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# Identifying parameter regions for multistationarity

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## Abstract

Mathematical modeling has become an established tool for studying biological dynamics. Current applications range from building models that reproduce quantitative data to identifying models with predefined qualitative features, such as switching behavior, bistability or oscillations. Mathematically, the latter question amounts to identifying parameter values associated with a given qualitative feature.

We introduce an algorithm to partition the parameter space of a parameterized system of ordinary differential equations into regions for which the system has a unique or multiple equilibria. The algorithm is based on a simple idea, the computation of the Brouwer degree, and creates a multivariate polynomial with parameter depending coefficients. Using algebraic techniques, the signs of the coefficients reveal parameter regions with and without multistationarity.

We demonstrate the algorithm on models of gene transcription and cell signaling, and argue that the parameter constraints defining each region have biological meaningful interpretations.

**Keywords:** biological dynamics; reaction networks; algebraic parameterization; qualitative analysis; Newton polytope; dissipative system

Mathematical models in the form of parameterized systems of ordinary differential equations (ODEs) are valuable tools in biology. Often qualitative properties of the ODEs are associated with macroscopic properties and biological functions [1–4]. It is therefore increasingly important that mathematical modeling in biology not only focuses on obtaining accurate models, but even more so on understanding the qualitative properties of these models. With the growing adaptation of differential equations in biology, it is expected that an automated screening of ODE models for parameter dependent properties and discrimination of parameter regions with different properties would be a very useful tool for biology, and perhaps even more for synthetic biology [5]. Even though it is currently not conceivable how and if this task can be efficiently formalized we view this paper as a first step in this direction.

*Multistationarity*, that is, the possibility of the system to rest in different positive equilibria depending on the initial state of the system, is an important qualitative property. Biologically, multistationarity is linked to cellular decision making and ‘memory’-related on/off responses to graded input [2–4]. Consequently the existence of multiple equilibria is often a design objective in synthetic biology [6, 7]. Various mathematical methods, developed in the context of reaction network theory, can be applied to decide whether multistationarity exists for some parameter values or not at all, or to pinpoint specific values for which it does occur [8–14]. Some of these methods are freely available as software tools [15, 16].

Delimiting parameter regions for which multistationarity occurs is a hard mathematical problem. Often it is solved by numerical investigations and parameter sampling, guided by

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Motif	Discriminating expression	Condition
$A \xrightleftharpoons[F]{K} A_p$	$b(k) = (k_{c1}k_{c4} - k_{c2}k_{c3}) \cdot$	<b>Multiple:</b> $b(k) < 0$
$B \xrightleftharpoons[F]{K} B_p$	$\left( \frac{k_{c1}k_{c4}}{k_{M1}k_{M4}} - \frac{k_{c2}k_{c3}}{k_{M2}k_{M3}} \right)$	<b>Unique:</b> $b(k) \geq 0$
$A \xrightleftharpoons[F]{K} A_p$ $A_p \xrightleftharpoons[F]{K} A_{pp}$	$b_1(k) = (k_{c1}k_{c4} - k_{c2}k_{c3})$ $b_2(k) = k_{c1}k_{c4}(k_{M2} + k_{M3})$ $- k_{c2}k_{c3}(k_{M1} + k_{M4})$	<b>Multiple:</b> $b_1(k) < 0$ <b>Unique:</b> $b_1(k) \geq 0$ and $b_2(k) \geq 0$

Table 1: Conditions for multiple and unique equilibria in post-translational modification of proteins. Symbols  $k_{ci}$  and  $k_{Mi}$  denote the catalytic and Michaelis-Menten constants of the  $i$ -th modification step ( $i = 1$ : phosphorylation of  $A$ ,  $i = 2$ : dephosphorylation of  $A_p$ ,  $i = 3$ : phosphorylation of  $B$  (resp.  $A_p$ ),  $i = 4$ : dephosphorylation of  $B_p$  (resp.  $A_{pp}$ )). All parameter values satisfying the conditions in column 3 yield multiple (unique) equilibria for some (all) values of the conserved quantities. See **Supporting Information** for details.

biological intuition or by case-by-case mathematical approaches. Alternatively, for polynomial ODEs, a decomposition of the parameter space into regions with different numbers of equilibria could be achieved by Cylindrical Algebraic Decomposition (a version of quantifier elimination) [17]. This method, however, scales very poorly and is thus only of limited help in biology, where models tend to be large in terms of the number of variables and parameters.

Here we present an algorithm to identify regions of the parameter space for which multiple equilibria exist and regions for which only one equilibrium exists. In many cases we achieve a complete partitioning of the parameter space. The input is a system of ODEs, commonly used to model biological processes, and the output is a single polynomial. The capacity for multiple (unique) equilibria is encoded in the signs of the polynomial as a function of the parameters and the variables. The algorithm originates from ideas in [18, 19].

Table 1 shows two examples of network motifs that occur frequently in intracellular signaling: a two-site protein modification by a kinase–phosphatase pair and a one-site modification of two proteins by the same kinase–phosphatase pair. The conditions discriminating between a unique and multiple equilibria highlight a delicate relationship between the catalytic and Michaelis-Menten constants of the kinase and the phosphatase with the modified protein as a substrate (the  $k_c$ - and  $k_M$ -values). If the condition for multiple equilibria is met, then multiple equilibria occur provided the total concentrations of kinase, phosphatase and substrate are in suitable ranges (values thereof can be computed as part of the algorithm).

The paper is organized as follows: we first introduce notation and mathematical background material. We then give a theorem that links the number of equilibria to the sign of the determinant of the Jacobian of a certain function, related to the ODE system. Using the theorem we state the algorithm and give examples. Larger examples and proofs are given in the **Supporting Information**, together with a detailed analysis of the motifs in Table 1.

## Results

**Reaction networks.** A *reaction network*, or simply a *network*, consists of a set of species  $\{X_1, \dots, X_n\}$  and a set of reactions of the form:

$$R_j: \sum_{i=1}^n \alpha_{ij} X_i \rightarrow \sum_{i=1}^n \beta_{ij} X_i, \quad j = 1, \dots, \ell \quad (1)$$

where  $\alpha_{ij}, \beta_{ij}$  are non-negative integers. We let  $N = (N_{ij}) \in \mathbb{R}^{n \times \ell}$  be the *stoichiometric matrix* of the network, defined as  $N_{ij} = \beta_{ij} - \alpha_{ij}$ , that is, the  $(i, j)$ -th entry encodes the net production of species  $X_i$  in reaction  $R_j$ . We refer to the ‘running example’ in Fig. 1 for illustration of definitions.

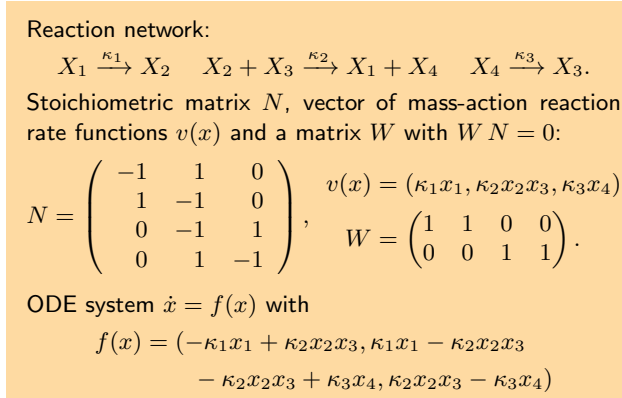


Figure 1: **Running example.** Simplified model of a two-component system, consisting of a histidine kinase HK and a response regulator RR. Both occur unphosphorylated and phosphorylated (subscript p);  $X_1 = \text{HK}$ ,  $X_2 = \text{HK}_p$ ,  $X_3 = \text{RR}$  and  $X_4 = \text{RR}_p$ .

The concentration of the species  $X_1, \dots, X_n$  are denoted by lower-case letters  $x_1, \dots, x_n$ . We denote by  $\mathbb{R}_{>0}^n$  ( $\mathbb{R}_{\geq 0}^n$ ), the positive (non-negative) orthant in  $\mathbb{R}^n$ . The evolution of the concentrations with respect to time is modeled as an ODE system with initial condition  $x(0) = x_0$  and

$$\dot{x} = f(x), \quad f(x) = Nv(x), \quad x \in \mathbb{R}_{\geq 0}^n, \quad (2)$$

where  $x = (x_1, \dots, x_n)$ ,  $v = (v_1, \dots, v_\ell)$  and  $v_j: \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}_{\geq 0}$  is a  $C^1$ -function, called the *reaction rate function* of reaction  $R_j$ . Typical choices are mass-action kinetics, Michaelis-Menten and Hill kinetics.

Under the assumption

$$v_j(x) = 0 \quad \Leftrightarrow \quad x_i = 0 \quad \text{for some } i \text{ such that } \alpha_{ij} > 0, \quad (3)$$

the orphans  $\mathbb{R}_{>0}^n$  and  $\mathbb{R}_{\geq 0}^n$  are *forward-invariant* under  $f$  in (19) [20, Section 16]. The above mentioned kinetics fulfil the assumption in (3).

In the following, by a (reaction) network we implicitly assume it comes together with an ODE system.

The trajectories of the ODEs in (19) are confined to the so-called *stoichiometric compatibility classes (SCCs)*, which are defined as follows. Let  $s = \text{rank}(N)$  and  $d = n - s$ . Further, let  $W \in \mathbb{R}^{d \times n}$  be a matrix whose rows form a basis of  $\text{im}(N)^\perp$ , see Fig. 1. Then, each  $c \in \mathbb{R}^d$  defines a SCC by

$$\mathcal{P}_c := \{x \in \mathbb{R}_{\geq 0}^n \mid Wx = c\},$$

which is empty if  $c \notin W(\mathbb{R}_{\geq 0}^n)$ . The *positive SCC* is defined as the relative interior of  $\mathcal{P}_c$ ,

$$\mathcal{P}_c^+ := \{x \in \mathbb{R}_{>0}^n \mid Wx = c\} = \mathcal{P}_c \cap \mathbb{R}_{>0}^n.$$

The sets  $\mathcal{P}_c^+$  and  $\mathcal{P}_c$  are convex and forward-invariant, hence by definition  $Wx$  is conserved through time.

An equation of the form  $\omega \cdot x = c'$  for some  $\omega \in \text{im}(N)^\perp$  and  $c' \in \mathbb{R}$  is called a *conservation law*. In particular,  $Wx = c$  forms a system of  $d$  conservation laws. The matrix  $W$  in Fig. 1 leads to the conservation laws  $x_1 + x_2 = c_1$ ,  $x_3 + x_4 = c_2$ . Here we have  $\mathcal{P}_{(c_1, c_2)}^+ \neq \emptyset$  if and only if  $c_1, c_2 > 0$ .

**Dissipative and conservative reaction networks.** A reaction network is *dissipative* if every trajectory eventually ends up in a compact set, which depends only on the SCC (see **Supporting Information**). A reaction network is *conservative* if all concentrations participate in at least one conservation law with non-negative coefficients. This is equivalent to the SCCs

being compact sets [21]. Hence, in particular, a conservative reaction network is dissipative. The running example is conservative.

A criterion to decide on dissipativity is the following proposition (see the **Supporting Information**).

**Proposition (Dissipative network).** Let  $\|\cdot\|$  be a norm in  $\mathbb{R}^n$ . Assume that for each  $c$  with  $\mathcal{P}_c^+ \neq \emptyset$ , there exists a vector  $\omega_c \in \mathbb{R}_{>0}^n$  and a number  $R > 0$  such that  $\omega_c \cdot f(x) < 0$  for all  $x \in \mathcal{P}_c$  with  $\|x\| > R$ . Then the network is dissipative.

**Equilibria.** Given the ODE in (19), the set of non-negative equilibria is

$$V = \{x \in \mathbb{R}_{\geq 0}^n \mid f(x) = 0\}.$$

With conservation laws, there are at most  $s$  linearly independent equations in at most  $s < n$  unknown variables.

We assume that  $V \cap \mathbb{R}_{>0}^n$  admits a positive parameterization

$$\begin{aligned} \Phi: \mathbb{R}_{>0}^m &\rightarrow \mathbb{R}_{>0}^n \\ \hat{x} = (x_1, \dots, x_m) &\mapsto (x_1, \dots, x_m, \Phi_{m+1}(\hat{x}), \dots, \Phi_n(\hat{x})), \end{aligned} \quad (4)$$

for some  $m < n$ , such that  $\hat{x} \in \mathbb{R}_{>0}^m$  is the vector of free variables. In other words,  $x_{m+1}, \dots, x_n$  are expressed at equilibrium as functions of  $\hat{x}$ :

$$x_i = \Phi_i(\hat{x}), \quad i = m+1, \dots, n,$$

such that  $x_{m+1}, \dots, x_n$  are positive for positive  $\hat{x}$ . Typically,  $m = d$ . We will say that a parameterization is *algebraic* if the components of  $\Phi$  are polynomials or rational functions (quotients of polynomials). See Fig. 2 (Step 4) for an example.

We are interested in the positive equilibria in each SCC, that is, the set  $V \cap \mathcal{P}_c$ . Generically, this set consists of isolated points obtained as the positive solutions to the equations

$$f(x) = 0, \quad Wx = c. \quad (5)$$

We choose the matrix of conservation laws  $W \in \mathbb{R}^{d \times n}$  to be row reduced (as in Fig. 1) and let  $i_1, \dots, i_d$  be the indices of the first non-zero coordinate of each row. For  $c \in \mathbb{R}^d$ , we define a  $\mathcal{C}^1$ -function  $\varphi_c(x): \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}^n$  by

$$\varphi_c(x)_i = \begin{cases} f_i(x) & i \notin \{i_1, \dots, i_d\} \\ (Wx - c)_i & i \in \{i_1, \dots, i_d\}. \end{cases} \quad (6)$$

Fig. 2 (Step 5) shows  $\varphi_c(x)$  for the running example in Fig. 1. The function  $\varphi_c(x)$  is obtained by replacing redundant equations in  $f(x) = 0$  by components corresponding to conservation laws. Thus

$$V \cap \mathcal{P}_c = \{x \in \mathbb{R}_{\geq 0}^n \mid \varphi_c(x) = 0\}.$$

A positive  $\hat{x}$  determines uniquely a positive equilibrium  $\Phi(\hat{x})$ . This equilibrium belongs to the SCC  $\mathcal{P}_c$  with

$$c := W\Phi(\hat{x}). \quad (7)$$

Given  $c$ , the positive solutions to (5) are in one-to-one correspondence with the positive solutions to (7).

We conclude this subsection with some definitions: a network admits *multiple equilibria* (or is *multistationary*) if there exists  $c \in \mathbb{R}^d$  such that  $V \cap \mathcal{P}_c^+$  contains at least two points. Equivalently, if the equation  $\varphi_c(x) = 0$  has at least two positive solutions for some  $c \in \mathbb{R}^d$ . Equilibria belonging to  $V \cap \mathcal{P}_c$  but not to  $V \cap \mathcal{P}_c^+$  for some  $c$  are *boundary equilibria*. A boundary equilibrium has at least one coordinate equal to zero.

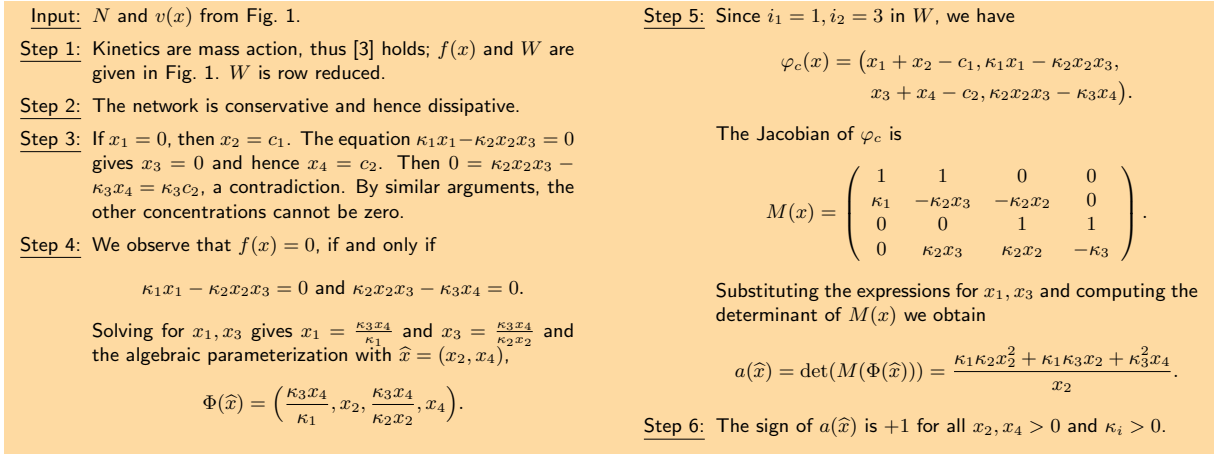


Figure 2: The algorithm applied to the example in Fig. 1.

**Unique and multiple equilibria.** Let  $M(x) \in \mathbb{R}^{n \times n}$  be the Jacobian matrix of  $\varphi_c(x)$ , which does not depend on  $c$ , see (6). An equilibrium  $x^* \in V \cap \mathcal{P}_c$  is *non-degenerate* if the Jacobian of  $\varphi_c$  at  $x^*$ ,  $M(x^*)$ , is non-singular, that is,  $\det(M(x^*)) \neq 0$  [22].

We consider the determinant of  $M(x)$  evaluated at  $\Phi(\hat{x})$ ,

$$a(\hat{x}) = \det(M(\Phi(\hat{x}))), \quad \hat{x} \in \mathbb{R}_{>0}^m. \quad (8)$$

By means of the following theorem,  $a(\hat{x})$  can be used to discriminate between multiple and unique equilibria. The theorem is based on relating  $a(\hat{x})$ ,  $\hat{x} \in \mathbb{R}_{>0}^m$ , to the Brouwer degree of  $\varphi_c$  at 0 (see the **Supporting Information**).

**Theorem (Unique and multiple equilibria).** Assume (3) on the reaction rate functions is fulfilled and let  $s = \text{rank}(N)$ . Further, assume that

- (1) The network is dissipative.
- (2) The set of positive equilibria admits a positive parameterization as in (25).
- (3)  $\mathcal{P}_c$  has no boundary equilibria, if  $\mathcal{P}_c^+ \neq \emptyset$  for  $c \in \mathbb{R}^d$ .

Then the following holds.

(A) **Uniqueness of equilibria.** If

$$\text{sign}(a(\hat{x})) = (-1)^s \quad \text{for all } \hat{x} \in \mathbb{R}_{>0}^m,$$

then there is exactly one positive equilibrium in each  $\mathcal{P}_c$  with  $\mathcal{P}_c^+ \neq \emptyset$ . Further, this equilibrium is non-degenerate.

(B) **Multiple equilibria.** If

$$\text{sign}(a(\hat{x})) = (-1)^{s+1} \quad \text{for some } \hat{x} \in \mathbb{R}_{>0}^m,$$

then there are at least two positive equilibria in  $\mathcal{P}_c$ , at least one of which is non-degenerate, where  $c := W\Phi(\hat{x})$ . If all positive equilibria in  $\mathcal{P}_c$  are non-degenerate, then there are at least three and always an odd number.

The conclusion of part (A) is also true if  $\text{sign}(\det(M(x))) = (-1)^s$  for all  $x$  in a set containing the positive equilibria, thereby removing the requirement of the existence of a parameterisation. In addition, the theorem holds for a given SCC (that is, for  $c$  fixed) with  $\hat{x} \in \mathbb{R}_{>0}^m$  replaced by  $\Phi(\hat{x}) \in \mathcal{P}_c^+$ . Also note that the only situation not covered by the theorem is when  $\text{sign}(a(\hat{x}))$  takes the value 0 for some  $\hat{x}$ , but never the value  $(-1)^{s+1}$ .

**The algorithm.** Since the reaction rate functions  $v(x)$  depend on the parameters  $\kappa$ , the theorem can be used to find parameter inequalities that discriminate between unique and multiple equilibria. Essentially, parameter regions for which either part (A) or part (B) of the theorem

is fulfilled, can be established. We propose an algorithm that takes  $v(x)$  and  $N$  (the stoichiometric matrix) as input. The reaction rate functions are assumed to be polynomials (as for mass-action kinetics) or quotients of polynomials (as for Michaelis-Menten or Hill kinetics with integer exponents).

**Algorithm (Identification of parameter regions)**

**Input:**  $N$  and  $v(x)$ .

1. Find  $f(x)$ , a row reduced matrix  $W$  such that the rows form a basis of  $\text{im}(N)^\perp$  and check that  $v(x)$  satisfies (3).
2. Check that the network is dissipative.
3. Check for boundary equilibria in  $\mathcal{P}_c$  with  $\mathcal{P}_c^+ \neq \emptyset$ .
4. Obtain an algebraic parameterization  $\Phi(\hat{x})$  of the set of positive equilibria, as in (25).
5. Construct  $\varphi_c(x)$ ,  $M(x)$ , compute  $\det(M(x))$  and  $a(\hat{x})$ .
6. By the algebraic hypothesis,  $a(\hat{x})$  can be written as the quotient of two polynomials, typically with positive denominator (a consequence of the positive parameterization). If so only the numerator needs to be analyzed. If not, special care needs to be taken to avoid division by zero.

**6a.** Identify coefficients that can change the sign of  $a(\hat{x})$ .

**6b.** Use these terms to construct parameter inequalities such that, whenever these inequalities hold, one has either  $\text{sign}(a(\hat{x})) = (-1)^s$  for all  $\hat{x}$  or  $\text{sign}(a(\hat{x})) = (-1)^{s+1}$  for at least one  $\hat{x}$ .

There is no guarantee that all steps of the algorithm can be carried out successfully. While steps 1 and 5 usually are straightforward, steps 2, 3, 4 and 6 might require case specific approaches. Before we comment further on this, we apply the algorithm to selected examples, and identify parameter regions where unique or multiple equilibria occur.

**Examples.**

**Running example in Fig. 1** A description of the steps of the algorithm is given in Fig. 2. The determinant  $a(\hat{x})$  obtained in Step 6 has sign +1 for all  $x_2, x_4 > 0$  and  $\kappa_i > 0$ . Hence, applying part (A) of the theorem with  $s = 2$ , we conclude that there exists a unique positive non-degenerate equilibrium in each stoichiometric compatibility class with  $c_1, c_2 > 0$ , for all choice of reaction rate constants. Multiple equilibria are thus excluded.

**Hybrid histidine kinase.** This example is an extension of the running example. Specifically, the histidine kinase is assumed to be *hybrid*, that is, it has two ordered phosphorylation sites [23]. Whenever the second phosphorylation site is occupied, the phosphate group can be transferred to a response protein, see Fig. 3. With mass-action kinetics the network is known to be multistationary for specific choices of reaction rate constants [23]. An application of the algorithm provides precise conditions for when it occurs.

We have  $s = 4$ . Let  $X_1, \dots, X_6$  be  $\text{HK}_{00}$ ,  $\text{HK}_{p0}$ ,  $\text{HK}_{0p}$ ,  $\text{HK}_{pp}$ ,  $\text{RR}$  and  $\text{RR}_p$ , respectively. The network is conservative (hence also dissipative), due to the linearly independent conservation laws  $x_1 + x_2 + x_3 + x_4 = c_1$  and  $x_5 + x_6 = c_2$ . It has no boundary equilibria for  $c_1, c_2 > 0$ , and admits a positive parameterization with free variables  $\hat{x} = (x_4, x_5)$ . Fig. 3 shows  $a(\hat{x})$  (for  $W$  derived from the given conservation laws). See §6.1 in the **Supporting Information** for details.

Only one of the coefficients of the polynomial  $a(\hat{x})$  can be negative. If  $\kappa_3 \leq \kappa_1$ , then  $\text{sign}(a(\hat{x})) = (-1)^4 = 1$  for all  $x_4, x_5 > 0$ . Part (A) of the theorem implies that there is a unique positive non-degenerate equilibrium in each SCC for  $c_1, c_2 > 0$ . Oppositely, if  $\kappa_3 > \kappa_1$ , define  $\hat{x}(T) = (T, T)$ . Then  $a(\hat{x}(T))$  is a polynomial in  $T$  with negative leading coefficient of degree 3. Therefore, for  $T$  large enough,  $a(\hat{x}(T))$  is negative and  $\text{sign}(a(\hat{x}(T))) = (-1)^5 = -1$ . Part (B) of the theorem implies that there exists  $(c_1, c_2)$  such that  $\mathcal{P}_c$  contains at least two positive

Reaction network	$a(\hat{x})$	Condition	Newton polytope
$\text{HK}_{00} \xrightarrow{\kappa_1} \text{HK}_{p0} \xrightarrow{\kappa_2} \text{HK}_{0p} \xrightarrow{\kappa_3} \text{HK}_{pp}$ $\text{HK}_{0p} + \text{RR} \xrightarrow{\kappa_4} \text{HK}_{00} + \text{RR}_p$ $\text{HK}_{pp} + \text{RR} \xrightarrow{\kappa_5} \text{HK}_{p0} + \text{RR}_p$ $\text{RR}_p \xrightarrow{\kappa_6} \text{RR}$	$\kappa_1 \kappa_2 \kappa_3 \kappa_6 + (\kappa_1 + \kappa_2) \kappa_4 \kappa_5 \kappa_6 x_5^2$ $+ \kappa_2 \kappa_4 \kappa_5^2 \left( \frac{\kappa_1}{\kappa_3} - 1 \right) x_4 x_5^2$ $+ 2 \kappa_1 \kappa_2 \kappa_4 \kappa_5 x_4 x_5$ $+ \kappa_1 (\kappa_2 + \kappa_3) \kappa_5 \kappa_6 x_5$ $+ \kappa_1 \kappa_2 \kappa_3 \kappa_5 x_4$	<p>multiple equilibria: <math>\kappa_3 &gt; \kappa_1</math></p> <p>unique equilibria: <math>\kappa_3 \leq \kappa_1</math></p>	
$X_1 \xrightarrow{\kappa_1} X_1 + P_1 \quad P_1 \xrightarrow{\kappa_3} 0$ $X_2 \xrightarrow{\kappa_2} X_2 + P_2 \quad P_2 \xrightarrow{\kappa_4} 0$ $X_2 + P_1 \xrightleftharpoons[\kappa_6]{\kappa_5} X_2 P_1 \quad 2P_2 \xrightleftharpoons[\kappa_8]{\kappa_7} P_2 P_2$ $X_1 + P_2 P_2 \xrightleftharpoons[\kappa_{10}]{\kappa_9} X_1 P_2 P_2$	$\frac{\kappa_3 \kappa_6}{x_4} (\kappa_2 \kappa_7 \kappa_9 x_4^2 x_5$ $- \kappa_4 \kappa_7 \kappa_9 x_4^3 - \kappa_2 \kappa_8 \kappa_{10} x_5$ $- \kappa_4 \kappa_8 \kappa_{10} x_4)$	<p>multiple equilibria possible for all <math>\kappa_i &gt; 0</math></p>	

Figure 3: The polynomial  $a(\hat{x})$ , conditions for multiple/unique equilibria and the Newton polytope for the hybrid histidine kinase and the gene transcription networks. *Column 2*: Monomials with coefficient with constant sign  $(-1)^s$  are in blue, those that can have sign  $(-1)^{s+1}$  in red. *Column 4*: Each point corresponds to the exponent vector of a monomial of the numerator of  $a(\hat{x})$ , e.g.  $(1, 2)$  is the exponent vector of the monomial  $x_4 x_5^2$ . Blue points are vertices of the Newton polytope. Red vectors correspond to the red monomials in column 2.

equilibria. Specific values of  $c_1, c_2 > 0$  for which this is the case can be found from (7) with  $\hat{x}$  chosen such that  $a(\hat{x})$  is negative.

In summary, the region of the parameter space for which multistationarity exists is completely characterized by the inequality  $\kappa_3 > \kappa_1$ . This condition states that the reaction rate constant for phosphorylation of the first site of the hybrid kinase is larger when the second site is already phosphorylated.

**Gene transcription network.** We consider a gene transcription motif with two proteins  $P_1, P_2$ , produced by their respective genes  $X_1, X_2$ , and such that  $P_2$  dimerises [24]. Further, the proteins cross regulate each other as depicted in Fig. 3.

We let  $X_3, \dots, X_7$  denote  $P_1, P_2, X_2 P_1, P_2 P_2$ , and  $X_1 P_2 P_2$ , respectively. We have  $s = 5$  and two linearly independent conservation laws,  $x_1 + x_7 = c_1$  and  $x_2 + x_5 = c_2$ . The network is not conservative. However, with mass-action kinetics the network is dissipative [24]. This also follows from the proposition with  $\omega_c = (1, 1, 1, 1, 2, 2, 3) \in \mathbb{R}_{>0}^7$ . Indeed, we have

$$\omega_c \cdot f(x) = \kappa_1 x_1 + \kappa_2 x_2 - \kappa_3 x_3 - \kappa_4 x_4.$$

Note that  $x_1, x_2$  are bounded (due to the conservation laws) while  $x_3, x_4$  can be arbitrarily large. Then, for  $x_3, x_4$  large enough,  $\omega_c \cdot f(x) < 0$  and the network is dissipative.

The network has no boundary equilibria if  $c_1, c_2 > 0$ , and admits a positive parameterization with free variables  $\hat{x} = (x_4, x_5)$ . The function  $a(\hat{x})$  is given in Fig. 3 (for  $W$  derived from the given conservation laws). See §6.2 in the **Supporting Information** for details.

In this case, part (B) of the theorem holds for all choices of reaction rate constants. Indeed, let  $\hat{x}(T) = (T, T^2)$ . Then  $a(\hat{x}(T))$  is a polynomial of degree 4 in  $T$  with positive leading term. Thus for large  $T$ ,  $\text{sign}(a(\hat{x}(T))) = (-1)^{s+1} = 1$  since  $s = 5$ . We conclude that for all  $\kappa_i > 0$  there exists  $c$  such that  $\mathcal{P}_c$  contains at least two positive equilibria.

## Comments on the algorithm.

**Step 2: establishing dissipativity.** If the network is not dissipative, then at least one concentration grows to infinity over time. This is typically not the case for realistic networks, but it needs to be ruled out in order to apply the theorem.

We start by checking whether the network is conservative. This implies solving the linear system  $\omega^t N = 0$  with the constraint  $\omega > 0$ . As  $N$  does not contain parameters, this requires only



numerical computation and hence can be done efficiently. Alternatively, conservation laws are often easily established by inspection of the reactions. For example, in many signaling networks, the total concentration of enzyme (free and bounded) and of substrate (phosphoforms) are conserved.

If the network is not conservative, we proceed by checking dissipativity using the proposition stated above. We look for  $\omega_c > 0$  such that  $\omega_c \cdot f(x) < 0$  for large  $x$ . As we do not wish to make any assumptions on the parameter values, this computation has to be done symbolically. Here computer algebra software may assist. The task is computationally demanding, especially if there is a large number of variables and parameters.

**Step 3: absence of boundary equilibria.** For systems of moderate size it is often possible to establish nonexistence of boundary equilibria by arguments similar to those employed in the analysis of the running example. For each  $i$ , assume  $x_i = 0$ , and show that it leads to a contradiction. A systematic procedure relies on computing the so-called *minimal siphons* of the network [25] (see §5.1 in the **Supporting Information** for details). The complexity of this computation can often be substantially reduced [26].

**Step 4: finding an algebraic parameterization.** Computer algebra systems like Maple or Mathematica can be used to find a parameterization. The strategy is to solve the equations  $f_i(x) = 0$ ,  $i \notin \{i_1, \dots, i_d\}$  for some subset of (at most)  $s$  variables, treating the remaining (at least)  $d$  variables as coefficients of the system. If a parameterization exists but is not positive, another set of variables should be tried out.

Existing results on positive parameterization of equilibria may be useful. Cascades of post-translational modification networks admit a positive parameterization in terms of the concentrations of the enzymes and one substrate form [27, 28].

A general setting is based on *non-interacting sets of species* [29]. A set, say  $\{X_{m+1}, \dots, X_n\}$ , is non-interacting if two species never appear on the same side of a reaction and they have coefficient at most one in all reactions. If the sum  $x_{m+1} + \dots + x_n$  is not conserved, then a positive parameterization exists with the species  $x_1, \dots, x_m$  as free variables [29]. In the running example,  $\{X_1, X_3\}$  is a non-interacting set.

A systematic way to obtain a positive parameterization is to try all possible subsets of variables and to check whether one of them gives a positive parameterization. It requires computation and analysis of at most  $\binom{n}{d}$  parameterizations. This can, for example, be achieved by computing the circuits of degree one of the matroid associated with the equilibrium equations [30].

**Step 6: the sign of  $a(\hat{x})$  and the Newton polytope.** This is perhaps the hardest step of all. Assume the denominator of  $a(\hat{x})$  is always positive and hence the sign of  $a(\hat{x})$  is determined by the polynomial of the numerator, say  $p(\hat{x})$ . We first look for conditions that ensure uniqueness of positive equilibria by imposing that all coefficients of  $p(\hat{x})$  have sign  $(-1)^s$ .

We next identify coefficients that can have the sign  $(-1)^{s+1}$  (for some parameter choice) and check whether the associated monomial can “dominate” the sign of  $p(\hat{x})$ . This is to say, we determine whether  $p(\hat{x})$  has the sign  $(-1)^{s+1}$  for some  $\hat{x}$ , if the coefficient, say  $\beta$ , of a specific monomial in  $p(\hat{x})$  has sign  $(-1)^{s+1}$ . If that is the case, then the condition  $\text{sign}(\beta) = (-1)^{s+1}$  is a sufficient condition for multiple equilibria.

Given a coefficient with sign  $(-1)^{s+1}$ , it might not be straightforward to decide if the polynomial can attain the same sign for some values of  $\hat{x}$ . (For example, the polynomial  $x^2 - 2xy + y^2 = (x - y)^2$  has a negative monomial but the polynomial itself can never be negative.) The strategy we apply in the examples determines whether the monomial of interest corresponds to a vertex of the *Newton polytope* (see §5.2 in the **Supporting Information**). The Newton polytope of  $p(\hat{x})$  is defined as the convex hull of the exponent vectors  $\alpha = (\alpha_1, \dots, \alpha_m) \in \mathbb{R}^m$  corresponding to the monomials  $x_1^{\alpha_1} \cdots x_m^{\alpha_m}$  of  $p(\hat{x})$ . If  $\alpha$  is a vertex of the Newton polytope,

then there exists  $\hat{x} \in \mathbb{R}_{>0}^m$  such that the sign of  $p(\hat{x})$  agrees with the sign of the coefficient of the monomial.

Fig. 3 shows the Newton polytopes associated with the networks in the figure. The vertex corresponding to the monomial of interest is shown in red. To find the vertices of the Newton polytope from the exponent vectors, one can for example use the software Polymake [31] or Maple, as we demonstrate in the **Supporting Information**.

## Discussion

The main result of this paper, the algorithm to identify parameter regions for unique and multiple equilibria, combines Brouwer degree theory and algebraic geometry. In particular, under the assumptions of the theorem, we show that there exist SCCs with at least two equilibria if, and only if, a certain multivariate polynomial can attain a specific sign.

Discriminating regions of the parameter space where multistationarity occurs is a hard mathematical problem, theoretically addressable by computationally expensive means [17]. Our algorithm beautifully overcomes these difficulties by building on a simple idea, the computation of the Brouwer degree of a function related to a dissipative network. Additionally, not only closed-form expressions in the parameters are obtained, but, as illustrated in examples, these expressions are often interpretable in biochemical terms, providing an explanation of *why* multistationarity occurs.

Previous work based on so-called *injectivity* [10, 22, 32–36] is closely related to part (A) of the theorem. A general version of the injectivity theorems state that multistationarity is precluded if  $\det(M(x)) = (-1)^s$  for all  $x \in \mathbb{R}_{>0}^n$  and all  $\kappa \in \mathbb{R}_{>0}^\ell$ . In this case, part (A) guarantees additionally the existence of exactly one equilibrium in each  $\mathcal{P}_c$  (for each  $\kappa$ ). It might also be the case that for a particular  $\kappa$ ,  $\det(M(x)) = (-1)^{s+1}$  for some  $x \in \mathbb{R}_{>0}^n$  (injectivity theorems are silent in this case), while (A) holds for a given parameterization (see the **Supporting Information**, §7.3 for an example). In [37] conditions for the existence of degenerate equilibria, where one expects  $\det(M(x))$  to change sign, are given.

Several natural questions remain outside the reach of our algorithm. Firstly one would like to determine the particular SCCs for which there are multiple equilibria. As stated in the theorem, if  $\text{sign}(a(\hat{x})) = (-1)^{s+1}$ , then  $c := W\Phi(\hat{x})$  defines a SCC with multiple equilibria. However, this only establishes  $c$  indirectly through  $\hat{x}$ .

Secondly, one could ask for parameter regions differentiating between the precise number of equilibria (that is, 0, 1, 2, ...). This question should be seen in conjunction with the previous question: in typical examples, when there are two equilibria in a particular SCC, then there exists another class for which there are three. Hence the number of equilibria cannot be separated from the SCCs.

A third question concerns the stability of the equilibria, which cannot be obtained from our algorithm. It is, however, known that if the sign of the Jacobian evaluated at an equilibrium is  $(-1)^{s+1}$ , then it is unstable [22].

We consider our research a step in the direction of providing ‘black box tools’ to analyse complex dynamical systems. Such tools would easily find their use in systems and synthetic biology, where it is commonplace to consider (many) competing models. A particular problem is to exclude models that cannot explain observed qualitative features, such as multistationarity.

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# Identifying parameter regions for multistationarity

## Supplementary Information

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In this document we prove the claims of the main text. The document is self-contained and does not require parallel reading of the main text. For this reason some parts of the main text are repeated here for convenience.

Sections 1 to 4 focus on the proofs of the proposition and theorem in the main text. We start by introducing some preliminaries before recapitulating the main facts about Brouwer degree theory. Then we compute the Brouwer degree for a special class of functions (Theorem 2). We proceed to introduce the necessary background on reaction networks and to state and prove a key result regarding the Brouwer degree of a reaction network with a dissipative semiflow (Theorem 3). In Section 4 we use Theorem 3 to prove the theorem of the main text.

Subsequently in Section 5, we provide details on how to check the steps in the procedure of the main text. In Section 6 we give details of the two examples in the main text (hybrid histidine kinase and a gene transcription network). Finally, in Section 7 we work out three more examples: the two examples in Table 1 of the main text, and an additional one.

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# 1 Preliminaries

## 1.1 Convex sets

We let  $\mathbb{R}_{\geq 0}^n$  denote the non-negative orthant of  $\mathbb{R}^n$  and  $\mathbb{R}_{> 0}^n$  denote the positive orthant of  $\mathbb{R}^n$ .

For a subset  $B$  of  $\mathbb{R}^n$ , we let  $\text{bd}(B)$  denote the boundary of  $B$  and  $\text{cl}(B)$  the closure of  $B$ , such that  $\text{cl}(B) = \text{bd}(B) \cup B$ . If  $B$  is open, then  $\text{bd}(B) \cap B = \emptyset$ . If  $B$  is bounded, then  $\text{cl}(B)$  is compact.

A set  $B$  is *convex* if the following holds:

$$\text{if } x_1, x_2 \in B \text{ then } \lambda x_1 + (1 - \lambda)x_2 \in B \text{ for all } 0 \leq \lambda \leq 1.$$

Let  $B \subseteq \mathbb{R}^n$  be a convex set. We say that  $v \in \mathbb{R}^n$  *points inwards*  $B$  at  $x \in \text{bd}(B)$  if  $x + \epsilon v \in \text{cl}(B)$  for all  $\epsilon > 0$  small enough. In particular,  $v = 0$  points inwards  $B$  at all  $x \in \text{bd}(B)$ . If  $v$  points inwards  $B$  at  $x \in \text{bd}(B)$ , then it also points inwards  $\text{cl}(B)$  at  $x \in \text{bd}(B)$ . The vector  $v$  *points outwards*  $B$  at  $x \in \text{bd}(B)$ , if it does not point inwards  $B$  at  $x \in \text{bd}(B)$ .

We will use the following facts about convex sets.

**Lemma 1.** *Let  $B \subseteq \mathbb{R}^n$  be a convex set. Then the following holds:*

(i) *The closure  $\text{cl}(B)$  of  $B$  is convex.*

(ii) *Assume  $B$  is open and consider  $x_1 \in B$ ,  $x_2 \in \text{bd}(B)$ . Let*

$$[x_1, x_2) = \{tx_1 + (1 - t)x_2 \mid 0 < t \leq 1\}$$

*be the half-closed line segment between  $x_1$  and  $x_2$ . Then  $[x_1, x_2) \in B$ .*

(iii) *Let  $x_1 \in B$  and  $x_2 \in \text{bd}(B)$ . Then the vector  $x_1 - x_2$  points inwards  $B$  at  $x_2$ . If  $B$  is open, then the vector  $x_2 - x_1$  points outwards  $B$  at  $x_2$ .*

*Proof.* (i) See Theorem 6.2 in [38]. (ii) See Theorems 6.1 in [38]. (iii) Consider  $x = x_2 + \epsilon(x_1 - x_2) = (1 - \epsilon)x_2 + \epsilon x_1$  with  $0 < \epsilon < 1$ . By convexity,  $x$  belongs to  $\text{cl}(B)$ , hence  $x_1 - x_2$  points inwards  $B$  at  $x_2 \in \text{bd}(B)$ . Assume that  $x_2 - x_1$  also points inwards  $B$  at  $x_2$  and that  $B$  is open. Then, for small  $\epsilon$  we have  $x = x_2 + \epsilon(x_1 - x_2) \in B$  by (ii) (which is stronger than  $x \in \text{cl}(B)$ ), and  $x' = x_2 + \epsilon(x_2 - x_1) \in \text{cl}(B)$  by definition of pointing inwards. Again by (ii),  $\frac{1}{2}x + \frac{1}{2}x' = x_2 \in B$ , contradicting that  $x_2 \in \text{bd}(B)$  ( $B$  is open). Hence  $x_2 - x_1$  points outwards  $B$  at  $x_2$ .  $\square$

## 1.2 Functions

Given an open set  $B \subseteq \mathbb{R}^n$ , we let  $\mathcal{C}^k(B, \mathbb{R}^m)$  denote the set of  $\mathcal{C}^k$ -functions from  $B$  to  $\mathbb{R}^m$ . If  $B$  is open and bounded, then we let  $\mathcal{C}^k(\text{cl}(B), \mathbb{R}^m)$  denote the subset of  $\mathcal{C}^k(B, \mathbb{R}^m)$ -functions  $f$  whose  $j$ -th derivative  $d^j f$ ,  $j = 0, \dots, k$ , extends continuously to the boundary of  $B$ . Equivalently,  $d^j f$  is uniformly continuous in  $B$  for  $j = 0, \dots, k$ , since  $\text{cl}(B)$  is compact.

For  $f \in \mathcal{C}^1(B, \mathbb{R}^n)$  and  $x^* \in B$ , we let  $J_f(x^*) \in \mathbb{R}^{n \times n}$  be the *Jacobian* of  $f$  evaluated at  $x^*$ , that is,  $J_f(x^*)$  is the matrix with  $(i, j)$ -entry  $\partial f_i(x^*) / \partial x_j$ . We say that  $y \in \mathbb{R}^n$  is a *regular value* for  $f$  if  $J_f(x)$  is non-singular for all  $x \in B$  such that  $y = f(x)$ . If this is not the case, then we say that  $y$  is a *critical value* for  $f$ .

If  $B \subseteq \mathbb{R}^n$  is open and bounded,  $f \in \mathcal{C}^1(\text{cl}(B), \mathbb{R}^n)$  and  $y$  is a regular value for  $f$  such that  $y \notin f(\text{bd}(B))$ , then the set

$$\{x \in B \mid f(x) = y\}.$$

is finite [39, Lemma 1.4].

## 2 Brouwer degree and a theorem

### 2.1 Brouwer degree

We first recall basic facts about the *Brouwer degree*. We refer to Section 14.2 in [40] for background and fundamental properties of the Brouwer degree. See also the lecture notes by Vandervorst [39].

In this section we let  $B \subseteq \mathbb{R}^n$  be an open bounded set. We use the symbol  $\deg(f, B, y)$  to denote the Brouwer degree (which is an integer number) of a function  $f \in \mathcal{C}^0(\text{cl}(B), \mathbb{R}^n)$  with respect to  $(B, y)$ ,  $y \in \mathbb{R}^n \setminus f(\text{bd}(B))$ .

A main property of the Brouwer degree is that if  $y \notin f(\text{cl}(B))$ , then  $\deg(f, B, y) = 0$  (but not vice versa) and if  $\deg(f, B, y) \neq 0$ , then there exists at least one  $x \in B$  such that  $y = f(x)$ . In particular, the Brouwer degree can be used to study the number of solutions to the equation

$$f(x) = y, \quad x \in B,$$

provided  $y \notin f(\text{bd}(B))$  and  $f \in \mathcal{C}^0(\text{cl}(B), \mathbb{R}^n)$ .

The Brouwer degree  $\deg(f, B, y)$  is characterized by the following properties:

(A1) **Normalization.** Let  $\text{id}_B$  denote the identity map from  $B$  to itself. If  $y \in B$ , then

$$\deg(\text{id}_B, B, y) = 1.$$

(A2) **Additivity.** If  $B_1$  and  $B_2$  are disjoint open subsets of  $B$  such that  $y \notin f(\text{cl}(B) \setminus (B_1 \cup B_2))$ , then

$$\deg(f, B, y) = \deg(f, B_1, y) + \deg(f, B_2, y).$$

(A3) **Homotopy invariance.** Let  $f, g: \text{cl}(B) \rightarrow \mathbb{R}^n$  be two homotopy equivalent  $\mathcal{C}^0$ -functions via a continuous homotopy  $H: \text{cl}(B) \times [0, 1] \rightarrow \mathbb{R}^n$  such that  $H(x, 0) = f(x)$  and  $H(x, 1) = g(x)$ . If  $y \notin H(\text{bd}(B) \times [0, 1])$ , then

$$\deg(f, B, y) = \deg(g, B, y).$$

(A4) **Translation invariance.**  $\deg(f, B, y) = \deg(f - y, B, 0)$ .

To prove our main result (Theorem 4 below) we need the following well-known property of the Brouwer degree, see e.g. [40, Theorem 14.4]:

**Theorem 1.** *Let  $f \in \mathcal{C}^1(\text{cl}(B), \mathbb{R}^n)$  with  $B \subseteq \mathbb{R}^n$  an open bounded set. If  $y$  is a regular value for  $f$  and  $y \notin f(\text{bd}(B))$ , then*

$$\deg(f, B, y) = \sum_{\{x \in B \mid f(x) = y\}} \text{sign}(\det(J_f(x))), \quad (9)$$

where the sum over an empty set is defined to be zero.

**Corollary 1.** *Under the assumptions of Theorem 1, assume  $\deg(f, B, y) = \pm 1$ . Then the equation  $f(x) = 0$  has at least one solution  $x \in B$  and the number of solutions in  $B$  is odd.*

### 2.2 The Brouwer degree for a special class of functions

In this section we use Theorem 1 and the homotopy invariance of the Brouwer degree (A3) to compute the Brouwer degree of certain functions. Specifically, we are concerned with  $\mathcal{C}^1$ -functions

$$f: \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}^n, \quad (10)$$

and matrices  $W \in \mathbb{R}^{d \times n}$  of maximal rank  $d$ . A priori there is no relationship between  $f$  and  $W$ .

Assume that  $W$  is row reduced and let  $i_1, \dots, i_d$  be the indices of the first non-zero coordinate of each row,  $i_1 < \dots < i_d$ . Let  $c \in \mathbb{R}^d$  and define the  $\mathcal{C}^1$ -function

$$\varphi_c(x): \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}^n$$

by

$$\varphi_c(x)_i = \begin{cases} f_i(x) & i \notin \{i_1, \dots, i_d\} \\ (Wx - c)_i & i \in \{i_1, \dots, i_d\}. \end{cases} \quad (11)$$

We say that  $\varphi_c$  is constructed from  $f$  and  $W$ . The dependence of  $\varphi_c$  on  $f$  and  $W$  is omitted in the notation. We will make use of this construction with different choices of  $f$  and  $W$ .

Define the positive closed and open level sets of  $W$  by

$$\mathcal{P}_c = \{x \in \mathbb{R}_{\geq 0}^n \mid Wx = c\}, \quad \mathcal{P}_c^+ = \{x \in \mathbb{R}_{> 0}^n \mid Wx = c\}. \quad (12)$$

It follows readily that the two sets are convex. By reordering the columns of  $W$ , the vector  $(x_1, \dots, x_n)$  and the coordinates of  $f$  simultaneously, if necessary, we can assume without loss of generality that  $\{i_1, \dots, i_d\} = \{1, \dots, d\}$ . In this case,  $W$  has the block form

$$W = (I_d \quad \widehat{W}), \quad (13)$$

where  $\widehat{W} \in \mathbb{R}^{d \times s}$ ,  $s := n - d$ , and  $I_d$  is the identity matrix of size  $d$ . The last  $s$  coordinates of the function  $\varphi_c$  come from  $f$ .

Assuming this reordering, let  $\pi: \mathbb{R}^n \rightarrow \mathbb{R}^s$  be the projection onto the last  $s$  coordinates. Using (13), it follows that

$$Wx = c \quad \text{if and only if} \quad (x_1, \dots, x_d)^T = c - \widehat{W}(\pi(x)). \quad (14)$$

In particular, for  $x, y \in \mathbb{R}^n$  fulfilling  $Wx = Wy$ , we have that

$$x = y \quad \text{if and only if} \quad \pi(x) = \pi(y). \quad (15)$$

If  $Wf(x) = 0$ , then it follows from (15) that  $f(x) = 0$  if and only if  $\pi(f(x)) = 0$ .

Our first result concerns the Brouwer degree of  $\varphi_c$ . The proof of the theorem is adapted from the proof of Lemma 2 in [41] in order to account for the reduction in dimension introduced by  $\mathcal{P}_c$ .

**Theorem 2.** *Let  $f: \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}^n$  be a  $\mathcal{C}^1$ -function and  $W \in \mathbb{R}^{d \times n}$  a matrix of rank  $d$ . Let  $s := n - d$ ,  $c \in \mathbb{R}^d$ ,  $\mathcal{P}_c$  as in (12) and  $\varphi_c$  as in (11). Let  $B_c$  be an open, bounded and convex subset of  $\mathbb{R}_{> 0}^n$  such that*

- (i)  $B_c \cap \mathcal{P}_c \neq \emptyset$ .
- (ii)  $f(x) \neq 0$  and  $Wf(x) = 0$  for  $x \in \text{bd}(B_c) \cap \mathcal{P}_c$ .
- (iii) for every  $x \in \text{bd}(B_c) \cap \mathcal{P}_c$ , the vector  $f(x)$  points inwards  $B_c$  at  $x$ .

Then

$$\deg(\varphi_c, B_c, 0) = (-1)^s.$$

*Proof.* Without loss of generality, we might assume that  $W$  has the block form in (13). Choose an arbitrary point  $\bar{x} \in B_c \cap \mathcal{P}_c$ , which exists by assumption (i), and consider the continuous function  $G: \text{cl}(B_c) \rightarrow \mathbb{R}^n$  defined by

$$G(x) = (Wx - c, \pi(\bar{x} - x)) \in \mathbb{R}^d \times \mathbb{R}^s \cong \mathbb{R}^n,$$

where  $\pi$  is the projection map onto the last  $s$  coordinates of  $\mathbb{R}^n$ . By (13), the Jacobian of  $G$  has the block form

$$J_G(x) = \begin{pmatrix} I_d & \widehat{W} \\ 0 & -I_s \end{pmatrix}.$$

Therefore,  $\det(J_G(x)) = (-1)^s$  for all  $x$ . In particular, 0 is a regular value for  $G$ . Furthermore, if  $G(x) = 0$ , then  $x \in \mathcal{P}_c$  since  $Wx = c$  and  $\pi(\bar{x}) = \pi(x)$ . Using (15), we conclude that  $\bar{x} = x$ . Since  $\bar{x} \notin \text{bd}(B_c)$ , it follows that  $G$  does not vanish on the boundary. We apply Theorem 1 to compute the degree of  $G$  for 0:

$$\deg(G, B_c, 0) = \text{sign}(\det(J_G(\bar{x}))) = (-1)^s.$$

Consider now the following homotopy between the functions  $\varphi_c$  and  $G$ :

$$\begin{aligned} H: \text{cl}(B_c) \times [0, 1] &\rightarrow \mathbb{R}^n \\ (x, t) &\mapsto t\varphi_c(x) + (1-t)G(x). \end{aligned}$$

Clearly,  $H$  is continuous. To apply (A3) to find the degree of  $\varphi_c$ , we need to show that  $H(\text{bd}(B_c) \times [0, 1]) \neq 0$  for all  $t \in [0, 1]$ . Since

$$H(x, t) = (Wx - c, t\pi(f(x)) + (1-t)\pi(\bar{x} - x)),$$

$H(x, t) = 0$  implies that  $Wx = c$  and hence  $x \in \mathcal{P}_c$ . Thus, we need to show that

$$t\pi(f(x)) + (1-t)\pi(\bar{x} - x) \neq 0 \quad \text{for all } x \in \text{bd}(B_c) \cap \mathcal{P}_c. \quad (16)$$

For  $t = 1$ , (16) follows from (15) using that  $f(x) \neq 0$  and  $Wf(x) = 0$  for  $x \in \text{bd}(B_c) \cap \mathcal{P}_c$  by assumption (ii). For  $t = 0$ , we have already shown that  $G$  does not vanish on the boundary of  $B_c$ .

Assume now that for  $t \in (0, 1)$ , (16) does not hold. That is, there exists  $x' \in \text{bd}(B_c) \cap \mathcal{P}_c$  such that

$$\pi(f(x')) = \frac{t-1}{t}\pi(\bar{x} - x').$$

Since  $x' \in \text{bd}(B_c) \cap \mathcal{P}_c$ , we have that  $Wf(x') = 0$  and  $W(\bar{x} - x') = 0$ . We conclude using (15) that

$$f(x') = \frac{t-1}{t}(\bar{x} - x'). \quad (17)$$

Since  $\frac{t-1}{t} < 0$ ,  $\bar{x} \in B_c$  and  $x' \in \text{bd}(B_c)$ , it follows from Lemma 1(iii) that  $f(x')$  points outwards  $B_c$  at  $x'$ , contradicting assumption (iii).

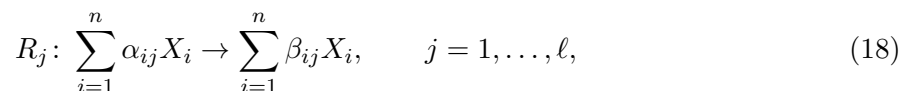
Therefore,  $H(x, t) \neq 0$  for all  $x \in \text{bd}(B_c)$  and  $t \in [0, 1]$ . As a consequence, the homotopy invariance of the Brouwer degree (A3), gives the desired result

$$\deg(\varphi_c, B_c, 0) = \deg(G, B_c, 0) = (-1)^s. \quad \square$$

### 3 Chemical reaction networks

#### 3.1 Setting

Consider a chemical reaction network with species set  $\{X_1, \dots, X_n\}$  and reactions:



where  $\alpha_{ij}, \beta_{ij}$  are non-negative integers.

The ODE system associated with the chemical reaction network  $G$  (as described in the main text) takes the form

$$\dot{x} = f(x) = Nv(x), \quad f: \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}^n, \quad (19)$$

where  $N \in \mathbb{R}^{n \times \ell}$  is the stoichiometric matrix and  $v(x)$  is the vector of rate functions, which are assumed to be  $\mathcal{C}^1$ -functions (e.g. mass-action monomials).

We say that the network has rank  $s$  if the rank of the stoichiometric matrix is  $s$ . The stoichiometric compatibility classes are the convex sets  $\mathcal{P}_c$  defined in (12), where  $W$  is a matrix such that the rows form a basis of  $\text{im}(N)^\perp$ . By construction, a trajectory of (19) is confined to the stoichiometric compatibility class where its initial condition belongs to. The positive stoichiometric compatibility classes  $\mathcal{P}_c^+$  are defined accordingly.

The positive solutions to the system of equations  $\varphi_c(x) = 0$  with  $\varphi_c$  as in (11), are precisely the positive equilibria of the network in the stoichiometric compatibility class  $\mathcal{P}_c$ .

