Algebraic methods for bistability and oscillations in reaction networks

PhD thesis by

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Summary

The research presented in this thesis lies in the field of applied algebraic geometry. The main focus is on developing algebraic methods for studying the local dynamics of steady states in systems of polynomial differential equations with parametric coefficients. In particular, in systems arising from chemical reaction network models of biochemical processes.

In this thesis we present the contributions made in two topics: effective methods for detecting bistability and Hopf bifurcations in a chemical reaction network, and detection of Absolute Concentration Robustness in chemical reaction networks.

This document has three parts. In the first part we present the background required for our contributions. It contains the basic definitions of chemical reaction network theory, a small survey on methods for assessing the presence of multistationarity, and background in polytopes and the Newton polytope of a polynomial. The second part contains our contributions regarding bistability and Hopf bifurcations. We outline an algorithm to guarantee or preclude bistability in chemical reaction networks satisfying certain conditions. This algorithm is symbolic and can be used to find parameter regions for bistability. Additionally, we use the Newton polytope and its outer normal fan to effectively compute a set of parameters and a steady state where a Hopf bifurcation arises in a model of ERK regulation and in a MAPK cascade. Finally, in the third part, we present our contributions related with Absolute Concentration Robustness (ACR). In particular, we present a graphic method for finding ACR in small networks and then, we study whether ACR is preserved under structural modifications of the network.

Dansk resumé

Forskningen præsenteret i denne afhandling ligger inden for anvendt algebraisk geometri. Hovedfokus er på at udvikle algebraiske metoder til undersøgelse af den lokale dynamik i stabile tilstande i systemer med polynomale differentialligninger med parametriske koefficienter. Især i systemer, der stammer fra kemiske reaktionsnetværksmodeller af biokemiske processer.

I denne afhandling præsenterer vi bidragene, der er fremsat i to emner: effektive metoder til at detektere bistabilitet og Hopf bifurcationer i et kemisk reaktionsnetværk, og påvisning af Absolute Concentration Robustness i kemiske reaktionsnetværk.

Dette dokument har tre dele. I den første del præsenterer vi den nødvendige baggrund for vores bidrag. Det indeholder de grundlæggende definitioner af kemisk reaktionsnetværksteori, en lille undersøgelse af metoder til vurdering af til stedeværelsen af multistationatitet og baggrund i polytoper og Newton polytopen af et polynom. Den anden del indeholder vores bidrag vedrørende bistabilitet og Hopf-bifurcationer. Vi skitserer en algoritme til at garantere eller udelukke bistabilitet i kemiske reaktionsnetværk, der opfylder visse betingelser. Denne algoritme er symbolsk og kan bruges til at finde parameterregioner for bistabilitet. Derudover bruger vi Newton polytopen og dens ydre normale ventilator til effektivt at beregne et sæt parametre og en stabil tilstand, hvor en Hopf-bifurcation opstår i en model af ERK-regulering og i en to-lags kaskade. Endelig i tredje del præsenterer vi vores bidrag relateret til Absolute Concentration Robustness (ACR). Især præsenterer vi en grafisk metode til at finde ACR i små netværk, og derefter undersøger vi, om ACR er bevaret under strukturelle ændringer af netværket.

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Introduction

This work lies on the field of applied algebraic geometry. The main focus is on developing algebraic methods for studying the local dynamics of equilibrium points in systems of polynomial differential equations with parametric coefficients. In particular in systems arising from chemical reaction network models of biochemical processes.

The field of Chemical Reaction Network Theory (CRNT) was started in the decade of the seventies, by Feinberg, Horn and Jackson, in works such as [17, 18, 33], as a way to provide mechanisms to study the behaviour of biochemical systems. In this setting, a biochemical process is represented as a graph, indicating the interactions of different species. For instance, the activation of a substrate S with an enzyme E to produce a product P, could be represented in a graph as

$$\mathrm{E} + \mathrm{S} \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{\kappa_3} \mathrm{E} + \mathrm{P}$$

and it is known as the Michaelis-Menten mechanism. The reaction labelled as κ_1 represents an association of the substrate and the enzyme, and the reaction labelled as κ_3 represents the catalysis of the process. An association could also break, leading to the reaction labelled as κ_2. In this process it is said that E catalyses the transformation of S into P.

Once the graph representation of a biochemical process is done, the main goal is to study how the concentration of each species in the system changes through time. This study is done by associating a system of ordinary differential equations (ODEs) to the graph, and studying its dynamics. The definition of this system of ODEs depends on assumptions on the rates at which the reactions occur. In practice, one of the most common assumptions is that the rate of occurrence of a reaction is proportional to the product of the concentrations of the species at the beginning of each reaction. This assumption is called *mass action kinetics* and generates an autonomous polynomial system of ODEs.

With a system of ODEs in place, tools coming from dynamical systems are key to understand properties on these systems. However, this is not an easy task. Some of the issues that arise from this approach are

- The size of the network: some biochemical processes involve a large amount of species and reactions that are complex. This generates large dynamical systems that, in practice, are very difficult to study.

- Estimation of parameters in the system: as mentioned above, the system of ODEs depends on the rate of occurrence of each reaction, and as the rates change, the behaviour of the system does too. Depending on the rates, a system could have oscillatory behaviour or just one equilibrium point, and finding the value of these parameters in general is a difficult task.

With these issues in mind, how can we answer questions regarding the dynamic behaviour of a system? In particular, could we guarantee the existence of a steady state in a system of ODEs? Could we preclude the presence of oscillatory behaviour for some reaction rate constants? Can we explore the presence of two stable steady states in the system of ODEs?

Under the assumption of mass action kinetics, these questions can be addressed with tools of algebraic geometry as the ODEs are polynomial and the steady states form an algebraic variety. In this case, the parameters are regarded as symbolic and, since we are interested in concentration variables, we only study the intersection of the algebraic variety with the positive orthant [17]. We call this intersection the positive steady state variety. For the systems of ODEs in this setting it is possible to prove that, given an initial condition $x_0 \in \mathbb{R}^n_{>0}$, the trajectories starting at x_0 are confined to a linear space determined by this point. These spaces are called stoichiometric compatibility classes, and depend on x_0. Since the dynamics of the system occurs within each stoichiometric compatibility class, questions regarding the existence of steady states or oscillatory behaviour are considered within each class.

With this in mind, one question that has been extensively studied is whether it is possible for the positive steady state variety to intersect some stoichiometric compatibility class in more than one point. Since the systems of ODEs depend on different parameters, the answer to this question depends on their value, but in case of an affirmative answer for a set of parameters, it is said that the network admits multistationarity. Several methods have been developed to preclude or guarantee multistationarity. These methods rely on different tools. For example, in [15] and [12] the positive steady state variety is completely understood for chemical reaction networks with deficiency zero and deficiency one. The deficiency is a structural property that depends on the graph associated with the network, and not on the rate of occurrence of the reactions. Using the implications of having small deficiency, the deficiency one and deficiency zero theorems, preclude the existence of multistationarity for all reactions of deficiency zero and for reactions with deficiency one that satisfy additional conditions. In works as [11, 19, 35, 36, 44], the approach to study multistationarity is different, as it is more algebraic. In [11, 35, 44] multistationarity in systems with mass action kinetics, can be linked to the sign of a polynomial arising from the determinant of the Jacobian of a polynomial function.

The results presented in this work, explore mainly three properties of the systems of ODEs coming from chemical reaction networks with mass action kinetics: Bistability, oscillations and Absolute Concentrarion Robustness.

Bistability is the existence of two stable steady states in a dynamical system. This property is linked to switch-like behaviour in different biological networks and to cellular decision making. It has been observed experimentally in a variety of systems [29, 45, 47]. However, proving the presence of bistability remains a difficult problem.

In previous works, stability of steady states has been studied using a variety of methods. In [1], the stability analysis comes from Piecewise Linear in Rates Lyapunov Functions and establishing necessary conditions for their existence. In [8–10] the approach is based on the Hurwitz criterion and graphical methods to detect sign changes on the Hurwitz determinants. In addition to this, as part of the zero deficiency Theorem, asymptotic stability has been established for mass action systems that are complex balanced [2, 13, 14, 32]. However, ensuring the presence of bistability, is much harder and typically solved by first deciding whether the network admits multiple steady states, and then numerically computing the steady states and their stability for a suitable choice of parameter values. Formal proofs of bistability require advanced analytical arguments such as bifurcation theory and geometric singular perturbation theory, as in [20, 30] for futile cycles.

One of the main contributions in this work, is to outline an algorithm for detecting bistability. This algorithm uses algebraic parametrizations of the positive steady state variety, the Center Manifold Theorem and the Hurwitz criterion for polynomials, and provides a symbolic proof of the presence of bistability in chemical reaction networks satisfying certain conditions. This method is computationally challenging, due to the large amount of parameters in the networks. Since all the computations are symbolic, the Hurwitz determinants can be large functions. In our method, we get around this issue relying on structural reduction techniques, in particular removal of intermediates and reversible reactions [23, 36].

In addition to the method mentioned above, we explore the presence of bistability and oscillations in two specific networks where the method mentioned above cannot be applied. These networks are a model of ERK regulation and a MAPK cascade.

The first of these networks, comprises extracellular signal-regulated kinase (ERK) regulation by dual-site phosphorylation by the kinase MEK and dephosphorylation by the phosphatase MKP3. This network,which we call the ERK network, has an important role in regulating many cellular activities, with deregulation implicated in many cancers [55]. It was proven by Rubinstein, Mattingly, Berezhkovskii and Shvartsman [52] that the ERK network is bistable and exhibits oscillations (for some choices of rate constants). However, some reductions made on this network yield monostationary systems. The main contribution in this project is to find a set of reactions necessary for bistability and to explore whether oscillations are lost when doing structural modifications of the network. The oscillatory behaviour in the ERK network and its reductions, came from a simple Hopf bifurcation detected through the analysis of Hurwitz determinants. Due to the amount of variables and parameters, one of the main tools for finding a set of parameters where a Hopf bifurcation arises, was the Newton polytope.

Regarding our contributions on the MAPK cascade, bistability was precluded when it was proven that this network is monostationary for every set of parameters [21], and oscillations in the network had not been proven. However, when considering MAPKK and MAPKKK cascades [34, 42, 51], containing the MAPK cascade as a subnetwork, bistability and oscillations had been found. Using the Newton polytope and its outer normal cone, we were able to find a set of parameters where a Hopf bifurcation of the MAPK cascade arises.

The final part of this thesis is devoted to the exploration of Absolute Concentration Robustness. A network has Absolute Concentration Robustness (ACR) in a species X_i if the positive steady state variety is constant in x_i. This is equivalent to say that for every pair of positive steady states of the system, the value for the concentration of x_i is the same. This property had been precluded in networks with deficiency zero where the positive steady state variety is nonempty [57]. However, there are not many sufficient conditions to ensure the presence of ACR in a network. One of the main results is proved in [38], and is applicable on networks only with deficiency one. ACR has also been explored using translation networks in [60] as part of a more general study of robust ratios. Our contribution is to characterize ACR in small networks and study whether ACR is preserved under certain structural modifications of the network.

This document is structured as follows. In Chapter 1, we introduce the background in chemical reaction networks necessary for setting our contributions. We start by defining a chemical reaction network and all its structure, then we present the main results for assessing multistationarity. We also include in this chapter an introduction to polytopes and define the Newton polytope of a polynomial. In Chapter 2, we present our contributions related to detection of bistability and Hopf bifurcations in a chemical reaction network. This chapter starts with some background on dynamical systems and stability of steady states, then we summarize the contributions made in papers I and II. Finally in this chapter, we present the advances in ongoing projects, also aimed to study the stability of positive steady states. Finally in Chapter 3, we present our contributions related to Absolute Concentration Robustness, first we explore ACR in small networks; afterwards we study how ACR is preserved under structural modifications of the network.

Contributions

The contributions presented in this work correspond to research exploring options for solving two problems: detection of bistability and oscillations, and detection of Absolute Concentration Robustness (ACR) in chemical reaction networks.

Regarding the detection of bistability, we outline a procedure to assess the existence of at least two exponentially stable positive steady states in the same stoichiometric compatibility class of a Chemical Reaction Network. This procedure relies on algebraic parametrizations of the positive steady state variety and on the Theorems (9) and (10) about structural reductions and multistarionarity. The procedure is symbolic and can be used for detecting parameter regions for bistability. This work corresponds to the first paper called *Detecting parameter regions for bistability*[61], with Elisenda Feliu and currently under review.

A second contribution regarding bistability is presented in paper II called *Oscillations and bistability in a model of ERK regulation*[46], with Nida Obatake and Anne Shiu from University of Texas A&M and with Xiaoxian Tang from Beihang University. In this work we analysed a model for ERK regulation in search of key features for the presence of bistability and oscillations. It was proven before that the model was bistable [52]; however, it was also proven that the network limits to a network that is not bistable. We used the reduction techniques in Section 1.4 and effective parametrizations of the positive steady state variety to detect two reactions characterizing the presence of bistability. Additionally, we used the Hurwitz criterion and Newton polytopes to effectively compute sets of parameters and steady states where the system exhibits oscillatory behaviour arising from a Hopf bifurcation.

These two contributions correspond to papers I and II enclosed at the end of the thesis. We present the arXiv version of paper I and the published version of paper II.

An additional contribution regarding oscillations is connected with the MAPK cascade. Using the Newton polytope and its outer normal fan, we also explore the presence of oscillatory behaviour in a MAPK cascade [34], a network that is monostationary for every set of parameters [21]. For this network we found a set of parameters where a simple Hopf bifurcation arises.

Finally, we explore whether the stability of steady states of small networks, like the

Michaelis-Menten mechanism, is the same as the stability of the steady states of the reduction of these networks obtained by removing one reverse reaction. This analysis consists on studying the coefficients of the characteristic polynomials associated with the Jacobian of both networks, and establishing whether there are sets of parameters for both networks such that they yield the same characteristic polynomial.

Contributions in the area of ACR are presented in Chapter 3. The first contribution is to prove that ACR in a core species is preserved under the removal or addition of intermediates. The second contribution consists in giving a graphic way for identifying ACR in networks with two reactions and n species.

1

Framework

Notation: Given a vector $\kappa \in \mathbb{R}^m$, $\operatorname{diag}(\kappa)$ denotes the diagonal matrix of size m whose diagonal entries correspond to the entries of κ. Given vectors $x, b \in \mathbb{R}^n$, $x^b = x_1^{b_1} x_2^{b_2} \cdots x_n^{b_n}$.

1.1 Chemical Reaction Networks

A Chemical Reaction Network $\mathcal{N} = (\mathcal{S}, \mathcal{R}, \mathcal{C})$ is a finite directed graph with no loops, whose nodes are linear combinations of the elements in a set $\mathcal{S} = \{X_1, \ldots, X_n\}$. The set $\mathcal{S}$ is called the set of *species*, the nodes of the graph are called *complexes* and form the set $\mathcal{C}$, and each arrow is called a *reaction*. According to this definition, and assuming that the graph has m reactions $\mathcal{R} = \{r_1, \ldots, r_m\}$, every reaction r_j has the form

$$r_j = \sum_{i=1}^{n} \alpha_{ij} X_i \longrightarrow \sum_{i=1}^{n} \beta_{ij} X_i \quad \text{for } j = 1, \ldots, m \tag{1.1}$$

with $\alpha_{ij} \neq \beta_{ij}$ for some i. The complex on the left side of the arrow is called the *reactant* and the complex on the right side is called the *product* of the reaction.

The concentration of X_i at time t is denoted by $x_i(t)$, and we refer to the vector $x(t) = (x_1(t), \ldots, x_n(t))$ as the *vector of concentrations*. In order to understand the behaviour of the system, we assign to each reaction r_j a function $v_j(x) : \Omega_v \to \mathbb{R}_{\geq 0}$, where its domain is such that $\mathbb{R}^n_{>0} \subset \Omega_v \subset \mathbb{R}^n_{\geq 0}$. The vector $v = (v_1, \ldots, v_m)$ is called the *vector of kinetics*. Intuitively, each function $v_j(x)$ represents the rate to which the reaction r_j occurs. Some of the commonly used kinetics are

1. **Mass action kinetics:** With the assumption of these kinetics, each reaction r_j is assigned a positive real number κ_j, called *reaction rate constant* and each entry of the vector of kinetics is $v_j(x) = \kappa_j x_1^{\alpha_{1j}} \cdots x_n^{\alpha_{nj}}$. This definition means that the rate of occurrence of each reaction is proportional to the product of the concentrations of its reactants. In this case a reaction cannot occur if the concentration of one of the reactants is zero.

2. **Power-law kinetics:** Similarly to mass action, each reaction r_j is assigned a positive reaction rate constant κ_j and the function $v_j(x)$ is equal to $\kappa_j x_1^{b_{1j}} \cdots x_n^{b_{nj}}$ for some $(b_{1j}, \dots, b_{nj}) \in \mathbb{R}^n$. However, the choice of b_j does not depend on the reactant complex and can have negative entries. In this case Ω_v corresponds to $\mathbb{R}^n_{\geq 0}$ minus the hyperplanes $x_i = 0$ for every i such that $b_{ij} < 0$ for some j.

3. **Michaelis-Menten kinetics:** For this choice of kinetics a function $v_j(x)$ is defined as $v_j(x) = \frac{Vx_i}{K_M + x_i}$ for some i and some $V, K_M > 0$. This kind of function arises when the network is obtained by doing simplifications, like a quasi-steady state approximation [27], on more complex networks. In the vector of kinetics, these functions can be combined with mass action.

Note that whenever a reaction r_j occurs, the amount of units of X_i changes in $\beta_{ij} - \alpha_{ij}$. This, together with a vector of kinetics, allows to model the variation of $x_i(t)$ through time with the differential equation

$$\dot{x}_i = \sum_{j=1}^{m} (\beta_{ij} - \alpha_{ij}) v_j(x) \quad \text{for} \quad i = 1, \dots, n \text{ and } x \in \mathbb{R}^n_{\geq 0}, \tag{1.2}$$

where $\dot{x}_i = \frac{dx_i}{xt}$. The system of Ordinary Differential Equations (ODEs) defined by the equations above, models the evolution of the whole system through time. Since these ODEs are autonomous, we normally write x_i instead of $x_i(t)$ to denote the concentration of species X_i. Defining N as the matrix whose j-th column is the vector $(\beta_{1j} - \alpha_{1j}, \dots, \beta_{nj} - \alpha_{nj})$, the system of ODEs can be written as $\dot{x} = Nv(x)$. N is called *the stoichiometric matrix* and the function $f(x) := Nv(x)$ is called *the rate function*. The column span of N is called the *stoichiometric subspace* S of the network.

With the stoichiometric subspace, define an equivalence relation in $\mathbb{R}^n$ as follows.

