# Sign patterns for chemical reaction networks

**J. William Helton · Vitaly Katsnelson · Igor Klep**

**Abstract**    Most differential equations found in chemical reaction networks (CRNs) have the form:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = f(x) = Sv(x),$$

where $x \geq 0$, that is, $x$ lies in the nonnegative orthant $\mathbb{R}^d_{\geq 0}$, where $S$ is a real $d \times d'$ matrix (*stoichiometric* matrix) and $v$ is a column vector consisting of $d'$ real-valued functions having a special relationship to $S$. Our main interest will be in the Jacobian matrix, $f'(x)$, of $f(x)$, in particular in whether or not each entry $f'(x)_{ij}$ has the same sign for all $x$ in the orthant, i.e., the Jacobian respects a sign pattern. In other words species $x_j$ always acts on species $x_i$ in an inhibitory way or its ac-

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tion is always excitatory. In Helton et al. (SIAM J. Matrix Anal. Appl. 31:732–754, 2009) we gave necessary and sufficient conditions on the species-reaction graph naturally associated to $S$ which guarantee that the Jacobian of the associated CRN has a sign pattern. In this paper, given $S$ we give a construction which adds certain rows and columns to $S$, thereby producing a stoichiometric matrix $\widehat{S}$ corresponding to a new CRN with some added species and reactions. The Jacobian for this CRN based on $\widehat{S}$ has a sign pattern. The equilibria for the $S$ and the $\widehat{S}$ based CRN are in exact one to one correspondence with each equilibrium $e$ for the original CRN gotten from an equilibrium $\widehat{e}$ for the new CRN by removing its added species. In our construction of a new CRN we are allowed to choose rate constants for the added reactions and if we choose them large enough the equilibrium $\widehat{e}$ is locally asymptotically stable if and only if the equilibrium $e$ is locally asymptotically stable. Further properties of the construction are shown, such as those pertaining to conserved quantities and to how the deficiencies of the two CRNs compare.

**Keywords**   Sign pattern · Signed matrix · Chemical reaction network

## 1 Introduction

In this paper we are concerned with polynomial systems of equations arising from systems of ordinary differential equations (ODEs) which act on the nonnegative orthant $\mathbb{R}^d_{\geq 0}$ in $\mathbb{R}^d$:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = f(x), \tag{1.1}$$

where $f : \mathbb{R}^d_{\geq 0} \to \mathbb{R}^d$. The differential equations we address are of a special form found in chemical reaction kinetics:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = Sv(x), \tag{1.2}$$

where $S$ is a real $d \times d'$ matrix and $v$ is a column vector consisting of $d'$ real-valued functions. An ODE (1.1) has **reaction form** provided it is represented as in (1.2) with $v(x) = \begin{bmatrix} v_1 & \cdots & v_{d'} \end{bmatrix}^t$ and

$$v_j \text{ depends exactly on variables } x_i \text{ for which } S_{ij} < 0. \tag{1.3}$$

Since such ODEs are identified with chemical reaction networks, we often refer to these as CRNs. Call $S$ the **stoichiometric matrix** and the entries of $v(x)$ the **fluxes**. We always assume the fluxes are continuously differentiable. Furthermore, in many situations all fluxes $v_j(x)$ are monotone nondecreasing in each $x_i$ when the other variables are fixed, that is, $v'(x) = \left[ \frac{\partial v_i(x)}{\partial x_j} \right]_{ij}$, the Jacobian of $v$, has all entries nonnega-

tive for all $x \in \mathbb{R}^d_{>0}0$. This happens in classical mass action kinetics or for Michaelis-Menten-Hill type fluxes. See [4,18] for an exposition. We shall develop a few matrix theoretic phenomena bearing on the properties of $f'(x) = Sv'(x)$, called the **Jacobian of the reaction**. For monotone nondecreasing fluxes the reaction form property (1.3) is equivalent to

$$\frac{\partial v_j(x)}{\partial x_i} \not\equiv 0 \quad \Leftrightarrow \quad \left( \frac{\partial v_j(x)}{\partial x_i} \geq 0 \text{ and } \not\equiv 0 \right) \quad \Leftrightarrow \quad S_{ij} < 0. \tag{1.4}$$

and this is what we shall mostly be using.

The property analyzed in this paper is whether or not each entry $f'(x)_{ij}$ of the Jacobian of the CRN has an unambiguous sign, that is it is the same for all $x \in \mathbb{R}^d_{>0}$. If the sign $f'(x)_{ij}$ is minus (resp. plus), then the effect of species $j$ on species $i$ is always inhibitory (resp. excitatory). If such is the case we say that $f'$ respects a sign pattern.

## 1.1 Sign pattern of $AA^t$

Given this monotone property, we employ the language of signed matrices [5]. Call a **sign pattern** a matrix $A$ with entries which are $\pm a_{ij}$ or 0, where $a_{ij}$ are free variables. To a *real* matrix $B$ we can associate its sign pattern $A = \text{SP}(B)$ with $\pm a_{ij}$ or 0 in the correct locations. Given a matrix with *symbolic* entries (i.e., polynomials) we might or might not be able to associate a sign pattern. Here, we think of the free variables as being *positive*.

*Example 1.1* If $B = \begin{bmatrix} 0 & 6 \\ -2 & -5 \end{bmatrix}$, then $A = \text{SP}(B) = \begin{bmatrix} 0 & +a_{12} \\ -a_{21} & -a_{22} \end{bmatrix}$ and

$$AA^t = \begin{bmatrix} a_{12}^2 & -a_{12}a_{22} \\ -a_{12}a_{22} & a_{21}^2 + a_{22}^2 \end{bmatrix}.$$

Observe that $AA^t$ respects a sign pattern.

On the other hand, if $B = \begin{bmatrix} -1 & 6 \\ -2 & -5 \end{bmatrix}$, then $A = \text{SP}(B) = \begin{bmatrix} -a_{11} & +a_{12} \\ -a_{21} & -a_{22} \end{bmatrix}$ and

$$AA^t = \begin{bmatrix} a_{11}^2 + a_{12}^2 & a_{11}a_{21} - a_{12}a_{22} \\ a_{11}a_{21} - a_{12}a_{22} & a_{21}^2 + a_{22}^2 \end{bmatrix}$$

does *not* respect a sign pattern. Namely, the off-diagonal entries of $AA^t$ are not positive linear combinations of monomials in the $a_i$. They may attain positive and negative values when evaluated at appropriate positive values of the $a_{ij}$.

**Theorem 1.2** (cf. [15, Theorem 5.1]) *Let $A$ be a sign pattern. The hermitian square $AA^t$ of $A$ respects a sign pattern if and only if $A$ does not contain a $2 \times 2$ submatrix whose rows and columns can be permuted to obtain a matrix whose sign pattern agrees with the one of*

$$\begin{bmatrix} +1 & -1 \\ -1 & -1 \end{bmatrix} \quad or \quad \begin{bmatrix} -1 & +1 \\ +1 & +1 \end{bmatrix}. \tag{1.5}$$

*Such* $2 \times 2$ *matrices either contain* $3$ *minus signs and* $1$ *plus sign, or they contain* $1$ *minus sign and* $3$ *plus signs.*

*Proof of Theorem 1.2* Suppose the entry $(AA^t)_{ij}$ of $AA^t$ fails to respect a sign. As

$$(AA^t)_{ij} = \sum_k A_{ik} A^t_{kj} = \sum_k A_{ik} A_{jk},$$

this is equivalent to not all terms of the last sum having the same sign. Which is equivalent to the existence of $k, \ell$ with sign $A_{ik} = $ sign $A_{jk} \neq 0$ and sign $A_{i\ell} = -$ sign $A_{j\ell} \neq 0$. Hence the $2 \times 2$ submatrix

$$\begin{bmatrix} A_{ik} & A_{jk} \\ A_{i\ell} & A_{j\ell} \end{bmatrix}$$

of $A$ will (after a possible permutation of rows and columns) have the same sign pattern as one of the matrices in (1.5). □

1.2 Sign pattern for the Jacobian

In the language of chemical reaction networks Theorem 1.2 has an interpretation as follows:

**Theorem 1.3** *The Jacobian* $f'(x) = Sv'(x)$ *of the right hand side of a reaction form ODE (1.2) with monotone nondecreasing fluxes respects a sign pattern in the positive orthant whenever* $S$ *does not have a* $2 \times 2$ *submatrix whose rows and columns can be permuted to obtain a matrix whose sign pattern agrees with the one of*

$$\begin{bmatrix} +1 & -1 \\ -1 & -1 \end{bmatrix}. \tag{1.6}$$

*Conversely, under mass action kinetics (cf. [4,18] or Sect. 3.2), if a reversible stoichiometric matrix* $S$ *contains such a submatrix, then* $f'(x) = Sv'(x)$ *fails to respect a sign pattern on an open dense set of reversible matrices having the same sign pattern as* $S$.

Since, it is brief we review why this is true. Write $S = S_+ - S_-$ for real matrices $S_+$, $S_-$ with nonnegative entries satisfying the complimentarity property $(S_+)_{ij}(S_-)_{ij} = 0$. If the $(i, j)$th entry of $f'(x) = Sv'(x)$ does not have a sign pattern, then $(S_+v'(x))_{ij} \neq 0$ and $(S_-v'(x))_{ij} \neq 0$. As

$$(S_+v'(x))_{ij} = \sum_k (S_+)_{ik} v'(x)_{kj},$$

$(S_+ v'(x))_{ij} \neq 0$ if and only if for some $k$, $S_{ik} > 0$ and $v'(x)_{kj} \not\equiv 0$, so from the reaction form property (1.4), we get $S_{jk} < 0$. To summarize: $S_{ik} > 0$ and $S_{jk} < 0$. Similarly, $(S_- v'(x))_{ij} \neq 0$ if and only if there is some $\ell$ with $(S_-)_{i\ell} \neq 0$ and $v'(x)_{\ell j} \not\equiv 0$, so we get $S_{j\ell} < 0$. To summarize: $S_{i\ell} < 0$ and $S_{j\ell} < 0$. Taken together this implies that the $2 \times 2$ submatrix of $S$ given by rows $i$, $j$ and columns $k$, $\ell$ has the same sign pattern as the matrix (1.6), up to a permutation of rows and columns. The converse reverses this line of reasoning, with the hypothesis requiring robustness under small perturbations of $S$ to rule out fluke cancellations.

See [15, 3.1] for details and extensions.

That many CRNs have Jacobians with sign patterns is the basis for the works of Thomas [20], Kaufman [21], Soulé [19], Gouzé [13], Cinquin and Demongeot [6], Sontag [1–3], and many subsequent publications. Typically Sontag and collaborators assume this and something considerably stronger to obtain results on *globally stable* equilibria. Sontag has had the philosophy for many years that any CRN can be modelled carefully to have Jacobians respecting sign patterns.

This paper concerns CRNs whose Jacobians *do not* have a sign pattern and describes a method for transforming such a system of ODEs into a system of ODEs whose Jacobian *does* have a sign pattern, and for which the equilibria of both CRNs remain "the same" (see Sect. 3.2 for a precise formulation). We shall refer to this as the sign fixing algorithm.

## 2 Fixing the sign pattern for the Jacobian

In the first part of of this Sect. 2.1, we describe our sign fixing algorithm. In subsequent (sub)sections we show that the algorithm has several (pleasant) properties. Sections 3 and 4 show how equilibria and the classical notion of deficiency behave with respect to our algorithm.

### 2.1 An algorithm for eliminating non-signed entries of $f'$

Let $S$ be a stoichiometric matrix associated to a chemical network with a submatrix whose sign pattern coincides with that of (1.6), which Theorem 1.3 demonstrates is an obstruction for having a sign pattern. Let $A$, $B$ be the species representing the two rows of $S$ corresponding to this **bad submatrix**. Consider the two columns of $S$ belonging to this bad submatrix.

$$
S = \begin{array}{c} \\ A \\ \\ B \end{array}
\begin{array}{c} (2.1) \quad (2.2) \\ \left[ \begin{array}{cc} -p_1 & -p_3 \\ \\ p_2 & -p_4 \end{array} \right] \end{array}
$$

These yield two reactions in the network of the following form:

$$p_1 A + C_1 \rightarrow p_2 B + C_2 \qquad (2.1)$$

$$p_3 A + p_4 B + C_3 \rightarrow C_4, \qquad (2.2)$$
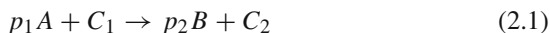
where $p_i \in \mathbb{N}$ and $C_i$ are some (possibly empty) positive linear combinations of species (avoiding $A$ and $B$).

We will construct a new network from $S$ to eliminate this bad submatrix. Consider the following network, where each reaction of the original CRN remains the same except that we add a new species $B'$, reaction (2.1) is replaced by

$$p_1 A + C_1 \rightarrow B' + C_2, \qquad (2.3)$$

and we create an additional reaction

$$B' \rightarrow p_2 B. \qquad (2.4)$$

Notice that the stoichiometric matrix $\check{S}$ associated to this new chemical network will not have the bad submatrix we started with. Also, no new bad submatrices have been added in this process. Thus we have reduced the number of bad submatrices.

$$
\check{S} =
\begin{array}{c}
\\
A \\
\\
B \\
\\
B'
\end{array}
\begin{array}{c}
(2.3) \quad (2.2) \quad (2.4)
\end{array}
$$

|  | (2.3) | (2.2) | (2.4) |
|---|---|---|---|
|  |  |  | $0$ |
| $A$ | $-p_1$ | $-p_3$ | $0$ |
| $B$ | $0$ | $-p_4$ | $p_2$ |
|  |  |  | $0$ |
| $B'$ | $0$ | $1$ | $0$ | $0$ | $-1$ |

We continue applying the same procedure (on this new network) to eliminate any other existing bad $2 \times 2$ submatrices.

In matrix terms, each time we apply this procedure to eliminate a bad submatrix, we change one column [e.g., changing reaction (2.1) into (2.3)] of $S$ and we append one additional row (e.g., for the "species" $B'$) and column [e.g., for the reaction (2.4)] to $S$. We will call this procedure the **sign fixing algorithm**.

**Definition 2.1** Let $S$ be a stoichiometric matrix corresponding to a chemical network, and suppose $S$ has bad submatrices. We write $\widehat{S}$ for a new stoichiometric matrix with no bad submatrices obtained by the sign fixing algorithm applied to each bad submatrix as explained above. $\widehat{S}$ is called a **sign fixing matrix** of $S$.

Each step of the sign fixing algorithm has the interpretation that we are keeping track of additional information. Namely, we measure how much of species $B$ is produced

by reaction (2.1) thereby obtaining $B'$ and also we measure how much of species $B$ is consumed by reaction (2.2). Of course, in the original CRN we just kept track of the net amount of $B$, a single species, now we have replaced this with two species. The added reaction has the effect of identifying the two species asymptotically.

The above definition implies that if $S$ has no bad submatrices, then $S = \widehat{S}$ is the sign fixing matrix of itself. If $S$ has multiple bad submatrices, we obtain $\widehat{S}$ by a finite number of applications of the sign fixing algorithm.

We emphasize what we have found so far by stating.

**Theorem 2.2** *Given a CRN one can derive a sign fixed CRN. The Jacobian of the sign fixed CRN respects a sign pattern, provided each flux $v_j$ is monotone increasing on each $x_i$.*

This follows from Theorem 1.3 because we have produced a sign fixed CRN $\widehat{S}$ not containing the forbidden pattern (1.6).

*Example 2.3* We now present a modification of Example [8, Table 1.1.(v)] of a CRN who's Jacobian fails to have a sign pattern, and we illustrate use of the sign fixing algorithm to create a network who's Jacobian has a sign pattern. Consider the network:

$$A + B \rightarrow F \tag{2.5}$$
$$A + C \rightarrow G \tag{2.6}$$
$$C + D \rightleftharpoons B \tag{2.7}$$
$$C + E \rightleftharpoons 2D \tag{2.8}$$

The stoichiometric matrix for this network (with species arranged in alphabetical order) is

$$S = \begin{bmatrix} -1 & -1 & 0 & 0 & 0 & 0 \\ -1 & 0 & 1 & -1 & 0 & 0 \\ 0 & -1 & \mathbf{-1} & 1 & \mathbf{-1} & \mathbf{1} \\ 0 & 0 & \mathbf{-1} & 1 & \mathbf{2} & \mathbf{-2} \\ 0 & 0 & 0 & 0 & -1 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \end{bmatrix}.$$

The boldface entries are those appearing in bad submatrices of $S$, of which there are two:

- $B_1$, corresponds to species $C$, $D$ and the forward reactions of (2.7) and (2.8).
- $B_2$, corresponds to species $C$, $D$ and the forward reaction of (2.7) and the reverse reaction of (2.8).

The first step of the sign fixing algorithm eliminates $B_2$ and gives

$$\check{S} = \begin{bmatrix}
-1 & -1 & 0 & 0 & 0 & 0 & 0 \\
-1 & 0 & 1 & -1 & 0 & 0 & 0 \\
0 & -1 & -\mathbf{1} & 1 & -\mathbf{1} & \mathbf{1} & 0 \\
0 & 0 & -\mathbf{1} & 1 & 0 & -\mathbf{2} & 2 \\
0 & 0 & 0 & 0 & -1 & 1 & 0 \\
1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & -1
\end{bmatrix}.$$

The second step eliminates $B_1$ and gives

$$\widehat{S} = \check{S} = \begin{bmatrix}
-1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
-1 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\
0 & -1 & -\mathbf{1} & 1 & -\mathbf{1} & 0 & 0 & 1 \\
0 & 0 & -\mathbf{1} & 1 & 0 & -\mathbf{2} & 2 & 0 \\
0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 \\
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & -1 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & -1
\end{bmatrix},$$

Thus $\widehat{S}$ is a sign fixing matrix for $S$. □

## 2.2 The story in terms of graphs

To $S$ one often associates a bipartite graph $\mathcal{G}_S$. One set of nodes is rows R$i$ (chemical species) the other set of nodes is columns C$j$ (reactions). If $S_{ij}$ has a $-$ sign (resp. $+$), then the arrow points from R$i$ into C$j$ (resp. out of C$j$ into R$i$). The arrow is from R$i$ into C$j$ (resp. out of C$j$ into R$i$) if species $i$ is consumed (resp. produced) in reaction $j$, respectively. Which leads us to refer to **consumed** and **produced edges**. Also we make a distinction between dotted and solid edges, the convention being that all dotted edges touching a particular C$j$ are either all consumed or all produced. Likewise for solid edges. This does not determine the choice solid versus dotted uniquely, for example, completely switching the choice of solid and dotted carries the same information. If no reaction is reversible this would be redundant information with the direction of arrows, but for reversible reactions dotted versus solid is needed.

   This graph is a simplified version of the species-reaction graph used in [9]. Theorem 1.2 (see also the paragraph following it) in this languages says

**Theorem 2.4** *The Jacobian $f'(x) = Sv'(x)$ of the right hand side of a reaction form ODE (1.2) with monotone nondecreasing fluxes respects a sign pattern in the positive orthant whenever the graph $\mathcal{G}_S$ does not contain a cycle of length four with three consumed edges and one produced edge. That is the CRN contains two reactions and*

*two species, one reaction consumes both species while the other reaction consumes one species and produces the other.*

The sign fixing algorithm takes a length four cycle $\mathcal{C}$ as in the theorem and "breaks it" by

1. removing an edge from the cycle
2. adding a reaction node C* and species node R* and two edges to the graph

thereby converting $\mathcal{C}$ to "harmless" a cycle of length six.

*Example 2.5* We return to Example 2.3 and observe that its graph is
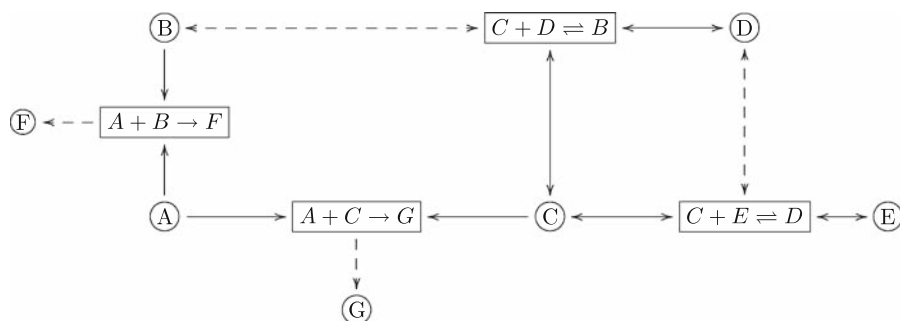


(Instead of R$i$ and C$j$ more descriptive names have been used to denote the nodes.)

## 2.3 Uniqueness of the sign fixing matrix

Suppose we have a CRN with stoichiometric matrix $S$. The sign fixing matrix $\widehat{S}$ of $S$ is non-unique, since it depends on the indexing of the bad submatrices of $S$. It turns out that this non-uniqueness is easy to classify. One finds that any two sign fixing matrices for $S$ can be gotten from the other by "conjugation" with a permutation matrix in a certain class which we shall describe completely.

Suppose $B = \{b_1, \ldots, b_n\}$ are the bad submatrices of $S$. Then $\widehat{S}$ is determined by the finite sequence $S = S_0, S_1, \ldots, S_{n-1}, S_n = \widehat{S}$, where $S_j$ is the sign fixing matrix of $S_{j-1}$ with respect to $b_j$. Another problem arises if $b_i$ and $b_j$ share the same positive entry in $S$. Namely, in this case, $S_j$ will equal $S_{j-1}$ by construction, if $i < j$. To resolve this problem, we introduce an equivalence relation on $B$: $b_i \sim b_j$ if and only if $b_i$ and $b_j$ share a positive entry of $S$. This is an equivalence relation by construction. We will use $\mathcal{B}$ to denote the set of all equivalence classes of bad submatrices of $S$, and from now on, we identify each bad submatrix of $S$ with its equivalence class. Thus, the number of new columns and rows in $\widehat{S}$ is precisely card$(\mathcal{B})$.

Suppose $\mathcal{B} = \{b_1, \ldots, b_n\}$. Let Sym$_n$ be the symmetric group on $\{1, 2, \ldots, n\}$, i.e., the set of all permutations of $n$ elements. Every $\sigma \in$ Sym$_n$ determines a sign fixing matrix $\widehat{S}_\sigma$ of $S$ by the finite sequence $S, S_{\sigma,1}, \ldots, S_{\sigma,n-1}, S_{\sigma,n} = \widehat{S}_\sigma$, where $S_{\sigma,1}$ is the sign fixing matrix of $S$ with respect to $b_{\sigma(1)}$, and $S_{\sigma,j}$ is the sign fixing matrix of $S_{\sigma,j-1}$ with respect to $b_{\sigma(j)}$. Clearly, each sign fixing matrix of $S$ is determined by a

permutation of $\mathcal{B}$, and hence by an element in $\mathrm{Sym}_n$. We thus identify the set of all sign fixing matrices of $S$ with the elements of $\mathrm{Sym}_n$.

Our result in this subsection gives a map from one sign fixing matrix to another.

**Theorem 2.6** *Suppose S is a d × d′ stoichiometric matrix corresponding to a CRN with n pairwise nonequivalent bad submatrices. If σ, τ ∈ $\mathrm{Sym}_n$ then*

$$\widehat{S}_\sigma = \begin{bmatrix} I_d & 0 \\ 0 & P \end{bmatrix} \widehat{S}_\tau \begin{bmatrix} I_{d'} & 0 \\ 0 & P \end{bmatrix}^t,$$

*where P is the n × n permutation matrix associated to $\tau^{-1}\sigma \in \mathrm{Sym}_n$.*

Let $C$ be the matrix obtained by substituting each of the $n$ positive entries of $S$ corresponding to the $n$ bad submatrices by 0. By construction, each sign fixing matrix of $S$ will have the form

$$S_\sigma = \begin{bmatrix} C & M_\sigma \\ N_\sigma & -I_n \end{bmatrix},$$

where $N_\sigma$ is a $n \times d'$ matrix whose rows correspond to the bad submatrices in the order determined by $\sigma$, and $M_\sigma$ is a $d \times n$ matrix whose columns correspond to the bad submatrices in the order determined by $\sigma$. More precisely, the $i$th row of $N_\sigma$ is the unit vector $e'_j$ of length $d'$ if the bad submatrix $b_{\sigma(i)}$ has a positive entry in column $j$ of $S$. Similarly, the $i$th column of $M_\sigma$ is a multiple of the unit vector $e_j$ of length $d$ if the bad submatrix $b_{\sigma(i)}$ has a positive entry in row $j$ of $S$. The multiple is the value of $S$ at this positive entry.

*Proof of Theorem 2.6* Note that

$$\begin{bmatrix} I & 0 \\ 0 & P \end{bmatrix} \widehat{S}_\tau \begin{bmatrix} I & 0 \\ 0 & P \end{bmatrix}^t = \begin{bmatrix} C & M_\tau P^t \\ P N_\tau & -I_n \end{bmatrix},$$

so we only need to prove $M_\tau P^t = M_\sigma$ and $P N_\tau = N_\sigma$ for the given $P$.

Suppose $\mathcal{B} = \{b_1, \ldots, b_n\}$, and $\varepsilon \in \mathrm{Sym}_n$ is the identity permutation. With the notation above, let

$$N_\varepsilon = \begin{bmatrix} \alpha_1 \\ \vdots \\ \alpha_n \end{bmatrix} \quad \text{and} \quad M_\varepsilon = \begin{bmatrix} \beta_1 & \cdots & \beta_n \end{bmatrix},$$

where $\alpha_i$, $i = 1, \ldots, n$ are the rows of $N_\varepsilon$, and $\beta_i$, $i = 1, \ldots, n$ are the columns of $M_\varepsilon$. Given $\sigma \in \mathrm{Sym}_n$, we have

$$N_\sigma = \begin{bmatrix} \alpha_{\sigma(1)} \\ \vdots \\ \alpha_{\sigma(n)} \end{bmatrix} \quad \text{and} \quad M_\sigma = \begin{bmatrix} \beta_{\sigma(1)} & \cdots & \beta_{\sigma(n)} \end{bmatrix}.$$

The $n \times n$ permutation matrix associated to $\tau^{-1}\sigma$ is

$$P = \begin{bmatrix} e_{(\tau^{-1}\sigma)(1)} \\ \vdots \\ e_{(\tau^{-1}\sigma)(n)} \end{bmatrix},$$

where $e_i$ denotes the unit vector of length $n$ with a one in the $i$th coordinate and 0's elsewhere. By construction, $PN_\tau$ is a matrix whose $i$th row is the $(\tau^{-1}\sigma)(i)$th row of $N_\tau$, so the $i$th row of $PN_\tau$ is $\alpha_{\tau((\tau^{-1}\sigma)(i))} = \alpha_{\sigma(i)}$. Hence $N_\sigma = PN_\tau$ as desired.

To conclude the proof let us verify $M_\sigma = M_\tau P^t$. Notice that $M_\tau P^t$ is a matrix whose $i$th column is the $(\tau^{-1}\sigma)(i)$th column of $M_\tau$, so the $i$th column of $M_\tau P^t$ is $\beta_{\tau((\tau^{-1}\sigma)(i))} = \beta_{\sigma(i)}$. This implies $M_\sigma = M_\tau P^t$.                          □

## 3 Equilibria behave well under sign fixing

Our next goal is to analyze how equilibria for the original CRN compare to equilibria for a sign fixed CRN. We shall find that the equilibria are in perfect correspondence. The key to this is a simple fact in linear algebra which constitutes the next subsection.

### 3.1 Linear algebra associated to sign fixing

We now show how the nullspace of $S$ and the nullspace of $\check{S}$ are related. Likewise for the range of $S$ versus the range of $\check{S}$.

**Proposition 3.1** *Let $S$ be a stoichiometric matrix with a bad submatrix. Let $\check{S}$ be obtained from $S$ by applying the sign fixing algorithm to eliminate this bad submatrix. Then* $\dim \ker S = \dim \ker \check{S}$ *and* $\dim \ker S^t = \dim \ker \check{S}^t$.

*Indeed, there is a precise correspondence: given $v \in \ker S$ there exists a unique $v_\infty \in \mathbb{R}$ with $\check{v} = \begin{bmatrix} v^t & v_\infty \end{bmatrix}^t \in \ker \check{S}$. Conversely, for $\check{v} = \begin{bmatrix} v^t & v_\infty \end{bmatrix}^t \in \ker \check{S}$, one has $v \in \ker S$. A similar statement holds for the left kernels. Furthermore, under this correspondence $v$ has positive (resp. nonnegative) entries if and only if $\check{v}$ does.*

*Proof* Let $p$ and $q$ be the rows of $S$, and let $k$ and $\ell$ be the columns of $S$ corresponding to the bad submatrix. Without loss of generality assume that the bad submatrix has the same sign pattern as $\begin{bmatrix} -1 & -1 \\ -1 & +1 \end{bmatrix}$. For the sake of exposition, here is a picture:

Suppose $S \in \mathbb{R}^{d \times d'}$ and let $v \in \ker S$. Then clearly $\check{v} = \begin{bmatrix} v^t & v_\ell \end{bmatrix}^t \in \ker \check{S}$. Moreover, if $v \in \mathbb{R}_{>0}^{d'}$, then $\check{v} \in \mathbb{R}_{>0}^{d'+1}$. Conversely, every $\check{v} = \begin{bmatrix} v^t & v_\infty \end{bmatrix}^t \in \ker \check{S}$ satisfies $\check{v}_\ell = v_\infty$ and so gives rise to $v \in \ker S$. Again, positivity is preserved. The corresponding analogous statements and proofs for the left kernel are left as an exercise for the reader.                                                                         □

*Remark 3.2* After applying one step of the sign fixing algorithm the number of bad submatrices decreases. More precisely, let $S$ be a stoichiometric matrix with a bad submatrix. Let $\check{S}$ be obtained from $S$ by applying the sign fixing algorithm to eliminate this bad submatrix. Then the number of bad submatrices in $\check{S}$ is less than the number of those in $S$.

Indeed, the form of $\check{S}$, because the added row and column have all entries but two equal to zero, guarantees $\check{S}$ does not contain the one bad submatrix under attack, and at the same time no new bad submatrices have been added. Thus we have reduced the number of bad submatrices by at least one.                                          □

*Remark 3.3* The sign fixing algorithm for the situation of the hermitian square $AA^t$ of a sign pattern $A$, goes just as in Sect. 2.1 with $A$ replacing $S$ in the picture in the proof of Proposition 3.1.

Details are left as an exercise for the interested reader.                          □

## 3.2 Behavior of equilibria and steady states in mass action kinetics under sign fixing

This subsection uses the linear algebra result of the previous subsection to show that the equilibria of a CRN and of its sign fixed CRN are in perfect correspondence. We shall show this for mass action kinetics, although as one will see from the arguments here it works for a much more general class of CRNs.

We now review mass action kinetics with the primary aim of introducing our notation. The postulate of mass action kinetics is *"the reaction rate is proportional to reactant concentrations"*. For instance, for the chemical reaction

$$2A + B \to 4C$$

the reaction rate is $k_{2A+B \to 4C} x_A^2 x_B$, where $x$ denotes the **concentration** of a species and $k_{2A+B \to 4C} > 0$ is the **rate constant**. The corresponding ODE is

$$\begin{bmatrix} \dot{x}_A \\ \dot{x}_B \\ \dot{x}_C \end{bmatrix} = \begin{bmatrix} -2k_{2A+B \to 4C} x_A^2 x_B \\ -k_{2A+B \to 4C} x_A^2 x_B \\ 4k_{2A+B \to 4C} x_A^2 x_B. \end{bmatrix} = \begin{bmatrix} -2 \\ -1 \\ 4 \end{bmatrix} \begin{bmatrix} k_{2A+B \to 4C} x_A^2 x_B \end{bmatrix}.$$

In general, for $S \in \mathbb{R}^{d \times d'}$ the flux vector $v(x)$ is given by

$$v(x)_i = k_i \prod_{j=1}^{d} x_j^{-\min\{0, S_{ji}\}}, \quad i = 1, \dots, d'.$$

(Here $k_i > 0$ is the rate constant associated to the $i$th reaction and $x_j$ is the concentration of the $j$th species.)

If the ODE (1.1) admits a positive vector in the left kernel (i.e., there exists $m \in \mathbb{R}^d_{>0}$ with $m \cdot \dot{x} = m \cdot f(x) = 0$), then the ODE is called **conserving**. This reflects quantities (like the mass or the number of carbon atoms) being conserved. An obvious sufficient condition for ODEs of the form (1.2) is ker $S^t \cap \mathbb{R}^d_{>0} \neq \{0\}$. If this is satisfied, we say that $S$ is **conserving**. By Proposition 3.1 this condition is preserved under the sign fixing algorithm.

**Corollary 3.4** *Let S be a stoichiometric matrix, and suppose S has a bad submatrix. Let $\check{S}$ be obtained from S by applying the sign fixing algorithm to eliminate this bad submatrix. If S is conserving, then so is $\check{S}$. Moreover, the reaction form differential equations (1.2) associated under mass action kinetics to S and to $\check{S}$, respectively, have the same equilibria in the following sense.*

*Suppose $S \in \mathbb{R}^{d \times d'}$. If $\check{x} = \begin{bmatrix} x^t & x_\infty \end{bmatrix}^t \in \mathbb{R}^{d+1}_{>0}$ (resp. $\check{x} \in \mathbb{R}^{d+1}_{\geq 0}$) satisfies $\check{S}\check{v}(\check{x}) = 0$, then $Sv(x) = 0$. Conversely, if $x \in \mathbb{R}^d_{>0}$ (resp. $x \in \mathbb{R}^d_{\geq 0}$) satisfies $Sv(x) = 0$, then there exists a unique $x_\infty \in \mathbb{R}_{>0}$ (resp. $x_\infty \in \mathbb{R}_{\geq 0}$) with $\check{S}\check{v}(\check{x}) = 0$ for $\check{x} = \begin{bmatrix} x^t & x_\infty \end{bmatrix}^t$. (Here $\check{v}$ will be used to denote a flux vector associated under mass action kinetics to $\check{S}$.)*

*Proof* This is essentially a consequence of Proposition 3.1 and the figure contained in its proof describes the notation we now use. Let $p$ and $q$ be the rows of $S$, and let $k$ and $\ell$ be the columns of $S$ corresponding to the bad submatrix, and assume without loss of generality $S_{q\ell} > 0$.

If $\check{v}(\check{x}) \in$ ker $\check{S} \cap \mathbb{R}^{d'+1}_{>0}$ and $\check{x} = \begin{bmatrix} x^t & x_\infty \end{bmatrix}^t$, then (by construction) the first $d'$ entries of $\check{v}(\check{x})$ coincide with $v(x)$, that is,

$$\check{v}(\check{x})_i = v(x)_i, \quad i = 1, \ldots, d'.$$

Additionally,

$$0 = \dot{\check{x}}_{d+1} = \check{v}(\check{x})_\ell - \check{v}(\check{x})_{d'+1} \tag{3.1}$$

so we obtain

$$\check{v}(\check{x})_{d'+1} = \check{v}(\check{x})_\ell = v(x)_\ell. \tag{3.2}$$

Note that by construction, $\check{v}(\check{x})_{d'+1}$ depends only on $x_\infty$ and thus we can solve (3.2) for $x_\infty$ uniquely. Hence

$$0 = \dot{\check{x}}_q = \sum_{i=1}^{d'+1} \check{S}_{qi} \check{v}(\check{x})_i = \sum_{\substack{i=1 \\ i \neq \ell}}^{d'} S_{qi} v(x)_i + S_{q\ell} \check{v}(\check{x})_{d'+1}$$

$$= \sum_{\substack{i=1 \\ i \neq \ell}}^{d'} S_{qi} v(x)_i + S_{q\ell} v(x)_\ell = \sum_{i=1}^{d'} S_{qi} v(x)_i.$$

For $s \neq q$,

$$0 = \dot{\check{x}}_s = \sum_{i=1}^{d'+1} \check{S}_{si} \check{v}(\check{x})_i = \sum_{i=1}^{d'} S_{si} v(x)_i$$

proving $Sv(x) = 0$ (Alternatively, the conclusion can be reached by the proof of Proposition 3.1). The calculation above reverses to show that converses of these implications hold as well.                                                                                      □

**Theorem 3.5** *Let S be a stoichiometric matrix corresponding to a chemical reaction network. Then the reaction form differential equations corresponding to S and to its sign fixing matrix $\widehat{S}$ under mass action kinetics have the equilibria which are equivalent under the correspondence in Corollary 3.4.*

*Proof* This follows easily from Corollary 3.4 and Remark 3.2 by an induction on the number of bad submatrices of $S$.                                                                      □

*Remark 3.6* Theorem 3.5 and Corollary 3.4 extend to more general, reaction form ODEs (1.2) with monotone fluxes. In one step of the algorithm the key is to add a reaction consuming exactly one (new) species (variable) $x_\infty$. Since this is an artificial reaction we can specify a flux $\check{v}(\check{x})_{d'+1}$ and the key is to pick it to be monotone and *surjective*, e.g., it depends only on $x_\infty$ and is linear. This ensures the solvability of (3.2) for $x_\infty$. The uniqueness of $x_\infty$ is then guaranteed by the monotone property. Under these assumptions both proofs work verbatim.                                                                      □

3.3 Local stability is preserved by sign fixing

In the previous subsection we showed that the equilibria of the original CRN sit in a perfect correspondence with those of the sign fixed CRN. An important question is whether or not stability of an equilibrium of the original CRN implies stability of the corresponding equilibrium of the sign fixed CRN. This question is open to interpretation because the sign fixing CRN contains a rate constant which we are allowed to define. Let us call this rate constant $k$. A natural version of the question would be: is there an *a priori* choice of $k$ such that the equilibrium of the original CRN is stable if and only if the corresponding equilibrium is stable for the sign fixed CRN. While we have not analyzed global stability, we have analyzed and answered the question for *local* asymptotic stability. We found that if we choose $k$ large enough, then one of the eigenvalues of the sign fixed Jacobian will be very negative, and all the others will be close to the eigenvalues of the Jacobian of the original CRN. Recall that a matrix is said to be **stable** if all its eigenvalues have negative real part. An equilibrium $x_0 \in \mathbb{R}^d_{\geq 0}$ of an ODE of the form (1.1) is **locally asymptotically stable** if the matrix $f'(x_0)$ is stable.

As before, we assume mass action kinetics although this assumption can be weakened to reaction form ODEs (1.2) with monotone entrywise surjective fluxes.

**Theorem 3.7** *Let S be a $d \times d'$ stoichiometric matrix with a bad submatrix. Let $\check{S}$ be obtained from S by applying the sign fixing algorithm to eliminate this bad submatrix. Write $J(x) = Sv'(x)$ and $\check{J}_k(\check{x}) = \check{S}\check{v}'(\check{x})$. Here k denotes the rate constant assigned to the additional reaction created in the sign fixing algorithm. Fix a point $\check{x} \in \mathbb{R}_{\geq 0}^{d+1}$ and let $x \in \mathbb{R}_{\geq 0}^{d}$ denote its first d components. Furthermore, let $J = J(x)$ and $\check{J}_k = \check{J}_k(\check{x})$.*

*Then d of the eigenvalues of the $(d+1) \times (d+1)$ Jacobian matrix $\check{J}_k$ (counting multiplicity) converge (as $k \to \infty$) to the d eigenvalues of J and the remaining eigenvalue is real and converges to $-\infty$.*

Without loss of generality, we may assume the bad submatrix in $S$ is the $2 \times 2$ bottom right block and $S_{d,d'} > 0$. Then the relationship between the $d \times d$ matrix $J = Sv'(x)$ and the $(d+1) \times (d+1)$ matrix $\check{J}_k = \check{S}\check{v}'(\check{x})$ is as follows:

$$
\check{J}_k = \begin{bmatrix}
J_{1,1} & \cdots & J_{1,d} & 0 \\
\vdots & \ddots & \vdots & \vdots \\
J_{d-1,1} & \cdots & J_{d-1,d} & 0 \\
J_{d,1} - S_{d,d'}\frac{\partial v_{d'}}{\partial x_1} & \cdots & J_{d,d} - S_{d,d'}\frac{\partial v_{d'}}{\partial x_d} & kS_{d,d'} \\
\frac{\partial v_{d'}}{\partial x_1} & \cdots & \frac{\partial v_{d'}}{\partial x_d} & -k
\end{bmatrix}
$$

$$
= \begin{bmatrix}
 & & 0 \\
 & J & 0 \\
 & & 0 \\
0 & 0 & 0 & 0
\end{bmatrix} + \begin{bmatrix}
0 \\
0 \\
-S_{d,d'} \\
1
\end{bmatrix} \begin{bmatrix} \frac{\partial v_{d'}(x)}{\partial x_1} & \cdots & \frac{\partial v_{d'}(x)}{\partial x_d} & -k \end{bmatrix}.
$$

Let $c(\lambda) = \det(J - \lambda I_d) \in \mathbb{R}[\lambda]$ and $c_k(\lambda) = \det(\check{J}_k - \lambda I_{d+1}) \in \mathbb{R}[\lambda]$ denote the characteristic polynomials (in $\lambda$) of $J$ and $\check{J}_k$, respectively.

**Lemma 3.8** *The degree $d + 1$ polynomials $\frac{1}{k}c_k$ converge uniformly on compact subsets of $\mathbb{C}$ to the degree d polynomial $-c$.*

*Proof* Let us consider $\check{J}_k - \lambda I$ and its determinant. For notational convenience let us write $s = S_{d,d'}$ and $v_{d',j} = \frac{\partial v_{d'}}{\partial x_j}$. Then

$$
c_k = \det(\check{J}_k - \lambda I) = \det \begin{bmatrix}
J_{1,1} - \lambda & \cdots & J_{1,d} & 0 \\
\vdots & \ddots & \vdots & \vdots \\
J_{d-1,1} & \cdots & J_{d-1,d} & 0 \\
J_{d,1} - sv_{d',1} & \cdots & J_{d,d} - sv_{d',d} - \lambda & ks \\
v_{d',1} & \cdots & v_{d',d} & -k - \lambda
\end{bmatrix}
$$

$$
= \det \begin{bmatrix}
J_{1,1} - \lambda & \cdots & J_{1,d} & 0 \\
\vdots & \ddots & \vdots & \vdots \\
J_{d-1,1} & \cdots & J_{d-1,d} & 0 \\
J_{d,1} & \cdots & J_{d,d} - \lambda & -s\lambda \\
v_{d',1} & \cdots & v_{d',d} & -k - \lambda
\end{bmatrix}
$$

$$= (-k - \lambda)c + s\lambda \ \det \underbrace{\begin{bmatrix} J_{1,1} - \lambda & \cdots & J_{1,d-1} & J_{1,d} \\ \vdots & \ddots & \vdots & \vdots \\ J_{d-1,1} & \cdots & J_{d-1,d-1} - \lambda & J_{d-1,d} \\ v_{d',1} & \cdots & v_{d',d-1} & v_{d',d} \end{bmatrix}}_{h}$$

Thus

$$\det(\check{J}_k - \lambda I) = c_k = (-k - \lambda)c + s\lambda h = (-k - \lambda) \ \det J \ + \ s\lambda h. \quad (3.3)$$

Note $h \in \mathbb{R}[\lambda]$ is a polynomial of degree $\leq d - 1$ in $\lambda$ and does not contain $k$. Thus

$$\frac{1}{k}c_k = \frac{-k - \lambda}{k}c - \frac{1}{k}s\lambda h \xrightarrow{k \to \infty} -c$$

uniformly on compact subsets of $\mathbb{C}$. □

In fact, the polynomial $h$ from the proof of Lemma 3.8 is of degree $\leq d - 2$. Since the ODEs are in reaction form, $S_{d,d'} > 0$ implies $v_{d',d} = \frac{\partial v_{d'}}{\partial x_d} = 0$, cf. (1.4).

*Proof of Theorem 3.7* Let $x_j^k, m_j^k$ denote the zeroes of $c_k$ together with their multiplicities and $x_j, m_j$ denote the zeroes of $c$. Certainly $c_k$ is analytic in the complex variable $\lambda$, thus $d$ zeroes of $c_k$ (counting multiplicity) converge to the zeroes of $c$. This is a standard consequence of the argument principle, since we can put a small circle $C_\varepsilon$ around a zero of $x_j$ and for large enough $k$ the winding number (with respect to 0) of $\frac{1}{k}c_k$ on $C_\varepsilon$ equals that of $c$. Thus $c$ and $c_k$ have the same number of zeroes inside $C_\varepsilon$.

Similarly, to analyze the point at infinity, one can draw a circle $C_R$ of arbitrarily large radius $R$ containing all zeroes of $c$. The winding number (with respect to 0) of $c$ around $R$ is $d$, so for large enough $k$ the winding number of $\frac{1}{k}c_k$ is also $d$, thus one zero of $c_k$, without loss of generality denote it $x_{d+1}^k$ lies outside of $C_R$. Hence the sequence $x_{d+1}^k$ diverges to infinity. Since all coefficients of the polynomial $c_k$ are real, its zeroes are either real or occur in conjugate pairs. So $x_{d+1}^k$ must be real, since if not $c_k$ would have two zeroes outside of $C_R$.

Let us retain the notation from the proof of Lemma 3.8. Then

$$c_k = (-k - \lambda)c + s\lambda h \in \mathbb{R}[\lambda],$$

where $c \in \mathbb{R}[\lambda]$ is of degree $d$, $h \in \mathbb{R}[\lambda]$ is of degree $\leq d - 1$, and $s \in \mathbb{R}$. Thus for $\lambda > 0$ big enough, $-\lambda c$ dominates $s\lambda h$. For such $\lambda > 0$ the sign of $c_k(\lambda)$ will equal the sign of $-c(\lambda)$ for any $k > 0$. This shows that with $R$ big, the zero $x_{d+1}^k$ of $c_k$ outside of $C_R$ must be negative, thereby concluding the proof. □

**Theorem 3.9** *Let $S$ be a stoichiometric matrix corresponding to a CRN and let $\widehat{S}$ be one of its sign fixing matrices. Then there exists a choice of rate constants for the added reactions such that the equilibria of the reaction form ODEs corresponding to*

*S are locally asymptotically stable if and only if the same holds for the equilibria of the reaction form ODEs corresponding to* $\widehat{S}$.

*Proof* This follows easily from Theorem 3.7 by an induction on the number of bad submatrices in *S*. □

### 3.4 Craciun–Feinberg theory: determinants of Jacobians

This brief subsection is for those familiar with the Craciun–Feinberg (CF) theory [8,9] and we observe that it behaves well under sign fixing. Recall the key requirement of the CF theory in order to invoke its consequences is that the determinant $\Delta$ of the Jacobian has an unambiguous sign. Note from (3.3) with $\lambda = 0$ that the determinant of the Jacobian of the sign fixed CRN is a scalar times $\Delta$. Thus one determinant has an unambiguous sign if and only if the other one does.

The conclusion is that if the CF theory applies to a CRN, then it applies to the closely related CRN whose Jacobian respects a sign pattern.

## 4 Deficiency versus sign patterns

An important notion in chemical networks is that of **deficiency**. In this section we show that sign fixing might increase the deficiency of a CRN by at the most the number of bad submatrices for the stoichiometric matrix of the original CRN.

We follow the notation and terminology of Gunawardena [14] (or see [7,12]). Thus, we denote:

$$n := \text{the number of complexes of the network,}$$
$$\ell := \text{the number of linkage classes of the network,}$$
$$s := \text{the rank of the stoichiometric matrix,}$$

and the **topological deficiency** of the network is

$$\delta := n - \ell - s.$$

### 4.1 Zero deficiency versus sign patterns

A natural question is whether the sign pattern of $f'(x) = Sv'(x)$ has any correlation to the CRN having zero deficiency. The answer is no, and in this subsection we give examples of

1.  Chemical networks *S* with zero deficiency and no sign pattern for $Sv'(x)$;
2.  Chemical networks *S* with nonzero deficiency and a sign pattern for $Sv'(x)$.

*Example 4.1* (see [17, 4.3] for more details) Consider the reaction network

$$A \rightarrow B$$

$$B \to C$$
$$C \rightleftharpoons A + B.$$
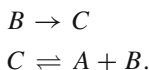
The deficiency of the CRN is easily seen to be zero. However,

$$S = \begin{bmatrix} -1 & 0 & -1 & 1 \\ 1 & -1 & -1 & 1 \\ 0 & 1 & 1 & -1 \end{bmatrix}$$

so $Sv'(x)$ will not respect a sign pattern (Theorem 1.2). It will have exactly one entry without a sign. In order to obtain an example of a deficiency zero network with an arbitrary number of non-signed entries in the Jacobian, one simply considers a network with the following stoichiometry:

$$\begin{bmatrix} S & & & \\ & S & & \\ & & \ddots & \\ & & & S \end{bmatrix}.$$

Conversely, having a sign pattern will not yield any information about the deficiency of the network.

*Example 4.2* Consider the chemical reaction network

$$B + C \rightleftharpoons A \rightleftharpoons B' + C'$$
$$B \rightleftharpoons B' \rightleftharpoons C \rightleftharpoons C'$$
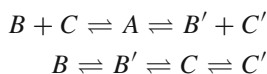
with stoichiometric matrix

$$S = \begin{bmatrix} -1 & -1 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 1 & 0 & -1 & 0 & 0 & -1 & 0 & 1 & 0 & 0 \\ 0 & 1 & 1 & -1 & 0 & 0 & -1 & -1 & 1 & 0 \\ 1 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & -1 & 1 \\ 0 & 1 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & -1 \end{bmatrix}.$$

By Theorem 1.2, $Sv'(x)$ respects an unambiguous sign pattern but the deficiency of the network is one. To achieve arbitrary deficiency one can employ a block diagonal construction as above.

## 4.2 Deficiency and the sign fixing algorithm

In this subsection we consider how the deficiency of a CRN changes after we apply the sign fixing algorithm to produce a new CRN.

Let $S_1$ be the stoichiometric matrix for a CRN, and let $S_2$ be the sign fixing matrix of $S_1$ with respect to some bad submatrix. All variables with subscript 1 refer to the

original CRN and variables with subscript 2 refer to the new CRN unless otherwise noted. Also, $\mathcal{C}$ denotes the set of all complexes of a network and $\mathcal{L}$ denotes the set of all linkage classes of a network. We also denote $\Delta\delta := \delta_2 - \delta_1$, $\Delta n := n_2 - n_1$, $\Delta\ell := \ell_2 - \ell_1$, and $\Delta s = s_2 - s_1$. Assuming that $S_1$ has a bad submatrix corresponding to the species $A$, $B$, then this network has 2 reactions of the form

$$p_1 A + C_1 \to p_2 B + C_2 \tag{4.1}$$

$$p_3 A + p_4 B + C_3 \to C_4, \tag{4.2}$$
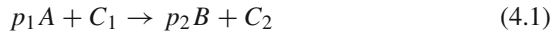
where $p_1, p_2, p_3, p_4 \in \mathbb{N}$ and $C_1, C_2, C_3, C_4$ are some (possibly empty) positive linear combination of species. The only changes to the new network are we add a new species $B'$, reaction (4.1) is replaced by

$$p_1 A + C_1 \to B' + C_2 \tag{4.3}$$

and we create an additional reaction

$$B' \to p_2 B. \tag{4.4}$$

By Proposition 3.1, we always have $\Delta s = 1$. To get a better handle on the change of deficiency, we proceed as follows.

**Lemma 4.3** *If $S_1$ is the stoichiometric matrix of a CRN and $S_2$ is its sign fixing matrix with respect to a bad submatrix, then the following inequalities are sharp:*

$$\Delta\ell \leq 2 \text{ and } 1 \leq \Delta n \leq 3. \tag{4.5}$$

*Proof* With the notation above, the only possible new complexes are $B' + C_2$, $B'$, and $p_2 B$. Hence $\Delta n \leq 3$. Also, the only possibly new linkage classes are $[B' + C_2]_2$ and $[p_2 B]_2$, so $\Delta\ell \leq 2$. The fact that $\Delta n \geq 1$ is obvious.

To show that the inequalities (4.5) are sharp, consider the following network:
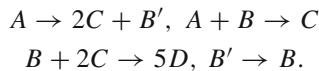
$$A \to B + 2C \to 5D$$
$$A + B \to C.$$
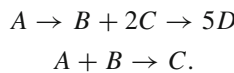
This CRN has the stoichiometric matrix

$$S_1 = \begin{bmatrix} -1 & -1 & 0 \\ 1 & -1 & -1 \\ 2 & 1 & -2 \\ 0 & 0 & 5 \end{bmatrix}.$$

Hence $n_1 = 5$ and $\ell_1 = 2$. The sign fixing matrix for $S_1$ (with respect to the species $A, B$) is

$$S_2 = \begin{bmatrix} -1 & -1 & 0 & 0 \\ 0 & -1 & -1 & 1 \\ 2 & 1 & -2 & 0 \\ 0 & 0 & 5 & 0 \\ 1 & 0 & 0 & -1 \end{bmatrix},$$

and our new chemical network is

$$A \to 2C + B', \quad A + B \to C$$
$$B + 2C \to 5D, \quad B' \to B.$$

We thus see that $n_2 = 8$ and $\ell_2 = 4$, so $\Delta n = 3$ and $\Delta \ell = 2$. $\qquad\square$

We now need an efficient way to determine which complexes and reactions of the new CRN affect $\Delta n$ and $\Delta \ell$. We shall define functions which will precisely determine which complexes and linkage classes in the new CRN increase $\Delta n$ and $\Delta \ell$. Define $\phi : \{B' + C_2, B', p_2 B\} \to \{0, 1\}$ by

$$\phi(B' + C_2) = \begin{cases} 2 & \text{if } C_2 \neq \emptyset \text{ and } p_2 B + C_2 \in \mathcal{C}_2 \\ 1 & \text{otherwise} \end{cases}$$

$$\phi(p_2 B) = \begin{cases} 1 & \text{if } p_2 B \notin \mathcal{C}_1 \\ 0 & \text{otherwise} \end{cases}.$$

We also define $\psi : \{[B' + C_2]_2, [B']_2\} \to \{0, 1\}$ such that

$$\psi([B' + C_2]_2) = \begin{cases} 1 & \text{if } p_2 B + C_2 \in \mathcal{C}_2 \text{ and } [B' + C_2]_2 \bigcap [p_2 B + C_2]_2 = \emptyset \\ 0 & \text{otherwise} \end{cases}$$

$$\psi([B']_2) = \begin{cases} 1 & \text{if } p_2 B \notin \mathcal{C}_1 \text{ and } C_2 \neq \emptyset \\ 0 & \text{otherwise} \end{cases}.$$

The advantage of this new notation is that we now have a succinct way to measure $\Delta n$ and $\Delta \ell$:

$$\Delta n = \phi(B' + C_2) + \phi(p_2 B) \tag{4.6}$$
$$\Delta \ell = \psi([B' + c_2]_2) + \psi([B']_2). \tag{4.7}$$

Equation (4.7) follows directly from the definition of $\Delta \ell$ and the construction of the new network. However, (4.6) needs more justification.

**Lemma 4.4** *With the setup described above, (4.6) holds.*

*Proof* By construction, notice that we always have $C_1 \subseteq C_2$ or $C_1 \setminus \{p_2 B + C_2\} \subseteq C_2$. Also, $\{B' + C_2, B', p_2 B\}$ are the only possible new complexes that are not in $C_1$. Observe that if $p_2 B + C_2 \in C_2$, then $C_2 = C_1 \bigcup \{B' + C_2, B', p_2 B\}$, whence

$$
\begin{aligned}
\Delta n &= \text{card}(C_1 \cup \{B' + C_2, B', p_2 B\}) - \text{card}(C_1) \\
&= \big(\text{card}(C_1 \cup \{B' + C_2, B'\}) - \text{card}(C_1)\big) + (\text{card}(C_1 \cup \{p_2 B\}) - \text{card}(C_1)) \\
&= \text{card}(C_1 \cup \{B' + C_2, B'\}) - \text{card}(C_1) + \phi(p_2 B),
\end{aligned}
$$

where the last equality follows by the definition of $\phi$. Also, if $C_2 = \emptyset$, then

$$
\text{card}(C_1 \cup \{B' + C_2, B'\}) - \text{card}(C_1) = 1; \text{ otherwise, } \text{card}(C_1 \cup \{B' + C_2, B'\}) \\
-\text{card}(C_1) = 2,
$$

so $\phi(B' + C_2) = \text{card}(C_1 \cup \{B' + C_2, B'\}) - \text{card}(C_1)$ by construction. This implies $\Delta n = \phi(B' + C_2) + \phi(p_2 B)$, as desired. On the other hand, suppose $p_2 B + C_2 \notin C_2$. If $C_2 = \emptyset$, then $p_2 B + C_2 = p_2 B \in C_2$, which is a contradiction. Thus we must have $C_2 \neq \emptyset$. A simple count shows

$$
\Delta n = \text{card}(C_1 \setminus \{p_2 B + C_2\} \cup \{B' + C_2, B', p_2 B\}) - \text{card}(C_1) = \begin{cases} 2 & \text{if } p_2 B \notin C_1 \\ 1 & \text{otherwise} \end{cases}
$$

$$
= \phi(B' + C_2) + \phi(p_2 B),
$$

where the last equality follows directly from the definition of $\phi$. $\square$

**Theorem 4.5** *Let $S_1$ be the stoichiometric matrix to a chemical network with a bad submatrix, and let $S_2$ be the sign fixing matrix with respect to this bad submatrix. Then $0 \leq \Delta\delta \leq 1$, and this inequality is sharp.*

*Proof* First, Lemma 4.3 shows $\Delta\delta = \Delta n - \Delta\ell - 1 \leq 3 - \Delta\ell - 1 \leq 2$. Notice that if $\Delta\delta = 2$, then $\Delta n = 3$ and $\Delta\ell = 0$, so $\phi(B' + C_2) = 2$, $\phi(p_2 B) = 1$ and $\psi([B' + C_2]_2) = \psi([B']_2) = 0$. However, $\phi(B' + C_2) = 2$ implies $C_2 \neq \emptyset$, and $\phi(p_2 B) = 1$ implies $p_2 B \notin C_1$ by construction. Hence $\psi([B']_2) = 1$ by the definition of $\psi$ and this is a contradiction. Thus, we cannot have $\Delta n = 3$ and $\Delta\ell = 0$. This proves $\Delta\delta \leq 1$.

Now suppose $\Delta\delta < 0$ to derive a contradiction. By Lemma 4.3, $\Delta n = 2$ and $\Delta\ell = 2$, or $\Delta n = 1$ and $\Delta\ell \geq 1$.

*Case 1:* Suppose $\Delta n = 2$ and $\Delta\ell = 2$. Notice if $\Delta\ell = 2$ then $\psi([B' + C_2]_2) = \psi([B']_2) = 1$ and thus $C_2 \neq \emptyset$, $p_2 B + C_2 \in C_2$, and $p_2 B \notin C_1$ by construction. Hence $\phi(p_2 B) = 1$ and $\phi(p_2 B + C_2) = 2$ by construction, so $\Delta n = 2 + 1 = 3$ by (4.6) and this is a contradiction.

*Case 2:* Suppose $\Delta n = 1$ and $\Delta\ell \geq 1$. Notice, $\Delta n = 1$ and (4.6) imply $\phi(p_2 B) = 0$ and $p_2 B \in C_1$, so $\psi([B']_2) = 0$ and $\psi([B' + c_2]_2) = 1$ since $\Delta\ell \geq 1$ implies $p_2 B + C_2 \in C_2$ and $[B' + C_2]_2 \cap [p_2 B + C_2]_2 = \emptyset$. If $C_2 = \emptyset$, then $[B' + C_2]_2 = [B']_2 = [p_2 B]_2 = [p_2 B + C_2]_2$. Hence $[B' + C_2]_2 \cap [p_2 B + C_2]_2 \neq \emptyset$ and this is a

contradiction. Thus $C_2 \neq \emptyset$. But then $\phi(B' + C_2) = 2$ by construction, and $\Delta n \geq 2$ by (4.6); contradiction.

To show that these inequalities are sharp, consider the following network:

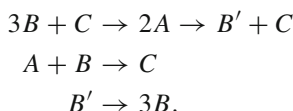$$2A \rightleftharpoons 3B + C$$
$$A + B \rightarrow C.$$
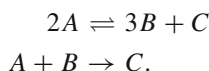
This CRN has the stoichiometric matrix

$$S_1 = \begin{bmatrix} -2 & -1 & 2 \\ 3 & -1 & -3 \\ 1 & 1 & -1 \end{bmatrix}.$$

Hence $n_1 = 4$ and $\ell_1 = 2$. The sign fixing matrix for $S_1$ with respect to $A$, $B$ is

$$S_2 = \begin{bmatrix} -2 & -1 & 2 & 0 \\ 0 & -1 & -3 & 3 \\ 1 & 1 & -1 & 0 \\ 1 & 0 & 0 & -1 \end{bmatrix},$$

and our new chemical network is

$$3B + C \rightarrow 2A \rightarrow B' + C$$
$$A + B \rightarrow C$$
$$B' \rightarrow 3B.$$

We thus see that $n_2 = 7$ and $\ell_2 = 3$. So $\Delta n = 3$ and $\Delta \ell = 1$. Thus, $\Delta \delta = \Delta n - \Delta \ell - \Delta s = 3 - 1 - 1 = 1$.                                                                 □

**Corollary 4.6** *Suppose S is the stoichiometric matrix for some chemical network, and it contains k bad submatrices. If $\widehat{S}$ is its sign fixing matrix, then $0 \leq \Delta \delta \leq k$.*

*Proof* Recall that by definition, $\widehat{S}$ is determined by a recursive sequence of at most $k$ sign fixing matrices, each with respect to a certain bad submatrix from the previous matrix in the sequence. An application of Theorem 4.5 at each step yields our desired result.                                                                 □

In fact, the upper bound for $\Delta \delta$ in Corollary 4.6 is the number of equivalence classes of bad submatrices of $S$ as defined in Sect. 2.3. Also, all sign fixed matrices $\widehat{S}$ obtained from our algorithm have the same deficiency (independent of the order in which the sign fixing algorithm is applied).

Theorem 4.5 together with formulas (4.6) and (4.7) helps determining necessary conditions for $\Delta \delta = 1$ for a stoichiometric matrix and its sign fixing matrix with respect to a certain bad submatrix. Notice that $\Delta \delta = 1$ implies $\Delta n = 2$ and $\Delta \ell = 0$ or $\Delta n = 3$ and $\Delta \ell = 1$. If $C_2 = \emptyset$, then $p_2 B = p_2 B + C_2 \in C_1$, so $\phi(p_2 B) = 0$. Also, $C_2 = \emptyset$ implies $\phi(B' + C_2) = 1$ by construction, So $\Delta n = 1$ by (4.6), and hence, $\Delta \delta = 0$ by Theorem 4.5. This observation yields the following:

**Theorem 4.7** *Let S be the stoichiometric matrix for a chemical network. Suppose that the column corresponding to each bad submatrix of S with the positive entry has only one positive entry. Then if $\widehat{S}$ is a sign fixing matrix for S, we have $\Delta\delta = 0$.*

*Proof* By assumption, each bad submatrix of $S$ corresponds to 2 reactions of the form:

$$p_1 A + C_1 \rightarrow p_2 B \tag{4.8}$$

$$p_3 A + p_4 B + C_2 \rightarrow C_3, \tag{4.9}$$

where $A$, $B$ are species, $p_1, p_2, p_3, p_4 \in \mathbb{N}$ and $c_1, c_2, c_3$ are some (possibly empty) positive linear combination of species. As already shown, the deficiency for the sign fixing matrix of $S$ with respect to this bad submatrix does not change. An inductive procedure yields our desired result.                    □

## 5 An alternative sign fixing algorithm?

Given a stoichiometric matrix $S$ with bad submatrices, there is an easier way of eliminating these. Instead of performing the sign fixing algorithm for each submatrix separately and thus adding a row and a column in every step, we can add only one row and column and eliminate all bad submatrices in a single step. Unfortunately, this construction changes the dimension of ker $S$; thus the two matrices yield reaction networks with very different equilibria structure. We illustrate this with an example.

*Example 5.1* Suppose

$$S = \begin{bmatrix} -2 & -1 & 4 & 4 & -4 \\ -12 & 4 & 4 & 0 & 0 \\ 4 & -1 & -2 & 0 & 0 \\ 10 & -2 & -6 & -4 & 4 \end{bmatrix}.$$

Notice that $S$ has several bad submatrices. We start by adding a row and column of zeros to $S$. Pick a bad $2 \times 2$ submatrix of $S$. Replace the positive entry $S_{p\ell}$ of $S$ by 0, add $+1$ to the $\ell$th entry of the new row and add $S_{p\ell}$ to the $p$th entry of the new column. Repeat this for all the bad submatrices. After all the bad submatrices have been eliminated, the bottom right entry is changed into the negative sum of all the entries in the last row. We obtain a matrix $\widetilde{S}$ with no bad submatrices. In our example this is

$$\widetilde{S} = \begin{bmatrix} -2 & -1 & 0 & 0 & -4 & 8 \\ -12 & 0 & 4 & 0 & 0 & 4 \\ 0 & -1 & -2 & 0 & 0 & 4 \\ 0 & -2 & -6 & -4 & 0 & 14 \\ 1 & 1 & 1 & 1 & 1 & -5 \end{bmatrix}.$$

We note that

$$\ker S = \text{span}\left\{ \begin{bmatrix} 0 & 0 & 0 & 1 & 1 \end{bmatrix}^t, \begin{bmatrix} 1 & 2 & 1 & 0 & 0 \end{bmatrix}^t \right\}$$

and

$$\ker S^t = \operatorname{span}\left\{\begin{bmatrix} 1 & 1 & 1 & 1 \end{bmatrix}^t\right\}.$$

The corresponding kernels for $\widetilde{S}$ are:

$$\ker \widetilde{S} = \operatorname{span}\left\{\begin{bmatrix} 2 & 0 & 4 & 1 & 3 & 2 \end{bmatrix}^t\right\}$$

and

$$\ker \widetilde{S}^t = \{0\}.$$

Given its kernel, the ODE $\dot{\widetilde{x}} = \widetilde{S}\widetilde{v}(\widetilde{x})$ can have equilibria only on the boundary. In fact, each solution to $\widetilde{S}\widetilde{v}(\widetilde{x}) = 0$ can be shown to satisfy $\widetilde{v}(\widetilde{x}) = 0$.

Also, assuming mass action kinetics,

$$v(x) = \begin{bmatrix} k_1 x_1^2 x_2^{12} & k_2 x_1 x_3 x_4^2 & k_3 x_3^2 x_4^6 & k_4 x_4^4 & k_5 x_1^4 \end{bmatrix}^t$$

so for every $x_2 \in \mathbb{R}_{>0}$,

$$x_1 = \frac{k_2 \sqrt[4]{k_4}}{2\sqrt{k_1}\sqrt{k_3}\sqrt[4]{k_5}x_2^6}, \quad x_3 = \frac{4k_1^{3/2}\sqrt{k_3}\sqrt[4]{k_4}x_2^{18}}{k_2^2\sqrt[4]{k_5}}, \quad x_4 = \frac{k_2}{2\sqrt{k_1}\sqrt{k_3}x_2^6}$$

yields a positive solution to $Sv(x) = 0$.

Hence it is not possible to recover positive equilibria for the chemical CRN by $S$ from those obtained by $\widetilde{S}$.

Also note that there is a nonnegative vector orthogonal to the range of $S$, thus the corresponding reaction form dynamics has a conserved quantity. On the other hand, $\widetilde{S}$ is not conserving.

## 6 Software

The discovering of the results in this paper was considerably facilitated by computer experiments. The programs we wrote to do this might be of value to a broad community, so we documented them and provided tutorial examples. They are found on the web site: http://www.math.ucsd.edu/~chemcomp/

The Mathematica files provided contain software for dealing with equations that come from CRNs; $dx/dt = f(x) = Sv(x)$ as in (1.2). Some of our commands focus on the Jacobian, $f'$, of $f$; they do the following

1. compute the Jacobian $f'$ of $f$ (given say the stoichiometric matrix $S$);
2. check existence of a sign pattern for $f'(x)$ which remains unchanged for all $x \geq 0$, using Theorem 1.2 in this paper;
3. implement the sign fixing algorithm in Sect. 2.1;

4. compute the Craciun–Feinberg (CF) determinant [8,9,11] of $f'$ (governs CRNs with outflows for all species with outflow rate constants equal to one);

5. compute the more general Helton–Klep–Gomez core determinant [15] of $f'$ (governs CRNs with any number of outflows).

The CF determinant and core determinants are used in tests to count the number of positive equilibria, namely $x^* > 0$ such that $f(x^*) = 0$ (For more information, please look at the papers [10] and [15] and the original Craciun, Feinberg et al. papers, [8,9,11]).

Another part of our Mathematica package deals with deficiency of reaction form differential equations, as discussed in Sect. 4. The software allows us to compute the deficiency of a CRN as well as conversion of representations as follows. One starts with the traditional representation $f(x) = Sv(x)$. Our program produces the representation

$$Sv(x) = Y A_k \psi(x)$$

where $A_k$ is the Laplacian of the "complexes graph" of the chemical reaction network. $Y$ is the matrix whose columns are indexed by complexes and which contain nonzero entries corresponding to chemical species which enter the complex. $\psi$ is a list of monomials in the chemical concentrations. For details, see [14,12,16].

Our commands also compute the components of the complexes graph. Capability to automatically plot planar graphs is under development and should be available soon.

## 7 Conclusions

1. The effect of any species on another species is always (for all concentrations) inhibitory or always excitatory, if (generically only if for reversible CRNs) the species-reaction graph of $S$ has no bad cycle.

2. If a CRN has bad cycles, then *the sign fixing algorithm produces a CRN whose Jacobian respects a sign pattern*. The key properties are:

   (a) Equilibria in perfect correspondence;
   (b) Local asymptotic stability corresponds perfectly;
   (c) Conservation laws correspond;
   (d) The determinant of the sign fixed Jacobian is a constant multiple of the original Jacobian, thus the theory of Craciun and Feinberg applies to both CRNs or to neither;
   (e) Deficiency does not drop and its increase has a simple bound.

Possibly the tests and constructions outlined here will be useful to some experimentalists. In an experiment where one aims to understand which reactions occur and the pattern of inhibition and excitation, one "first" obtains the species-reaction graph $\mathcal{G}$. Based on measuring some limited number of concentrations one determines the inhibitory or excitatory effects. An issue is whether or not enough concentrations were measured, for a possibility is that species $x_i$ has an excitatory effect at some concentrations and an inhibitory effect at others.

The results here give a simple way to sort out this problem. First suppose the CRN does not contain two reactions involving two species A and B, one species being consumed by both reactions while the other species is consumed by one reaction and produced by the other. Then there is no such problem (see Theorem 1.3); in principle, one concentration measurement (of all species) suffices. If $\mathcal{G}$ has such a bad pair of reactions, probably there is trouble. Our sign fixing algorithm shows additional measurements which, if they can be made, fix this trouble.

We emphasize that our analysis does not say what effect a species $i$ has on species $j$ in a single reaction but it bears on its effect inside the entirety of the CRN.

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