Let  $\phi(x, t)$  denote the flow of the ODE system and let the semiflow of the ODE system be the restriction of the flow to  $t \geq 0$ . It is assumed that the choice of rate functions  $v(x)$  is such that

$$v_j(x) = 0 \quad \text{if} \quad x_i = 0 \quad \text{for some } i \text{ with } \alpha_{ij} > 0. \quad (20)$$

In particular, mass-action kinetics fulfil this condition. Under this assumption, the non-negative and the positive orthants,  $\mathbb{R}_{\geq 0}^n$  and  $\mathbb{R}_{> 0}^n$ , are forward invariant under the ODE system (19), cf. [20, Section 16]. That is, if  $x_0 \in \mathbb{R}_{\geq 0}^n$  (resp.  $\mathbb{R}_{> 0}^n$ ), then the solution to the ODE system (19) with initial condition  $x_0$  is confined to  $\mathbb{R}_{\geq 0}^n$  (resp.  $\mathbb{R}_{> 0}^n$ ):

$$x_0 \in \mathbb{R}_{\geq 0}^n \Rightarrow \phi(x_0, t) \in \mathbb{R}_{\geq 0}^n, \quad \forall t \geq 0 \quad \text{in the interval of definition.} \quad (21)$$

Forward invariance implies that the semiflow  $\phi(x, t)$  maps  $\mathbb{R}_{\geq 0}^n$  to itself for any fixed  $t \geq 0$  for which the solution is defined.

Since the dynamics is confined to the stoichiometric compatibility classes, this implies that for a point  $x'$  at the relative boundary of  $\mathcal{P}_c$ , the vector  $f(x')$  points inwards  $\mathcal{P}_c$ . Further, both  $\mathcal{P}_c$  and  $\mathcal{P}_c^+$  are also forward invariant sets. Recall that these are convex sets.

### 3.2 Conservative and dissipative networks

**Definition 1.** A chemical reaction network is conservative if  $\text{im}(N)^\perp$  contains a positive vector, that is, if  $\mathbb{R}_{> 0}^n \cap \text{im}(N)^\perp \neq \emptyset$ .

A network is *conservative* if and only if the stoichiometric compatibility classes  $\mathcal{P}_c$  are compact subsets of  $\mathbb{R}_{\geq 0}^n$  [21].

**Definition 2.** Consider a network with associated ODE system  $\dot{x} = Nv(x)$ . The semiflow of the network is *dissipative* if for all  $c \in \mathbb{R}^d$  such that  $\mathcal{P}_c^+ \neq \emptyset$ , there exists a compact set  $K_c \subseteq \mathcal{P}_c$  such that  $\phi(x, t) \in K_c$  for all  $x \in \mathcal{P}_c$  and  $t \geq t(x)$ , for some  $t(x) \geq 0$ . That is, all trajectories in  $\mathcal{P}_c$  enter  $K_c$  at some point.

The set  $K_c$  is called *attracting* (and sometimes *absorbing*) [42]. Equivalently, the semiflow of a network is dissipative if all trajectories are *eventually uniformly bounded*, that is, there exists a constant  $k > 0$  such that

$$\limsup_{t \rightarrow +\infty} x_i(t) \leq k$$

for all  $i = 1, \dots, n$  and all initial conditions in  $\mathcal{P}_c$ , provided that  $\mathcal{P}_c^+ \neq \emptyset$  for  $c$  arbitrary.

If the semiflow of the network is dissipative, then the unique solution to the ODE system (19) for a given initial condition is defined for all  $t \geq 0$ , in which case the semiflow is said to be forward complete.

**Lemma 2.** Consider a network with a dissipative semiflow and let  $c \in \mathbb{R}^d$  such that  $\mathcal{P}_c^+ \neq \emptyset$ . Further, let  $K_c \subseteq \mathcal{P}_c$  be an attracting set. Then the following holds:

- (i) The set  $K_c \cap \mathbb{R}_{> 0}^n$  is non-empty, that is,  $K_c \not\subseteq \text{bd}(\mathbb{R}_{\geq 0}^n)$ .
- (ii) All  $\omega$ -limit points in  $\mathcal{P}_c$  of the system are contained in  $K_c$ . In particular, all positive equilibria in  $\mathcal{P}_c$  belong to  $K_c$ .
- (iii) There exists an attracting set  $K'_c$  such that  $K_c \subseteq K'_c$ ,  $K'_c$  is forward invariant and all  $\omega$ -limit points outside the boundary of  $\mathbb{R}_{\geq 0}^n$  are in the interior of  $K'_c$  (relatively to  $\mathcal{P}_c$ ).



*Proof.* (i) It follows from the fact that  $\mathcal{P}_c^+ \neq \emptyset$  and that the positive orthant is forward invariant under the ODE system  $\dot{x} = Nv(x)$ .

(ii) If it were not the case, there would exist an  $\omega$ -limit point  $x' \in \mathcal{P}_c \setminus K_c$ , a trajectory  $\phi(x, t)$  and a sequence of time points  $t_i$  such that  $\lim_{i \rightarrow \infty} t_i = \infty$  and  $\lim_{i \rightarrow \infty} \phi(x, t_i) = x'$ . As  $K_c$  is closed, there exists an open ball  $B_\epsilon(x')$  in  $\mathbb{R}^n$  such that  $B_\epsilon(x') \cap K_c = \emptyset$  and  $\phi(x, t) \in B_\epsilon(x')$  for arbitrary many time points. However, this contradicts that  $K_c$  is an attracting set.

(iii) By (ii) and choosing  $K_c$  potentially larger, all  $\omega$ -limit points outside the boundary of  $\mathbb{R}_{\geq 0}^n$  are in the interior of  $K_c$  (relatively to  $\mathcal{P}_c$ ). The existence of an attracting set  $K'_c$  that includes  $K_c$  and is forward-invariant is proven in the first part of the proof of Lemma 2 in [42]. In the notation of [42],  $K'_c = K^+$ .  $\square$

The semiflow of a conservative network is dissipative. Indeed, it is sufficient to take  $K_c = \mathcal{P}_c$ , since  $\mathcal{P}_c$  is compact. If the network is not conservative, then the semiflow associated with the network might still be dissipative (see Example ‘‘Gene transcription network’’ [15] in the main text). However, in general, it is not straightforward to show that. In some cases it is possible to prove dissipativity by constructing a suitable Lyapunov function. It is the idea underlying the proof of the next proposition.

**Proposition 1.** *Assume that for all  $c \in \mathbb{R}^d$  such that  $\mathcal{P}_c^+ \neq \emptyset$ , there exists a vector  $\omega \in \mathbb{R}_{> 0}^n$  and a real number  $r > 0$  such that  $\omega \cdot f(x) < 0$  for all  $x \in \mathcal{P}_c$  with  $\|x\| \geq r$ , where  $\|\cdot\|$  is any norm. (Note that  $\omega$  and  $r$  might depend on  $c$ .) Then the semiflow of the network is dissipative.*

*Proof.* Let  $c \in \mathbb{R}^d$  with  $\mathcal{P}_c^+ \neq \emptyset$  and let  $\omega$  be as given in the statement. Define

$$V(x) = \sum_{i=1}^n \omega_i x_i \quad \text{for } x \in \mathbb{R}_{\geq 0}^n.$$

The function  $V(x)$  satisfies  $V(0) = 0$  and  $V(x) > 0$  for all  $x \in \mathbb{R}_{\geq 0}^n$ , different from 0. Further, for  $\|x\| \geq r$  and  $x \in \mathcal{P}_c$ ,  $\dot{V}(x) = \nabla V \cdot f(x) = \omega \cdot f(x) < 0$  by assumption. Thus,  $V(x)$  is a strict Lyapunov function and  $V(\phi(x, t))$  is strictly decreasing along trajectories  $\phi(x, t)$  in  $\mathcal{P}_c$  as long as  $\|\phi(x, t)\| \geq r$ . Choose  $R > 0$  such that

$$\{x \in \mathbb{R}_{\geq 0}^n \mid \|x\| \leq r\} \subseteq \{x \in \mathbb{R}_{\geq 0}^n \mid V(x) \leq R\},$$

and define  $K_c = \{x \in \mathbb{R}_{\geq 0}^n \mid V(x) \leq R\} \cap \mathcal{P}_c$ . The set  $K_c$  is compact by construction and forward invariant since  $\dot{V}(x) < 0$  for all  $\|x\| \geq r$ . Further, all trajectories eventually enter  $K_c$  within finite time, that is,  $K_c$  is attracting. Indeed, if this were not the case, then there would exist  $x \in \mathcal{P}_c$ ,  $x \notin K_c$  (hence  $\|x\| > r$ ) such that  $V(\phi(x, t))$  is decreasing for all  $t \geq 0$  in the interval of definition and bounded above by  $R$ . As a consequence, the trajectory is defined for all  $t \geq 0$  and  $(*) \lim_{t \rightarrow \infty} V(\phi(x, t)) = R' \geq R$  for some  $R'$ . Hence  $\phi(x, t)$  is in  $B_\epsilon := \{x \mid V(x) \leq R' + \epsilon\}$  for large  $t$  (and any  $\epsilon > 0$ ). Since  $B_\epsilon$  is compact it follows that the semiflow  $\phi(x, t)$  has at least one  $\omega$ -limit point in  $B_\epsilon$ . By virtue of  $(*)$ , all  $\omega$ -limit points  $x'$  of  $\phi(x, t)$  must fulfil  $V(x') = R'$ . Further, the set of  $\omega$ -limit points is forward invariant and since  $V(x') = R'$  it must be that  $\dot{V}(x') = 0$ . This contradicts the assumption that  $\dot{V}(x) < 0$  for all  $x$  with  $\|x\| \geq r$ . We conclude that there exists  $t(x) \geq 0$  such that  $\phi(x, t) \in K_c$  for all  $x \in \mathcal{P}_c$  and  $t \geq t(x)$ . Hence, the semiflow is dissipative.  $\square$

### 3.3 Degree for dissipative semiflows

The main results to establish a characterization of regions of multistationarity (Theorem 4) are Theorem 1 and the theorem below. The proof of the theorem relies on Theorem 2 and ideas developed in [42].

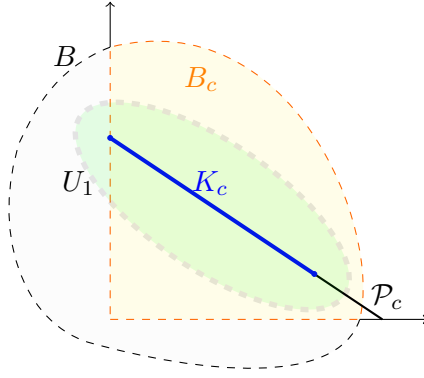


Figure 4: Step (A). The set  $\mathcal{P}_c$  is the straight line connecting the two axis. The compact attracting set  $K_c$  is depicted in blue. The set  $B \subseteq \mathbb{R}^n$  is an open set containing  $K_c$  and  $B_c = B \cap \mathbb{R}_{>0}^n$  is the restriction of  $B$  to the positive orthant (shown in orange), such that  $B_c$  is open. Hence  $K_c$  is contained in  $B_c$ , except for points on the boundary  $K_c \cap \text{bd}(\mathbb{R}_{\geq 0}^n)$ , hence also  $B_c \cap \mathcal{P}_c \neq \emptyset$ . Step (B). The open set  $U_1 \subseteq \mathbb{R}^n$  (in green) is chosen such that  $K_c \subseteq U_1 \subseteq B$ . In the  $\mathcal{C}^1$ -partition of unit, the support of  $\psi_1$  is in  $U_1$  and that of  $\psi_2$  is in  $\mathbb{R}^n \setminus K_c$ .

**Theorem 3.** Consider a network of rank  $s$  with an associated ODE system  $\dot{x} = f(x)$  where  $f(x) = Nv(x)$  as in (19). Assume (20) holds on the rate functions and let  $W \in \mathbb{R}^{d \times n}$ ,  $d = n - s$ , be a row reduced matrix such that the rows of  $W$  form a basis of  $\text{im}(N)^\perp$ . Let  $c \in \mathbb{R}^d$  such that  $\mathcal{P}_c^+ \neq \emptyset$ . Further, assume that:

- The semiflow of the network is dissipative, and that
- $f(x) \neq 0$  for all  $x \in \text{bd}(\mathbb{R}_{\geq 0}^n) \cap \mathcal{P}_c$ . That is, there are no boundary equilibria in  $\mathcal{P}_c$ .

Then there exists an open bounded and convex set  $B_c \subseteq \mathbb{R}_{>0}^n$  that contains all positive equilibria of the network in the stoichiometric compatibility class  $\mathcal{P}_c$ , and such that

$$\deg(\varphi_c, B_c, 0) = (-1)^s,$$

where  $\varphi_c$  is defined in (11) from  $f$  and  $W$ .

*Proof.* The idea of the proof is to construct a function  $g$  defined on  $\mathbb{R}_{\geq 0}^n$  and a set  $B_c \subseteq \mathbb{R}_{>0}^n$  such that the conditions of Theorem 2 are fulfilled for  $g, W$  and  $B_c$ . If we let  $\varphi_c^g$  be the function  $\varphi_c$  in (11) constructed from the function  $g$  and  $W$ , this will imply that  $\deg(\varphi_c^g, B_c, 0) = (-1)^s$ . Subsequently, we will use homotopy invariance to conclude that also  $\deg(\varphi_c, B_c, 0) = (-1)^s$ .

The function  $g$  will be defined as

$$g(x) = \frac{1}{T}(\phi(x, T) - x) + T\rho(x),$$

where  $\phi(x, t)$  is the semiflow of  $\dot{x} = f(x)$ ,  $K_c$  is a suitably chosen attracting set,  $T$  is the maximum entrance time into  $K_c$  from a specific set, and  $\rho(x)$  is an auxiliary function with certain useful properties (see below).

The proof is divided into four steps. In step (A) we define the set  $B_c$ , choose  $K_c$  and find basic properties of  $B_c$  and  $K_c$ . In step (B), we construct the function  $\rho$ . In step (C), we properly define  $g$  and show that  $g, B_c$  and  $W$  have the required properties to apply Theorem 2. In step (D) we show that  $\varphi_c^g$  and  $\varphi_c$  are homotopy equivalent and conclude the proof of the theorem using the homotopy invariance of the Brouwer degree.

**(A)** Let  $K_c \subseteq \mathcal{P}_c$  be as in Definition 2, that is, a compact attracting set of all trajectories with initial condition in  $\mathcal{P}_c$ . According to Lemma 2,  $K_c$  can be chosen such that all  $\omega$ -limit points in  $\mathcal{P}_c^+$  are interior points of  $K_c$  (relatively to  $\mathcal{P}_c$ ) and such that  $K_c$  is forward invariant. Further, by Lemma 2(i),  $K_c \cap \mathbb{R}_{>0}^n \neq \emptyset$ .

Let  $B \subseteq \mathbb{R}^n$  be an open, bounded and convex set containing  $K_c$ , that is,  $K_c \subseteq B$ . Let  $B_c = \mathbb{R}_{>0}^n \cap B$ . Then  $B_c$  is also open, bounded and convex. Since  $K_c \subseteq \mathbb{R}_{\geq 0}^n \cap B$ , then  $B_c$  contains all points in  $K_c$  except those on the boundary  $K_c \cap \text{bd}(\mathbb{R}_{\geq 0}^n)$ . Further,

$$K_c \subseteq \text{cl}(B_c) \subseteq \mathbb{R}_{\geq 0}^n, \quad \text{and} \quad K_c \cap \text{bd}(B_c) = K_c \cap \text{bd}(\mathbb{R}_{\geq 0}^n),$$

see Figure 4. Since  $\emptyset \neq K_c \cap \mathbb{R}_{>0}^n = B \cap K_c \cap \mathbb{R}_{>0}^n = B_c \cap K_c \subseteq B_c \cap \mathcal{P}_c$ , then

$$B_c \cap \mathcal{P}_c \neq \emptyset.$$

Since  $f(x) \neq 0$  for all  $x \in \text{bd}(\mathbb{R}_{\geq 0}^n) \cap \mathcal{P}_c$  by assumption and  $K_c$  contains all zeros of  $f$  in  $\mathcal{P}_c$ , then  $B_c$  contains all zeros of  $f$  in  $\mathcal{P}_c$ , that is

$$\{x \in \mathcal{P}_c \mid f(x) = 0\} \subseteq B_c. \quad (22)$$

**(B)** The function  $\rho: \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}^n$  in the definition of  $g$  is defined such that it has the following properties:

- (i)  $\rho(x)$  points inwards  $B_c$  for all  $x \in \text{bd}(B_c) \cap \mathcal{P}_c$ .
- (ii)  $\rho(x) = 0$  for  $x \in \mathbb{R}_{>0}^n \cap \text{bd}(B_c) \cap \mathcal{P}_c$ .
- (iii)  $\rho(x) \neq 0$  for  $x \in K_c \cap \text{bd}(B_c)$ .
- (iv)  $W\rho(x) = 0$  for all  $x \in \text{bd}(B_c) \cap \mathcal{P}_c$ .

We first construct two other functions  $\tilde{\rho}$  and  $\psi_1$ , and subsequently define  $\rho: \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}^n$  as the product  $\rho = \tilde{\rho}\psi_1$ . Let  $\tilde{x} \in K_c \cap \mathbb{R}_{>0}^n$  and define  $\tilde{\rho}: \mathbb{R}^n \rightarrow \mathbb{R}^n$  as  $\tilde{\rho}(x) := \tilde{x} - x$ . Let  $U_1 \subsetneq B$  be an open set containing  $K_c$  (which exists since  $B$  is open), see Figure 4. Consider the open cover of  $\mathbb{R}^n$  given by  $U_1$  and  $U_2 = \mathbb{R}^n \setminus K_c$ , such that  $U_1 \cap U_2 \neq \emptyset$  and  $U_1 \cup U_2 = \mathbb{R}^n$ . Choose a  $\mathcal{C}^1$ -partition of unit  $\psi_1, \psi_2: \mathbb{R}^n \rightarrow [0, 1]$  associated with this open cover. This implies in particular that the support of  $\psi_i$  is included in  $U_i$  and  $\psi_1(x) + \psi_2(x) = 1$  for all  $x$ .

Define  $\rho: \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}^n$  by  $\rho(x) = \psi_1(x)\tilde{\rho}(x)$ ,  $x \in \mathbb{R}_{\geq 0}^n$  (note the restriction to  $\mathbb{R}_{\geq 0}^n$ ). This function fulfils properties (i)-(iv) above. Property (i): Follows by definition of  $\rho(x) = \psi_1(x)(\tilde{x} - x)$ ,  $\psi_1(x) \geq 0$  and Lemma 1(iii), using that  $\tilde{x} \in B_c$  and  $x \in \text{bd}(B_c)$ . Property (ii): Since the support of  $\psi_1$  is contained in  $U_1$ ,  $\psi_1(x) = 0$  for all  $x \notin U_1$ , in particular for all  $x \in \mathbb{R}_{>0}^n \cap \text{bd}(B_c) \cap \mathcal{P}_c$ , since  $\mathbb{R}_{>0}^n \cap \text{bd}(B_c) \subseteq \text{bd}(B)$  and  $\text{bd}(B) \cap U_1 = \emptyset$ . Property (iii): Similarly,  $\psi_1(x) = 1$  (since  $\psi_2(x) = 0$ ) for all  $x \notin U_2 = \mathbb{R}^n \setminus K_c$ , that is, for all  $x \in K_c$ ; hence  $\rho(x) \neq 0$  for  $x \in K_c \cap \text{bd}(B_c)$  since  $\tilde{x} \notin \text{bd}(B_c)$ . Property (iv):  $W\rho(x) = \psi_1(x)W(\tilde{x} - x) = 0$  as  $x, \tilde{x} \in \mathcal{P}_c$ .

**(C)** Let  $T$  be defined as the maximum of the entry times to  $K_c$  from any  $x \in \text{cl}(B_c) \cap \mathcal{P}_c$ . The time  $T$  is finite because  $\text{cl}(B_c) \cap \mathcal{P}_c$  is compact and the semiflow is dissipative with respect to  $K_c$ . Note that once a trajectory is in  $K_c$ , it stays there since  $K_c$  is forward invariant. Redefine  $T$  to be any positive number if  $T = 0$ .

We define

$$g: \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}^n, \quad g(x) := \frac{1}{T}(\phi(x, T) - x) + T\rho(x),$$

Observe that  $Wg(x) = 0$  for all  $x \in \text{bd}(B_c) \cap \mathcal{P}_c$ , using property (iv) in step (B) and that  $\phi(x, T), x \in \mathcal{P}_c$ . By definition of  $T$ ,  $\phi(x, T) \in \text{cl}(B_c) \cap \mathcal{P}_c$  if  $x \in \text{cl}(B_c) \cap \mathcal{P}_c$  and hence  $\frac{1}{T}(\phi(x, T) - x)$  points inwards  $B_c$  at  $x \in \text{bd}(B_c) \cap \mathcal{P}_c$  by convexity of  $\text{cl}(B_c)$ . Also  $T\rho(x)$  points inwards  $B_c$  at  $x$  by property (i) in step (B). Hence,  $g(x)$  points inwards  $B_c$  at  $x \in \text{bd}(B_c) \cap \mathcal{P}_c$  by convexity again.

Therefore, the function  $g$ , together with  $B_c$  and  $W$ , fulfil the conditions of Theorem 2. By letting  $\varphi_c^g$  be the function  $\varphi_c$  in (11) constructed from  $g$  and  $W$ , we conclude that

$$\deg(\varphi_c^g, B_c, 0) = (-1)^s.$$

(D) We define a homotopy between  $\varphi_c$  and  $\varphi_c^g$  on  $\text{cl}(B_c) \times [0, T]$  by

$$H(x, t) = \begin{cases} \varphi_c(x) & \text{if } t = 0 \\ (Wx - c, \frac{1}{t}\pi(\phi(x, t) - x) + t\pi(\rho(x))) & \text{if } 0 < t \leq T. \end{cases}$$

The function  $H(x, t)$  is continuous since  $\phi(x, t)$  is differentiable and is the semiflow of  $\dot{x} = f(x)$ . Note that  $H(x, 0) = \varphi_c(x)$  and  $H(x, T) = (Wx - c, \pi(g(x))) = \varphi_c^g(x)$ . Thus  $H(x, t)$  is a homotopy between  $\varphi_c(x)$  and  $\varphi_c^g(x)$ . We need to show that  $H(x, t)$  does not vanish on the boundary  $\text{bd}(B_c)$ .

If  $H(x, 0) = \varphi_c(x) = 0$ , then  $x \in \mathcal{P}_c$  is an equilibrium of the ODE system. Hence  $H(x, 0)$  does not vanish on  $\text{bd}(B_c)$  since  $B_c$  contains all zeros of  $f$  in  $\mathcal{P}_c$ , see (22). Now let  $x' \in \text{bd}(B_c)$  and assume that  $H(x', t) = 0$  for some  $t \in (0, T]$ . It follows that  $x' \in \mathcal{P}_c$ , hence

$$x' \in \text{bd}(B_c) \cap \mathcal{P}_c, \quad \text{and} \quad \pi(\phi(x', t) - x') = -t^2\pi(\rho(x')). \quad (23)$$

Using (15) and property (iv) in step (B) we have that

$$\phi(x', t) = x' - t^2\rho(x'). \quad (24)$$

By construction of  $K_c$ , all fixed points and periodic orbits are contained in  $K_c$ . If  $\rho(x') = 0$ , then (24) implies  $x' \in K_c \cap \text{bd}(B_c)$  as  $x' \in \text{bd}(B_c)$  by assumption. However, this contradicts property (iii) in step (B). Hence, it must be the case that  $\rho(x') \neq 0$ .

Using that  $x' \in \text{bd}(B_c) \cap \mathcal{P}_c$  from (23) and  $\rho(x') \neq 0$ , we conclude that  $x' \in \text{bd}(\mathbb{R}_{\geq 0}^n)$  by property (ii) in step (B), since  $x' \notin \mathbb{R}_{> 0}^n \cap \text{bd}(B_c) \cap \mathcal{P}_c$ . It follows that there exists  $i$  such that  $x'_i = 0$  and we have

$$\phi(x', t)_i = x'_i - t^2\rho(x')_i = x'_i - t^2\psi_1(x)\tilde{\rho}(x)_i = -t^2\psi_1(x)\tilde{x}_i < 0.$$

That is,  $\phi(x', t)$  does not belong to  $\mathbb{R}_{\geq 0}^n$ . However, this contradicts the forward invariance of  $\mathbb{R}_{\geq 0}^n$  with respect to the flow. Therefore,  $H$  does not vanish on  $\text{bd}(B_c) \times [0, T]$ .

With this in place, homotopy invariance of the Brouwer degree implies that

$$\deg(\varphi_c, B_c, 0) = \deg(H(x, 0), B_c, 0) = \deg(H(x, T), B_c, 0) = \deg(\varphi_c^g, B_c, 0) = (-1)^s.$$

□

**Remark 1.** The statement and proof of the theorem focus exclusively on one stoichiometric compatibility class, that is, on a fixed value  $c \in \mathbb{R}^d$ . Therefore, if a semiflow admits an attracting set in one specific stoichiometric compatibility class (and not necessarily in all), then the theorem and computation of the Brouwer degree holds for this specific class.

## 4 Multistationarity in dissipative networks

In this section we prove the main theorem stated in the main text, which is a consequence of Theorem 3 from the previous section.

We assume that the positive solutions to the system  $f(x) = 0$  (with  $f(x)$  as in (19)) admit a parameterisation

$$\begin{aligned} \Phi: \mathbb{R}_{> 0}^m &\rightarrow \mathbb{R}_{> 0}^n \\ (x_1, \dots, x_m) &\mapsto (x_1, \dots, x_m, \Phi_{m+1}(x_1, \dots, x_m), \dots, \Phi_n(x_1, \dots, x_m)), \end{aligned} \quad (25)$$

for some  $m < n$ . That is, we assume that we can express  $x_{m+1}, \dots, x_n$  at equilibrium as functions in  $x_1, \dots, x_m$ :

$$x_i = \Phi_i(x_1, \dots, x_m), \quad i = m + 1, \dots, n,$$

such that  $x_{m+1}, \dots, x_n$  are positive if  $x_1, \dots, x_m$  are positive. Clearly, if a parameterisation exists as a function of some other  $m$  variables, we can always reorder the coordinates such that the parameterisation is in  $x_1, \dots, x_m$ .

For mass-action kinetics, the equation  $f(x) = 0$  results in  $s = n - d$  polynomial equations in  $n$  unknowns, which generically would lead to a  $d$ -dimensional parameterisation and  $m = d$  (if such a parameterisation exists).

When such a parameterisation exists, then positive values of  $x_1, \dots, x_m$  determine uniquely a positive equilibrium. This equilibrium then belongs to the stoichiometric compatibility class given by

$$c := W\Phi(x_1, \dots, x_m).$$

Reciprocally, given  $c$ , the positive solutions to  $\varphi_c(x) = 0$  are in one-to-one correspondence with the positive solutions to the equation  $c = W\Phi(x_1, \dots, x_m)$ .

As before, we let  $W \in \mathbb{R}^{d \times n}$  be a row-reduced matrix whose rows form a basis of  $\text{im}(N)^\perp$ . Let  $i_1, \dots, i_d$  be the indices of the first non-zero coordinate of each row. Let  $\pi: \mathbb{R}^n \rightarrow \mathbb{R}^s$  denote the projection onto the coordinates with indices different from  $i_1, \dots, i_d$ . We do not reorder the coordinates now to ensure that  $\{i_1, \dots, i_d\} = \{1, \dots, d\}$ , because we have already chosen a convenient order of the free variables of the parameterisation.

Consider the Jacobian of the map  $\varphi_c(x)$ . Because  $\varphi_c(x)$  is independent of  $c$ , we denote the Jacobian by  $M(x)$ . The  $i$ -th row of this matrix is given as

$$M(x)_i := J_{\varphi_c(x)}_i = \begin{cases} J_{f_i}(x) & i \notin \{i_1, \dots, i_d\} \\ W_i & i \in \{i_1, \dots, i_d\}, \end{cases}$$

where  $W_i$  is the  $i$ -th row of  $W$ . That is, one can think of  $M(x)$  as being the matrix obtained from the Jacobian of  $f(x)$ , with the  $i_j$ -th row,  $j = 1, \dots, d$ , replaced by the  $j$ -th row of  $W$ .

An equilibrium  $x^*$  of the network is said to be *non-degenerate* if  $M(x^*)$  has rank  $n$ , that is, if  $\det(M(x^*)) \neq 0$ .

We next consider the determinant of  $M(x)$  and use the parameterisation (25) to substitute the values of  $x_{m+1}, \dots, x_n$  by their expressions as functions of  $x_1, \dots, x_m$ . For simplicity, we let

$$\hat{x} = (x_1, \dots, x_m) \in \mathbb{R}^m$$

be the vector of free variables and define

$$a(\hat{x}) = \det(M(\Phi(\hat{x}))). \quad (26)$$

**Theorem 4.** *Let  $G$  be a network of rank  $s$  with associated ODE system (19) such that assumption (20) on the rate functions holds. Assume that*

- (1) *The semiflow of the network is dissipative.*
- (2) *The set of positive equilibria admit a positive parameterisation as in (25).*
- (3) *There are no equilibria in  $\text{bd}(\mathbb{R}_{\geq 0}^n) \cap \mathcal{P}_c$ , as long as  $\mathcal{P}_c^+ \neq \emptyset$ .*

Then

(A) **Uniqueness of equilibria.** *Assume that*

$$\text{sign}(a(\hat{x})) = (-1)^s \quad \text{for all } \hat{x} \in \mathbb{R}_{> 0}^m.$$

*Then there is exactly one positive equilibrium in each stoichiometric compatibility class  $\mathcal{P}_c$  such that  $\mathcal{P}_c^+ \neq \emptyset$ . Further, this equilibrium is non-degenerate.*

(B) **Multiple equilibria.** *Assume that*

$$\text{sign}(a(\hat{x})) = (-1)^{s+1} \quad \text{for some } \hat{x} \in \mathbb{R}_{> 0}^m,$$

*and let  $c \in \mathbb{R}^d$  be given by*

$$c := W\Phi(\hat{x}).$$

*Then there are at least two positive equilibria in the stoichiometric compatibility class  $\mathcal{P}_c$ . In particular, if all positive equilibria are non-degenerate, then there is an odd number strictly greater than one of positive equilibria. If this is not the case, then there are at least two positive equilibria (and possibly infinitely many), of which at least one is non-degenerate.*

*Proof.* The hypotheses ensure that we can apply Theorem 3. Therefore, for each  $c \in \mathbb{R}^d$  such that  $\mathcal{P}_c^+ \neq \emptyset$  we can choose an open bounded convex set  $B_c \subset \mathbb{R}_{>0}^n$  that contains all positive equilibria of the network in the stoichiometric compatibility class  $\mathcal{P}_c$  and such that

$$\deg(\varphi_c, B_c, 0) = (-1)^s.$$

Given  $c$ , note that there is a bijection between the sets

$$\{x \in B_c \mid \varphi_c(x) = 0\} \quad \text{and} \quad \mathcal{S}_c := \{\hat{x} \in \mathbb{R}_{>0}^m \mid c = W\Phi(\hat{x})\}.$$

An element of  $\mathcal{S}_c$  corresponds to a positive equilibrium in the stoichiometric compatibility class  $\mathcal{P}_c$ .

(A) Consider a stoichiometric compatibility class  $\mathcal{P}_c$  defined by  $c$  such that  $\mathcal{P}_c^+ \neq \emptyset$ . For all  $\hat{x} \in \mathbb{R}_{>0}^m$ , we have that  $\det(J_{\varphi_c}(\Phi(\hat{x}))) = a(\hat{x}) \neq 0$ , and hence 0 is a regular value for  $\varphi_c$ . We can therefore apply Theorem 1 and obtain

$$(-1)^s = \sum_{\hat{x} \in \mathcal{S}_c} \text{sign}(a(\hat{x})) = (-1)^s (\#\mathcal{S}_c),$$

where  $\#\mathcal{S}_c$  is the cardinality of  $\mathcal{S}_c$ . We conclude that  $\#\mathcal{S}_c = 1$  and therefore that there exists a unique positive equilibrium in the stoichiometric compatibility class. Furthermore, since  $\text{sign}(a(\hat{x})) \neq 0$ , the equilibrium is non-degenerate.

(B) Let  $\hat{x}$  be such that  $\text{sign}(a(\hat{x})) = (-1)^{s+1}$  and let  $c$  be defined as in the statement of the theorem. Then  $\hat{x} \in \mathcal{S}_c$  and further  $\Phi(\hat{x}) \in \mathcal{P}_c^+$ . Therefore,  $\mathcal{P}_c^+ \neq \emptyset$ . If 0 is a regular value for  $\varphi_c(\cdot)$ , then the equality

$$(-1)^s = \sum_{\hat{x} \in \mathcal{S}_c} \text{sign}(a(\hat{x})) = (-1)^{s+1} + \sum_{\hat{x}' \neq \hat{x}, \hat{x}' \in \mathcal{S}_c} \text{sign}(a(\hat{x}'))$$

implies that there must exist at least two other points  $\hat{x}', \hat{x}'' \in \mathcal{S}_c$ , that is, there are at least three positive equilibria in  $\mathcal{P}_c$ , all of which are non-degenerate. In this case by Corollary 1, there is an odd number of equilibria and they are all non-degenerate.

Assume now that 0 is not a regular value for  $\varphi_c$ . By construction,  $\hat{x}$  defines a positive equilibrium in  $\mathcal{P}_c$  and  $a(\hat{x}) \neq 0$  by hypothesis. Therefore, if 0 is not regular, then there must exist another positive equilibrium  $x^*$  in  $\mathcal{P}_c$  for which the Jacobian of  $\varphi_c(x^*)$  is singular. This implies that there are at least two positive equilibria in  $\mathcal{P}_c$ , one of which is non-degenerate.  $\square$

In typical applications we find an odd number of equilibria ( $\geq 3$ ), all of which are non-degenerate. Observe that the hypothesis for Part (A) holds if the sign of  $\det(M(x))$  is  $(-1)^s$  for all  $x$  in a set containing the positive equilibria. In particular, this is the case for all  $x \in \mathbb{R}_{>0}^n$ .

## 5 Details on the steps of the procedure

In this section we expand further on how to check step 3 and 6 of the algorithm.

### 5.1 On siphons and boundary equilibria

A *siphon* is a set of species  $Z \subseteq \{X_1, \dots, X_n\}$  fulfilling the following closure property: if  $X_i \in Z$  and  $X_i$  is produced in reaction  $R_j$  (that is,  $\beta_{ij} > 0$ ), then there exists  $X_k \in Z$  such that  $X_k$  is consumed in the same reaction (that is,  $\alpha_{kj} > 0$ ). A *minimal siphon* is a siphon that does not properly contain any other siphon.

**Proposition 2.** *If for every minimal siphon  $Z$  there exists a subset  $\{X_{i_1}, \dots, X_{i_k}\} \subseteq Z$ , and a conservation law  $\lambda_1 x_{i_1} + \dots + \lambda_k x_{i_k} = c$  for some positive  $\lambda_1, \dots, \lambda_k$ , then the network has no boundary equilibria in any stoichiometric compatibility class  $\mathcal{P}_c$  with non-empty relative interior,  $\mathcal{P}_c^+ \neq \emptyset$ .*

A proof of this proposition for mass-action kinetics can be found in [43], where strategies to find siphons are also detailed. The proof in [43] is however valid for general kinetics fulfilling assumption (20) (see [26, Prop. 2]). Different algorithms developed in Petri Net theory can be applied to find the siphons of a reaction network.

The hypothesis of the proposition is summarised by saying that each minimal siphon contains the support of a positive conservation law.

**Example 1.** The minimal siphons of the running example in the main text are  $\{X_1, X_2\}$  and  $\{X_3, X_4\}$ . The conservation laws  $x_1 + x_2 = c_1$ ,  $x_3 + x_4 = c_2$  fulfil the requirements of the proposition, and hence the network has no boundary equilibria in any  $\mathcal{P}_{(c_1, c_2)}$  with  $c_1, c_2 > 0$ .

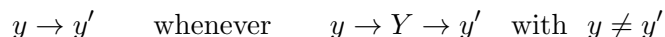
For large networks, the task of finding the siphons can be daunting. A way to reduce the complexity of the computation is by the removal of intermediate species and catalysts [26]. We explain the key aspects of this reduction method here. The method is used in the examples below.

The first reduction concerns **removal of intermediates**. Intermediates are species in the network that do not appear interacting with any other species, are produced in at least one reaction, and consumed in at least one reaction. For example the species  $ES_0$  in the reaction network



is an intermediate.

Given a network, we obtain a reduced network by “removing” some intermediates, one at a time. This is done in the following way. Say we want to remove an intermediate  $Y$  from the network. We remove all reactions of the original network that involve  $Y$  and add a reaction

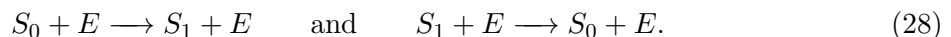


belongs to the original network. Here  $y$  and  $y'$  are the reactant of a reaction  $y \rightarrow Y$  and product of a reaction  $Y \rightarrow y'$ , respectively.

To illustrate this, we consider the removal of the intermediate  $ES_0$  in the network (27). The reactions of the reduced network are obtained by considering all length 2 paths of the original network that go through  $ES_0$ . We have two such paths:

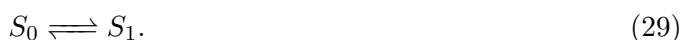


By “collapsing” these paths we obtain the reactions



Clearly the process could be repeated now by choosing other intermediates of the network (if any). In this way we can obtain reduced networks by removing several intermediates.

The second reduction concerns **removal of catalysts**. Catalysts are species that whenever they appear in a reaction, then they appear at both sides and with the same stoichiometric coefficient. For example,  $E$  in the reaction network (28) is a catalyst. Catalysts are actually defined in more generality in [26], but we restrict to this scenario to keep the discussion simple. Catalysts are removed from a network by literally removing them from the reactions where they appear. Removal of  $E$  in the reaction network (28) yields the reaction network



This network has one minimal siphon, namely  $\{S_0, S_1\}$ , and  $s_0 + s_1 = c$  is a conservation law. By Proposition 2, it does not admit boundary equilibria in stoichiometric compatibility classes with non-empty positive part. The next proposition allows us to conclude that the original network in (27) neither admits boundary equilibria in stoichiometric compatibility classes with non-empty positive part.

**Proposition 3** (Theorems 1 and 2 in [26]). *Let  $G$  be a network and  $G'$  be a network obtained after iterative removal of intermediates or catalysts from  $G$  as described above. Each minimal siphon of  $G$  contains the support of a positive conservation law if and only if this is the case for  $G'$ .*

In several cases, removal of intermediates and catalysts yields a so-called *monomolecular network*. That is, a network whose complexes agree with some species or the complex zero. For example, the network in (29) is monomolecular. In this case, checking the hypothesis of Proposition 2 is straightforward, in view of the next lemma.

**Lemma 3** (Proposition 3 in [26]). *Let  $G$  be a monomolecular network. Each minimal siphon of  $G$  contains the support of a positive conservation law if and only if all connected components of  $G$  are strongly connected.*

The network in (29) is clearly strongly connected. Thus, we do not need to find the siphons of the network to conclude that each of its minimal siphons contains the support of a positive conservation law and thereby conclude that (27) does not admit boundary equilibria in stoichiometric compatibility classes with non-empty positive part.

**Corollary 2.** *Let  $G$  be a network and  $G'$  be a network obtained after iterative removal of intermediates or catalysts from  $G$  as described above. If  $G'$  is a monomolecular network with all connected components strongly connected, then  $G$  has no boundary equilibria in any stoichiometric compatibility class  $\mathcal{P}_c$  such that  $\mathcal{P}_c^+ \neq \emptyset$ .*

## 5.2 Newton polytope

We write a multivariate polynomial  $f(x) \in \mathbb{R}[x_1, \dots, x_n]$  as a sum of monomials:

$$f(x) = \sum_{\alpha \in \mathbb{N}^n} c_\alpha x^\alpha,$$

where  $x^\alpha = x_1^{\alpha_1} \dots x_n^{\alpha_n}$  and  $c_\alpha \in \mathbb{R}$ , for which only a finite number are non-zero.

The *Newton polytope* of  $f(x)$ , denoted by  $\mathcal{N}(f)$ , is a closed convex set in  $\mathbb{R}^n$ , defined as the convex hull of the exponents  $\alpha \in \mathbb{N}^n$  for which  $c_\alpha \neq 0$  (See [38, Section 2] for a definition of convex hull). The set of vertices of  $\mathcal{N}(f)$  is a subset of the set of points  $\alpha$  for which  $c_\alpha \neq 0$ .

The following is a well-known fact about the Newton polytope of a polynomial. The proof of the fact is constructive and provides an explicit way to find  $\hat{x}$  in Theorem 4(B). Thus it offers a way to find stoichiometric compatibility classes (i.e. values of  $c$ ) for which multistationarity exists.

**Proposition 4.** *Let  $f(x) = \sum_{\alpha \in \mathbb{N}^n} c_\alpha x^\alpha$  and let  $\alpha'$  be a vertex of  $\mathcal{N}(f)$ . Then there exists  $x' \in \mathbb{R}_{>0}^n$  such that*

$$\text{sign}(f(x')) = \text{sign}(c_{\alpha'}).$$

*Proof.* By hypothesis  $c_{\alpha'} \neq 0$ . Since  $\alpha'$  is a vertex in a bounded convex polytope, there exists a separating hyperplane  $\omega \cdot x = T$  that intersects the polytope only in  $\alpha'$  and such that  $\omega \cdot x' < T$  for any other point  $x'$  of the polytope (see e.g. Definition 3.5 and Theorem 3.8 in [44]). In particular,  $\omega \cdot \alpha < \omega \cdot \alpha'$  for all vertices  $\alpha \neq \alpha'$ .

For  $y = t^\omega$ , we have

$$f(y) = \sum_{\alpha \in \mathbb{N}^n} c_\alpha (t^\omega)^\alpha = \sum_{\alpha \in \mathbb{N}^n} c_\alpha t^{\omega \cdot \alpha} = c_{\alpha'} t^{\omega \cdot \alpha'} + \sum_{\alpha \in \mathbb{N}^n, \alpha \neq \alpha'} c_\alpha t^{\omega \cdot \alpha}.$$

Let  $\omega'' \cdot \alpha''$  be the smallest of the exponents of  $t$  in  $f(y)$  (this exponent might be negative). After multiplication of  $f(y)$  by  $t^{\omega'' \cdot \alpha''}$ , we obtain a univariate polynomial in  $t$  with leading coefficient equal to  $c_{\alpha'}$ . By letting  $t$  be large enough, the sign of  $f(y)$  agrees with the sign of  $c_{\alpha'}$ .  $\square$



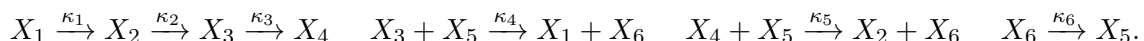
**Finding the vertices in practice.** In the examples below, we find the vertices of the Newton polytope of the polynomial of interest as follows. We use Maple (version 2015). We construct first the polytope using the command *PolyhedralSet* and subsequently use the command *VerticesAndRays*, from the package *PolyhedralSets*, to find the vertices.

## 6 Details on the examples in the main text

In this section we present the details of the algorithm for the two examples in the main text: the hybrid histidine kinase model and a gene transcription network.

### 6.1 Hybrid histidine kinase

Using the notation  $X_1 = \text{HK}_{00}$ ,  $X_2 = \text{HK}_{p0}$ ,  $X_3 = \text{HK}_{0p}$ ,  $X_4 = \text{HK}_{pp}$ ,  $X_5 = \text{RR}$  and  $X_6 = \text{RR}_p$ , the reaction network is



The stoichiometric matrix  $N$  of the network and a row reduced matrix  $W$  whose rows form a basis of  $\text{im}(N)^\perp$  are

$$N = \begin{pmatrix} -1 & 0 & 0 & 1 & 0 & 0 \\ 1 & -1 & 0 & 0 & 1 & 0 \\ 0 & 1 & -1 & -1 & 0 & 0 \\ 0 & 0 & 1 & 0 & -1 & 0 \\ 0 & 0 & 0 & -1 & -1 & 1 \\ 0 & 0 & 0 & 1 & 1 & -1 \end{pmatrix}, \quad W = \begin{pmatrix} 1 & 1 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 \end{pmatrix}. \quad (30)$$

The matrix  $W$  gives rise to the conservation laws

$$x_1 + x_2 + x_3 + x_4 = c_1, \quad x_5 + x_6 = c_2. \quad (31)$$

With mass-action kinetics, the vector of reaction rates is

$$v(x) = (\kappa_1 x_1, \kappa_2 x_2, \kappa_3 x_3, \kappa_4 x_3 x_5, \kappa_5 x_4 x_5, \kappa_6 x_6).$$

The function  $f(x) = Nv(x)$  is thus

$$f(x) = (-\kappa_1 x_1 + \kappa_4 x_3 x_5, \kappa_1 x_1 - \kappa_2 x_2 + \kappa_5 x_4 x_5, -\kappa_3 x_3 + \kappa_2 x_2 - \kappa_4 x_3 x_5, \\ \kappa_3 x_3 - \kappa_5 x_4 x_5, -\kappa_4 x_3 x_5 - \kappa_5 x_4 x_5 + \kappa_6 x_6, \kappa_4 x_3 x_5 - \kappa_6 x_6 + \kappa_5 x_4 x_5).$$

We apply the algorithm to this network with the matrix  $N$  and the vector  $v(x)$ .

**Step 1.** Mass-action kinetics fulfils assumption [3] in the main text. The function  $f(x)$  and  $W$  are given above. The matrix  $W$  in (30) is row reduced.

**Step 2.** The network is conservative since  $(1, 1, 1, 1, 1, 1) \in \text{im}(N)^\perp$ . Therefore the semiflow of the network is dissipative.

**Step 3.** The minimal siphons of the network are  $\{X_1, X_2, X_3, X_4\}$  and  $\{X_5, X_6\}$ . These two sets are the supports of the conservation laws in (31). By Proposition 2, there are no boundary equilibria in any  $\mathcal{P}_c$  as long as  $\mathcal{P}_c^+ \neq \emptyset$ .

**Step 4.** We solve the equilibrium equations  $f_2 = f_3 = f_4 = f_6 = 0$  for  $x_1, x_2, x_3, x_6$ . This gives the following algebraic parameterization  $\Phi: \mathbb{R}_{>0}^2 \rightarrow \mathbb{R}_{>0}^6$  of the set of equilibria in terms of  $\hat{x} = (x_4, x_5)$ :

$$\Phi(x_4, x_5) = \left( \frac{\kappa_4 \kappa_5 x_4 x_5^2}{\kappa_1 \kappa_3}, \frac{\kappa_5 (\kappa_4 x_5 + \kappa_3) x_4 x_5}{\kappa_2 \kappa_3}, \frac{\kappa_5 x_4 x_5}{\kappa_3}, x_4, x_5, \frac{\kappa_5 (\kappa_4 x_5 + \kappa_3) x_4 x_5}{\kappa_3 \kappa_6} \right).$$

**Step 5.** With our choice of  $W$ , we have  $i_1 = 1, i_2 = 5$ . Hence  $\varphi_c$  is obtained by replacing the components  $f_1(x), f_5(x)$  of  $f(x)$  by the expressions derived from the two conservation laws:

$$\varphi_c(x) = (x_1 + x_2 + x_3 + x_4 - c_1, \kappa_1 x_1 - \kappa_2 x_2 + \kappa_5 x_4 x_5, -\kappa_3 x_3 + \kappa_2 x_2 - \kappa_4 x_3 x_5, \\ \kappa_3 x_3 - \kappa_5 x_4 x_5, x_5 + x_6 - c_2, \kappa_4 x_3 x_5 - \kappa_6 x_6 + \kappa_5 x_4 x_5).$$

The Jacobian of  $\varphi_c(x), M(x)$ , is

$$M(x) = \begin{pmatrix} 1 & 1 & 1 & 1 & 0 & 0 \\ \kappa_1 & -\kappa_2 & 0 & \kappa_5 x_5 & \kappa_5 x_4 & 0 \\ 0 & \kappa_2 & -\kappa_3 - \kappa_4 x_5 & 0 & -\kappa_4 x_3 & 0 \\ 0 & 0 & \kappa_3 & -\kappa_5 x_5 & -\kappa_5 x_4 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & \kappa_4 x_5 & \kappa_5 x_5 & \kappa_4 x_3 + \kappa_5 x_4 & -\kappa_6 \end{pmatrix}.$$

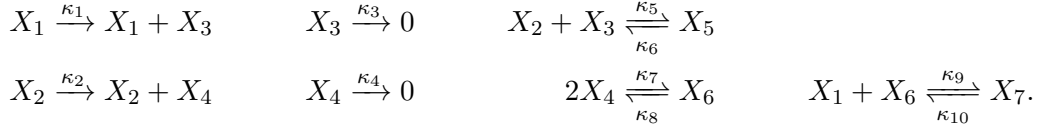
We compute the determinant of  $M(x)$  and evaluate it in  $\Phi(x_4, x_5)$  to obtain the polynomial

$$a(\hat{x}) = \kappa_1 \kappa_2 \kappa_3 \kappa_6 + (\kappa_1 + \kappa_2) \kappa_4 \kappa_5 \kappa_6 x_5^2 + \kappa_2 \kappa_4 \kappa_5^2 \left( \frac{\kappa_1}{\kappa_3} - 1 \right) x_4 x_5^2 \\ + 2\kappa_1 \kappa_2 \kappa_4 \kappa_5 x_4 x_5 + (\kappa_2 + \kappa_3) \kappa_1 \kappa_5 \kappa_6 x_5 + \kappa_1 \kappa_2 \kappa_3 \kappa_5 x_4.$$

**Step 6.** Proceed as in the main text.

## 6.2 Gene transcription network

We use the notation  $X_1 = X_1, X_2 = X_2, X_3 = P_1, X_4 = P_2, X_5 = X_2 P_1, X_6 = P_2 P_2$ , and  $X_7 = X_1 P_2 P_2$ . The reaction network is



The stoichiometric matrix  $N$  of the network and a row reduced matrix  $W$  whose rows form a basis of  $\text{im}(N)^\perp$  are

$$N = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\ 1 & 0 & -1 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & -1 & 0 & 0 & -2 & 2 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{pmatrix}, \quad W = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 \end{pmatrix}. \quad (32)$$

The matrix  $W$  gives rise to the conservation laws

$$x_1 + x_7 = c_1, \quad x_2 + x_5 = c_2. \quad (33)$$

With mass-action kinetics, the vector of reaction rates is

$$v(x) = (\kappa_1 x_1, \kappa_2 x_2, \kappa_3 x_3, \kappa_4 x_4, \kappa_5 x_2 x_3, \kappa_6 x_5, \kappa_7 x_4^2, \kappa_8 x_6, \kappa_9 x_1 x_6, \kappa_{10} x_7).$$

The function  $f(x) = Nv(x)$  is thus

$$f(x) = (-\kappa_9 x_1 x_6 + \kappa_{10} x_7, -\kappa_5 x_2 x_3 + \kappa_6 x_5, \kappa_1 x_1 - \kappa_3 x_3, -\kappa_5 x_2 x_3 + \kappa_6 x_5 \kappa_2 x_2 - \kappa_4 x_4 \\ - 2\kappa_7 x_4^2 + 2\kappa_8 x_6, \kappa_5 x_2 x_3 - \kappa_6 x_5, \kappa_7 x_4^2 - \kappa_8 x_6 - \kappa_9 x_1 x_6 + \kappa_{10} x_7, \kappa_9 x_1 x_6 - \kappa_{10} x_7).$$

We apply the algorithm to this network with the matrix  $N$  and the vector  $v(x)$ .

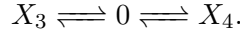
**Step 1.** Mass-action kinetics fulfils assumption [3] in the main text. The function  $f(x)$  and  $W$  are given above. The matrix  $W$  in (32) is row reduced.

**Step 2.** It is shown in the main text that the semiflow of the network is dissipative.

**Step 3.** The species  $X_5, X_6, X_7$  are intermediates. Removing them from the network yields the reaction network



For this network,  $X_1, X_2$  are catalysts. Its removal yields the reaction network



This is a strongly connected monomolecular network. By Corollary 2, there are no boundary equilibria in any  $\mathcal{P}_c$  as long as  $\mathcal{P}_c^+ \neq \emptyset$ .

**Step 4.** We solve the equilibrium equations  $f_3 = f_4 = f_5 = f_6 = f_7 = 0$  for  $x_1, x_2, x_3, x_6, x_7$ . This gives the following algebraic parameterization  $\Phi: \mathbb{R}_{>0}^2 \rightarrow \mathbb{R}_{>0}^7$  of the set of equilibria in terms of  $\hat{x} = (x_4, x_5)$ :

$$\Phi(x_4, x_5) = \left( \frac{\kappa_2 \kappa_3 \kappa_6 x_5}{\kappa_1 \kappa_4 \kappa_5 x_4}, \frac{\kappa_4 x_4}{\kappa_2}, \frac{\kappa_2 \kappa_6 x_5}{\kappa_4 \kappa_5 x_4}, x_4, x_5, \frac{\kappa_7 x_4^2}{\kappa_8}, \frac{\kappa_2 \kappa_3 \kappa_6 \kappa_7 \kappa_9 x_4 x_5}{\kappa_1 \kappa_4 \kappa_5 \kappa_8 \kappa_{10}} \right).$$

**Step 5.** With our choice of  $W$ , we have  $i_1 = 1, i_2 = 2$ . Hence  $\varphi_c$  is:

$$\begin{aligned} \varphi_c(x) = & (x_1 + x_7 - c_1, x_2 + x_5 - c_2, \kappa_1 x_1 - \kappa_3 x_3, -\kappa_5 x_2 x_3 + \kappa_6 x_5 \kappa_2 x_2 - \kappa_4 x_4 \\ & - 2\kappa_7 x_4^2 + 2\kappa_8 x_6, \kappa_5 x_2 x_3 - \kappa_6 x_5, \kappa_7 x_4^2 - \kappa_8 x_6 - \kappa_9 x_1 x_6 + \kappa_{10} x_7, \kappa_9 x_1 x_6 - \kappa_{10} x_7). \end{aligned}$$

We compute the Jacobian of  $\varphi_c(x)$ ,  $M(x)$ , its determinant and evaluate it in  $\Phi(x_4, x_5)$  to obtain the polynomial

$$a(x_4, x_5) = \frac{\kappa_3 \kappa_6}{x_4} (\kappa_2 \kappa_7 \kappa_9 x_4^2 x_5 - \kappa_4 \kappa_7 \kappa_9 x_4^3 - \kappa_2 \kappa_8 \kappa_{10} x_5 - \kappa_4 \kappa_8 \kappa_{10} x_4).$$

**Step 6.** Proceed as in the main text.

## 7 Further examples

In this section we obtain the conditions for multiple and unique equilibria for the two networks in Table 1 in the main text. We also provide an extra example (that is not multistationary), involving a two-substrate enzyme catalysis.

### 7.1 Phosphorylation of two substrates

In this subsection we consider the network in the first row of Table 1 in the main text.

We consider a system in which two substrates can be either unphosphorylated,  $A, B$  or phosphorylated  $A_p, B_p$ . Phosphorylation of both substrates is catalyzed by the same kinase  $K$  and dephosphorylation of  $A_p, B_p$  is catalyzed by the same phosphatase  $F$ . That is, the system consists of two futile cycles sharing kinase and phosphatase.

The reactions of the system are:



It was shown in [45] that this network with mass-action kinetics is multistationary. Here we find the necessary and sufficient condition on the reaction rate constants for having multistationarity in some stoichiometric compatibility class. We let

$$\begin{array}{lllll} X_1 = K, & X_3 = A, & X_5 = B, & X_7 = AK, & X_9 = A_p F, \\ X_2 = F, & X_4 = A_p, & X_6 = B_p, & X_8 = BK, & X_{10} = B_p F. \end{array}$$

The stoichiometric matrix  $N$  of the network and a row reduced matrix  $W$  whose rows form a basis of  $\text{im}(N)^\perp$  are

$$N = \begin{pmatrix} -1 & 1 & 1 & 0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 & -1 & 1 & 1 \\ -1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 \end{pmatrix},$$

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

The rank of  $N$  is  $s = 6$ . The matrix  $W$  gives rise to the conservation laws

$$\begin{array}{ll} c_1 = x_1 + x_7 + x_8, & c_3 = x_3 + x_4 + x_7 + x_9, \\ c_2 = x_2 + x_9 + x_{10}, & c_4 = x_5 + x_6 + x_8 + x_{10}, \end{array}$$

where  $c_1, c_2, c_3, c_4$  correspond to the total amounts of kinase, phosphatase, substrate  $A$  and substrate  $B$ , respectively.

With mass-action kinetics, the vector of reaction rates is

$$v(x) = (\kappa_1 x_1 x_3, \kappa_2 x_7, \kappa_3 x_7, \kappa_4 x_2 x_4, \kappa_5 x_9, \kappa_6 x_9, \kappa_7 x_1 x_5, \kappa_8 x_8, \kappa_9 x_8, \kappa_{10} x_2 x_6, \kappa_{11} x_{10}, \kappa_{12} x_{10}).$$

The function  $f(x) = Nv(x)$  is thus

$$\begin{aligned} f(x) = & (-\kappa_1 x_1 x_3 - \kappa_7 x_1 x_5 + \kappa_2 x_7 + \kappa_3 x_7 + \kappa_8 x_8 + \kappa_9 x_8, \\ & -\kappa_4 x_2 x_4 - \kappa_{10} x_2 x_6 + \kappa_5 x_9 + \kappa_6 x_9 + \kappa_{11} x_{10} + \kappa_{12} x_{10}, -\kappa_1 x_1 x_3 + \kappa_2 x_7 + \kappa_6 x_9, \\ & -\kappa_7 x_1 x_5 + \kappa_8 x_8 + \kappa_{12} x_{10}, -\kappa_{10} x_2 x_6 + \kappa_9 x_8 + \kappa_{11} x_{10}, \kappa_1 x_1 x_3 - \kappa_2 x_7 - \kappa_3 x_7, \\ & \kappa_7 x_1 x_5 - \kappa_8 x_8 - \kappa_9 x_8, \kappa_4 x_2 x_4 - \kappa_5 x_9 - \kappa_6 x_9, \kappa_{10} x_2 x_6 - \kappa_{11} x_{10} - \kappa_{12} x_{10}). \end{aligned}$$

We apply the algorithm to this network with the matrix  $N$  and the vector  $v(x)$ .

**Step 1.** Mass-action kinetics fulfils assumption [3] in the main text. The function  $f(x)$  and  $W$  are given above and the matrix  $W$  is row reduced.

**Step 2.** The network is conservative since the concentration of every species is in the support of a conservation law with positive coefficients. Therefore the semiflow of the network is dissipative.

**Step 3.** The network has four intermediates  $AK, A_p F, BK, B_p F$ . After their elimination, we are left with the reaction network



This network has two catalysts:  $K, F$ . Their elimination yields the reaction network



This is a monomolecular network with two strongly connected components. By Corollary 2, there are no boundary equilibria in any  $\mathcal{P}_c$  for which  $\mathcal{P}_c^+ \neq \emptyset$ .

**Step 4.** By solving the equilibrium equations  $f_4 = f_6 = f_7 = f_8 = f_9 = f_{10} = 0$  in the variables  $x_4, x_6, x_7, x_8, x_9, x_{10}$ , we find the following positive parameterization of the set of equilibria in terms of  $\hat{x} = (x_1, x_2, x_3, x_5)$ :

$$\begin{aligned} x_4 &= \frac{(\kappa_6 + \kappa_5) \kappa_3 x_3 x_1 \kappa_1}{\kappa_6 (\kappa_3 + \kappa_2) x_2 \kappa_4}, & x_7 &= \frac{\kappa_1 x_1 x_3}{\kappa_3 + \kappa_2}, & x_9 &= \frac{\kappa_3 x_3 x_1 \kappa_1}{\kappa_6 (\kappa_3 + \kappa_2)}, \\ x_6 &= \frac{(\kappa_{12} + \kappa_{11}) \kappa_9 x_5 x_1 \kappa_7}{\kappa_{12} (\kappa_9 + \kappa_8) \kappa_{10} x_2}, & x_8 &= \frac{\kappa_7 x_1 x_5}{\kappa_9 + \kappa_8}, & x_{10} &= \frac{\kappa_9 x_5 x_1 \kappa_7}{\kappa_{12} (\kappa_9 + \kappa_8)}. \end{aligned}$$

**Step 5.** For our choice of  $W$ , we have  $i_1 = 1, i_2 = 2, i_3 = 3, i_4 = 5$ . The function  $\varphi_c(x)$  is thus

$$\begin{aligned} \varphi_c(x) &= (x_1 + x_7 + x_8 - c_1, x_2 + x_9 + x_{10} - c_2, x_3 + x_4 + x_7 + x_9 - c_3, \\ &\quad -\kappa_4 x_2 x_4 + \kappa_3 x_7 + \kappa_5 x_9, x_5 + x_6 + x_8 + x_{10} - c_4, -\kappa_{10} x_2 x_6 + \kappa_9 x_8 + \kappa_{11} x_{10}, \\ &\quad \kappa_1 x_1 x_3 - \kappa_2 x_7 - \kappa_3 x_7, \kappa_7 x_1 x_5 - \kappa_8 x_8 - \kappa_9 x_8, \kappa_4 x_2 x_4 - \kappa_5 x_9 - \kappa_6 x_9, \\ &\quad \kappa_{10} x_2 x_6 - \kappa_{11} x_{10} - \kappa_{12} x_{10}). \end{aligned}$$

The Jacobian matrix  $M(x) = J_{\varphi_c}(x)$  is

$$\begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & 1 & 1 & 0 & 0 & 1 & 0 & 1 & 0 \\ 0 & -\kappa_4 x_4 & 0 & -\kappa_4 x_2 & 0 & 0 & \kappa_3 & 0 & \kappa_5 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 0 & 1 & 0 & 1 \\ 0 & -\kappa_{10} x_6 & 0 & 0 & 0 & -\kappa_{10} x_2 & 0 & \kappa_9 & 0 & \kappa_{11} \\ \kappa_1 x_3 & 0 & \kappa_1 x_1 & 0 & 0 & 0 & -\kappa_2 - \kappa_3 & 0 & 0 & 0 \\ \kappa_7 x_5 & 0 & 0 & 0 & \kappa_7 x_1 & 0 & 0 & -\kappa_8 - \kappa_9 & 0 & 0 \\ 0 & \kappa_4 x_4 & 0 & \kappa_4 x_2 & 0 & 0 & 0 & 0 & -\kappa_5 - \kappa_6 & 0 \\ 0 & \kappa_{10} x_6 & 0 & 0 & 0 & \kappa_{10} x_2 & 0 & 0 & 0 & -\kappa_{11} - \kappa_{12} \end{pmatrix}.$$

We compute the determinant of  $M(x)$  and substitute  $x_4, x_6, x_7, x_8, x_9, x_{10}$  with the terms of the parameterization in Step 4, that is, we find  $a(\hat{x})$ .

**Step 6.** The function  $a(\hat{x})$  is a large rational function with positive denominator. Therefore, the numerator of this function, a polynomial  $p(\hat{x})$ , determines the sign of  $a(\hat{x})$ . The coefficients are polynomials in  $\kappa_1, \dots, \kappa_{10}$ . All but one of the coefficients are polynomials in  $\kappa_1, \dots, \kappa_{10}$  with positive coefficients. Therefore, all coefficients but one are always positive, independently of the values of the reaction rate constants  $\kappa_1, \dots, \kappa_{10}$ .

The only coefficient with sign depending on the specific values of  $\kappa_1, \dots, \kappa_{10}$  is

$$\begin{aligned} \alpha(\kappa) &= \kappa_1 \kappa_7 (\kappa_3 \kappa_{12} - \kappa_6 \kappa_9) (\kappa_1 \kappa_3 \kappa_5 \kappa_8 \kappa_{10} \kappa_{12} + \kappa_1 \kappa_3 \kappa_5 \kappa_9 \kappa_{10} \kappa_{12} + \kappa_1 \kappa_3 \kappa_6 \kappa_8 \kappa_{10} \kappa_{12} \\ &\quad + \kappa_1 \kappa_3 \kappa_6 \kappa_9 \kappa_{10} \kappa_{12} - \kappa_2 \kappa_4 \kappa_6 \kappa_7 \kappa_9 \kappa_{11} - \kappa_2 \kappa_4 \kappa_6 \kappa_7 \kappa_9 \kappa_{12} - \kappa_3 \kappa_4 \kappa_6 \kappa_7 \kappa_9 \kappa_{11} - \kappa_3 \kappa_4 \kappa_6 \kappa_7 \kappa_9 \kappa_{12}). \end{aligned}$$

If  $\alpha(\kappa) \geq 0$ , then all coefficients of  $p(\hat{x})$  are positive, and hence  $a(\hat{x})$  is positive for all positive  $\hat{x}$ . Using  $(-1)^s = (-1)^6 = 1$ , Theorem 4(A) gives that there is a unique positive equilibrium in each stoichiometric compatibility class with non-empty positive relative interior.

When this coefficient is negative, then we need to check whether  $p(\hat{x})$  is negative for some  $\hat{x}$ . We analyse this by finding the Newton polytope and using Proposition 4.

The coefficient  $\alpha(\kappa)$  corresponds to the monomial  $x_1^2 x_2 x_3 x_5$ . The exponent vectors of the monomials of  $p(\hat{x})$  are:

$$\begin{aligned} (0, 3, 0, 0), & (0, 3, 0, 1), & (0, 3, 1, 0), & (1, 2, 0, 0), & (1, 2, 0, 1), & (1, 2, 1, 0), & (1, 3, 0, 0), \\ (1, 3, 0, 1) & (1, 3, 1, 0), & (2, 1, 0, 0), & (2, 1, 0, 1), & (2, 1, 1, 0), & (2, 1, 1, 1), & (2, 2, 0, 0), \\ (2, 3, 0, 0), & (3, 0, 0, 1), & (3, 0, 1, 0), & (3, 1, 0, 1), & (3, 1, 1, 0). \end{aligned}$$

We find the vertices of the convex hull of the exponent vectors, and find that they are

$$(0, 3, 0, 1), \quad (0, 3, 1, 0), \quad (\mathbf{2}, \mathbf{1}, \mathbf{1}, \mathbf{1}), \quad (0, 3, 0, 0), \quad (2, 1, 0, 0), \quad (3, 0, 0, 1), \\ (3, 0, 1, 0), \quad (1, 3, 0, 1), \quad (1, 3, 1, 0), \quad (2, 3, 0, 0), \quad (3, 1, 0, 1), \quad (3, 1, 1, 0).$$

Thus the exponent vector of the monomial of interest,  $(2, 1, 1, 1)$  (highlighted in bold), is a vertex of the Newton polytope. Therefore, by Proposition 4, there exists  $\hat{x}$  such that  $p(\hat{x})$  is negative. Theorem 4(B) gives that there is a stoichiometric compatibility class with multiple positive equilibria.

The condition  $\alpha(\kappa) < 0$  can be rewritten as:

$$(\kappa_3\kappa_{12} - \kappa_6\kappa_9)(\kappa_3\kappa_{12}\kappa_1\kappa_{10}(\kappa_5 + \kappa_6)(\kappa_8 + \kappa_9) - \kappa_6\kappa_9\kappa_4\kappa_7(\kappa_2 + \kappa_3)(\kappa_{11} + \kappa_{12})) < 0,$$

which in turn can be written as

$$(\kappa_3\kappa_{12} - \kappa_6\kappa_9) \left( \kappa_3\kappa_{12} \cdot \frac{\kappa_1}{\kappa_2 + \kappa_3} \cdot \frac{\kappa_{10}}{\kappa_{11} + \kappa_{12}} - \kappa_6\kappa_9 \cdot \frac{\kappa_4}{\kappa_5 + \kappa_6} \cdot \frac{\kappa_7}{\kappa_8 + \kappa_9} \right) < 0,$$

Note that  $\kappa_3, \kappa_6, \kappa_9, \kappa_{12}$  are the catalytic constants of phosphorylation/dephosphorylation of  $A$  and  $B$  ( $k_{c1}, k_{c2}, k_{c3}, k_{c4}$  in the main text), and

$$k_{M1}^{-1} = \frac{\kappa_1}{\kappa_2 + \kappa_3}, \quad k_{M2}^{-1} = \frac{\kappa_4}{\kappa_5 + \kappa_6}, \quad k_{M3}^{-1} = \frac{\kappa_7}{\kappa_8 + \kappa_9}, \quad k_{M4}^{-1} = \frac{\kappa_{10}}{\kappa_{11} + \kappa_{12}}$$

are the inverses of the Michaelis-Menten constants of  $K$  and  $F$  for each substrate. Therefore, the necessary and sufficient condition for multistationarity can be written in terms of the catalytic constants and the Michaelis-Menten constants,

$$(\kappa_3\kappa_{12} - \kappa_6\kappa_9) \left( \frac{\kappa_3\kappa_{12}}{k_{M1}k_{M4}} - \frac{\kappa_6\kappa_9}{k_{M2}k_{M3}} \right) < 0.$$

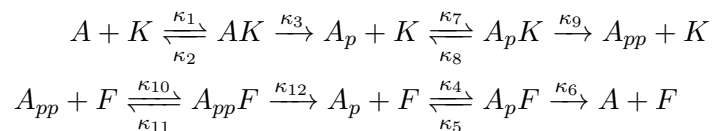
This proves the condition for multiple and unique equilibria given in the first row of Table 1 in the main text. In particular, we have that

- If  $\kappa_3\kappa_{12} > \kappa_6\kappa_9$ , then we need  $\frac{\kappa_3\kappa_{12}}{k_{M1}k_{M4}} < \frac{\kappa_6\kappa_9}{k_{M2}k_{M3}}$  for multiple equilibria to occur.
- If  $\kappa_3\kappa_{12} < \kappa_6\kappa_9$ , then we need  $\frac{\kappa_3\kappa_{12}}{k_{M1}k_{M4}} > \frac{\kappa_6\kappa_9}{k_{M2}k_{M3}}$  for multiple equilibria to occur.

## 7.2 Two-site phosphorylation system

In this subsection we consider the network in the second row of Table 1 in the main text. The conditions given here were also found in [18], the paper that lay the foundations of this algorithm. In this work we consider a direct route using the function  $\varphi_c$  and avoiding changes of variables. We explain here how to find the conditions using the algorithm in the main text.

We consider a system in which one substrate undergoes sequential and distributive phosphorylation by a kinase  $K$  and sequential and distributive dephosphorylation by a phosphatase  $F$ . The three phosphoforms of the substrate are  $A, A_p, A_{pp}$ . The reactions of the system are:



We let

$$X_1 = K, \quad X_3 = A, \quad X_5 = A_{pp}, \quad X_6 = AK, \quad X_7 = A_pF, \\ X_2 = F, \quad X_4 = A_p, \quad X_8 = A_pK, \quad X_9 = A_{pp}F.$$

The stoichiometric matrix  $N$  of the network and a row reduced matrix  $W$  whose rows form a basis of  $\text{im}(N)^\perp$  are

$$N = \begin{pmatrix} -1 & 1 & 1 & 0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 & -1 & 1 & 1 \\ -1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 1 & 0 & -1 & 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 \end{pmatrix},$$

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

The rank of  $N$  is  $s = 6$ . The matrix  $W$  gives rise to the conservation laws

$$c_1 = x_1 + x_6 + x_8, \quad c_2 = x_2 + x_7 + x_9, \quad c_3 = x_3 + x_4 + x_5 + x_6 + x_7 + x_8 + x_9,$$

where  $c_1, c_2, c_3$  correspond to the total amounts of kinase, phosphatase and substrate  $A$ , respectively.

With mass-action kinetics, the vector of reaction rates is

$$v(x) = (\kappa_1 x_1 x_3, \kappa_2 x_6, \kappa_3 x_6, \kappa_4 x_2 x_4, \kappa_5 x_7, \kappa_6 x_7, \kappa_7 x_1 x_4, \kappa_8 x_8, \kappa_9 x_8, \kappa_{10} x_2 x_5, \kappa_{11} x_9, \kappa_{12} x_9).$$

The function  $f(x) = Nv(x)$  is thus

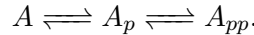
$$\begin{aligned} f(x) = & (-\kappa_1 x_1 x_3 - \kappa_7 x_1 x_4 + \kappa_2 x_6 + \kappa_3 x_6 + \kappa_8 x_8 + \kappa_9 x_8, \\ & -\kappa_4 x_2 x_4 - \kappa_{10} x_2 x_5 + \kappa_5 x_7 + \kappa_6 x_7 + \kappa_{11} x_9 + \kappa_{12} x_9, -\kappa_1 x_1 x_3 + \kappa_2 x_6 + \kappa_6 x_7, \\ & -\kappa_4 x_2 x_4 - \kappa_7 x_1 x_4 + \kappa_3 x_6 + \kappa_5 x_7 + \kappa_8 x_8 + \kappa_{12} x_9, -\kappa_{10} x_2 x_5 + \kappa_9 x_8 + \kappa_{11} x_9, \\ & \kappa_1 x_1 x_3 - \kappa_2 x_6 - \kappa_3 x_6, \kappa_4 x_2 x_4 - \kappa_5 x_7 - \kappa_6 x_7, \kappa_7 x_1 x_4 - \kappa_8 x_8 - \kappa_9 x_8, \\ & \kappa_{10} x_2 x_5 - \kappa_{11} x_9 - \kappa_{12} x_9). \end{aligned}$$

We apply the algorithm to this network with the matrix  $N$  and the vector  $v(x)$ .

**Step 1.** Mass-action kinetics fulfils assumption [3] in the main text. The function  $f(x)$  and  $W$  are given above and the matrix  $W$  is row reduced.

**Step 2.** The network is conservative since the concentration of every species is in the support of a conservation law with positive coefficients. Therefore the semiflow of the network is dissipative.

**Step 3.** The network has four intermediates  $AK, A_p K, A_p F, A_{pp} F$ . After their elimination, we are left with a reaction network with two catalysts:  $K, F$ . Their elimination yields the reaction network



This is a monomolecular network with two strongly connected components. By Corollary 2, there are no boundary equilibria in any  $\mathcal{P}_c$  for which  $\mathcal{P}_c^+ \neq \emptyset$ .

**Step 4.** By solving the equilibrium equations  $f_4 = f_5 = f_6 = f_7 = f_8 = f_9 = 0$  in the variables  $x_4, \dots, x_9$ , we find the following positive parameterization of the set of equilibria in terms of  $\hat{x} = (x_1, x_2, x_3)$ :

$$\begin{aligned} x_4 &= \frac{\kappa_1 \kappa_3 (\kappa_5 + \kappa_6) x_1 x_3}{(\kappa_2 + \kappa_3) \kappa_4 \kappa_6 x_2}, & x_5 &= \frac{\kappa_1 \kappa_3 (\kappa_5 + \kappa_6) \kappa_7 \kappa_9 (\kappa_{11} + \kappa_{12}) x_1^2 x_3}{(\kappa_2 + \kappa_3) \kappa_4 \kappa_6 (\kappa_8 + \kappa_9) \kappa_{10} \kappa_{12} x_2^2}, \\ x_6 &= \frac{\kappa_1 x_1 x_3}{\kappa_2 + \kappa_3}, & x_7 &= \frac{\kappa_1 \kappa_3 x_1 x_3}{(\kappa_2 + \kappa_3) \kappa_6}, \\ x_8 &= \frac{\kappa_1 \kappa_3 (\kappa_5 + \kappa_6) \kappa_7 x_1^2 x_3}{(\kappa_2 + \kappa_3) \kappa_4 \kappa_6 (\kappa_8 + \kappa_9) x_2}, & x_9 &= \frac{\kappa_1 \kappa_3 (\kappa_5 + \kappa_6) \kappa_7 \kappa_9 x_1^2 x_3}{(\kappa_2 + \kappa_3) \kappa_4 \kappa_6 (\kappa_8 + \kappa_9) \kappa_{12} x_2}. \end{aligned}$$

**Step 5.** For our choice of  $W$ , we have  $i_1 = 1, i_2 = 2, i_3 = 3$ . The function  $\varphi_c(x)$  is thus

$$\begin{aligned} \varphi_c(x) = & (x_1 + x_6 + x_8 - c_1, x_2 + x_7 + x_9 - c_2, x_3 + x_4 + x_5 + x_6 + x_7 + x_8 + x_9 - c_3, \\ & -\kappa_4 x_2 x_4 - \kappa_7 x_1 x_4 + \kappa_3 x_6 + \kappa_5 x_7 + \kappa_8 x_8 + \kappa_{12} x_9, -\kappa_{10} x_2 x_5 + \kappa_9 x_8 + \kappa_{11} x_9, \\ & \kappa_1 x_1 x_3 - \kappa_2 x_6 - \kappa_3 x_6, \kappa_4 x_2 x_4 - \kappa_5 x_7 - \kappa_6 x_7, \kappa_7 x_1 x_4 - \kappa_8 x_8 - \kappa_9 x_8, \\ & \kappa_{10} x_2 x_5 - \kappa_{11} x_9 - \kappa_{12} x_9). \end{aligned}$$

The Jacobian matrix  $M(x) = J_{\varphi_c}(x)$  is

$$\begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \\ 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ -\kappa_7 x_4 & -\kappa_4 x_4 & 0 & -\kappa_4 x_2 - \kappa_7 x_1 & 0 & \kappa_3 & \kappa_5 & \kappa_8 & \kappa_{12} \\ 0 & -\kappa_{10} x_5 & 0 & 0 & -\kappa_{10} x_2 & 0 & 0 & \kappa_9 & \kappa_{11} \\ \kappa_1 x_3 & 0 & \kappa_1 x_1 & 0 & 0 & -\kappa_2 - \kappa_3 & 0 & 0 & 0 \\ 0 & \kappa_4 x_4 & 0 & \kappa_4 x_2 & 0 & 0 & -\kappa_5 - \kappa_6 & 0 & 0 \\ \kappa_7 x_4 & 0 & 0 & \kappa_7 x_1 & 0 & 0 & 0 & -\kappa_8 - \kappa_9 & 0 \\ 0 & \kappa_{10} x_5 & 0 & 0 & \kappa_{10} x_2 & 0 & 0 & 0 & -\kappa_{11} - \kappa_{12} \end{pmatrix}.$$

We compute the determinant of  $M(x)$  and substitute  $x_4, \dots, x_9$  with their expressions in the parameterization, that is, find  $a(\hat{x})$ .

**Step 6.** The function  $a(\hat{x})$  is a large rational function with positive denominator. The numerator of this function, a polynomial  $p(\hat{x})$ , determines therefore the sign of  $a(\hat{x})$ . The coefficients are polynomials in  $\kappa_1, \dots, \kappa_{10}$ .

The polynomial has 15 terms, 9 of which are positive for all values of the reaction rate constants. The remaining 6 coefficients are polynomials in  $\kappa_1, \dots, \kappa_{10}$  that can either be positive or negative.

Five of the six coefficients are of the form  $\beta(\kappa)b_1(\kappa)$ , where  $\beta(\kappa)$  is a positive polynomial in  $\kappa$  and

$$b_1(\kappa) = \kappa_3 \kappa_{12} - \kappa_6 \kappa_9$$

(thus  $b_1(\kappa)$  is the same for all five coefficients). These five coefficients correspond to the monomials  $x_1^3 x_2^2 x_3$ ,  $x_1^2 x_2^2 x_3^2$ ,  $x_1^3 x_2 x_3^2$ ,  $x_1^2 x_3^3 x_3$  and  $x_1^4 x_3^2$ .

The remaining coefficient is of the form  $\gamma(\kappa)\alpha(\kappa)$ , where  $\gamma(\kappa)$  is a positive polynomial in  $\kappa$  and

$$\begin{aligned} \alpha(\kappa) = & \kappa_1 \kappa_3 \kappa_4 \kappa_8 \kappa_{10} \kappa_{12} + \kappa_1 \kappa_3 \kappa_4 \kappa_9 \kappa_{10} \kappa_{12} + \kappa_1 \kappa_3 \kappa_5 \kappa_7 \kappa_{10} \kappa_{12} + \kappa_1 \kappa_3 \kappa_6 \kappa_7 \kappa_{10} \kappa_{12} \\ & - \kappa_1 \kappa_4 \kappa_6 \kappa_7 \kappa_9 \kappa_{11} - \kappa_1 \kappa_4 \kappa_6 \kappa_7 \kappa_9 \kappa_{12} - \kappa_2 \kappa_4 \kappa_6 \kappa_7 \kappa_9 \kappa_{10} - \kappa_3 \kappa_4 \kappa_6 \kappa_7 \kappa_9 \kappa_{10}. \end{aligned}$$

It corresponds to the monomial  $x_1^2 x_2^2 x_3$ .

Since  $(-1)^6 = 1$ , part Theorem 4(A) tells us that there is a unique positive equilibrium in each stoichiometric compatibility class with non-empty positive part, if

$$b_1(\kappa) \geq 0 \quad \text{and} \quad \alpha(\kappa) \geq 0.$$

The condition  $\alpha(\kappa) \geq 0$  can be rewritten as:

$$\kappa_1 \kappa_3 \kappa_{10} \kappa_{12} (\kappa_4 (\kappa_9 + \kappa_8) + \kappa_7 (\kappa_6 + \kappa_5)) - \kappa_4 \kappa_6 \kappa_7 \kappa_9 (\kappa_1 (\kappa_{12} + \kappa_{11}) + \kappa_{10} (\kappa_3 + \kappa_2)) \geq 0.$$

Dividing the expression by  $\kappa_1 \kappa_4 \kappa_7 \kappa_{10}$ , the condition can be rewritten as

$$\kappa_3 \kappa_{12} (k_{M2} + k_{M3}) - \kappa_6 \kappa_9 (k_{M1} + k_{M4}) \geq 0,$$

where

$$k_{M1} = \frac{\kappa_2 + \kappa_3}{\kappa_1}, \quad k_{M2} = \frac{\kappa_5 + \kappa_6}{\kappa_4}, \quad k_{M3} = \frac{\kappa_8 + \kappa_9}{\kappa_7}, \quad k_{M4} = \frac{\kappa_{11} + \kappa_{12}}{\kappa_{10}}$$



are the Michaelis-Menten constants of  $K$  and  $F$  for each site. Note that  $\kappa_3, \kappa_6, \kappa_9, \kappa_{12}$  are the catalytic constants of phosphorylation of  $A$ , dephosphorylation of  $A_p$ , phosphorylation of  $A_p$  and dephosphorylation of  $A_{pp}$ . These are denoted by  $k_{c1}, k_{c2}, k_{c3}, k_{c4}$  in the main text.

By letting

$$b_2(\kappa) = \kappa_3 \kappa_{12} (k_{M2} + k_{M3}) - \kappa_6 \kappa_9 (k_{M1} + k_{M4}),$$

$\alpha(\kappa) \geq 0$  if and only if  $b_2(\kappa) \geq 0$ . Thus we have proven the condition for unique equilibria given in the second row of Table 1 in the main text.

Let us consider whether Theorem 4(B) applies if  $b_1(\kappa) < 0$  and/or  $\alpha(\kappa) < 0$ . The exponent vectors of the monomials of  $p(\hat{x})$  are:

$$\begin{array}{cccccccc} (3, 1, 1) & (1, 3, 1) & (2, 2, 1) & (2, 2, 2) & (2, 3, 0) & (2, 2, 0) & (1, 3, 0) & (3, 1, 2) \\ (2, 3, 1) & (3, 2, 1) & (4, 0, 2) & (4, 0, 1) & (0, 4, 1) & (1, 4, 0) & (0, 4, 0) & \end{array}$$

The vertices of the convex hull of the exponent vectors are

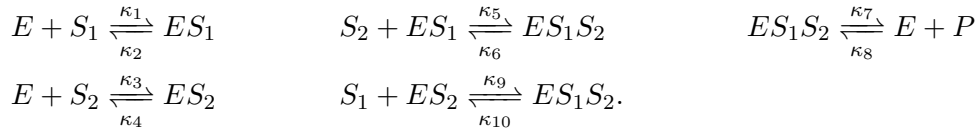
$$(2, 3, 0) \quad (4, 0, 1) \quad (2, 2, 0) \quad (0, 4, 0) \quad (1, 4, 0) \quad \mathbf{(3, 2, 1)} \quad (4, 0, 2) \quad (0, 4, 1) \quad (2, 3, 1) \quad (2, 2, 2).$$

The vertex highlighted in bold corresponds to the monomial  $x_1^3 x_2^2 x_3$ , whose sign depends on  $b_1(\kappa)$ . By Proposition 4, if  $b_1(\kappa) < 0$ , then there exists  $\hat{x}$  such that  $p(\hat{x})$  is negative. Theorem 4(B) gives that there is a stoichiometric compatibility class that admits positive multiple equilibria. This proves the condition for multistationarity given in the second row of Table 1 in the main text.

The exponent vector of the monomial corresponding to the coefficient  $\alpha(\kappa)$ ,  $(2, 2, 1)$ , is not a vertex of the Newton polytope. In this case it is uncertain whether the condition  $\alpha(\kappa) < 0$  is sufficient for multistationarity.

### 7.3 Two-substrate enzyme catalysis

We consider here a mechanism in which an enzyme  $E$  binds two substrates,  $S_1, S_2$ , in an unordered manner in order to catalyze the reversible conversion to the product  $P$ . A variation of this system was considered in [46]. The reactions of the system are:



We let

$$X_1 = E, \quad X_2 = S_1, \quad X_3 = ES_1, \quad X_4 = S_2, \quad X_5 = ES_2, \quad X_6 = ES_1 S_2, \quad X_7 = P.$$

The stoichiometric matrix  $N$  of the network and a row reduced matrix  $W$  whose rows form a basis of  $\text{im}(N)^\perp$  are

$$N = \begin{pmatrix} -1 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 1 & -1 \\ -1 & 1 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 1 & -1 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 1 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & -1 & 1 & -1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{pmatrix}$$

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

The rank of  $N$  is  $s = 4$ . The matrix  $W$  gives rise to the conservation laws

$$c_1 = x_1 + x_3 + x_5 + x_6, \quad c_2 = x_2 + x_3 + x_6 + x_7, \quad c_3 = x_4 + x_5 + x_6 + x_7,$$

where  $c_1, c_2, c_3, c_4$  correspond to the total amounts of kinase, substrate  $S_1$  and substrate  $S_2$ , respectively.

With mass-action kinetics, the vector of reaction rates is

$$v(x) = (\kappa_1 x_1 x_2, \kappa_2 x_3, \kappa_3 x_1 x_4, \kappa_4 x_5, \kappa_5 x_4 x_3, \kappa_6 x_6, \kappa_7 x_6, \kappa_8 x_2 x_5, \kappa_9 x_6, \kappa_{10} x_1 x_7).$$

The function  $f(x) = Nv(x)$  is

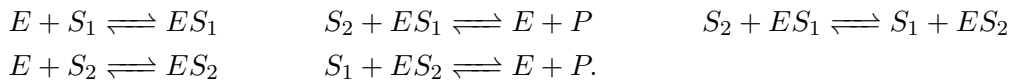
$$\begin{aligned} f(x) = & (-\kappa_1 x_1 x_2 - \kappa_3 x_1 x_4 - \kappa_{10} x_1 x_7 + \kappa_2 x_3 + \kappa_4 x_5 + \kappa_9 x_6, \\ & -\kappa_1 x_1 x_2 - \kappa_8 x_2 x_5 + \kappa_2 x_3 + \kappa_7 x_6, \kappa_1 x_1 x_2 - \kappa_5 x_4 x_3 - \kappa_2 x_3 + \kappa_6 x_6 \\ & -\kappa_3 x_1 x_4 - \kappa_5 x_4 x_3 + \kappa_4 x_5 + \kappa_6 x_6, \kappa_3 x_1 x_4 - \kappa_8 x_2 x_5 - \kappa_4 x_5 + \kappa_7 x_6, \\ & \kappa_5 x_4 x_3 + \kappa_8 x_2 x_5 + \kappa_{10} x_1 x_7 - \kappa_6 x_6 - \kappa_7 x_6 - \kappa_9 x_6, -\kappa_{10} x_1 x_7 + \kappa_9 x_6). \end{aligned}$$

We apply the algorithm to this network with the matrix  $N$  and the vector  $v(x)$ .

**Step 1.** Mass-action kinetics fulfils assumption [3] in the main text. The function  $f(x)$  and  $W$  are given above and the matrix  $W$  is row reduced.

**Step 2.** The network is conservative since the concentration of every species is in the support of a conservation law with positive coefficients. Therefore the semiflow of the network is dissipative.

**Step 3.** This network has only one intermediate  $ES_1 S_2$ . Its removal yields the reaction network



The conservation laws of this new network are (with the notation above):

$$c_1 = x_1 + x_3 + x_5, \quad c_2 = x_2 + x_3 + x_7 \quad c_3 = x_4 + x_5 + x_7.$$

The minimal siphons of the network are

$$\{E, ES_1, ES_2\}, \{S_1, ES_1, P\}, \{S_2, ES_2, P\}.$$

These siphons contain the support of the conservation laws for  $c_1, c_2, c_3$  respectively. Thus, by Propositions 2 and 3 the original network does not have boundary equilibria in any stoichiometric compatibility class that intersects the positive orthant.

**Step 4.** By solving the equilibrium equations  $f_3 = f_5 = f_6 = f_7 = 0$  in the variables  $x_3, x_5, x_6, x_7$ , we find the following positive parameterization of the set of equilibria in terms of  $\hat{x} = (x_1, x_2, x_4)$ :

$$\begin{aligned} x_3 &= \frac{x_2 x_1 (\kappa_1 \kappa_6 \kappa_8 x_2 + \kappa_3 \kappa_6 \kappa_8 x_4 + \kappa_1 \kappa_4 \kappa_6 + \kappa_1 \kappa_4 \kappa_7)}{\kappa_2 \kappa_6 \kappa_8 x_2 + \kappa_4 \kappa_5 \kappa_7 x_4 + \kappa_2 \kappa_4 \kappa_6 + \kappa_2 \kappa_4 \kappa_7}, \\ x_5 &= \frac{x_1 x_4 (\kappa_1 \kappa_5 \kappa_7 x_2 + \kappa_3 \kappa_5 \kappa_7 x_4 + \kappa_2 \kappa_3 \kappa_6 + \kappa_2 \kappa_3 \kappa_7)}{\kappa_2 \kappa_6 \kappa_8 x_2 + \kappa_4 \kappa_5 \kappa_7 x_4 + \kappa_2 \kappa_4 \kappa_6 + \kappa_2 \kappa_4 \kappa_7}, \\ x_6 &= \frac{x_2 x_4 (\kappa_1 \kappa_5 \kappa_8 x_2 + \kappa_3 \kappa_5 \kappa_8 x_4 + \kappa_1 \kappa_4 \kappa_5 + \kappa_2 \kappa_3 \kappa_8) x_1}{\kappa_2 \kappa_6 \kappa_8 x_2 + \kappa_4 \kappa_5 \kappa_7 x_4 + \kappa_2 \kappa_4 \kappa_6 + \kappa_2 \kappa_4 \kappa_7}, \\ x_7 &= \frac{\kappa_9 x_2 x_4 (\kappa_1 \kappa_5 \kappa_8 x_2 + \kappa_3 \kappa_5 \kappa_8 x_4 + \kappa_1 \kappa_4 \kappa_5 + \kappa_2 \kappa_3 \kappa_8)}{(\kappa_2 \kappa_6 \kappa_8 x_2 + \kappa_4 \kappa_5 \kappa_7 x_4 + \kappa_2 \kappa_4 \kappa_6 + \kappa_2 \kappa_4 \kappa_7) \kappa_{10}}. \end{aligned}$$

**Step 5.** For our choice of  $W$ , we have  $i_1 = 1, i_2 = 2, i_3 = 4$ . The function  $\varphi_c(x)$  is thus

$$\begin{aligned} \varphi_c(x) = & \left( x_1 + x_3 + x_5 + x_6 - c_1, x_2 + x_3 + x_6 + x_7 - c_2, \kappa_1 x_1 x_2 - \kappa_5 x_4 x_3 - \kappa_2 x_3 + \kappa_6 x_6, \right. \\ & x_4 + x_5 + x_6 + x_7 - c_3, \kappa_3 x_1 x_4 - \kappa_8 x_2 x_5 - \kappa_4 x_5 + \kappa_7 x_6, \\ & \left. \kappa_5 x_4 x_3 + \kappa_8 x_2 x_5 + \kappa_{10} x_1 x_7 - \kappa_6 x_6 - \kappa_7 x_6 - \kappa_9 x_6, -\kappa_{10} x_1 x_7 + \kappa_9 x_6 \right). \end{aligned}$$

The Jacobian matrix  $M(x) = J_{\varphi_c}(x)$  is

$$\begin{pmatrix} 1 & 0 & 1 & 0 & 1 & 1 & 0 \\ 0 & 1 & 1 & 0 & 0 & 1 & 1 \\ \kappa_1 x_2 & \kappa_1 x_1 & -\kappa_5 x_4 - \kappa_2 & -\kappa_5 x_3 & 0 & \kappa_6 & 0 \\ 0 & 0 & 0 & 1 & 1 & 1 & 1 \\ \kappa_3 x_4 & -\kappa_8 x_5 & 0 & \kappa_3 x_1 & -\kappa_8 x_2 - \kappa_4 & \kappa_7 & 0 \\ \kappa_{10} x_7 & \kappa_8 x_5 & \kappa_5 x_4 & \kappa_5 x_3 & \kappa_8 x_2 & -\kappa_6 - \kappa_7 - \kappa_9 & \kappa_{10} x_1 \\ -\kappa_{10} x_7 & 0 & 0 & 0 & 0 & \kappa_9 & -\kappa_{10} x_1 \end{pmatrix}.$$

The determinant of  $M(x)$  has terms with positive sign and terms with negative sign. After substituting  $x_3, x_5, x_6, x_7$  with their expressions in the parameterization, that is, after finding  $a(\hat{x})$ , the obtained rational function is always positive.

**Step 6.** By Theorem 4(A) (using  $s = 4$ ), there is a unique positive equilibrium in each stoichiometric compatibility class that intersects the positive orthant.

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