Definition 1. Two vectors $x, z \in \mathbb{R}^n$ are *stoichiometrically compatible* if $x - z \in S$. This is an equivalence relation and the intersection of each equivalence class with $\mathbb{R}^n_{\geq 0}$ is called a *stoichiometric compatibility class.*

Given $x_0 \in \mathbb{R}^n$, its stoichiometric compatibility class is

$$(x_0 + S) \cap \mathbb{R}^n_{\geq 0} = \{x \in \mathbb{R}^n_{\geq 0} : x \text{ is stoichiometrically compatible with } x_0\}.$$

The importance of the stoichiometric compatibility classes comes from the fact that the trajectories defined by an initial solution x_0 are confined to $(x_0 + S) \cap \mathbb{R}^n_{\geq 0}$, as long as the coordinates remain non-negative. This is proven in the following lemma.

Lemma 1. *Let $f(x) = Nv(x)$ be the rate function associated with a chemical reaction network with vector of kinetics $v(x)$, and denote the stoichiometric subspace as S. Then, the trajectories of the dynamical system $\dot{x} = Nv(x)$, $x \in \mathbb{R}^n_{\geq 0}$, are confined to the stoichiometric compatibility classes.*

Proof. Let $\omega \in \mathbb{R}^n$ be an element of $S^{\perp}$. Since every column of N is in S, we have that $\omega \cdot \dot{x} = \omega \cdot Nv(x) = 0$. This means that $\omega \cdot x$ is constant through time and the concentrations are always constrained to their stoichiometric compatibility classes. Proving that the trajectories also remain in the non-negative orthant is not trivial and we refer the reader to [62]. $\square$

The equations defining the stoichiometric compatibility classes are called *conservation laws* and, in order to find them, we choose a row reduced matrix W, whose rows form a basis of $S^{\perp}$. The stoichiometric compatibility class containing x_0 has equations $Wx_0 = Wx$, and the entries of the vector Wx_0 are called *total amounts*. Typically we denote the vector of total amounts as T, and the stoichiometric compatibility class defined by these equations as $\mathcal{P}_T$. Observe that the conservation laws do not depend on the choice of kinetics, they only depend on the stoichiometric subspace and are inherent to the structure of the network.

As an example, consider the reaction network

$$\mathrm{X}_1 \xrightarrow{\kappa_1} \mathrm{X}_2 \qquad \mathrm{X}_2 + \mathrm{X}_3 \xrightarrow{\kappa_2} \mathrm{X}_1 + \mathrm{X}_4 \qquad \mathrm{X}_4 \xrightarrow{\kappa_3} \mathrm{X}_3. \tag{1.3}$$

This network is a simplified model of a two-component system, consisting of a histidine kinase HK and a response regulator RR [11]. Both occur unphosphorylated and phosphorylated (subscript p) and they are represented in the system as follows: $X_1 = \mathrm{HK}, X_2 = \mathrm{HK}_p, X_3 = \mathrm{RR}$ and $X_4 = \mathrm{RR}_p$.

The set of species is $\mathcal{S} = \{X_1, X_2, X_3, X_4\}$, the set of complexes is

$$\{X_1, X_2, X_3, X_4, X_2 + X_3, X_1 + X_4\},$$

and the reaction rate constants are $\kappa_1, \kappa_2, \kappa_3$. With mass action kinetics, the system of ODEs modelling the dynamics of the network is

$$\begin{aligned} \dot{x}_1 &= -\kappa_1 x_1 + \kappa_2 x_2 x_3 & \qquad \dot{x}_3 &= -\kappa_2 x_2 x_3 + \kappa_3 x_4 \\ \dot{x}_2 &= \kappa_1 x_1 - \kappa_2 x_2 x_3 & \qquad \dot{x}_4 &= \kappa_2 x_2 x_3 - \kappa_3 x_4. \end{aligned}$$

This system can also be written as

$$\begin{pmatrix} \dot{x_1} \\ \dot{x_2} \\ \dot{x_3} \end{pmatrix} = \begin{pmatrix} -1 & 1 & 0 \\ 1 & -1 & 0 \\ 0 & -1 & 1 \\ 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} \kappa_1 x_1 \\ \kappa_2 x_2 x_3 \\ \kappa_3 x_4 \end{pmatrix}, \tag{1.4}$$

where the 4×3 matrix on the right side is the stoichiometric matrix N, and the vector is the vector of kinetics v. The stoichiometric subspace is

$$S = \langle (-1, 1, 0, 0), (1, -1, -1, 1), (0, 0, 1, -1) \rangle,$$

which has dimension two, and a basis for $S^\perp$ is $\{(0,0,1,1),(1,1,0,0)\}$. In this case, we choose

$$W = \begin{pmatrix} 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 1 \end{pmatrix} \tag{1.5}$$

and for a vector $x_0 = (c_1, c_2, c_3, c_4)$ of initial conditions its stoichiometric compatibility class is defined by the equations

$$x_1 + x_2 = c_1 + c_2 \qquad x_3 + x_4 = c_3 + c_4.$$

These are the conservation laws, and the amounts $c_1 + c_2$ and $c_3 + c_4$ correspond to the total amounts T_1 and T_2 of the system.

In our work we focus mainly in networks with mass action kinetics and, unless said otherwise, we assume that each network has the vector of kinetics defined by the mass action assumption. The reaction rate constant will be represented as a label on each arrow of the graph defining the chemical reaction network. For these systems the rate function has polynomial entries and hence the system of ODEs (1.2) is polynomial. Additionally, every trajectory starting at a point $x_0 \in \mathbb{R}^n_{>0}$ remains positive through time, which means that both he positive orthant and the nonnegative orthant are invariant under trajectories of the system [17, 62].

1.2 Steady states

Recall that $f(x) = Nv$. The *steady states* (or equilibrium points) of the system are the non-negative solutions of the system of equations

$$0 = \sum_{j=1}^{m} (\beta_{ij} - \alpha_{ij}) v_j(x) \quad \text{for } i = 1, \dots, n \tag{1.6}$$

or equivalently $0 = f(x)$. Note that only non-negative solutions are meaningful. We say that the *steady state is positive* if all the concentrations are strictly greater than zero. Under mass action kinetics, we refer to the set of positive steady states as the *positive steady state variety*, which is the intersection of the algebraic variety given by $0 = f(x)$ with the positive orthant.

Since the dynamics take place in the stoichiometric compatibility classes, steady states are regarded within each class. These positive steady states correspond to the positive solutions to

$$f(x) = 0 \qquad \text{and} \qquad Wx = T.$$

The existence of conservation laws implies that some of the steady state equations are linearly dependent. That is, if the conservation laws of a network are given by $Wx = T$

and ω is a row of W, then $\omega \cdot \dot{x} = \omega \cdot f(x) = 0$, which means that the steady state equations $f_i(x) = 0$ for $i \in \operatorname{Supp}(\omega)$ are linearly dependent. As W is row reduced, defining $\{i_1, \dots, i_d\}$ as the indices of the first non-zero coordinate of each row of W, the linear combinations determined by the rows of W allow to write the equations $f_{i_k} = 0$ for $k = 1, \dots, d$ as linear combinations of the other equations, hence they are redundant.

With this in mind, for every $T \in \mathbb{R}^d$, define the function F_T as

$$F_T(x)_i = \begin{cases} f_i(x) & i \notin \{i_1, \dots, i_d\} \\ (Wx - T)_i & i \in \{i_1, \dots, i_d\}. \end{cases} \tag{1.7}$$

This function replaces the redundant equations of the rate function f with the conservations laws for some set of total amounts T, and the solutions to these equations are the steady states in the stoichiometric compatibility class given by T.

In example (1.3) the positive steady states are the positive solutions to the equations

$$\begin{aligned} 0 &= -\kappa_1 x_1 + \kappa_2 x_2 x_3 & \qquad 0 &= -\kappa_2 x_2 x_3 + \kappa_3 x_4 \\ 0 &= \kappa_1 x_1 - \kappa_2 x_2 x_3 & \qquad 0 &= \kappa_2 x_2 x_3 - \kappa_3 x_4. \end{aligned}$$

For W in (1.5), we have $i_1 = 1$ and $i_2 = 3$, and for a vector T of total amounts, the function F_T is

$$F_T = \begin{pmatrix} x_1 + x_2 - T_1 \\ \kappa_1 x_1 - \kappa_2 x_2 x_3 \\ x_3 + x_4 - T_2 \\ \kappa_2 x_2 x_3 - \kappa_3 x_4 \end{pmatrix}.$$

By solving the two linearly independent steady state equations, we find that any positive solution has the form

$$\phi(x_2, x_4) = \left(\frac{\kappa_3 x_4}{\kappa_1}, x_2, \frac{\kappa_3 x_4}{\kappa_2 x_2}, x_4 \right). \tag{1.8}$$

This defines a parametrization of the positive steady states, by the concentrations of x_2 and x_4, satisfying that whenever x_2 and x_4 are positive, so are the first and third entries of the vector.

In general, when the positive steady state variety admits a parametrization (a surjective map) of the form

$$\begin{aligned} \phi \colon \mathbb{R}^d_{>0} &\to \{x \in \mathbb{R}^n_{>0} \mid f(x) = 0\} \\ \xi &\mapsto \phi(\xi), \end{aligned} \tag{1.9}$$

we say that the parametrization is positive. In practice, since f is polynomial, the entries of ϕ will be rational functions in ξ. A strategy that often works for realistic networks to find parametrizations is to consider a subset of variables of size d and solve the steady state equations with respect to this subset using software such as Maple or Mathematica. Then it is necessary to verify that the entries of the parametrization are positive rational functions in $\mathbb{R}^d_{>0}$. In this case, at most $\binom{n}{d}$ subsets must be considered, but there is no guarantee that this will work, nor is the existence of a parametrization.

The existence of positive parametrizations, and methods to find them, have been established for some networks relying on their structure [22, 24, 28, 49, 54, 59].

We now define non-degeneracy of a steady state and show how to determine whether a positive steady state is non-degenerate.

Definition 2. Given a chemical reaction network $\mathcal{N}$ with rate function f and a steady state x^*, we say that x^* is non-degenerate if the Jacobian matrix $J_f(x^*)$ is injective on the stoichiometric subspace S.

The injectivity on the previous definition refers to the injectivity of the linear map defined by $J_f(x^*)$. If the stoichiometric subspace has full dimension, then $J_f(x^*)$ is injective if and only if $\det(J_f(x^*)) \neq 0$. However, in our setting, the existence of conservation laws implies that this is not always the case, which means that a different criterion for checking the injectivity of $J_f(x^*)$ on the stoichiometric subspace is necessary. The following lemma relates the determinant of $J_{F_T}(x^*)$, defined in (1.7), with the injectivity that we require.

Lemma 2. *A positive steady state x^* is non-degenerate if and only if, for every choice of matrix W such that $Wx = T$ are the equations defining the stoichiometric compatibility class for total amounts T, the Jacobian of F_T is non-singular at x^*, that is,* $\det J_{F_T}(x^*) \neq 0$.

Proof. Since $f(x) = Nv(x)$, we have $J_f(x) = NJ_v(x)$. Define $g(x)$ as the function obtained by considering only $s = \dim(S)$ linearly independent steady state equations. This function can be written as $g(x) = N'v(x)$ for a matrix N' with $\operatorname{rank}(N) = \operatorname{rank}(N')$. By the definition of g we have that $J_g(x) = N'J_v(x)$, which implies that $\ker(J_f(x)) = \ker(J_g(x))$. The Jacobian $J_f(x^*)$ defines a linear map that is injective on S if and only if, $\ker(J_f(x^*)) \cap S = \{0\}$. This is equivalent to say that there is not a positive vector z such that $Wz = 0$ and $J_f(x^*)(z) = 0$, but the last equality holds if and only if $J_g(x^*)(z) = 0$. Note now that the conditions $Wz = 0$ and $J_g(x^*)(z) = 0$ are equivalent to $J_{F_T}(x^*)(z) = 0$, and no $z \in S$ satisfy this if and only if, $J_{F_T}(x^*)$ is injective in S and $\det J_{F_T}(x^*) \neq 0$, which is what we wanted. □

1.3 Multistationarity

A network exhibits *multistationarity* if there exist two distinct positive steady states in one stoichiometric compatibility class, that is, the system $F_T(x) = 0$ has at least two

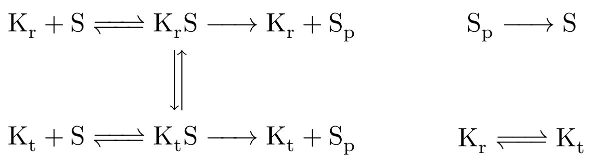


Figure 1.1: Allosteric network.

distinct positive solutions. If there is one positive steady state in each stoichiometric compatibility class, we say that the network is *monostationary.*

Deciding whether a network exhibits multistationarity is a problem that has been studied extensively [4, 6, 11, 19, 35, 36, 49, 53, 63]. Now we mention some results regarding multistationarity, focusing on those that played a significant role on this work. We start with the deficiency zero and deficiency one theorems, then we present results on injectivity of networks used to preclude multistationarity and, finally, we present an algebraic criteria for detecting parameter regions for multistationarity.

1.3.1 Deficiency zero and deficiency one theorems

As their names indicate, the deficiency zero and deficiency one theorems rely on the concept of deficiency. To define it, we start by defining the linkage classes of a network.

Definition 3 (Linkage class)**.** For a chemical reaction network $\mathcal{N} = (\mathcal{S}, \mathcal{R}, \mathcal{C},)$, a *linkage class* is defined as a connected component of the associated graph.

In our previous example (1.3), there are 6 complexes and the linkage classes are $\{X_1, X_2\}, \{X_2 + X_3, X_1 + X_4\}$ and $\{X_4, X_3\}$. In this example, the linkage classes are given exactly by the complexes that are adjacent.

As a second example, consider the network in Figure 1.1 Here, K corresponds to the kinase that exists in two conformations: K_r (relaxed state) and K_t (tensed state). Each conformation acts as a kinase for a common substrate S. We denote by S_p the phosphorylated form of the substrate. We assume that the intermediate kinase-substrate complexes, K_rS and K_tS, also undergo conformational change [25]. In this network we have 3 linkage classes given by $\{K_r + S, K_rS, K_r + S_p, K_tS, K_t + S, K_t + S_p\}, \{S_p, S\}$ and $\{K_r, K_t\}$.

Definition 4. A chemical reaction network is *weakly reversible* if every connected component of the associated graph is strongly connected.

Intuitively, being weakly reversible means that if one complex reacts to another, then it is possible to reverse this reaction through some sequence of complexes. The property of reacting through complexes is defined as *ultimately reacts to* and it allows us to define a strong linkage class. We now formalize these concepts.

Definition 5.

- A complex c *ultimately reacts to* c' if one of the following conditions holds: (i) they are equal, (ii) $c \to c'$ is a reaction in the network, or (iii) if there is a sequence of complexes $C_1, \ldots, C_k$ such that the sequence of reactions $c \to C_1 \to \cdots \to C_t \to c'$ is in the network.

- Two complexes c and c' are *strongly connected* if c ultimately reacts to c' and c' ultimately reacts to c. We denote this relation as $c \approx c'$. This is an equivalence relation over the set of complexes and each equivalence class is called a *strong-linkage class.*

- A *terminal strong-linkage class* is a strong-linkage class whose complexes do not react to complexes outside the class.

- A complex is called *terminal* if it belongs to a terminal strong-linkage class. Otherwise it is called non-terminal.

Having the previous definitions, we now present the definition of deficiency.

Definition 6 (Deficiency)**.** Given a chemical reaction network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$, its deficiency δ is defined as

$$\delta = \nu - \ell - s$$

where ν is the number of complexes, ℓ is the number of linkage classes and s is the dimension of the stoichiometric subspace.

In the previous examples, network (1.3) has deficiency $\delta = 6 - 3 - 2 = 1$, and network (1.1) has deficiency $\delta = 10 - 3 - 4 = 3$.

Note that the deficiency is independent of the kinetics, it is entirely a structural property of the network. However, under mass action kinetics, this structural property has implications on the existence of positive steady states.

Theorem 1 (Deficiency zero theorem)**.** *Let $\mathcal{N} = (\mathcal{S}, \mathcal{R}, \mathcal{C})$ be a network with deficiency zero and mass action kinetics.*

1. *If the network is not weakly reversible, then for every set of reaction rate constants and total amounts, the system of differential equations admits neither a positive steady state nor a periodic orbit.*

2. *If the network is weakly reversible, then for every set of reaction rate constants and total amounts each stoichiometric compatibility class contains precisely one positive steady state, which is asymptotically stable, and there is no nontrivial periodic orbit.*

This theorem was proved by Horn, Jackson and Feinberg [7, 15] and it characterizes the steady states of networks under mass action and with deficiency zero. Namely, the positive steady state variety of deficiency zero networks is nonempty for every set of parameters if, and only if, the network is weakly reversible. Furthermore, if this is the case, there is not multistationarity and the only steady state is asymptotically stable. We will go back to the stability property later.

This theorem cannot be used to study the positive steady state variety of examples (1.1) and (1.3) as they do not have deficiency zero. Consider the network in Figure 1.2 This network has 5 species, 5 complexes and 2 linkage classes. The stoichiometric subspace has dimension 3, hence the deficiency of the network is $\delta = 5 - 2 - 3 = 0$. Additionally, this network is weakly reversible. Therefore, the deficiency-zero theorem allows us to conclude that for every set of parameters, the network is monostationary.

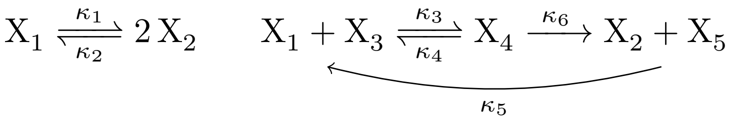


Figure 1.2: Network with deficiency zero

For networks with deficiency 1, the deficiency one theorem [15, 16] provides analogous conditions for a network to have a positive steady state.

Theorem 2 (Deficiency one theorem)**.** *Let $\mathcal{N} = (\mathcal{S}, \mathcal{R}, \mathcal{C})$ be a reaction network of deficiency δ, let $\delta_{\mathcal{L}}$ denote the deficiency of the linkage class $\mathcal{L}$ and suppose that the following conditions hold*

(i) $\delta_{\mathcal{L}} \leq 1$ *for every linkage class* $\mathcal{L}$*,*

(ii) $\sum_{\mathcal{L} \in \mathcal{C}/\sim} \delta_{\mathcal{L}} = \delta$*, where* $\mathcal{C}/\sim$ *represent the set of linkage classes of* $\mathcal{N}$*, and*

(iii) each linkage class contains only one terminal strong-linkage class.

If for a particular set of parameters, the mass action system admits a positive steady state, then each stoichiometric compatibility class contains precisely one steady state. If the network is weakly reversible, then, for every set of parameters the mass action system admits a positive steady state.

Similarly to the deficiency zero theorem, the deficiency one theorem characterizes the positive steady state variety of networks with mass action kinetics satisfying the deficiency conditions in *(i)* and *(ii)*. These conditions are structural and allow to preclude multistationarity without computing the steady states.

1.3.2 Injectivity

The deficiency zero and deficiency one theorems in the previous section rely on structural conditions of the network. However, for networks not satisfying them there is no insight on how the positive steady state variety is. We will now present some results [44] that preclude multistationarity using an algebraic approach.

The underlying idea behind the results in this section is to find sufficient conditions for the rate function f to be injective. If f is injective it is not possible for two different points $x, y \in \mathbb{R}^n$ to be positive steady states as $f(x) = 0 = f(y)$ cannot hold for $x \neq y$. The results presented in this section are valid, not only for mass action, but for more general kinetics as well; however, we will focus on mass action as it is the main focus of our work.

Since the rate function depends on a set of parameters κ and the injectivity property depends on κ, we define the family of functions

$$\mathcal{F}_{N,B} = \{f_\kappa : \kappa \in \mathbb{R}^m_{>0}\}$$

where $N \in \mathcal{M}_{n\times m}(\mathbb{R})$ and $B \in \mathcal{M}_{m\times n}(\mathbb{R})$ are defining the rate function, namely $f_\kappa(x) = N \operatorname{diag}(\kappa) x^B$ with $x^B = (x^{b_1}, x^{b_2}, \dots, x^{b_m})$ and b_i is the i-th row of B for $i = 1 \dots m$ and study the injectivity of these families. Note that N and B are fixed, so $\mathcal{F}_{N,B}$ just corresponds to the different rate functions obtained when varying the parameters of the system.

Definition 7. The family $\mathcal{F}_{N,B}$ is S-injective if for every $f_\kappa \in \mathcal{F}_{N,B}$ the following statement holds: for every $x, y \in \mathbb{R}^n_{>0}$ such that $0 \neq x - y$ and $x - y \in S$, $f_\kappa(x) \neq f_\kappa(y)$.

In this case we say that the network $\mathcal{N}$ is injective.

With the following theorem (Theorem 1.4 in [**?**]) it is proven that the injectivity of a function f_κ is related to the injectivity of a linear map, which depends on the sign of the entries of certain vectors. The sign function is defined as

$$\operatorname{sign}(a) = \begin{cases} -1 & \text{if } a < 0 \\ 0 & \text{if } a = 0 \\ 1 & \text{if } a > 0, \end{cases}$$

for every $a \in \mathbb{R}$. The sign of a vector $x \in \mathbb{R}^n$ is defined as $\sigma(x) = (\operatorname{sign}(x_1), \dots, \operatorname{sign}(x_n))$ and the sign set of $V \subset \mathbb{R}^n$ is $\sigma(V) = \{\operatorname{sign}(x) : x \in V\}$. Consider now the family of linear transformations

$$L_{N,B} = \{N \operatorname{diag}(\kappa) B \operatorname{diag}(\lambda) : \kappa \in \mathbb{R}^m_{>0} \text{ and } \lambda \in \mathbb{R}^n_{>0}\}.$$

In the same way as $\mathcal{F}_{N,B}$, the matrices N and B defining $L_{N,B}$, are fixed and the elements of $L_{N,B}$ vary depending on κ and λ.

Theorem 3. *Let* $N \in \mathcal{M}_{n\times m}(\mathbb{R})$*,* $B \in \mathcal{M}_{m\times n}(\mathbb{R})$ *and* S *be the stoichiometric subspace of a network* $\mathcal{N}$*. The following statements are equivalent:*

(i) *The family* $\mathcal{F}_{N,B}$ *is* S*-injective,*

(ii) *the linear transformation* $N\operatorname{diag}(\kappa)B\operatorname{diag}(\lambda) \in L_{N,B}$ *is injective on* S*, for every* $\kappa \in \mathbb{R}^m_{>0}$ *and* $\lambda \in \mathbb{R}^n_{>0}$*,*

(iii) $\sigma(\ker(N)) \cap \sigma(B(\Sigma(S^*))) = \emptyset$*,*

where $S^* = S \setminus \{0\}$ *and* $\Sigma(S^*) = \{x \in \mathbb{R}^n : \sigma(x) = \sigma(v) \text{ for some } v \in S^*\}$*.*

***Remark* 1.** The set $\Sigma(S^*)$ can be defined also for every subset $V \subset \mathbb{R}^n$ as $\Sigma(V) = \{x \in \mathbb{R}^n : \sigma(x) = \sigma(v) \text{ for some } v \in V\}$ and it can described also as $\Sigma(V) = \{\operatorname{diag}(\lambda)v : \lambda \in \mathbb{R}^n_{>0} \text{ and } v \in V\}$. This set contains all the vectors whose components have the same sign as one of the vectors in V.

Before proving the theorem, we prove a lemma on an alternative description of the set $\Sigma(V)$.

Lemma 3. *Given a subset* $V \subseteq \mathbb{R}^n$*, the following equality holds:*

$$\Sigma(V) = \{\ln(x) - \ln(y) : x, y \in \mathbb{R}^n_{>0} \text{ and } x - y \in V\}$$

where $\ln(x) = (\ln(x_1), \dots, \ln(x_n))$*.*

Proof. Denote the set on the right side of the equality as $\Lambda(V)$. We begin the proof by showing that $\Lambda(V) \subseteq \Sigma(V)$. Given $x, y \in \mathbb{R}^n_{>0}$ with $x - y \in V$, the monotonicity of the logarithm guarantees that $\sigma(x-y) = \sigma(\ln(x)-\ln(y))$, which means that $\ln(x)-\ln(y) \in \Sigma(V)$ and $\Lambda(V) \subseteq \Sigma(V)$. For the other inclusion, given $\operatorname{diag}(\lambda)v \in \Sigma(V)$ for some $\lambda \in \mathbb{R}^n_{>0}$ and $v \in V$, define the entries of x and y as follows:

$$y_i = \begin{cases} \frac{v_i}{e^{\lambda_i v_i}-1} & \text{if } v_i \neq 0 \\ 1 & \text{if } v_i \neq 0 \end{cases} \quad \text{and} \quad x_i = \begin{cases} y_i e^{\lambda_i v_i} & \text{if } v_i \neq 0 \\ 1 & \text{if } v_i \neq 0. \end{cases}$$

These vectors satisfy $\ln(x_i) - \ln(y_i) = \lambda_i v_i$ and $x - y \in V$, which means that $\operatorname{diag}(\lambda)v \in \Lambda(V)$ and $\Sigma(V) \subseteq \Lambda(V)$. □

With this lemma, we now prove Theorem 3.

Proof of Theorem 3. The proof of this theorem consists in showing that statements *(i)* and *(ii)* are equivalent to *(iii)*. We start proving that $(ii) \Leftrightarrow (iii)$. Since each $N\operatorname{diag}(\kappa)B\operatorname{diag}(\lambda)$ is linear, being injective in S means that the kernel of its restriction to S is equal to $\{0\}$, which holds if and only if $N\,\mathrm{diag}(\kappa)B\,\mathrm{diag}(\lambda)u \neq 0$ for every $u \in S^*$. This is equivalent to the statement

$$N\,\mathrm{diag}(\kappa)Bv \neq 0 \text{ for every } v \in \Sigma(S^*)$$

because λ has positive entries. Note now that a vector w is in $\ker(N\,\mathrm{diag}(\kappa))$ if and only if $\sigma(w) \in \sigma(\ker(N))$, due to the positivity of the entries of κ. With this remark we have now that $N\,\mathrm{diag}(\kappa)Bv \neq 0$ holds if and only if $\sigma(Bv) \notin \sigma(\ker(N))$ for every $v \in \Sigma(S^*)$, or equivalently $\sigma(B\Sigma(S^*)) \cap \sigma(\ker(N)) = \emptyset$, which is what we wanted to prove.

Now we show that $(i) \Leftrightarrow (iii)$. If $\mathcal{F}_{N,B}$ is S-injective, then for every pair of vectors $x, y \in S$, with $x \neq y$, $f_\kappa(x) \neq f_\kappa(y)$. This implies

$$0 \neq f_\kappa(x) - f_\kappa(y) = N\,\mathrm{diag}(\kappa)x^B - N\,\mathrm{diag}(\kappa)y^B = N\,\mathrm{diag}(\kappa)(x^B - y^B),$$

which holds if, and only if, $(x^B - y^B) \notin \ker(N\,\mathrm{diag}(\kappa))$. But, given that all the entries of κ are positive, this occurs if, and only if, $\sigma(x^B - y^B) \notin \sigma(\ker(N))$ for every $x \neq y$ in S. If we prove that $\sigma(\{x^B - y^B : x, y \in S \text{ and } x \neq y\}) = \sigma(B(\Sigma(S^*)))$, then we have that $\sigma(\ker(N)) \cap \sigma(B(\Sigma(S^*))) = \emptyset$ which is what we want to prove. Recall that given a vector in $\mathbb{R}^n_{>0}$, its logarithm is the vector obtained applying the logarithm function componentwise. From this definition, we have

$$\begin{aligned}
\ln(x^B) =& (\ln(x^{b_1}), \ln(x^{b_2}), \ldots, \ln(x^{b_m})) \\
=& \left(\sum_{j=1}^{n} b_{1j} \ln(x_j), \sum_{j=1}^{n} b_{2j} \ln(x_j), \ldots, \sum_{j=1}^{n} b_{mj} \ln(x_j) \right) \\
=& (b_1 \cdot \ln(x), b_2 \cdot \ln(x), \ldots, b_m \cdot \ln(x)) \\
=& B \ln(x).
\end{aligned}$$

Doing analogous computations for y^B, we get $\ln(y^B) = B\ln(y)$. Since the logarithm is a monotonous function,

$$\sigma(x^B - y^B) = \sigma(\ln(x^B) - \ln(y^B)) = \sigma(B(\ln(x) - \ln(y)))$$

for every $x, y \in \mathbb{R}^n_{>0}$ and $x - y \in S$. Using Lemma 3 we deduce that $\{\ln(x) - \ln(y) : x, \in \mathbb{R}^n_{>0} \text{ and } x - y \in S\} = \Sigma(S)$; therefore,

$$\sigma(\{x^B - y^B : x, y \in S \text{ and } x \neq y\}) = \sigma(B(\Sigma(S^*)))$$

and our proof is complete. □

Theorem 3 is an important tool to preclude multistationarity because checking whether a linear function is injective is computationally more efficient than verifying injectivity in a function with polynomial entries. Furthermore, this theorem guarantees that it is not necessary to find the kernel of a linear function, it is enough to study the signs of vectors in $\ker(N)$ and $B(\Sigma(S^*))$.

1.3.3 Parameter regions for multistationarity

Now we present a method for detecting parameter regions for multistationarity. This method will be connected with our contributions in bistability as multistationarity is a necessary condition for bistability.

The results proven in the previous section allow us to preclude multistationarty of a network. However, they do not give a sufficient condition to exhibit multistationarity. We now present the main results of [11] as they are a method for detecting the presence of multistationarity and, additionally, finding parameter regions where a network has multistationarity. The results rely on algebraic parametrizations of a variety and the sign of values attained by certain polynomials.

We start by giving some definitions on networks and then we present the criteria for detecting multistationarity.

Definition 8. Consider a chemical reaction network $\mathcal{N} = (\mathcal{S}, \mathcal{R}, \mathcal{C})$.

- $\mathcal{N}$ is *dissipative* if for every set of total amounts T such that $\mathcal{P}_T \neq \emptyset$, there exists a compact set $K_T \subset \mathcal{P}_T$ such that every trajectory starting in $x \in \mathcal{P}_T$ eventually ends up in K_T.
- $\mathcal{N}$ is *conservative* if $S^\perp$ contains a positive vector. This holds if, and only if, every concentration variable appears in at least one conservation law with all non-zero coefficients positive.

In [5] it was proven that a network is conservative if, and only if, the stoichiometric compatibility classes are compact subsets of $\mathbb{R}^n$. This implies that every conservative network is dissipative.

The following theorem gives a criterion for deciding whether a chemical reaction network exhibits multistationarity using an algebraic parametrization of the steady state variety as in (1.9) and the function F_T defined in (1.7).

Theorem 4. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{R}, \mathcal{C})$ be a chemical reaction network with mass action kinetics and assume that the stoichiometric subspace S has dimension s. Assume also that the network is dissipative, there are no boundary steady states in any stoichiometric compatibility class with nonempty intersection with the positive orthant, and the positive steady state variety admits an algebraic parametrization $\varphi\colon \mathbb{R}^d_{>0} \to \mathbb{R}^n_{>0}$. Then, the following holds:*

a) If $\operatorname{sign}(\det(J_F(\varphi(x)))) = (-1)^s$ for all $x \in \mathbb{R}^d_{>0}$, then there is exactly one positive steady state in each stoichiometric compatibility class. Furthermore, this steady state is non-degenerate.

b) If $\operatorname{sign}(\det(J_F(\varphi(\hat{x})))) = (-1)^{s+1}$ *for some* $\hat{x} \in \mathbb{R}^d_{>0}$*, then there are at least two positive steady states in the stoichiometric compatibility class containing* $\varphi(\hat{x})$*, at least one of which is non-degenerate. If all positive steady states are non-degenerate then there are at least three and always an odd number.*

This theorem corresponds to Corollary 1 in [11], which is obtained from Theorem 1 in [11], stated below.

Theorem 5. *Let* $\mathcal{N} = (\mathcal{S}, \mathcal{R}, \mathcal{C})$ *be a chemical reaction network with mass action kinetics and assume that the stoichiometric subspace* S *has dimension* s*. Assume also that the network is dissipative and there are no boundary steady states in any stoichiometric compatibility class* $\mathcal{P}_T$ *whose intersection with the positive orthant is nonempty. Then, the following holds:*

a) If $\operatorname{sign}(\det(J_F(x))) = (-1)^s$ *for all positive steady states* $x \in \mathcal{P}_T \cap \mathbb{R}^n_{>0}$*, then there is exactly one positive steady state in* $\mathcal{P}_T$*. Furthermore, this steady state is non-degenerate.*

b) If $\operatorname{sign}(\det(J_F(\hat{x}))) = (-1)^{s+1}$ *for some positive steady state* $\hat{x} \in \mathcal{P}_T \cap \mathbb{R}^n_{>0}$*, then there are at least two positive steady states in* $\mathcal{P}_T$*, at least one of which is non-degenerate. If all positive steady states in* $\mathcal{P}_T$ *are non-degenerate then there are at least three and always an odd number.*

The proof of this theorem relies on the Brouwer degree of a special class of functions. In general, the proof consists in using the Brouwer degree of F_T in an appropriate open set, to conclude that $(-1)^s = \Sigma_{x \in V_T} \operatorname{sign}(\det(J_F(x)))$, where V_T denotes the positive steady state variety intersected with $\mathcal{P}_T$. This equality allows us to estimate whether there are one or multiple positive steady states in V_T as the sum on the right hand side of the equality has as many terms as points in the variety.

***Remark* 2.** Recall that under mass action kinetics, the vector v depends on a set of parameters $\kappa \in \mathbb{R}^m_{>0}$, which means that the coefficients of $\det(J_F(\varphi(x)))$ are parametric and their variation might affect the sign of $\det(J_F(\varphi(x)))$. With this is mind, it is possible to detect parameter regions for multistationarity by studying the coefficients of $\det(J_F(\varphi(x)))$ for which is possible to guarantee that a value with sign $(-1)^{s+1}$ is attained. In practice, one of the most useful tools to find said regions has been the Newton polytope which we will explain in Section 1.5.

To illustrate the use of Theorem 5 for detecting multistationarity, we consider example (1.3). The conservation laws of the network are $x_1+x_2 = T_1$ and $x_3+x_4 = T_2$ and from them we see that every concentration variable appears in one conservation law with positive coefficient. Thus, the network is conservative. We now compute $\det(J_F(\varphi(x)))$ and study its sign.

$$\det(J_F(\varphi(x))) = \frac{\kappa_1\kappa_2 x_2^2 + \kappa_1\kappa_3 x_2 + \kappa_3^2 x_4}{x_2}$$

is a positive function for every positive value of x_2 and x_4. Since the dimension of the stoichiometric subspace is 2, we have that $(-1)^s = (-1)^2 = 1$, and thus this network is monostationary for every set of parameters.

An example of the use of Theorem 5 to establish mutistationarity is given at the end of Section 1.4.1 with network (1.14).

1.4 Reduction of networks

In the previous sections we went through some results for detecting multistationarity, and they range from methods depending on the structure of the network to the use of algebraic parametrizations and signs of polynomials to detect regions for multistationarity. These methods can be applied to each specific network; however, in doing so it is possible to encounter issues such as:

- Is it always possible to compute $\det(J_F(\varphi(x)))$ and detect whether it attains a value with sign $(-1)^{s+1}$?
- Do the conclusions of the theorems change when considering different models of one system?

The first issue is affected by the size of the network, and gives rise to computational problems. We now present results on how multistationarity is preserved when modifying the structure of the network. In particular, we consider the removal and addition of intermediate species [23, 53] and addition and removal of reactions [36]. These results are critical for the work presented in paper I and in 3.

1.4.1 Intermediates

Definition 9. Let $\mathcal{N} = (\mathcal{S}, \mathcal{R}, \mathcal{C})$ be a chemical reaction network. An intermediate $Y \in \mathcal{S}$ is a species that is produced and consumed, and additionally does not interact with any other species. That is, Y is the reactant of at least one reaction, the product of at least one reaction and the only complex containing Y is the complex Y itself.

As an example consider the following network:

$$\begin{aligned} \mathrm{X_1 + X_2} &\rightleftharpoons \mathrm{X_3} \longrightarrow \mathrm{X_1 + X_4} \\ \mathrm{X_3 + X_4} &\rightleftharpoons \mathrm{Y_1} \longrightarrow \mathrm{X_2 + X_3}. \end{aligned} \tag{1.10}$$

In this network, the species $\mathrm{Y_1}$, in red, is the only intermediate; it is both produced and consumed and it does not interact with any other species.

If a species Y is an intermediate of $\mathcal{N}$, it is possible to construct a new network $\mathcal{N}'$ that does not contain Y by collapsing all the reaction paths $\mathrm{y_i} \longrightarrow \mathrm{Y} \longrightarrow \mathrm{y_j}$, where Y is an intermediate into a reaction $\mathrm{y_i} \longrightarrow \mathrm{y_j}$, and keeping only meaningful reactions, that is reactions where $\mathrm{y_i} \neq \mathrm{y_j}$.

In the example above, the removal of $\mathrm{Y_1}$ leads to the reduced network

$$\begin{aligned} \mathrm{X_1 + X_2} &\rightleftharpoons \mathrm{X_3} \longrightarrow \mathrm{X_1 + X_4} \\ \mathrm{X_3 + X_4} &\longrightarrow \mathrm{X_2 + X_3}. \end{aligned} \tag{1.11}$$

Note that reactions like $\mathrm{X_3+X_4} \rightleftharpoons \mathrm{Y_1} \longrightarrow \mathrm{X_2+X_3}$ are a graphic way to represent the sequences of reactions $\mathrm{X_3+X_4} \longrightarrow \mathrm{Y_1} \longrightarrow \mathrm{X_2+X_3}$ and $\mathrm{X_3+X_4} \longrightarrow \mathrm{Y_1} \longrightarrow \mathrm{X_3+X_4}$ that, after the removal of $\mathrm{Y_1}$, become $\mathrm{X_3+X_4} \longrightarrow \mathrm{X_2+X_3}$ and $\mathrm{X_3+X_4} \longrightarrow \mathrm{X_3+X_4}$ respectively. Since the last of these reactions is not meaningful, we only keep the first one in the reduced model.

Removing all the intermediate species produces a network called the *core network* and all its complexes are called *core complexes*. It is also said that the original network is an *extension model* of the core network. In our example, (1.11) is the core network of (1.10) and the core complexes are $\mathrm{X_1+X_2}$,$\mathrm{X_3}$, $\mathrm{X_1+X_4}$, $\mathrm{X_3+X_4}$ and $\mathrm{X_2+X_3}$. It is also said that (1.10) is an extension model of (1.11). Extension models are defined in general as follows:

Definition 10. A network $\mathcal{N} = (\mathcal{S}, \mathcal{R}, \mathcal{C})$ is an extension model of $\mathcal{N}' = (\mathcal{S}', \mathcal{R}', \mathcal{C}')$ if

- the set of core complexes of $\mathcal{N}$ is equal to the set of complexes of $\mathcal{N}'$,
- every reaction in $\mathcal{R}$ occurs between two core complexes, two intermediates or between an intermediate and a core complex; and
- $\mathcal{N}'$ can be obtained from $\mathcal{N}$ by collapsing all reaction paths

$$\mathrm{c_i} \longrightarrow \mathrm{Y_1} \longrightarrow \cdots \longrightarrow \mathrm{Y_t} \longrightarrow \mathrm{c_j} \qquad \text{for } \mathrm{c_i} \neq \mathrm{c_j}$$

  into a single reaction $\mathrm{c_i} \longrightarrow \mathrm{c_j}$, where $\mathrm{Y_1}, \ldots, \mathrm{Y_t}$ are intermediate species in $\mathcal{S} \setminus \mathcal{S}'$, and there might be none.

The removal or addition of intermediates is an operation that is purely structural and does not depend on the kinetics. However, we are interested in studying the relation between the positive steady state varieties of extension models and core models, when the systems are considered under mass action kinetics. This analysis can be found in [23], but here we highlight the main results.

We start by establishing the relation between the steady state equations of both networks. First denote the species in the core complexes of $\mathcal{N}$ by $X_1, \dots, X_n$ and the intermediates as $Y_1, \dots, Y_\ell$. The set of species is the union of species in core complexes and intermediates.

Since the intermediates do not interact with any other species, they appear as linear terms in the steady state equations and, solving the equations $\dot{y}_i = 0$, it is possible to solve the concentration of each intermediate in terms of the concentration of the core complexes that ultimately react to them. This corresponds to the following theorem.

Theorem 6. *The system of equations formed by the steady state equations of the intermediates y_i for $i = 1, \dots, \ell$ in $\mathcal{N}$, can be solved in terms of the core species, and y_i is expressed at steady state as a sum*

$$y_i = \Sigma_{c\in\bar{\mathcal{C}}} \mu_{Y_i,c} x^c \tag{1.12}$$

where $\mu_{Y_i,c}$ is a non-negative constant that is different from zero if, and only if, there is a reaction path $c \to \cdots \to Y_i$ involving exclusively intermediates, and $\bar{\mathcal{C}}$ denotes the set of core complexes.

The proof of this theorem relies on the Cramer's rule for solving systems of linear equations, and on the Matrix-Tree theorem. The outline of this proof is to write the system of steady state equations for the intermediates, as system of linear equations in the concentration of the intermediates and use the Matrix-Tree theorem to guarantee that the system has a unique solution. Then, this same theorem is used to find the constants $\mu_{Y_i,c}$. For a detailed proof we refer the reader to the proof of Theorem 3.1 in [23].

Using these solutions to substitute the value of the intermediates at steady state in the equations of the core complexes, we are left with a system depending only on the concentrations of the core species. The following theorem, corresponds to Theorem 3.2 proved in [23], ensures that this system is actually a mass action system for the core network.

Theorem 7. *After substituting the expressions $y_i = \Sigma_{c\in\bar{\mathcal{C}}} \mu_{Y_i,c} x^c$ into the ODEs for non intermediate species x_i of the extension model, we obtain a mass action system for the core model whose reaction rate constants are derived from the paths connecting the complexes in the extension model.*

This theorem is proved by defining a function

$$\psi(\kappa)\colon \mathbb{R}^m_{>0} \to \mathbb{R}^{m'}_{>0} \tag{1.13}$$

from the set of parameters of the extension model to the set of parameters of the core model, such that the vectors $\kappa \in \mathbb{R}^m$ and $\psi(\kappa) \in \mathbb{R}^{m'}$ determine the same system of polynomial equations in the concentrations of the core species. Namely, for a reaction $\mathrm{c_i} \longrightarrow \mathrm{c_j}$ in $\mathcal{N}'$ define its reaction rate constant as

$$\tau_{c_i\to c_j} = \psi(\kappa)_{c_i\to c_j} = \kappa_{c_i\to c_j} + \Sigma_{k=1}^{\ell}\kappa_{Y_k\to c_j}\mu_{Y_k,c_i},$$

where some of the terms on the sum can be zero. With this definition, the positive steady state variety of the core model for parameters $\psi(\kappa)$, is equal to the positive solutions of the steady state equations of the extension model with parameters κ after doing the substitution in (1.12). The vector κ and $\psi(\kappa)$ are called *matching rate constants*. Given $\tau \in \mathbb{R}^{m'}_{>0}$, it is said that every element $\kappa \in \psi^{-1}(\tau)$ realizes τ and, if $\psi^{-1}(\tau) = \emptyset$, then the parameter τ is not realizable.

Recall example (1.10). Writing in the graph the labels corresponding to the reaction rate constants, the network is

$$\begin{gathered}
\mathrm{X_1} + \mathrm{X_2} \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} \mathrm{X_3} \xrightarrow{\kappa_3} \mathrm{X_1} + \mathrm{X_4} \\
\mathrm{X_3} + \mathrm{X_4} \underset{\kappa_5}{\overset{\kappa_4}{\rightleftharpoons}} \mathrm{Y_1} \xrightarrow{\kappa_6} \mathrm{X_2} + \mathrm{X_3},
\end{gathered}$$

and the system of ODEs is

$$\begin{aligned}
\dot{x}_1 &= -\kappa_1 x_1 x_2 + (\kappa_2 + \kappa_3)x_3 \\
\dot{x}_2 &= -\kappa_1 x_1 x_2 + \kappa_2 x_3 + \kappa_6 y_1 \\
\dot{x}_3 &= \kappa_1 x_1 x_2 - (\kappa_2 + \kappa_3)x_3 - \kappa_4 x_3 x_4 + (\kappa_5 + \kappa_6)y_1 \\
\dot{x}_4 &= \kappa_3 x_3 - \kappa_4 x_3 x_4 + \kappa_5 y_1 \\
\dot{y}_1 &= \kappa_4 x_3 x_4 - (\kappa_5 + \kappa_6)y_1.
\end{aligned}$$

The steady state equation for the intermediate is $0 = \kappa_4 x_3 x_4 - (\kappa_5 + \kappa_6)y_1$, and solving it for y_1 we have

$$y_1 = \frac{\kappa_4}{\kappa_5 + \kappa_6} x_3 x_4.$$

The concentration of Y_1 at steady state can be written in terms of the monomial x_3x_4 that corresponds to the complex $\mathrm{X_3} + \mathrm{X_4}$, which is the only core complex that ultimately reacts to $\mathrm{Y_1}$. With the notation of the theorem, this is equivalent to the sum $y_1 = \Sigma_{c\in\bar{\mathcal{C}}}\mu_{Y_1,c}x^c$, with $\mu_{Y_1,X_3+X_4} = \frac{\kappa_4}{\kappa_5+\kappa_6}$ and $\mu_{Y_1,c} = 0$ for any other complex c.

Substituting this value for y_1 in the differential equations for the core species we are left with the system

$$\begin{aligned}
\dot{x}_1 &= -\kappa_1 x_1 x_2 + (\kappa_2 + \kappa_3) x_3 \\
\dot{x}_2 &= -\kappa_1 x_1 x_2 + \kappa_2 x_3 + \frac{\kappa_6 \kappa_4}{\kappa_5 + \kappa_6} x_3 x_4 \\
\dot{x}_3 &= \kappa_1 x_1 x_2 - (\kappa_2 + \kappa_3) x_3 \\
\dot{x}_4 &= \kappa_3 x_3 - \frac{\kappa_6 \kappa_4}{\kappa_5 + \kappa_6} x_3 x_4 .
\end{aligned}$$

Defining $\tau_i = \kappa_i$ for $i = 1, 2, 3$ and $\tau_4 = \frac{\kappa_4 \kappa_6}{\kappa_5 + \kappa_6}$ we have that this system corresponds to the dynamics of the core network under mass action kinetics and with reaction rate constants as follows,

$$\mathrm{X}_1 + \mathrm{X}_2 \underset{\tau_2}{\overset{\tau_1}{\rightleftarrows}} \mathrm{X}_3 \xrightarrow{\tau_3} \mathrm{X}_1 + \mathrm{X}_4$$

$$\mathrm{X}_3 + \mathrm{X}_4 \xrightarrow{\tau_4} \mathrm{X}_2 + \mathrm{X}_3 .$$

The previous theorems shows that there is a natural projection from the steady state variety of the extended model, to the steady state variety of the core model, when the sets of parameters of each network satisfy the appropriate relations. However, for studying multistationarity, it is also necessary to analyse how the conservation laws change from the extension model to the core model. This question is answered in the following theorem.

Theorem 8. *The conservation laws in the core model $\mathcal{N}'$ are in one to one correspondence with the conservation laws in any extension model $\mathcal{N}$. The correspondence is obtained by adding a suitable linear combination of the concentration variables of the intermediates to each conservation law of the model.*

Proof. Let S and S' denote the stoichiometric subspace of $\mathcal{N}$ and $\mathcal{N}'$ respectively. The proof of this theorem consists on defining a correspondence between a basis of $S^{\perp}$ and a $S'^{\perp}$, which will define a correspondence between the conservation laws of the extension model and the conservation laws of the core model.

Let $\{\omega'_1, \ldots, \omega'_d\} \subseteq \mathbb{R}^n_{\geq 0}$ be a basis for $S'^{\perp}$. Assume additionally that $\mathcal{N}'$ has L linkage classes and for $k = 1, \ldots, L$ select a complex $\mathrm{c_k}$ belonging to the k-th linkage class. Now for each vector ω'_i with $i = 1, \ldots, d$ we define component wise a vector ω_i contained in $\mathbb{R}^{n+\ell}_{\geq 0}$ as follows

$$\omega_{ij} = \begin{cases} \omega'_{ij} & \text{for } j = i, \ldots, n \\ \omega'_i \cdot c_k & \text{if } j = n+1, \ldots, n+\ell \text{ and } \mathrm{Y_{j-n}} \text{ is in the } k\text{-th linkage class.} \end{cases}$$

With this definition, the vectors ω'_i and ω_i coincide in the first n components corresponding to the core species in the models. If we prove that the set $\{\omega_1, \ldots, \omega_d\}$ is a basis for $S^{\perp}$ then we have a correspondence between the conservation laws of both models. To prove it we show first that each $\omega_i \in S^{\perp}$, then we prove that the vectors are linearly independent and, finally, we prove that they generate $S^{\perp}$.

A vector ω_i is in $S^{\perp}$ if, and only if, for each reaction $\mathrm{c}_{\mathrm{j}_1} \longrightarrow \mathrm{c}_{\mathrm{j}_2}$, the inner product $\omega_i \cdot (\mathrm{c}_{\mathrm{j}_2} - \mathrm{c}_{\mathrm{j}_1}) = 0$. If $\mathrm{c}_{\mathrm{j}_1}$ and $\mathrm{c}_{\mathrm{j}_2}$ are core complexes, then $\omega_i \cdot (\mathrm{c}_{\mathrm{j}_2} - \mathrm{c}_{\mathrm{j}_1}) = \omega_i' \cdot (\mathrm{c}_{\mathrm{j}_2} - \mathrm{c}_{\mathrm{j}_1}) = 0$. If $\mathrm{c}_{\mathrm{j}_1}$ is a core complex and $\mathrm{c}_{\mathrm{j}_2} = \mathrm{Y}_\mathrm{j}$ for some intermediate Y_j, then

$$\begin{aligned} \omega_i \cdot (\mathrm{c}_{\mathrm{j}_2} - \mathrm{Y}_\mathrm{j}) &= \omega_i \cdot \mathrm{c}_{\mathrm{j}_2} - \omega_i \cdot \mathrm{Y}_\mathrm{j} \\ &= \omega_i' \cdot \mathrm{c}_{\mathrm{j}_2} - \omega_i' \cdot \mathrm{c}_\mathrm{k} \\ &= \omega_i'(\mathrm{c}_{\mathrm{j}_2} - \mathrm{c}_\mathrm{k}) \\ &= 0, \end{aligned}$$

where the last equality holds because both $\mathrm{c}_{\mathrm{j}_2}$ and c_k are in the same linkage class, hence $\mathrm{c}_{\mathrm{j}_2} - \mathrm{c}_\mathrm{k} \in S'^{\perp}$. Finally, if both $\mathrm{c}_{\mathrm{j}_1}$ and $\mathrm{c}_{\mathrm{j}_2}$ are intermediate complexes, then the reaction is $\mathrm{Y}_{\mathrm{j}_1} \longrightarrow \mathrm{Y}_{\mathrm{j}_2}$ and $\omega_i \cdot (y_{i_1} - y_{i_2}) = \omega_i' \cdot c_k - \omega_i' \cdot c_k = 0$. This proves that each $\omega_i \in S^{\perp}$.

The linear independence is deduced from the fact that $\{\omega_1', \ldots, \omega_d'\}$ is a basis. It is left to see that $\{\omega_1, \ldots, \omega_d\}$ generates $S^{\perp}$, and this will be done checking that $\dim(S^{\perp}) = d$. Since $S^{\perp}$ contains d linearly independent vectors $\dim(S^{\perp}) \geq d$, Now, note that for every reaction $\mathrm{c}_{\mathrm{j}_1} \longrightarrow \mathrm{c}_{\mathrm{c_j}2}$ in $\mathcal{N}'$, the natural inclusion of the vector $\mathrm{c}_{\mathrm{j}_2} - \mathrm{c}_{\mathrm{j}_1}$ into $\mathbb{R}^{n+\ell}$ is actually contained in S, due to the fact that in the extension model $\mathcal{N}$, this reaction comes from a path $\mathrm{c}_{\mathrm{j}_1} \longrightarrow \mathrm{Y}_1 \to \ldots \to \mathrm{Y}_\mathrm{t} \longrightarrow \mathrm{c}_{\mathrm{c}_{\mathrm{j}_2}}$. This implies that there is an inclusion $S' \hookrightarrow S$ and $\dim(S') \leq \dim(S)$. Which means that $d = \dim(S'^{\perp}) \geq \dim(S^{\perp})$. We conclude that $\dim(S^{\perp}) = d$ and the set $\{\omega_i, \ldots, \omega_d\}$ is a basis.

With this procedure we have established a correspondence between basis of $S'^{\perp}$ and $S^{\perp}$, and this correspondence can be reversed. That is, given a basis $\{\omega_1, \ldots, \omega_d\}$ of $S^{\perp}$, the natural projection on the first n coordinates forms a basis of $S'^{\perp}$. □

***Remark* 3.** In the previous proof, the value of $\omega_i \cdot \mathrm{c}_\mathrm{k}$ is independent of the selection of the complex c_k in each linkage class.

After understanding the positive steady state variety and the conservation laws, we proceed to establish how multistationarity is preserved or lost between core and extension models. The following theorem guarantees that multistationarity is preserved from core models to extension models, that is, that multistationarity in the core model implies multistationarity in any extension model subject to realization conditions on the reaction rate constants of both models.

Theorem 9. *If the core model has η non-degenerate positive steady states for some reaction rate constants and total amounts, then any extension model that realizes the* *reaction rate constants has at least η corresponding non-degenerate positive steady states for some reaction rate constants and total amounts. Oppositely, if the extension model has at most one positive steady state for any reaction rate constants and total amounts, then the core model has at most one positive steady state for any matching rate constants and total amounts. The reaction rate constants and total amounts can be chosen such that the correspondence preserves unstable steady states with at least one eigenvalue with positive real part and asymptotic stability for hyperbolic steady states.*

We now present an outline for the proof of this result. For a detailed proof we refer the reader to [23], where this result corresponds to Theorem 5.1. Denote by f_κ and f'_τ the rate functions of the extension model and core model respectively, for a pair of matching constants $\tau = \psi(\kappa)$. Consider now the functions F_T and $F'_{T'}$ as in (1.7) for both models. Since we are assuming that the core network exhibits multistationarity, this means that the system of equations $F'_{T'}(x) = 0$ has at least two positive solutions for certain set of total amounts T'. If we consider only the entries of $F'_{T'}$ corresponding to the steady state equations, their solutions are also solutions of the equations $f_\kappa(x) = 0$ arising from core species. As we saw before, with the concentrations of the core species at steady state, it is possible to find the concentrations of the intermediate species at steady state, and we get at least two points in the positive steady state variety of the extension model; however, these points are not necessarily in the same stoichiometric compatibility class. The proof then consists on using the solutions of $F'_{T'}(x) = 0$ to find a set of total amounts T and parameters κ' depending on T' and κ, in such a way that it is possible to find at least two positive solutions of $F_T(x) = 0$.

To illustrate how this theorem can be applied, recall example (1.10). We showed that the core network is (1.11) and that the function $\psi\colon \mathbb{R}^6_{\geq 0} \to \mathbb{R}^4_{\geq 0}$ was given by $(\kappa_1, \kappa_2, \kappa_3, \kappa_4, \kappa_5, \kappa_6) \mapsto (\kappa_1, \kappa_2, \kappa_3, \frac{\kappa_4\kappa_6}{\kappa_5+\kappa_6})$. The steady states of (1.10) can be parametrized as

$$\phi(x_1, x_2) = \left(x_1, x_2, \frac{\kappa_1 x_1 x_2}{\kappa_2 + \kappa_3}, \frac{\kappa_3(\kappa_5 + \kappa_6)}{\kappa_4\kappa_6}, \frac{\kappa_1\kappa_3 x_1 x_2}{(\kappa_2 + \kappa_3)\kappa_6}\right)$$

and doing some computations we have that

$$\det(J_{F_T}(\phi(x_1, x_2))) = \frac{(2\kappa_1\kappa_3 x_1 + \kappa_1\kappa_3 x_2 + \kappa_1\kappa_6 x_1 + \kappa_1\kappa_6 x_2 + \kappa_2\kappa_6 + \kappa_3\kappa_6)\kappa_1\kappa_4 x_1 x_2}{\kappa_2 + \kappa_3}.$$

Using Theorem (5), we conclude that the network (1.10) is monostationary and using Theorem (9) this implies that the network (1.11) is monostationary for matching sets of parameters.

As an additional example on the use of this theorem consider the following network

$$\begin{array}{ccc} \mathrm{X}_1 \xrightarrow{\kappa_1} \mathrm{X}_2 \xrightarrow{\kappa_2} \mathrm{X}_3 \xrightarrow{\kappa_3} \mathrm{X}_4 & \qquad & \mathrm{X}_3 + \mathrm{X}_5 \xrightarrow{\kappa_4} \mathrm{X}_1 + \mathrm{X}_6 \\ \mathrm{X}_6 \xrightarrow{\kappa_6} \mathrm{X}_5 & & \mathrm{X}_4 + \mathrm{X}_5 \xrightarrow{\kappa_5} \mathrm{X}_2 + \mathrm{X}_6. \end{array} \tag{1.14}$$

For this system we have

$$F_T = \begin{pmatrix} x_1 + x_2 + x_3 + x_4 - T_1 \\ \kappa_5 x_4 x_5 + \kappa_1 x_1 - \kappa_2 x_2 \\ -\kappa_4 x_3 x_5 + \kappa_2 x_2 - \kappa_3 x_3 \\ -\kappa_5 x_4 x_5 + \kappa_3 x_3 \\ x_5 + x_6 - T_2 \\ \kappa_4 x_3 x_5 + \kappa_5 x_4 x_5 - \kappa_6 x_6 \end{pmatrix}$$

for some total amounts T_1 and T_2, and the positive steady state variety can be parametrized as

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

For detecting whether this network exhibits multistationarity we compute $\det(J_{F_T}(\phi(x_4, x_5)))$ and study its sign:

$$\det(J_{F_T}(\phi(x_4, x_5))) = \frac{1}{\kappa_3} \big((\kappa_1 - \kappa_3)\kappa_2 \kappa_4 \kappa_5^2 x_4 x_5^2 + 2\kappa_1 \kappa_2 \kappa_3 \kappa_4 \kappa_5 x_4 x_5 + \kappa_1 \kappa_2 \kappa_3^2 \kappa_5 x_4 + \\ (\kappa_1 \kappa_3 \kappa_4 \kappa_5 \kappa_6 + \kappa_2 \kappa_3 \kappa_4 \kappa_5 \kappa_6) x_5^2 + (\kappa_1 \kappa_2 \kappa_3 \kappa_5 \kappa_6 + \kappa_1 \kappa_3^2 \kappa_5 \kappa_6) x_5 + \kappa_1 \kappa_2 \kappa_3^2 \kappa_6 \big).$$

Considering the numerator of the last function as a polynomial in x_4 and x_5, the first term is the term with highest total degree and it is the only one with a negative coefficient. For $\kappa_3 > \kappa_1$ it is possible to find values of x_4 and x_5 such that $\operatorname{sign}(\det(J_{F_T}(\phi(x_4, x_5)))) = -1 = (-1)^{s+1}$. By Theorem 5 this implies that the network exhibits multistationarity. Furthermore, Theorem 9 allows us to deduce that any extension model of this network obtained by adding intermediates, also exhibits multistationarity for matching reaction rate constants. For instance, models like

$$\begin{array}{cc} X_1 \longrightarrow X_2 \longrightarrow X_3 \longrightarrow X_4 & \qquad X_3 + X_5 \rightleftharpoons Y_1 \longrightarrow X_1 + X_6 \\ X_6 \rightleftharpoons Y_3 \longrightarrow X_5 & \qquad X_4 + X_5 \rightleftharpoons Y_2 \longrightarrow X_2 + X_6. \end{array}$$

or

$$\begin{array}{cc} X_1 \longrightarrow X_2 \longrightarrow X_3 \longrightarrow X_4 & \qquad X_3 + X_5 \longrightarrow X_1 + X_6 \\ X_6 \longrightarrow Y_2 \longrightarrow Y_3 \longrightarrow X_5 & \qquad X_4 + X_5 \longrightarrow X_2 + X_6 \rightleftharpoons Y_1. \end{array}$$

under mass action kinetics, exhibit multistationarity for matching sets of parameters.

1.4.2 Subnetworks and embedded networks

Now we present structural modifications on a network, corresponding to the removal or addition of reactions and removal or addition of species [36] . Similarly to the previous section, the goal is to understand how the steady state variety changes when doing this modifications and if the multistationarity property is preserved.

Definition 11. Let $\mathcal{N} = (\mathcal{S}, \mathcal{R}, \mathcal{C})$ be a chemical reaction network and let $\mathcal{S}' \subseteq \mathcal{S}$, $\mathcal{R}' \subseteq \mathcal{R}$ and $\mathcal{C}' \subseteq \mathcal{C}$.

- The restriction of $\mathcal{R}$ to $\mathcal{S}'$, denoted $\mathcal{R}|_{\mathcal{S}'}$, is the set of reactions obtained by taking the reactions in $\mathcal{R}$ and removing all species not in $\mathcal{S}'$ from the reactant and the product complexes. Trivial reactions and repeated copies of the same reaction are removed.
- The restriction of $\mathcal{C}$ to $\mathcal{R}'$, denoted $\mathcal{C}|_{\mathcal{R}'}$, is the set of complexes of the reactions in $\mathcal{R}'$, including both reactants and products.
- The restriction of $\mathcal{S}$ to $\mathcal{C}'$, denoted $\mathcal{S}|_{\mathcal{C}'}$, is the set of species that are in the complexes in $\mathcal{C}$.

***Remark* 4.** When removing repeated copies of the same reaction, the reaction rate constant of the reaction left in the restriction, is considered as the sum of the rate constants of all the copies.

Definition 12. Let $\mathcal{N} = (\mathcal{S}, \mathcal{R}, \mathcal{C})$ be a chemical reaction network.

1. Given a subset of reactions $\mathcal{R}' \subseteq \mathcal{R}$, the *subnetwork* defined by $\mathcal{R}'$ is $\mathcal{N}' = (\mathcal{S}|_{\mathcal{C}|_{\mathcal{R}'}}, \mathcal{C}|_{\mathcal{R}'}, \mathcal{R}')$.
2. Given a subset of species $\mathcal{S}' \subseteq \mathcal{S}$, the *embedded network* defined by $\mathcal{S}'$ is $\mathcal{N}' = (\mathcal{S}', \mathcal{C}|_{\mathcal{R}|_{\mathcal{S}'}}, \mathcal{R}|_{\mathcal{S}'})$.

To illustrate these definitions, recall example 1.10. The subnetwork determined by $\mathcal{R}' = \mathcal{R} \setminus \{\mathrm{X}_1 + \mathrm{X}_2 \longrightarrow \mathrm{X}_3, \mathrm{Y}_1 \longrightarrow \mathrm{X}_2 + \mathrm{X}_3\}$ is

$$\mathrm{X}_1 + \mathrm{X}_2 \longleftarrow \mathrm{X}_3 \longrightarrow \mathrm{X}_1 + \mathrm{X}_4$$

$$\mathrm{X}_3 + \mathrm{X}_4 \rightleftharpoons \mathrm{Y}_1,$$

with $\mathcal{C}|_{\mathcal{R}'} = \{\mathrm{X}_1{+}\mathrm{X}_2, \mathrm{X}_3, \mathrm{X}_1{+}\mathrm{X}_4, \mathrm{X}_3{+}\mathrm{X}_4, \mathrm{Y}_1\}$ and $\mathcal{S}|_{\mathcal{C}|_{\mathcal{R}'}} = \mathcal{S}$. The embedded network determined by $\mathcal{S}' = \mathcal{S} \setminus \{\mathrm{X}_1, \mathrm{X}_4\}$ is

$$\mathrm{X}_2 \rightleftharpoons \mathrm{X}_3 \longrightarrow 0$$

$$\mathrm{X}_3 + \mathrm{X}_4 \rightleftharpoons \mathrm{Y}_1 \longrightarrow \mathrm{X}_2 + \mathrm{X}_3,$$

with $\mathcal{C}|_{\mathcal{R}|_{\mathcal{S}'}} = \{\mathrm{X}_2, \mathrm{X}_3, \mathrm{X}_3 + \mathrm{X}_4, \mathrm{Y}_1, \mathrm{X}_2 + \mathrm{X}_3\}$.

In [36] some theorems regarding whether multistationarity of networks could be deduced from reduced or embedded networks were proven. We now mention some of them, corresponding to Theorems 3.1 and 4.2 in [36].

Theorem 10. *Let $\mathcal{N}'$ be a subnetwork of $\mathcal{N}$ such that both networks have the same stoichiometric subspace. Then the following holds:*

(i) If $\mathcal{N}'$ admits multiple non-degenerate positive steady states, then $\mathcal{N}$ does as well. Additionally, if $\mathcal{N}'$ admits finitely many such steady states, then $\mathcal{N}$ admits at least as many.

(ii) If $\mathcal{N}'$ admits multiple positive exponentially stable steady states, then $\mathcal{N}$ does as well. Additionally, if $\mathcal{N}'$ admits finitely many such steady states, then $\mathcal{N}$ admits at least as many.

The proof of this theorem relies on the following lemma.

Lemma 4. *Let $S \subseteq \mathbb{R}^n$ be a vector subspace, let $P \subset \mathbb{R}^n$ be a polyhedron contained in an affine translation of S, and let $\Omega \subset int(P)$ be a bounded domain in the relative interior of P. Assume that $g_\lambda : \bar{\Omega} \to S$, for $\lambda \in [0,1]$, is a continuously-varying family of smooth functions such that*

(i) for all $\lambda \in [0,1]$, g_λ has no zeros in the boundary of Ω, and

(ii) for $\lambda = 0$ and $\lambda = 1$, $\ker(J_{g_\lambda}(x)) \cap S = \{0\}$ for all $x \in \Omega$.

Then the number of zeros of g_0 in Ω equals the number of zeros of g_1 in Ω.

A definition of polyhedron is given in Section 1.5. With this lemma, the proof of Theorem 10 consists on defining a family of functions satisfying the hypotheses of the Lemma, in such a way that each positive solution of the rate function of $\mathcal{N}'$ is associated with a positive solution of the rate function of $\mathcal{N}$. Namely, let $f_\kappa(x)$ and $f'_{\kappa'}(x)$ be the rate functions of $\mathcal{N}$ and $\mathcal{N}'$ respectively. By hypothesis, $\mathcal{N}'$ and $\mathcal{N}$ have the same stoichiometric subspace S, which implies that the set of species for both networks, denoted $\mathcal{S}$, is the same. Additionally, since $\mathcal{N}'$ exhibits multistationarity there is a set of parameters κ' and a set of total amounts T' such that $F_{T'}(x) = 0$, with the reaction rate constants equal to κ', has at least two nondegenerate solutions x^* and x^{**}. The fact that x^* is nondegenerate means that there is an open set Ω containing x^* such that x^* is the only solution of $f'_{\kappa'}(x) = 0$ in Ω and such that $\ker(J_f(x)) \cap S = \{0\}$ for every $x \in \Omega$. Now, let $\kappa' = (\kappa'_1, \ldots, \kappa'_{m'})$ and denote by $\kappa_{m'+1}, \ldots, \kappa_m$ the labels of the reactions in $\mathcal{R} \setminus \mathcal{R}'$. Define the following family of functions for $\lambda \in [0,1]$

$$g^\kappa_\lambda(x) = f'_{\kappa'}(x) + \Sigma^m_{j=m'+1}(c'_j - c_j)\lambda\kappa_j x^c$$

where c'_j and c_j are the product and reactant of the reaction with label κ_j for $j = m'+1, \ldots, m$. Note that $g^\kappa_0(x) = f'_{\kappa'}(x)$ and $g^\kappa_1(x) = f_\kappa(x)$ for a set of parameters κ.

Applying Lemma 4 for this family of functions and adjusting the parameters if necessary, for it to satisfy the hypothesis of the Lemma, it is possible to prove that $f_\kappa(x) = 0$ has a solution in Ω. The lifting of multistationarity follows from doing this same procedure for x^{**} and choosing a new set Ω accordingly and noting that $\mathcal{P}$ corresponds to the intersection of the positive orthant with the stoichiometric compatibility class.

Regarding embedded networks, we prove Theorem 11 below. First we define a flow type subnetwork.

Definition 13. A mass action flow type subnetwork for a species $\mathrm{X_i}$ of a chemical reaction network $\mathcal{N}$ is a nonempty subnetwork $\mathcal{N}'$ of $\mathcal{N}$ such that

- the reactions in $\mathcal{N}'$ only involve the species $\mathrm{X_i}$, and
- there exists a choice of reaction rate constants for the reactions of $\mathcal{N}'$, such that for the resulting mass action system of $\mathcal{N}'$, $x_i = 1$ is a non-degenerate steady state.

Theorem 11. *Let $\mathcal{N}'$ be an embedded network of a network $\mathcal{N}$ such that the stoichiometric subspace of $\mathcal{N}'$ is full dimensional and for each species X_i that is in $\mathcal{N}$ not in $\mathcal{N}'$, there exists a mass action flow type subnetwork of $\mathcal{N}$ for $\mathrm{X_i}$. Then, the following holds*

(i) If $\mathcal{N}'$ admits multiple non-degenerate positive steady states, then $\mathcal{N}$ does as well. Additionally, if $\mathcal{N}'$ admits finitely many such steady states, then $\mathcal{N}$ admits at least as many.

(ii) If $\mathcal{N}'$ admits multiple positive exponentially stable steady states, then $\mathcal{N}$ does as well. Additionally, if $\mathcal{N}'$ admits finitely many such steady states, then $\mathcal{N}$ admits at least as many.

The proof of this theorem consists in proving the result first for embedded networks where the set of species of both networks differ only in one species. That is, for networks where $\mathcal{S} \setminus \mathcal{S}' = \{X_i\}$ with $\mathcal{S}$ and $\mathcal{S}'$ the set of species of $\mathcal{N}$ and $\mathcal{N}'$ respectively. In this case, given a steady state x^* of $\mathcal{N}'$, it is proven that $\mathcal{N}$ has a positive steady state in a neighbourhood of $(x^*, 1)$. With this case proven, multistationarity is lifted from embedded networks where $\mathcal{S} \setminus \mathcal{S}'$ has more than one element, one species at a time.

To illustrate the use of the previous theorems consider example (1.1) and consider the subnetwork depicted in Figure 1.3. Using Theorem 5 it is possible to prove that this network with mass action kinetics exhibits multistationarity. This implies that network (1.1) also exhibits multistationarity for a certain set of parameters. If instead of considering this subnetwork, we study the subnetwork obtained by removing all the

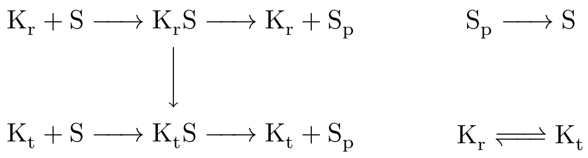


Figure 1.3: Subnetwork of the Allosteric network in 1.1

reverse reactions in (1.1), it is not possible to ensure multistationarity in the original network, because this subnetwork does not have positive steady states.

To illustrate Theorem 11 consider the network $\mathcal{N}$ given by

$$0 \rightleftharpoons X_1 \qquad\qquad 3\,X_1 \rightleftharpoons 2\,X_1$$

with mass action kinetics. The steady state equation for this network is a polynomial of degree 3 in x_1 and the stoichiometric subspace is equal to $\mathbb{R}$. It is possible to find a set of parameters for which the steady state equation has at least two positive roots and the network exhibits multistationarity. Theorem 11 implies that the network

$$X_2 \rightleftharpoons 0 \rightleftharpoons X_1 \qquad\qquad 3\,X_1 \rightleftharpoons 2\,X_1 + X_2$$

also exhibits multistationarity because $\mathcal{N}$ can be obtained from it by removing the species X_2 and there is a flow-type subnetwork involving only this species, namely $X_2 \rightleftharpoons 0$. If we consider the network

$$0 \rightleftharpoons X_1 \qquad\qquad 3\,X_1 \rightleftharpoons 2\,X_1 + X_2$$

it is not possible to guarantee multistationarity from $\mathcal{N}$ despite the fact that $\mathcal{N}$ can be obtained from it removing species X_2. This is due to the non-existence of a flow-type subnetwork involving X_2.

1.5 Newton Polytope

The Newton Polytope is going to be one of the main tools for finding Hopf bifurcations later in this work. In this section we present the background required to understand the Newton Polytope and then we present two results used in Paper II.

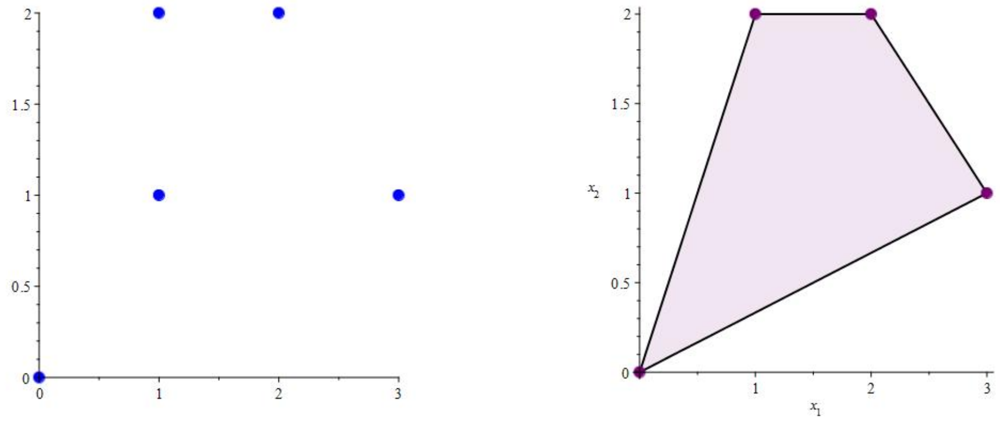


Figure 1.4: The set D is depicted on the left. On the right, conv(D).

1.5.1 Polytopes

Polytopes in $\mathbb{R}^n$ are sets that can be defined as the convex hull of a finite set of points or as the intersection of half spaces. We now present both definitions and study the basic structure of these sets.

Definition 14. Let $D \subset \mathbb{R}^n$ be a set of points. D is convex if for any pair of points $u, v \in D$, the segment joining u and v is contained in D, that is, if $tu + (1-t)v \in D$ for every $t \in [0,1]$. The convex hull of D, denoted conv(D), is defined as the smallest convex set containing D.

***Remark* 5.** If $D = \{v_1, \ldots, v_\ell\}$ is finite, then it is possible to define its convex hull constructively as

$$\mathrm{conv}(D) = \{\zeta_1 v_1 + \ldots + \zeta_\ell v_\ell : \zeta_i \geq 0 \text{ and } \Sigma_{i=1}^{\ell} \zeta_i = 1\}.$$

As an example, consider in $\mathbb{R}^2$ the set $D = \{(0,0),(1,1),(2,2),(3,1),(1,2)\}$. In Figure 1.4 we present the convex hull of the set D. Note that the point $(1,1)$ is an interior point of conv(D).

Definition 15. A hyperplane in $\mathbb{R}^n$ is defined as

$$\mathcal{H} = \{(x_1, \ldots, x_n) \in \mathbb{R}^n : a_1x_1 + \ldots + a_nx_n = b\}$$

for some $a_1, \ldots, a_n, b \in \mathbb{R}$. This can also be denoted as $\{x \in \mathbb{R}^n : a \cdot x = b\}$, which we denote $\mathcal{H}_{a,b}$.

Each hyperplane divides $\mathbb{R}^n$ in two regions, called the positive and negative half-spaces, defined as

$$\mathcal{H}^+_{a,b} = \{x \in \mathbb{R}^n : a \cdot x \geq b\} \qquad \mathcal{H}^-_{a,b} = \{x \in \mathbb{R}^n : a \cdot x \leq b\}$$

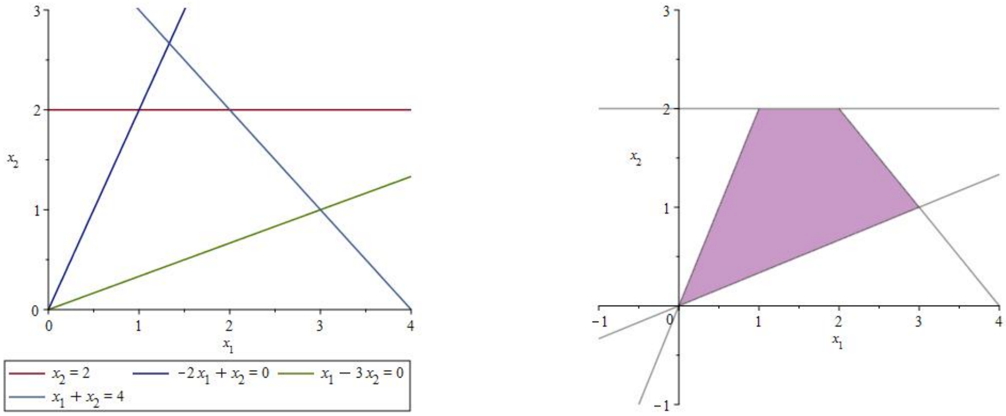


Figure 1.5: On the left the hyperplanes $\mathcal{H}_{(0,1),2}, \mathcal{H}_{(-2,1),0}, \mathcal{H}_{(1,-3),0}$and $\mathcal{H}_{(1,1),4}$. On the right the $\mathcal{H}$-polyhedron defined by the intersection of the negative half-spaces.

respectively. With this definition we define an $\mathcal{H}$-polyhedron, as the intersection of finitely many closed half spaces. Note that $\mathcal{H}^+_{a,b} = \mathcal{H}^-_{-a,b}$, which means that by choosing the vector a we are also choosing an orientation of the hyperplane.

As an example, consider the following hyperplanes

$$\mathcal{H}_{(0,1),2}, \qquad \mathcal{H}_{(-2,1),0}, \qquad \mathcal{H}_{(1,-3),0}, \qquad \mathcal{H}_{(1,1),4},$$

presented on the left side of Figure 1.5. If we consider the intersection of all the negative half-spaces defined by them, we obtain the $\mathcal{H}$-polyhedron on the right side of Figure (1.5).

Note that the $\mathcal{H}$-polyhedron in Figure 1.5 is equal to conv(D) in Figure 1.4. This is due to the fact that D is a finite set and, in this case, the convex hull defines a polytope that can be defined also as the intersection of some closed half-spaces. We now present the formal definition of a polytope and its representations.

Proposition 1 (See [65])**.** *Let $P \subseteq \mathbb{R}^n$. P is the convex hull of a finite set of points if, and only if, it is a bounded intersection of closed half-spaces.*

Definition 16. A set P satisfying any of the equivalent conditions of Proposition 1 is a *polytope*. The *$\mathcal{V}$-representation* of P is a set of points whose convex hull is equal to P, and the *$\mathcal{H}$-representation* of P is a list of closed half-spaces whose intersection is equal to P.

The proof of the equivalence between the $\mathcal{H}$-representation and the $\mathcal{V}$-representation of a polytope relies on the Minkowski sum of polyhedrons, and it can be found in [65]. A polyhedron is a set that is described as the intersection of half spaces, and polytopes are bounded polyhedrons.

We now define the faces of a polytope and then, we define the outer normal cone of a polytope.

Definition 17. Let P be a polytope. A linear inequality $a \cdot x \leq b$ is valid for P if it is satisfied for every $x \in P$. A face $\mathcal{F}$ of P is any set of the form

$$\mathcal{F} = P \cap \{x \in \mathbb{R}^n : a \cdot x = b\}$$

for a valid inequality $a \cdot x \leq b$ for P. Note that the inequalities $0 \cdot x \leq 0$ and $0 \cdot x \leq 1$ are valid for every P, and the equalities $0 \cdot x = 0$ and $0 \cdot x = 1$ define the faces P and $\emptyset$ respectively. If $\mathcal{F}$ is a face different from P and $\emptyset$, then $\mathcal{F}$ is called a proper face.

Every proper face of a polytope is contained in at least one hyperplane of its $\mathcal{H}$-representation [65]. For example, in the polytope P of Figure 1.5, if we intersect P with the hyperplane $x_2 = 2$, we obtain the segment on the top of the polytope connecting $(1, 2)$ and $(2, 2)$; this segment is a face of P. Similarly, if we intersect P with the hyperplane $x_1 + 2x_2 = 5$, we obtain the point $(2, 2)$ which is also a face.

Definition 18. Let P be a polytope. The dimension of P is defined as the dimension of the linear space generated by the vectors $\{u - v \in \mathbb{R}^n : u, v \in P\}$. Since a face of a polytope is a polytope itself, the dimension of a face $\mathcal{F}$ is the dimension of the linear space generated by $\{u - v \in \mathbb{R}^n : u, v \in \mathcal{F}\}$. For a polytope P with $\dim(P) = n$, all the faces of dimension $n - 1$ are called *facets*, all the faces of dimension one are called *edges*, and the faces of dimension zero are called *vertices*.

The dimension of the polytope in Figure (1.5) is 2, the facets are the edges as they have dimension 1, and the vertices are $\{(0, 0), (3, 1), (2, 2), (1, 2)\}$. Note that this polytope was obtained as the convex hull of $D = \{(0, 0), (1, 1), (2, 2), (3, 1), (1, 2)\}$; however not all of these points are vertices. In general, the set of vertices of a polytope P is the minimal set required to obtain P as a convex hull of points. Furthermore, the faces of P can be obtained as convex hulls of subsets of the set of vertices. This is summarized in the following proposition.

Proposition 2. *Let P be a polytope.*

(i) Every polytope is the convex hull of its vertices, and if $P = \operatorname{conv}(D)$ for some D, then D contains the vertices of P.

(ii) If $\mathcal{F}$ is a face of P, then the vertices of $\mathcal{F}$ are also vertices of P.

(iii) If $\mathcal{F}$ and $\mathcal{F}'$ are faces of P, then $\mathcal{F} \cap \mathcal{F}'$ is also a face of P.

(iv) If $\mathcal{F}$ is a face of P, then the set of faces of $\mathcal{F}$ is $\{\mathcal{F}' \cap \mathcal{F} : \mathcal{F}' \text{ is a face of } P\}$.

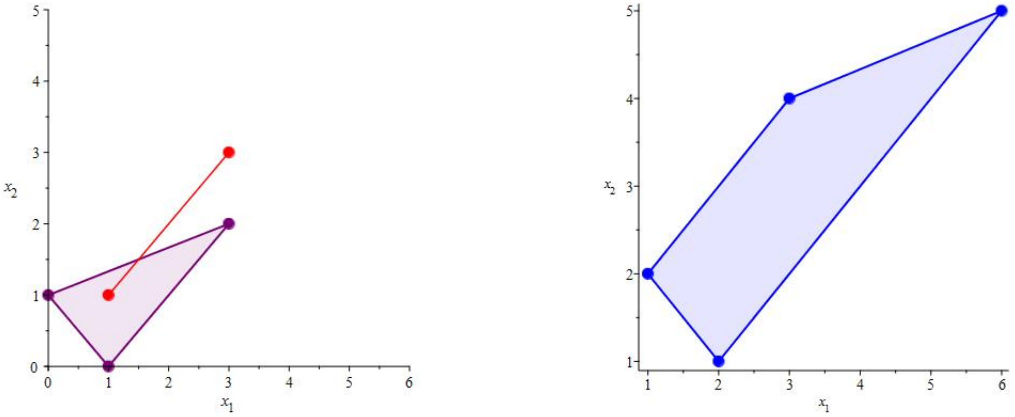


Figure 1.6: On the left two polytopes P and Q and on the right its Minkowski sum.

This proposition gives us an insight on the structure of a polytope. Their faces are polytopes themselves and any intersection of faces is again a face of the polytope. With this structure, the set of faces of a polytope, ordered by inclusion, forms a lattice called *the face lattice* of P.

We now define an operation to construct polytopes. This operation is the Minkowski sum.

Definition 19. Let $P, Q \subset \mathbb{R}^n$ be two polytopes. The Minkowski sum of P and Q, denoted $P + Q$, is defined as

$$P + Q \coloneqq \{u + v : u \in P \text{ and } v \in Q\}.$$

As an example consider the polytopes $P = \operatorname{conv}(\{(1,0),(0,1),(3,2)\})$ and $Q = \operatorname{conv}(\{(3,3),(1,1)\})$, depicted on the left part of Figure (1.6). Both polytopes are contained in $\mathbb{R}^2$ and $\dim(P) = 2$ and $\dim(Q) = 1$. Their Minkowski sum is the 2 dimensional polytope on the right side of the figure.

Proposition 3. *Let* $\operatorname{vert}(P)$ *and* $\operatorname{vert}(Q)$ *denote the set of vertices of* P *and* Q *respectively. Then,*

$$P + Q = \operatorname{conv}(\{u + v : u \in \operatorname{vert}(P) \text{ and } v \in \operatorname{vert}(Q)\}).$$

This proposition gives us a procedure for finding the Minkowski sum of two polytopes. As a remark, note that $\operatorname{vert}(P + Q) \subset \operatorname{vert}(P) + \operatorname{vert}(Q)$, but they are not necessarily equal.

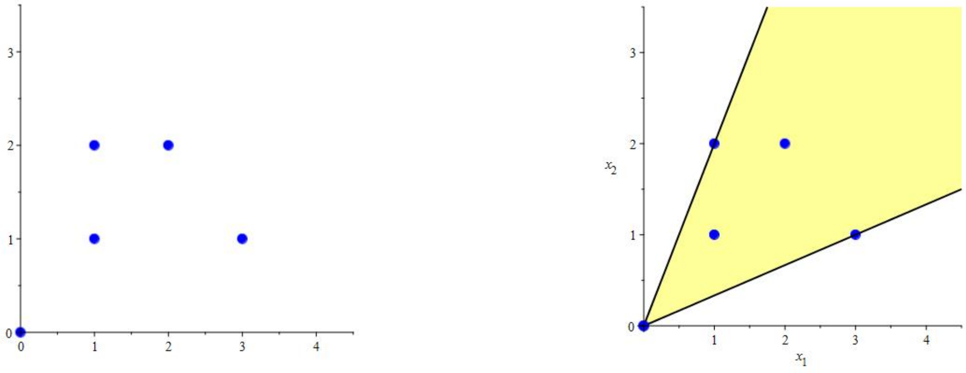


Figure 1.7: On the left the set D of points. On the right its conical hull cone(D) in yellow and the set D in blue.

Cones and fans

We now define a different kind of polyhedral sets. We start by defining a cone, then we proceed to define a fan, and finally we define the outer normal cone associated with a polytope.

Definition 20. A subset $C \subset \mathbb{R}^n$ is a cone if it contains all the finite non-negative linear combinations of its elements. For a set $Y \subset \mathbb{R}^n$, we define its *conical hull*, denoted cone(Y), as

$$\operatorname{cone}(Y) = \{\zeta_1 v_1 + \cdots + \zeta_t v_t : v_i \in Y \text{ and } \zeta_i \geq 0 \text{ for } i = 1, \ldots, t\}.$$

From the previous definition we deduce that every cone contains the origin and, if v is in the cone, then λv is also in the cone for every $\lambda \geq 0$. As an example consider again the set $D = \{(0,0),(1,1),(2,2),(3,1),(1,2)\}$, instead of computing its convex hull, we now show its conical hull on the right side of Figure 1.7. Note that this hull contains the origin and every positive multiple of each element.

A cone can also be obtained as the intersection of half spaces; however, the hyperplanes defining each half-space must contain the origin. In our example $\operatorname{cone}(D) = \mathcal{H}^-_{(1,-3),0} \cap \mathcal{H}^-_{(-2,1),0}$ and both hyperplanes $\mathcal{H}_{(1,-3),0}$ and $\mathcal{H}_{(-2,1),0}$ contain the origin. The faces of a cone can be defined in the same way as the faces of a polytope. In this case the faces are not bouded, for instance the faces of dimension 1 will be rays starting in the origin. In our example in Figure 1.7, the faces of dimension one are $\{\lambda(3,1) : \lambda \geq 0\}$ and $\{\lambda(1,2) : \lambda \geq 0\}$.

Definition 21. A *fan* in $\mathbb{R}^n$ is a family of nonempty cones $\mathfrak{F} = \{C_1, \ldots, C_t\}$ satisfying the following properties.

(i) Every nonempty face of a cone in $\mathfrak{F}$ is also a cone in $\mathfrak{F}$.

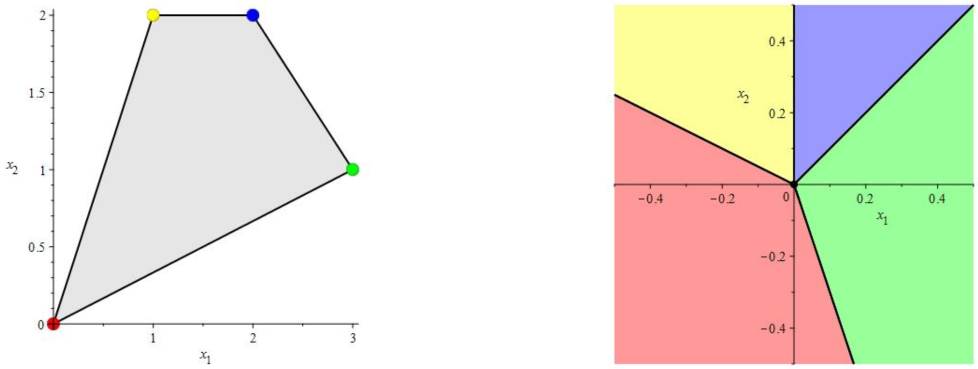


Figure 1.8: On the left the polytope P. On the right we see the outer normal fan of P, each cone has the same color as the vertex generating it.

(ii) The intersection of any two cones in $\mathfrak{F}$ is a face of both.

Now we define the outer normal fan associated with a polytope.

Definition 22. Let P be a full dimensional polytope in $\mathbb{R}^n$. The outer normal cone of a face $\mathcal{F}$ of P is defined as

$$C_{\mathcal{F}} := \{c \in (\mathbb{R}^n)^* : c \cdot v = \max\{c \cdot x : x \in P\} \text{ for every } v \in \mathcal{F}\}.$$

The outer normal fan of P is defined as $\{C_{\mathcal{F}} : \mathcal{F} \text{ is a face of } P\}$.

Note that the outer normal cone of a face $\mathcal{F}$ contains all the linear functions whose maximum value on P is attained at $\mathcal{F}$. If we come back to the polytope $P = \text{conv}(D)$ in Figure 1.5, the outer normal cone $C_{(0,0)}$ is the conical hull of $\{(-2,1),(1,-3)\}$. On the left part of Figure 1.8 we have the polytope P and on the right side we have the outer normal fan of P.

If a polytope P has full dimension and $\dim(\mathcal{F}) = m$, then $\dim(C_{\mathcal{F}}) = n - m$. In Figure 1.7, $\dim((0,0)) = 0$ and $\dim(C_{(0,0)}) = 2$. Note additionally that if u, v are adjacent vertices of P, then $C_u \cap C_v$ is the outer normal cone of the edge $[u,v]$. In general, the computation of the outer normal fan can be done by finding the outer normal cones of the vertices, and including the intersections of cones associated to the vertices defining each face of P.

1.5.2 Newton polytope

We now define a specific polytope associated with a multivariate polynomial. This polytope is an important tool in our work, as it allows to bound the number of complex solutions of a system of polynomial equations and, under certain conditions, it allows to find a solution in the positive orthant.

Definition 23. Let $p(x) = a_1 x^{\alpha_\ell} + \cdots + a_\ell x^{\alpha_\ell}$ be a multivariate polynomial with real coefficients, and where $x^{\alpha_i} = x_1^{\alpha_{i1}} \cdots x_n^{\alpha_{in}}$ for $\alpha_i \in \mathbb{Z}^n_{\geq 0}$. The Newton polytope associated with p, denoted $\mathrm{Newt}(p)$, is defined as the convex hull of the exponents:

$$\mathrm{Newt}(p) \coloneqq \mathrm{conv}(\{\alpha_1, \ldots, \alpha_\ell\}).$$

The Newton polytope is contained in $\mathbb{R}^n_{\geq 0}$, where n is the number of variables of p. As an example consider $p(x_1, x_2) = x_1^3 x_2 + 3x_1^2 x_2^2 - x_1 x_2^2 + 7x_1 x_2^3 - 8$, $\mathrm{Newt}(p) = \mathrm{conv}(\{(0,0),(2,2),(3,1),(1,2)\})$ which is precisely the polytope in Figure 1.4. Note that for $g(x_1, x_2) = 5x_1^3 x_2 - 10x_1^2 x_2^2 + x_1 x_2^2 - 11x_1 x_2^3 + 10$ and $h(x_1, x_2) = -x_1^3 x_2 + 3x_1^2 x_2^2 - x_1 x_2^2 + 7x_1 x_2^3 + x_1 x_2 + 7$, we have that $\mathrm{Newt}(g) = \mathrm{Newt}(h) = \mathrm{Newt}(p)$ even if the polynomials are different.

The Newton polytope can be used to determine whether a polynomial attains a positive or negative value. We will say that a vertex σ_i of $\mathrm{Newt}(p)$, is a *positive vertex* (respectively, *negative vertex*) if the corresponding monomial of p is positive, i.e., $a_i > 0$ (respectively, $a_i < 0$).

Proposition 4. *For a real, multivariate polynomial* $p(x) = a_1 x^{\alpha_1} + a_2 x^{\alpha_2} + \cdots + a_\ell x^{\alpha_\ell} \in \mathbb{R}[x_1, \ldots, x_n]$*, if* α_i *is a positive vertex (respectively, negative vertex) of* $\mathrm{Newt}(p)$*, then there exists* $x^* \in \mathbb{R}^n_{>0}$ *such that* $p(x^*) > 0$ *(respectively,* $p(x^*) < 0$*).*

Proof. Let α_i be a vertex of $\mathrm{Newt}(p)$. Pick $w = (w_1, w_2, \ldots, w_n)$ in the relative interior of the outer normal cone C_{σ_i}. Then, by construction, the linear functional $w \cdot -$ is maximized over the exponent vectors $\alpha_1, \alpha_2, \ldots, \alpha_\ell$ at α_i. Thus, we have the following univariate function in t:

$$p(t^{w_1}, t^{w_2}, \ldots, t^{w_n}) \;=\; a_1 t^{w\cdot\alpha_1} + a_2 t^{w\cdot\alpha_2} + \cdots + a_\ell t^{w\cdot\alpha_\ell} \;=\; a_i t^{w\cdot\sigma_i} + \text{lower-order terms} .$$

So, for t large, $\mathrm{sign}(p(t^{w_1}, t^{w_2}, \ldots, t^{w_n})) = \mathrm{sign}(a_i)$. Note that $(t^{w_1}, t^{w_2}, \ldots, t^{w_s}) \in \mathbb{R}^n_{>0}$. □

This proposition guarantees that the monomials whose exponents are vertices of $\mathrm{Newt}(p)$ are dominant monomials, in the sense that the polynomial attains values with the sign of the corresponding coefficients. With this in mind, it is possible to use the outer normal fan to find a solution of a polynomial, under certain conditions. Now we prove a proposition that allows us to use the previous result to find a solution of a multivariate polynomial, under certain conditions.

Proposition 5. *Let* $p, q \in \mathbb{R}[x_1, \ldots x_n]$*. Assume that* α *is a positive vertex of* $\mathrm{Newt}(p)$*,* β_+ *is a positive vertex of* $\mathrm{Newt}(q)$*, and* β_- *is a negative vertex of* $\mathrm{Newt}(q)$*. Then, if* $\mathrm{int}(C_\alpha) \cap \mathrm{int}(C_{\beta_+})$ *and* $\mathrm{int}(C_\alpha) \cap \mathrm{int}(C_{\beta_-})$ *are both nonempty, then there exists* $x^* \in \mathbb{R}^n_{>0}$ *such that* $p(x^*) > 0$ *and* $q(x^*) = 0$*.*

The proof of this result is included in the appendix of Paper II as Proposition B.2. This paper can be found at the end of this thesis. It is proved in the context of Hurwitz determinants for the detection of Hopf bifurcations. The proof is not constructive but we provide an algorithm to find an approximation of a point. We now present this algorithm with the notation $p_w(t) := p(t^{w_1}, t^{w_2}, \ldots, t^{w_n})$, for $t \in \mathbb{R}$ and $w = (w_1, w_2, \ldots, w_n) \in \mathbb{R}^n$.

Algorithm 1: Newton-polytope method

```
input  : polynomials $p, q$, and vertices $\alpha, \beta_+, \beta_-$, as in Proposition 5
output: a point $x^* \in \mathbb{R}^n_{>0}$ s.t. $p(x^*) > 0$ and $q(x^*) = 0$
define $L_0 := \operatorname{int}(C_\alpha) \cap \operatorname{int}(C_{\beta_+})$ and $L_1 := \operatorname{int}(C_\alpha) \cap \operatorname{int}(C_{\beta_-})$;
pick $\ell = (\ell_1, \ell_2, \ldots, \ell_n) \in L_0$ and $m = (m_1, m_2, \ldots, m_n) \in L_1$;
define $p_\ell(t) := p(t^{\ell_1}, t^{\ell_2}, \ldots, t^{\ell_n})$; define $p_m(t)$; define $q_\ell(t)$; define $q_m(t)$;
define $\tau_\ell := \inf\{t^* \in \mathbb{R}_{>0} \mid p_\ell(t) > 0$ and $q_\ell(t) > 0$ for all $t > t^*\}$;
define $\tau_m := \inf\{t^* \in \mathbb{R}_{>0} \mid p_m(t) > 0$ and $q_m(t) < 0$ for all $t > t^*\}$;
define $T := \max\{\tau_\ell, \tau_m\} + 1$;
define $h(r) := p_{r\ell+(1-r)m}(T)$;
while $\min\{h(r) \mid r \in [0,1]\} \leq 0$ do
    $T := 2T$;
    $h(r) := p_{r\ell+(1-r)m}(T)$;
define $r^* := \operatorname{argmin}\{\left(q_{r\ell+(1-r)m}(T)\right)^2 \mid r \in [0,1]\}$ (pick one $r^*$ if there are
  multiple);
return : $T^{r^*\ell+(1-r^*)m} := \left(T^{r^*\ell_1+(1-r^*)m_1}, T^{r^*\ell_2+(1-r^*)m_2}, \ldots, T^{r^*\ell_n+(1-r^*)m_n}\right)$
```

Remarks about Algorithm 1: First, ℓ and m exist by hypothesis. Additionally, τ_ℓ and τ_m exist by Proposition 4. Note now that $\min h(r)$ exists because h is a continuous univariate function defined on a compact interval.

By construction and by the convexity of the cones, the vector $r\ell + (1-r)m$, is in the relative interior of C_α for all $r \in [0,1]$. Thus, $(r\ell + (1-r)m) \cdot (\alpha - \alpha_i) > 0$ for the other exponents α_i of p and for all $r \in [0,1]$. This (together with a straightforward argument using continuity and compactness) implies the following:

$$\delta \ := \inf_{r \in [0,1]} \ \min_{\alpha_i \text{exponents of } p} (r\ell + (1-r)m) \cdot (\alpha - \alpha_i) > 0.$$

Next, let $\beta := \inf_{r \in [0,1]} (r\ell + (1-r)m) \cdot \alpha$. Then, for all $r \in [0,1]$ and $t > 0$,

$$\begin{aligned} f_{r\ell+(1-r)m}(t) &= a_+ t^{(r\ell+(1-r)m)\cdot\alpha} + \left(a_1 t^{(r\ell+(1-r)m)\cdot\alpha_1} + \cdots + a_d t^{(r\ell+(1-r)m)\cdot\alpha_d}\right) \\ &> a_+ t^{\beta} - (|a_1| + |a_2| + \cdots + |a_d|) t^{\beta-\delta} \ =: \ \widetilde{f}(t) \ . \end{aligned} \tag{1.15}$$

In $\widetilde{f}(t)$, the term $a_+ t^\beta$ dominates the other term, for t large, so there exists $T^* > 0$ such that $\widetilde{f}(t) \geq 0$ when $t \geq T^*$. This implies that the while loop ends when $T \geq T^*$ (or earlier).

Finally, r^*, τ_l, τ_m and the condition on the while loop are defined as argmin or minimums of functions, and finding an approximation for these numbers depends on numerical methods that might not halt. However, in practice, numerical methods can stop after achieving certain precision or after a specific number of iterations. Therefore, in practice it is possible to use Algorithm 1 to compute an approximation of these values.

Algorithm 1 is correct: For T fixed, the minimum of $\psi(r) := \left(q(T^{r\ell+(1-r)m})\right)^2$ over the compact set $[0,1]$ is attained, because ψ is continuous. Next we show that for $\chi(r) := q(T^{r\ell+(1-r)m})$ there exists some $r^* \in (0,1)$ such that $\chi(r^*) = 0$. But this follows from the Intermediate Value Theorem, because χ is continuous, $\chi(0) = q(T^m) < 0$ (because $T > \tau_m$), and $\chi(1) = q(T^\ell) > 0$ (because $T > \tau_\ell$).

Finally, the inequality $p(T^{r^*\ell+(1-r^*)m}) > 0$ holds by the definition of T, so taking $x^* := T^{r^*\ell+(1-r^*)m} \in \mathbb{R}^n_{>0}$ we obtain the vector satisfying $p(x^*) > 0$ and $q(x^*) = 0$.

2

Local dynamic behaviour of Chemical Reaction Networks

In the previous chapter we mentioned the stability property of steady states, specially in Theorems 9 and 10, where part of the results were related to this property. In this chapter we formally define what stability means and present an algebraic criterion for exploring stability. This criterion is going to be the main tool in our contributions and, the Hurwitz determinants will also be used for detecting Hopf bifurcations.

In what follows, we give an introduction to stability and Hopf bifurcations for any dynamical system, then we present the Hurwitz criterion. Afterwards, we use these tools to study the local dynamics of steady states of chemical reaction networks and present our contributions.

2.1 Background on Stable steady states and Hopf bifurcations

In this section we define stability and Hopf bifurcations for any system of ODEs given by $\dot{x} = f(x)$. We are particularly interested in non-linear systems of ODEs as these are the systems that often arise in our setting.

2.1.1 Stability of steady states

Consider an autonomous ODE $\dot{x} = f(x)$, with f a continuously differentiable function in an open set $\mathcal{E} \subset \mathbb{R}^n$.

Definition 24. A steady state $x^* \in \mathcal{E}$ is called *hyperbolic* if none of the eigenvalues of the matrix $J_f(x^*)$ have zero real part. The linear ODEs system given by $\dot{x} = J_f(x^*)x$ is called the linearization of $\dot{x} = f(x)$ at x^*.

The linearization of a system allows us to classify steady states with the definition below.

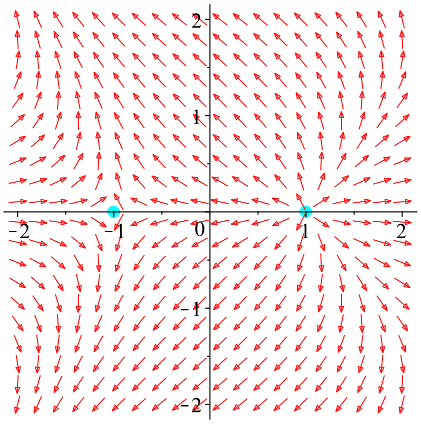


Figure 2.1: Vector field of a planar dynamic system. The horizontal axis corresponds to x_1 and the vertical axis to x_2.

Definition 25. A steady state x^* of $\dot{x} = f(x)$ is called a *sink* if all of the eigenvalues of the matrix $J_f(x^*)$ have negative real part; it is called a *source* if all of the eigenvalues of $J_f(x^*)$ have positive real part; and it is called a *saddle* if it is a hyperbolic steady sate and $J_f(x^*)$ has at least one eigenvalue with positive real part and at least one eigenvalue with negative real part.

As an example consider the ODEs system given by

$$\dot{x} = f(x) := \begin{pmatrix} x_1^2 - x_2^2 - 1 \\ 2x_2 \end{pmatrix}.$$

The steady states of the system are $(1, 0)$ and $(-1, 0)$. The Jacobian of f is given by

$$J_f(x) = \begin{bmatrix} 2x_1 & -2x_2 \\ 0 & 2 \end{bmatrix},$$

and the linearization of the system at $(1, 0)$ and $(-1, 0)$ are

$$\dot{x} = J_f(1,0)x = \begin{bmatrix} 2 & 0 \\ 0 & 2 \end{bmatrix} \begin{pmatrix} x_1 \\ x_2 \end{pmatrix} \text{ and } J_f(x) = \begin{bmatrix} -2 & 0 \\ 0 & 2 \end{bmatrix} \begin{pmatrix} x_1 \\ x_2 \end{pmatrix}$$

respectively. Additionally, from the eigenvalues of $J_f(1, 0)$ and $J_f(-1, 0)$ we conclude that $(1, 0)$ is a source and $(-1, 0)$ is a saddle. The vector field associated with the ODEs system is presented in Figure 2.1 and from it we see that the trajectories starting near the source will tend to move away from it as time increases. Sinks are going to be very important in our work as they can be detected by checking the eigenvalues of the linearization of the system at the steady state, in particular sinks are going to be a special case of stable steady states. We define what stability means in the following definition.

Definition 26. Let $\theta_t(x_0)$ be the trajectory of the ODEs system $\dot{x} = f(x)$ starting at a point $x_0 \in \mathbb{R}^n$. A steady state $x^* \in \mathbb{R}^n$ is *stable* if for every $\epsilon > 0$, there exists a $\delta > 0$ such that for all $x_0 \in \mathcal{B}_\delta(x^*)$ and $t \geq 0$ we have that $\theta_t(x_0) \in \mathcal{B}_\epsilon(x^*)$; the steady state is *unstable* if it is not stable. The steady state x^* is *asymptotically* stable if it is stable and if for every $\epsilon > 0$ there exists a $\delta > 0$ such that for all $x_0 \in \mathcal{B}_\delta(x^*)$ we have that

$$\lim_{t \to +\infty} \theta(x_0) = x^*.$$

Intuitively a steady state is stable if trajectories starting near it, remain in a neighbourhood of the steady state; and asymptotic stability holds when the trajectories not only remain in a neighbourhood of the steady state, but tend to it as time increases.

One of the main results for detecting whether a hyperbolic steady state is stable, unstable or a saddle, is the Stable Manifold theorem. Before stating it, we need some definitions regarding linear ODes systems.

Definition 27. Let A be a square matrix of size n, and consider the ODE system given by $\dot{x} = Ax$. Suppose that A has k real negative eigenvalues $\lambda_1, \dots, \lambda_k$ and $n - k$ real positive eigenvalues $\lambda_{k+1}, \dots, \lambda_n$ and that these eigenvalues are distinct. Let $\{v_1, \dots, v_n\}$ be a corresponding basis of eigenvectors. Then the *stable* and *unstable subspaces*, E^s and E^u, are the subspaces spanned by $\{v_1, \dots, v_k\}$ and $\{v_{k+1}, \dots, v_n\}$ respectively.

Note that under the assumptions of this definition, 0 is a steady state and the matrix A is diagonalizable, which implies that if we write the system $\dot{x} = Ax$ with respect to the basis formed by the eigenvectors, we obtain a diagonal system $\dot{y} = \text{diag}(\lambda_1, \dots, \lambda_n)y$ whose solution is given by $y_i(t) = \exp(\lambda_i t)y_{0i}$, where $y_0 \in \mathbb{R}^n$ is an initial condition. From this solution we have that E^s and E^u are invariant under trajectories of the system; specifically, if $y_0 \in E^s$, then the coordinates of y_0 in the basis given by the eigenvectors are zero for $\{v_{k+1}, \dots, v_n\}$, which means that all the trajectory lies on E^s and, additionally, all the entries of $y_i(t) = \exp(\lambda_i t)y_0$; tend to zero as t tends to infinity. Similarly, we see that E^u is invariant, as initial conditions $y_0 \in E^u$ have zero coordinates for the vectors $\{v_1, \dots, v_k\}$.

With this solution for the diagonal system and, doing the reverse change of basis, we obtain a solution for the original system $\dot{x} = Ax$. This change of basis preserves the direction of the trajectories, which gives an intuition as to why the subspaces E^s and E^u are called stable and unstable subspaces as the trajectories starting on E^s remain in E^s and tend to zero, whereas the trajectories starting in E^uremain in E^u but do not tend to zero.

As an example for the stable and unstable subspaces consider the ODEs system

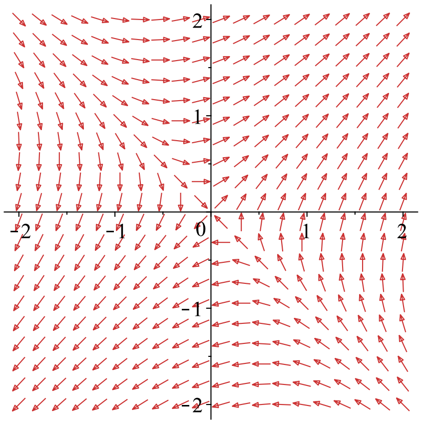


Figure 2.2: Vector field associated with the linear dynamic system $\dot{x_1} = x_1 + 3x_2$ and $\dot{x_2} = 3x_1 + x_2$. The horizontal axis corresponds to x_1 and the vertical axis to x_2.

given by

$$\dot{x} = \begin{bmatrix} 1 & 3 \\ 3 & 1 \end{bmatrix} x,$$

with $x \in \mathbb{R}^2$. The eigenvalues of the matrix are -2 and 4 and their corresponding eigenvectors are $(-1, 1)$ and $(1, 1)$ respectively. This means that the stable subspace is the subspace spanned by $(-1, 1)$, and the unstable subspace is the one spanned by $(1, 1)$. In Figure 2.2 we see the vector field associated with the system. Note that trajectories starting in the subspace given by $x_1 = -x_2$ tend to zero, and trajectories starting in the subspace $x_1 = x_2$ do not. We now extend these definitions to linear systems where the matrix A is not necessarily diagonalizable. In order to do so, we define the generalized eigenvectors for matrices with at least one eigenvalue with multiplicity larger than 1.

Definition 28. Let λ be an eigenvalue of a real matrix A of size n, of multiplicity $1 < m$. Then for $k = 1, \ldots, m$, any nonzero solution v of $(A - \lambda I)^k v = 0$ is called a *generalized eigenvector* of A.

Consider the matrix

$$A = \begin{bmatrix} 1 & 0 & 0 \\ -1 & 2 & 0 \\ 1 & 1 & 2 \end{bmatrix}. \tag{2.1}$$

This matrix has eigenvalues 1 and 2 with multiplicities 1 and 2 respectively. The eigenvector associated with $\lambda_1 = 1$ is $(1, 1, -1)$ and the eigenvector associated with $\lambda_2 = 2$ is $(0, 0, 1)$. Since λ_2 has multiplicity 2, we can find an additional generalized eigenvector associated with λ_2 as a solution of the equation $(A-2I)^2v=0$. In this case, one solution is given by the vector $(0,1,0)$ and this corresponds to a generalized eigenvector of A.

Note that, in the last definition, λ could be a complex number, in this case the entries of the generalized eigenvector can also be complex numbers, and the following theorem holds.

Theorem 12. *Let A be a real square matrix of size $2n$, with complex eigenvalues $\lambda_j = a_j + ib_j$ and $\bar{\lambda}_j = a_j - ib_j$ for $j = 1,\dots,n$. Then there exists generalized complex eigenvectors $w_j = u_j + iv_j$ and $\bar{w}_j = u_j - iv_j$, $j = 1,\dots,n$ such that $\{u_1, v_1, \dots, u_n, v_n\}$ is a basis for $\mathbb{R}^{2n}$.*

As an example of the generalized eigenvectors of a matrix with complex eigenvalues consider

$$A = \begin{bmatrix} 0 & -1 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 \\ 2 & 0 & 1 & 0 \end{bmatrix}. \tag{2.2}$$

The eigenvalues of A are $\lambda = i$ and $\lambda = -i$ each one with multiplicity 2. The eigenvector associated with $\lambda = i$ is $w_1 = (0,0,i,1)$ which can be written as $w_1 = (0,0,0,1) + i(0,0,1,0)$. A generalized eigenvector is obtained by solving $(A-iI)^2w = 0$, and one solution is given by $w_2 = (i,1,0,1)$ which can be written as $w_2 = (0,1,0,1)+i(1,0,0,0)$. In this case the basis obtained for $\mathbb{R}^4$ is $\{(0,0,0,1),(0,0,1,0),(0,1,0,1),(1,0,0,0)\}$.

With the definition of generalized eigenvalues and eigenvectors we define the stable, unstable and central subspaces of $\mathbb{R}^n$ associated with a matrix A.

Definition 29. Let $w_j = u_j + iv_j$ be a generalized eigenvector of the real matrix A corresponding to an eigenvalue $\lambda_j = a_j + ib_j$. Then the stable, central and unstable subspaces correspond to the following sets

$$E^s = \text{span}\{u_j, v_j : u_j + iv_j \text{ is the eigenvector corresponding to } a_j + ib_j \text{ and } a_j < 0\},$$

$$E^c = \text{span}\{u_j, v_j : u_j + iv_j \text{ is the eigenvector corresponding to } a_j + ib_j \text{ and } a_j = 0\},$$

$$E^u = \text{span}\{u_j, v_j : u_j + iv_j \text{ is the eigenvector corresponding to } a_j + ib_j \text{ and } a_j > 0\}.$$

The stable, central and unstable subspaces are invariant under trajectories of the ODEs system $\dot{x} = Ax$. Revisiting the previous matrices, for (2.2), all the eigenvalues are purely imaginary numbers. This implies that the stable and unstable subspaces are the trivial subspaces, and $E^c = \mathbb{R}^4$. For the matrix in (2.1), we have two positive eigenvalues. In this case, E^s and E^c are trivial and $E^u = \mathbb{R}^3$.

After giving the definition of the stable, unstable and central subspace we can now present the Center Manifold Theorem. This theorem allows to study the behaviour of the system near a steady state by exploring its linearization.

Theorem 13 (The Center Manifold Theorem)**.** *Let $\mathcal{E}$ be an open subset of $\mathbb{R}^n$ containing the origin, let $f \in \mathcal{C}^r(\mathcal{E})$ with $r > 1$, and let $\theta_t(x_0)$ represent the trajectory of the non linear system $\dot{x} = f(x)$ starting at x_0. Suppose that $f(0) = 0$ and that $J_f(0)$ has k eigenvalues with negative real part, j eigenvalues with positive real part, and $m = n-k-j$ eigenvalues with zero real part counted with multiplicity. Then there exists an m-dimensional center manifold $\mathfrak{C}$ of class C^r tangent to the center subspace E^c of the linear system $\dot{x} = J_f(0)x$ at zero, there exists a k-dimensional stable manifold $\mathfrak{S}$ of class C^r tangent to the stable subspace E^s of $\dot{x} = J_f(0)x$ at zero, and there exists a j-dimensional unstable manifold $\mathfrak{U}$ of class C^r tangent to the unstable subspace E^u of $\dot{x} = J_f(0)x$ at zero. Furthermore $\mathfrak{C}, \mathfrak{S}$ and $\mathfrak{U}$ are invariant under trajectories θ_t.*

This theorem guarantees the existence of three manifolds that near to 0, are tangent to the stable, unstable and center subspaces and such that the trajectories of the system starting in these manifolds have the same behaviour as the one of the linearization problem. We will not present the proof of this theorem as it is not the main goal of this work; however we refer the reader to [50]. However, as a remark we say that while the unstable and stable manifolds are unique, the center manifold is not necessarily unique.

This theorem and the definition of a sink, allow us to conclude that in a ODEs system $\dot{x} = f(x)$, a sink x^* is an asymptotically stable point. This holds because by definition $J_f(x^*)$ has only negative eigenvalues, which means that the stable subspace of the linearization is $\mathbb{R}^n$, and therefore all the trajectories starting near x^* remain in a neighbourhood of x^*. Furthermore, it is possible to find an upper bound, depending on an exponential function, for the distance between the points of the trajectory and x^*.

Theorem 14. *If x^* is a sink of the nonlinear system $\dot{x} = f(x)$, and $Re(\lambda_j) < \alpha < 0$ for all of the eigenvalues λ_j of the matrix $J_f(x^*)$, then given $\epsilon > 0$ there exists a $\delta > 0$ such that for all $x \in \mathcal{B}_\delta(x^*)$, the trajectories $\theta_t(x)$ satisfy*

$$|\theta_t(x) - x^*| \leq \epsilon e^{\alpha t}$$

for all $t \geq 0$.

Due to this bound, we will say that if the steady state is a sink, it is *exponentially stable.*

2.1.2 Hurwitz criterion

In the previous section we defined the sinks that are exponentially stable steady states of a ODEs system $\dot{x} = f(x)$. By definition, detecting whether a steady state is a sink (a source or saddle) corresponds to study the sign of the eigenvalues of $J_f(x^*)$. Depending on the system, this is not always an easy procedure, specially for parametric systems of ODEs as computationall problems arise. We now present a criterion for studying the sign of the real part of the roots of a polynomial. The criterion will be a fundamental tool in our work when applying it to the characteristic polynomial of $J_f(x^*)$ in papers I and II.

The Hurwitz criterion [3, 58, 64] is used in systems control to determine whether a polynomial is stable. A polynomial is stable if all its roots lay on the open left side of the complex plane, that is, have negative real part.

Theorem 15 (Hurwitz criterion). *Let $p(x) = a_s x^s + a_{s-1}x^{s-1} + \ldots + a_1 x + a_0$ be a polynomial with $a_i \in \mathbb{R}$, $a_s > 0$ and $a_0 \neq 0$. Define the Hurwitz matrix associated with p, as the matrix H whose entries are defined by $h_{i,j} = a_{s-2i+j}$ for $i, j = 1, \ldots, s$ and $a_k = 0$ if $k < 0$ or $k > s$:*

$$H = \begin{pmatrix} a_{s-1} & a_s & 0 & 0 & \cdots & 0 \\ a_{s-3} & a_{s-2} & a_{s-1} & a_s & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & a_{6-s} & \cdots & a_2 \\ 0 & 0 & 0 & 0 & \cdots & a_0 \end{pmatrix}.$$

Define the i-th Hurwitz determinant, denoted by H_i, as $H_i = \det(H_{I,I})$, with $I = \{1, \ldots, i\}$. All the roots of p have negative real part if, and only if, $H_i > 0$ for all $i = 1, \ldots, s$. Additionally, if $H_i < 0$ for some i, then there exists a root of p whose real part is positive.

This criterion guarantees that all roots of a polynomial p have negative real part if, and only if, the corresponding Hurwitz determinants are positive. This criterion is particularly useful when the ODEs system depends on certain parameters. In this case it is not always possible to compute the eigenvalues, but the Hurwitz criterion might allow to determine the sign of their real parts. We present an example of the the use of the criterion.

Consider the ODEs system given by

$$\begin{aligned} \dot{x} =& -y + x(\mu - x^2 - y^2) \\ \dot{y} =& x + y(\mu - x^2 - y^2). \end{aligned}$$

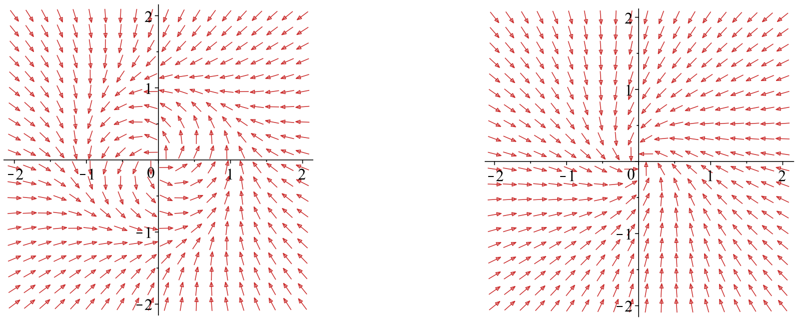


Figure 2.3: Vector field for the ODE system $\dot{x} = -y + x(\mu - x^2 - y^2)$, $\dot{y} = x + y(\mu - x^2 - y^2)$. The figure on the left corresponds to $\mu = 1$, and the figure on the right corresponds to $\mu = -1$.

The only steady state is the origin. The linearization of the system at the origin is determined by the Jacobian

$$J_f(0,0) = \begin{bmatrix} \mu & -1 \\ 1 & \mu \end{bmatrix}.$$

In this case, $J_f(0,0)$ depends on the parameter μ. We will apply the Hurwitz criterion to this polynomial to see if the dynamics near the origin change depending on μ. The characteristic polynomial is

$$p_{J_f}(\lambda) = \lambda^2 - 2\mu\lambda + \mu^2 + 1.$$

This is a polynomial of degree 2; therefore, the Hurwitz matrix has size 2 and corresponds to

$$H = \begin{bmatrix} -2\mu & 1 \\ 0 & \mu^2 + 1 \end{bmatrix}. \tag{2.3}$$

The Hurwitz determinants are $H_1 = -2\mu$ and $H_2 = -2\mu(\mu^2 + 1)$. According to the Hurwitz criterion, the roots of $p_{J_f}(\lambda)$ have negative real part if, and only if, H_1 and H_2 are positive. Notice that both H_1 and H_2 are positive for every $\mu < 0$, in which case the roots of p_{J_f} have negative real part which implies that $(0,0)$ is an exponentially stable steady state of the original system. On the other hand, for $\mu > 0$, the Hurwitz determinants are negative and the Hurwitz criterion guarantees that p_{J_f} has at least one root with positive real part. In the ODEs system, this means that $(0,0)$ would be an unstable steady state. In Figure 2.3 we see the vector field associated with this system for a positive value and a negative value of μ.

Note that the Hurwitz criterion does not say anything about the roots of the polynomial when the determinants are zero. Therefore, we cannot say anything about the dynamics of the system for $\mu = 0$, using this criterion. However, it is possible to study this particular case by assuming that $\mu = 0$ from the beginning. If $\mu = 0$, then p_{J_f} has a pair of imaginary roots. This generates a Hopf bifurcation, which we explore in the next subsection.

2.1.3 Hopf bifurcations

In the previous sections, we have studied the behaviour of a dynamical system $\dot{x} = f(x)$ in a neighbourhood of the steady states, and showed that it is linked with the sign of the real part of the eigenvalues of the Jacobian defining the linearization of the dynamical system. Additionally, we presented the Hurwitz criterion that, when used on the characteristic polynomial of $J_f(x^*)$, allow us to determine whether a steady state is exponentially stable or unstable. As we saw in example (2.3), if the system depends on a parameter, the dynamics could change as the parameter varies. In this example, when varying μ, the system always has one steady state (the origin); however its stability changes once $\mu = 0$. It is said that the system has a bifurcation in $\mu = 0$.

When talking about bifurcations, we are referring to a structural property of the system. That is, we are not studying the dynamics near a steady state, but we are studying how these dynamics change when a parameter of the system varies. In particular, in a Hopf bifurcation a steady state loses stability when the parameter reaches the bifurcation value. As we saw before, the stability of a steady state depends on the sign of the real part of the eigenvalues of $J_f(x^*)$, then it is not surprising if a bifurcation can be detected by analising again these eigenvalues. We define now these ideas formally.

A *simple Hopf bifurcation* is a bifurcation in which a single complex-conjugate pair of eigenvalues of the Jacobian matrix crosses the imaginary axis, while all other eigenvalues remain with negative real parts. Such a bifurcation, generates nearby oscillations, i.e. periodic orbits [41].

To detect simple Hopf bifurcations, we will use a criterion of Liu [41] that characterizes Hopf bifurcations in terms of Hurwitz determinants.

Consider an ODE system parametrized by $\mu \in \mathbb{R}$:

$$\dot{x} = f_\mu(x),$$

where $x \in \mathbb{R}^n$, and $f_\mu(x)$ varies smoothly in μ and x. Assume that $x_0 \in \mathbb{R}^n$ is a steady state of the system defined by μ_0, that is, $f_{\mu_0}(x_0) = 0$. Assume, furthermore, that we have a smooth curve of steady states:

$$\mu \;\mapsto\; x(\mu) \tag{2.4}$$

(that is, $f_\mu\left(x(\mu)\right) = 0$ for all μ) and that $x(\mu_0) = x_0$. Denote the characteristic polynomial of the Jacobian matrix of f_μ, evaluated at $x(\mu)$, as follows:

$$p_\mu(\lambda) \coloneqq \det\left(\lambda I - J_{f_\mu}\right)|_{x=x(\mu)} = \lambda^n + b_1(\mu)\lambda^{n-1} + \cdots + b_n(\mu),$$

and, for $i = 1, \dots, n$, define $H_i(\mu)$ to be the i-th Hurwitz matrix of $p_\mu(\lambda)$.

Proposition 6 (Liu's criterion [41]). *Assume the above setup. Then, there is a simple Hopf bifurcation at x_0 with respect to μ if and only if the following hold:*

(i) $b_n(\mu_0) > 0$,

(ii) $\det H_1(\mu_0) > 0$, $\det H_2(\mu_0) > 0$, $\dots$, $\det H_{n-2}(\mu_0) > 0$, *and*

(iii) $\det H_{n-1}(\mu_0) = 0$ *and* $\frac{d(\det H_{n-1}(\mu))}{d\mu}|_{\mu=\mu_0} \neq 0$.

This criterion will be used extensively in our work as it allows us to use the Hurwitz determinants for detecting Hopf bifurcations in parametric systems, specifically in a model for ERK regulation and in a MAPK cascade.

2.1.4 Jacobian of Chemical Reaction Networks

In the previous sections we have seen that the stability of a steady state can be explored using the Jacobian of the function. However, in the Chemical Reaction Networks setting, we know that the dynamics are constrained to a stoichiometric compatibility class and that the Jacobian has a special structure. We now present in more detail some properties of $J_f(x)$, when f is the rate function of a CRN, and how it can be restricted to the stoichiometric subspace. These properties will be very important in the results presented in the papers.

The first remark is that for a rate function $f(x) = Nv(x)$, its Jacobian can be written as

$$J_f(x) = NJ_v(x).$$

This is deduced straightforward from the definition of f. With this remark we consider now the projection of $J_f(x^*)$ onto the stoichiometric subspace S. That is, we write the ODE system restricted to the stoichiometric compatibility class $(x^* + S) \cap \mathbb{R}^n_{\geq 0}$ in local coordinates.

Consider a matrix $R_0 \in \mathbb{R}^{n \times s}$ whose columns form a basis of S. This basis is the system of coordinates in S, such that given coordinates $z = (z_1, \dots, z_s)$, the vector $R_0 z$ is the vector of coordinates in the canonical basis of $\mathbb{R}^n$. In order to write a vector $x \in S$ in local coordinates, we consider a matrix $R_1 \in \mathbb{R}^{s \times n}$ such that $R_1 R_0 = I_{s \times s}$. Now, using these matrices, the ODE system restricted to $(x^* + S) \cap \mathbb{R}^n_{\geq 0}$ in local S coordinates is

$$\dot{z} = R_1 f(R_0 z + x^*)$$

after translating the steady state x^* to the origin. The Jacobian matrix associated with this system at 0 is $R_1 J_f(x^*) R_0$.

The following proposition shows some basic properties of $R_1 J_f(x^*) R_0$.

Proposition 7. *Recall $f(x) = Nv(x)$. With the definitions above, we have:*

(i) $R_1 J_f(x^*) R_0 = L J_v(x^*) R_0$ *with* $L \in \mathcal{R}^{s\times m}$ *the matrix such that* $N = R_0 L$*. In particular,* $R_1 J_f(x^*) R_0$ *does not depend on the choice of* R_1*.*

(ii) Let $p(\lambda)$ *be the characteristic polynomial of* $J_f(x^*)$*. Then* $p(\lambda) = \lambda^d q(\lambda)$*, with* $q(\lambda)$ *the characteristic polynomial of* $L J_v(x^*) R_0$ *for any choice of* R_0*. Furthermore, the independent term of* $q(\lambda)$ *(or the coefficient of degree* d *of* $p(\lambda)$*) is the determinant of* $J_{F_T}(x^*)$*, with* F_T *given in (1.7).*

(iii) Given two matrices $R_0, R_0' \in \mathbb{R}^{n\times s}$ *whose columns form a basis of* S*, and* L, L' *as in (ii) for* R_0, R_0'*, then the matrices* $L' J_v(x^*) R_0'$ *and* $L J_v(x^*) R_0$ *are similar.*

Proof. (i) Since the columns of N belong to S, we can uniquely write $N = R_0 L$ with $L \in \mathcal{R}^{s\times m}$. Since $f(x) = Nv(x)$, we have $J_f = N J_v$. So

$$R_1 J_f(x^*) R_0 = R_1 N J_v(x^*) R_0 = R_1 R_0 L J_v(x^*) R_0 = L J_v(x^*) R_0.$$

(ii) Extend the matrix R_0 to a square matrix $R \in \mathcal{R}^{n\times n}$ by adding columns such that R has full rank n. Then the eigenvalues of the matrices $Q = R^{-1} J_f(x^*) R$ and $J_f(x^*)$ coincide. Since $\operatorname{Im}(J_f(x^*)) \subseteq S$, the matrix Q has the form

$$Q = \begin{pmatrix} Q' & Q'' \\ 0 & 0 \end{pmatrix},$$

where Q' is equal to $R_1 J_f(x^*) R_0 = L J_v(x^*) R_0$, after choosing R_1 as the first columns of R^{-1}. Clearly, the characteristic polynomial of Q in the variable λ agrees with the characteristic polynomial of Q' times λ^{n-s}. This proves the first part of (ii).

(iii) