stoichiometric compatibility class [12]. If it has three positive non-degenerate steady states, then two of them are exponentially stable and the other exponentially unstable. If it has only one, then it is exponentially stable [20].
Here we explore different partially open extensions that satisfy the assumptions of Theorem 8. To this end, we consider the following matrix of conservation laws for $F$, where the species set is ordered as $HK_{00}, HK_{p0}, HK_{0p}, HK_{pp}, Hpt_0, Hpt_p$:

$$W_F = \begin{pmatrix} 1 & 1 & 1 & 0 & 0 & 1 & 0 \\ 0 & 1 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 \end{pmatrix}.$$  

It follows that the sets $X_1^f = \{ HK_{00}, HK_{p0}, HK_{0p}, HK_{pp} \}$ and $X_2^f = \{ Hpt_0, Hpt_p \}$ both satisfy assumption (a) of Theorem 8. With $X_1^o = X_1^f$ and $X_2^o = X_2^f$, assumptions (b) and (c) of Theorem 8 hold for both sets. We obtain the following two partially open extensions

$$G_1: \begin{array}{c} HK_{00} \rightarrow HK_{p0} \rightarrow HK_{0p} \rightarrow HK_{pp} \rightarrow Hpt_p \rightarrow Hpt_0 \\ HK_{pp} + Hpt_0 \rightarrow HK_{p0} + Hpt_p \rightarrow HK_{0p} + Hpt_0 \rightarrow HK_{00} + Hpt_p \\ HK_{00} \rightarrow 0 \rightarrow HK_{p0} \rightarrow 0 \rightarrow HK_{0p} \rightarrow 0 \rightarrow HK_{pp} \rightarrow 0 \end{array}$$

$$G_2: \begin{array}{c} HK_{00} \rightarrow HK_{p0} \rightarrow HK_{0p} \rightarrow HK_{pp} \rightarrow Hpt_p \rightarrow Hpt_0 \\ HK_{pp} + Hpt_0 \rightarrow HK_{p0} + Hpt_p \rightarrow HK_{0p} + Hpt_0 \rightarrow HK_{00} + Hpt_p \\ Hpt_0 \rightarrow 0 \rightarrow Hpt_p \rightarrow 0 \end{array}$$

We conclude that for appropriate choices of flow reaction rate constants, these networks admit three positive steady states in some stoichiometric compatibility class, of which two are exponentially stable and the other is exponentially unstable. Network $G_1$ models the situation in which all phosphoforms of HK are degraded or exit the system, and the non-phosphorylated form is synthesized. Similarly, network $G_2$ models the situation in which both phosphoforms of Hpt are degraded, but only the non-phosphorylated form is synthesized or enters the system.

Adding inflow reactions for the species in $X_1^o$ or $X_2^o$ does not alter the conclusion. Additionally, by joining the inflow and outflow sets of both extensions, we obtain a new extension where Theorem 8 also applies.

We investigate what happens when flow reactions for Hpt, Hpt$_p$ are added in ways that do not satisfy assumptions (a)-(c). By analyzing the resulting systems in detail, we see that if the outflow from Hpt$_p$ in $G_2$ is removed or if only outflow from Hpt and input to Hpt$_p$ are considered, then the network has at most one positive steady state. If the outflow from Hpt is removed or if only inflow and outflow reactions for Hpt$_p$ are considered, then the network has at most two positive steady states. Hence, in all four cases, the conclusions of Theorem 8 do not hold.

**Example 14.** (Double phosphorylation cycle) We consider a double phosphorylation cycle comprising a substrate $S$ with two ordered phosphorylation sites admitting three phosphoforms $S_0, S_1, S_2$ with none, one, or two phosphate groups attached respectively. We assume phosphorylation and dephosphorylation are enzyme mediated and proceed in a sequential and distributive way. This gives rise to the following reaction network $F$:

$$E + S_0 \rightleftharpoons ES_0 \rightarrow E + S_1 \rightleftharpoons ES_1 \rightarrow E + S_2$$
$$F + S_2 \rightleftharpoons FS_2 \rightarrow F + S_1 \rightleftharpoons FS_1 \rightarrow F + S_0.$$  

We order the set of species $X = \{ E, F, S_0, S_1, S_2, ES_0, ES_1, FS_2, FS_1 \}$. Under mass-action kinetics, this network is known to admit up to three positive non-degenerate steady states [21], as well as parameter choices for which there are two exponentially stable positive steady states and one exponentially unstable positive steady state [10].

A matrix of conservation laws for $F$, is

$$W_F = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \end{pmatrix}.$$
The hypotheses of Theorem 8 hold for \( \mathcal{X}^0 = \mathcal{X} \) and \( \mathcal{X}^\iota = \{E, F, S_0\} \). Hence, in particular, bistability arises for this partially open extension. This implies that we need degradation of all species, but only production of \( E, F, S_0 \).

In order to obtain even smaller sets of inflow and outflow reaction that preserve bistability, we can consider the following reduced network:

\[
E + S_0 \rightarrow ES_0 \rightarrow E + S_1 \rightarrow E + S_2 \\
F + S_2 \rightarrow F + S_1 \rightarrow F + S_0.
\]

This network admits three positive non-degenerate steady states, and whenever this is the case, two of them are exponentially stable and the other unstable [20]. The original double phosphorylation network is obtained by the addition of the intermediates \( ES_1, FS_2, FS_1 \) and after making binding reactions reversible. These two modifications are known to preserve the number and stability of the steady states [8, 11]. This reduced network admits the following matrix of conservation laws:

\[
\begin{pmatrix}
1 & 0 & 0 & 0 & 0 & 1 \\
0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 1 & 1
\end{pmatrix}.
\]

Hence the hypotheses of Theorem 8 hold for \( \mathcal{X}^0 = \{E, S_0, S_1, S_2, ES_0\} \) and \( \mathcal{X}^\iota = \{E, S_0\} \). Hence, in particular, bistability arises for this partially open extension. We proceed now to add the intermediates \( ES_1, FS_2, FS_1 \) and make binding reactions reversible to obtain the following network:

\[
E + S_0 \leftrightarrow ES_0 \rightarrow E + S_1 \leftrightarrow ES_1 \rightarrow E + S_2 \\
F + S_2 \leftrightarrow FS_2 \rightarrow F + S_1 \leftrightarrow FS_1 \rightarrow F + S_0 \\
E \leftrightarrow 0 \quad S_0 \leftrightarrow 0 \quad S_1 \rightarrow 0 \quad S_2 \rightarrow 0 \quad ES_0 \rightarrow 0,
\]

which also admits three positive non-degenerate steady states, two of which are exponentially stable. By combining Theorem 8 with previously known operations that preserve bistability, we have obtained a smaller partially open extension of the double phosphorylation cycle that also admits bistability.

Actually, the outflow set can be made even smaller. The partially open extensions given by \( \mathcal{X}^0 = \{S_0, S_1, S_2\} \) and \( \mathcal{X}^\iota = \{S_0\} \) or \( \mathcal{X}^0 = \mathcal{X}^\iota = \{E\} \) admit also three positive non-degenerate steady states. These sets do not satisfy the assumptions of Theorem 8 as there is no conservation law with support in \( \mathcal{X}^0 \), and it is not evident how it could follow from Theorem 8 after first reducing the network as we did above. However, the partially open extension with respect to the sets \( \mathcal{X}^0 = \mathcal{X}^\iota = \{E, F\} \) does not admit three positive non-degenerate steady states. This shows the subtleties in obtaining general results with respect to how to lift properties of steady states of \( F \) to partially open extensions.

4 Proof of Theorem 8

This section is devoted to prove Theorem 8.

Consider a matrix \( W_F \) of conservation laws of \( F \) of the form [5]:

\[
W_F = \begin{pmatrix} W_1 & 0 \\ 0 & W_2 \end{pmatrix} \in \mathbb{R}^{d \times n},
\]

which exists by assumption (a), after appropriately reordering the set of species (c.f. Remark 5). In particular, we reorder the species such that the species in \( \mathcal{X}^\iota \) are the first \( n_1 \) species of \( \mathcal{X} \). Let \( d_1, d_2 \) be the number of rows of \( W_1 \) and \( W_2 \), respectively, and \( n_1, n_2 \) be the number of columns of \( W_1 \) and \( W_2 \), respectively. Consider the stoichiometric matrix \( \mathcal{N}_F \in \mathbb{R}^{n \times m} \) of \( F \) of rank \( s \), and write it in block form as

\[
\mathcal{N}_F = \begin{pmatrix} N_1 \\ N_2 \end{pmatrix}, \quad N_1 \in \mathbb{R}^{n_1 \times m}, \quad N_2 \in \mathbb{R}^{n_2 \times m}.
\]
Let $s_1$ and $s_2$ be the rank of $N_1$ and $N_2$, respectively. Fix two matrices $N'_1 \in \mathbb{R}^{s_1 \times m}$ and $N'_2 \in \mathbb{R}^{s_2 \times m}$ of rank $s_1, s_2$ and such that $\ker(N'_1) = \ker(N_1)$, $\ker(N'_2) = \ker(N_2)$. Then the matrix

$$N'_F = \begin{pmatrix} N'_1 \\ N'_2 \end{pmatrix}$$

satisfies $\ker(N'_F) = \ker(N_F)$. Since the rows of $W_1$ and $W_2$ generate the left kernel of $N_1$ and $N_2$ respectively, we have

$$s_1 + s_2 = n_1 - d_1 + n_2 - d_2 = n - d = s.$$

It follows that $N'_F$ has rank $s$.

Assume $F$ has $\ell$ non-degenerate positive steady states $c_1, \ldots, c_\ell$ in the stoichiometric compatibility class with equations $W_Fx = T$ with $T \in \mathbb{R}^d$, and let $C \subseteq \mathbb{R}^n_{>0}$ be an open subset containing the steady states. The steady state equations for $F$ in the given stoichiometric compatibility class are

$$N'_F K_F(x) = 0, \quad W_Fx = T,$$

which are equivalent to

$$N'_1 K_F(x) = 0, \quad N'_2 K_F(x) = 0, \quad W_Fx = T. \quad (6)$$

Let $E_O \in \mathbb{R}^{n_1 \times n_1}$ be the matrix with zero entries except for the diagonal entries $(i, i)$ with $X_i \in X^o$, which are equal to one. Similarly, let $E_I \in \mathbb{R}^{n_1 \times n_1}$ be the matrix with zero entries except for the diagonal entries $(i, i)$ with $X_i \in X^i$, which are equal to one. By assumption (b) of Theorem 8, if the $i$-th column of $W_1$ is not zero, then the entry $(i, i)$ of $E_O$ is 1. Thus, we have that $W_1 E_O = W_1$.

Let $T_1, T_2$ be the total amounts corresponding the the conservation laws given by $W_1, W_2$, respectively, that is, $T = (T_1, T_2)$. Let $\pi_1: \mathbb{R}^n \to \mathbb{R}^{n_1}$ and $\pi_2: \mathbb{R}^n \to \mathbb{R}^{n_2}$ be the projections on the first $n_1$ components and on the last $n_2$ components, respectively. Choose $\tilde{x} \in \mathbb{R}_{>0}^{n_2}$ with support in $X^i$ and such that $W_1 \tilde{x} = T_1$. Such an $\tilde{x}$ exists by assumption (c) of Theorem 8. Note that since the support of $\tilde{x}$ is in $X^i$, we have $W_1 \tilde{x} = W_1 E_I \tilde{x}$. With this choice, consider now the map

$$H: \mathbb{R} \times C \to \mathbb{R}^{s_1} \times \mathbb{R}^{s_2} \times \mathbb{R}^{d_1} \times \mathbb{R}^{d_2} \equiv \mathbb{R}^n$$

defined by

$$H(\theta, x) = \left( N'_1 K_F(x) - \theta A E_O \pi_1(x) + \theta A E_I \tilde{x}, \quad N'_2 K_F(x), \quad W_1 \pi_1(x) - T_1, \quad W_2 \pi_2(x) - T_2 \right).$$

The function $H$ is $C^1$ and we will prove statement (i) using the Implicit Function Theorem on $H$. For that, first note that when $\theta = 0$, the equation $H(0, x) = 0$ amounts to (6). Hence, $c_1, \ldots, c_\ell \in C$ satisfy the equation $H(0, x) = 0$. Moreover,

$$\partial_x H(\theta, x) = \begin{pmatrix} N'_1 \partial_{\pi_1(x)} K_F(x) - \theta A E_O \pi_1(x) + \theta A E_I \tilde{x} \\ N'_2 \partial_{\pi_1(x)} K_F(x) \\ W_1 \\ 0 \end{pmatrix}, \quad 0 \quad W_2$$

in particular, for $\theta = 0$ we have

$$\partial_x H(0, x) = \begin{pmatrix} N'_1 \partial_x K_F(x) \\ N'_2 \partial_x K_F(x) \\ W_F \end{pmatrix} = \begin{pmatrix} N'_F \partial_x K_F(x) \end{pmatrix}.$$

This matrix is non-singular when evaluated at $c_1, \ldots, c_\ell$ as the steady states are non-degenerate by assumption, see Lemma 5. For each $i = 1, \ldots, \ell$, we apply the Implicit Function Theorem to
such that the point $(0, c_i) \in (-\varepsilon, \varepsilon) \times C$ and the function $H$, to conclude that there exists an open interval $I_i \subseteq (-\varepsilon, \varepsilon)$ containing 0, an open set $U_i \subseteq C$ containing $c_i$, and a differentiable function

$$h_i : I_i \to U_i,$$

such that for all $\theta \in I_i$, $H(\theta, h_i(\theta)) = 0$ and $h_i(0) = c_i$. Further, $I_i$ can be chosen small enough such that the map $\partial_x H(\theta, h_i(\theta))$ is non-singular for every $\theta \in I_i$, since it is non-singular at $\theta = 0$. Since all points $c_i$ are distinct, there exist pairwise disjoint open sets $V_i \subseteq C$ containing $c_i$. We redefine $U_i$ to be $U_i \cap V_i$, which contains $c_i$, and $I_i$ to be the connected component of the anti-image of $U_i$ by $h_i$ that contains 0. With these definitions, the images of the maps $h_i : I_i \to U_i$ are pairwise disjoint and the components of $h_i(\theta)$ are positive.

Consider the open interval $I = \bigcap_{i=1}^{\ell} I_i$, which contains 0. All maps $h_i$ are defined on $I$. If $\theta \in I$, then by construction

$$H(\theta, h_i(\theta)) = 0$$

for all $i = 1, \ldots, \ell$ and all $h_i(\theta)$ are distinct.

Part (i) of the theorem will follow if we show that for $\theta$ small enough and positive, $h_1(\theta), \ldots, h_\ell(\theta)$ are positive non-degenerate steady states of $G$ for a choice of reaction rate constants of the flow reactions, and that they belong to the stoichiometric compatibility class $x_0 + S_G$.

So fix $\theta > 0$ with $\theta \in I$. By construction, the stoichiometric matrix $N_G$ of $G$ is

$$N_G = \begin{pmatrix} N_1 & -E_O & E_I \\ N_2 & 0 & 0 \end{pmatrix},$$

and the matrix

$$N_G' = \begin{pmatrix} N_1 & -E_O & E_I \\ N_2 & 0 & 0 \end{pmatrix} \in \mathbb{R}^{(n_1+s_2) \times n}$$

satisfies $\ker(N_G) = \ker(N_G')$. By Lemma 7, the matrix $W_G = (0 \ 2)$ is a matrix of conservation laws for $G$. Hence the rank of $N_G$ is $n - d_2 = n_1 + s_2$. This implies that $N_G'$ has maximal rank. Further, the stoichiometric compatibility class of $G$ containing $c_1, \ldots, c_\ell$ has equations

$$W_G x = T_2. \tag{8}$$

Let $K_G^\theta$ be the kinetics of $G$ agreeing with $K_F$ for the common reactions, such that the reaction rate constant of $X_j \to 0$ is $\theta$ if $X_j \in X^\circ$, and the reaction rate constant of $0 \to X_j$ is $\theta \hat{x}_j$ for $X_j \in \hat{X}$ (where $\hat{x}$ is as defined above). Then

$$N_G' K_G^\theta(x) = \begin{pmatrix} N_1 K_F(x) - \theta E_O \pi_1(x) + \theta E_I \hat{x} \\ N_2' K_F(x) \end{pmatrix} \in \mathbb{R}^{n_1+s_2}. \tag{8}$$

Hence, the steady states of $G$ in the class defined by $\mathfrak{S}$ are the solutions to the equations

$$N_1 K_F(x) - \theta E_O \pi_1(x) + \theta E_I \hat{x} = 0, \quad W_G x - T_2 = 0, \quad N_2' K_F(x) = 0. \tag{9}$$

Consider the matrix

$$P = \begin{pmatrix} W_1 \\ A \end{pmatrix} \in \mathbb{R}^{n_1 \times n_1},$$

where $A \in \mathbb{R}^{n_1 \times n_1}$ is such that $N_1' = AN_1$ and has full rank $s_1$. The matrix $P$ is invertible since $\ker(P) = 0$. To see this, note that $W_1 x = 0$ implies $x$ belongs to the column span of $N_1$, that is $x = N_1 y$ for $y \in \mathbb{R}^m$. Then, $0 = Ax = AN_1 y = N_1' y$. As $\ker(N_1') = \ker(N_1)$, this implies $x = 0$.

Hence, equation (9) is equivalent to

$$PN_1 K_F(x) - \theta P(E_O \pi_1(x)) + \theta PE_I \hat{x} = 0.$$
By construction, as \( W_1 E_O = W_1 \), we have
\[
\begin{align*}
PN_1K_F(x) &= \begin{pmatrix} 0 \\ N_1'K_F(x) \end{pmatrix}, \\
P(E_O\pi_1(x)) &= \begin{pmatrix} W_1\pi_1(x) \\ AE_O\pi_1(x) \end{pmatrix}, \\
PE_i\tilde{x} &= \begin{pmatrix} W_1E_i\tilde{x} \\ AE_i\tilde{x} \end{pmatrix} = \begin{pmatrix} T_1 \\ AE_i\tilde{x} \end{pmatrix}.
\end{align*}
\]

Then, equation (11) holds if and only if
\[
N_1'K_F(x) - \theta AE_O\pi_1(x) + \theta AE_i\tilde{x} = 0, \quad W_1\pi_1(x) - T_1 = 0. \tag{11}
\]

Now, let \( c^* \in C \) be such that \( H(\theta, c^*) = 0 \). Then by definition \( H \), both the equations in (11), which are equivalent to (10), and the equations (10) hold. It follows that \( c^* \) is a positive steady state of \( G \) for the kinetics \( K^d_G \) in the class defined by \( S_1 \).

This gives that \( h_1(\theta), \ldots, h_\ell(\theta) \) define \( \ell \) positive steady states of \( G \) for \( \theta \in I \) and positive. Using that \( \partial_x H(\theta, h_1(\theta)) \) is non-singular, we prove that these steady state also are non-degenerate provided \( \theta \in I \) and positive. For this, fix \( c^* \in C \) such that \( H(\theta, c^*) = 0 \) and satisfying that \( \partial_x H(\theta, c^*) \) is non-singular. By Lemma 3 \( c^* \) is non-degenerate if and only if
\[
J = \begin{pmatrix} N_1\partial_{\pi_1(x)}K_F(c^*) - \theta AE_O & N_1\partial_{\pi_2(x)}K_F(c^*) \\ N_2\partial_{\pi_1(x)}K_F(c^*) & N_2\partial_{\pi_2(x)}K_F(c^*) \\ 0 & W_2 \end{pmatrix}
\]
is non-singular. Note that
\[
\begin{pmatrix} P & 0 \\ 0 & \text{id}_{n_2} \end{pmatrix} J = \begin{pmatrix} -\theta W_1 & 0 \\ N_1\partial_{\pi_1(x)}K_F(c^*) - \theta AE_O & N_1\partial_{\pi_2(x)}K_F(c^*) \\ 0 & N_2\partial_{\pi_2(x)}K_F(c^*) \\ 0 & W_2 \end{pmatrix}.
\]

As \( \begin{pmatrix} P & 0 \\ 0 & \text{id}_{n_2} \end{pmatrix} \) is invertible and the matrix on the right-hand side is non-singular by hypothesis and (4), we conclude that \( J \) is non-singular. This shows that \( c^* \) is non-degenerate.

This concludes the proof of statement (i). Statement (iii) follows from statement (ii). So all we need is to show statement (ii). Consider a steady state \( c_i \) of \( F \) and the corresponding steady state \( h_i(\theta) \) of \( G \) as above. Let \( J_F \) be the Jacobian of the species formation rate function of \( F \) evaluated at \( c_i \) and \( J_G \) the Jacobian of the species formation rate function of \( G \) evaluated at \( h_i(\theta) \). Then
\[
J_F = \begin{pmatrix} N_1\partial_x K_F(c_i) \\ N_2\partial_x K_F(c_i) \end{pmatrix}, \quad J_G = \begin{pmatrix} N_1\partial_x K_F(h_i(\theta)) - \theta E_O & 0_n_{1\times n_2} \\ N_2\partial_x K_F(h_i(\theta)) \end{pmatrix},
\]
where \( 0_{n_1\times n_2} \) is the zero matrix of size \( n_1 \times n_2 \). Then \( J_F \) has the eigenvalue 0 with multiplicity at least \( d = d_1 + d_2 \), and assume further that it has \( r_1 \) eigenvalues with positive real part, and \( r_2 \) eigenvalues with negative real part. As \( h_i(0) = c_i \), for \( \theta > 0 \) small enough, \( J_G \) also has \( r_1 \) eigenvalues with positive real part, and \( r_2 \) eigenvalues with negative real part. All that remains is to show that \( J_G \) has \( d_1 \) additional eigenvalues with negative real part.

We consider left eigenvectors of \( J_F, J_G \) for convenience. Then, as \( N_1 \) has rank \( s_1 \), \( d \) distinct linearly independent left eigenvectors \( u_1, \ldots, u_d \) with eigenvalue 0 of \( J_F \) can be chosen such that for \( j = 1, \ldots, d_1 \) we have \( \pi_1(u_j)'N_1 = 0 \), \( \pi_2(u_j) = 0 \) (where superscript \( t \) denotes the transpose vector). That is, \( \pi_1(u_j) \), for \( j = 1, \ldots, d_1 \), form a basis of the left kernel of \( N_1 \). In
particular, \( \pi_1(u_j) \) belongs to the row span of \( W_1 \): \( \pi_1(u_j) = v_j^t W_1 \) for \( v_j \in \mathbb{R}^{d_1} \). Then the equality \( v_j^t W_1 E_0 = v_j^t W_1 \) gives \( \pi_1(u_j)^t E_0 = \pi_1(u_j)^t \). Now, for \( j = 1, \ldots, d_1 \), we have

\[
W_1^t J_G = u_j^t \left( \begin{bmatrix} N_1 \partial_x K_F(h_1(\theta)) \theta E_0 & 0_{n_1 \times n_2} \\ N_2 \partial_x K_F(h_2(\theta)) \end{bmatrix} \right) 
= \pi_1(u_j)^t N_1 \partial_x K_F(h_1(\theta)) + \pi_2(u_j)^t N_2 \partial_x K_F(h_2(\theta)) - \theta \pi_1(u_j)^t \left( E_0 \ 0_{n_1 \times n_2} \right) 
= -\theta \left( \pi_1(u_j)^t E_0 \ 0_{1 \times n_2} \right) = -\theta u_j^t,
\]

where in the last step we use that \( \pi_2(u_j) = 0 \). This shows that \(-\theta\) is an eigenvalue of \( J_G \) with multiplicity at least \( d_1 \), completing the proof of statement (iii) and thereby the proof of Theorem

\[ \square \]

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References


Addition of flow reactions preserving multistationarity and bistability


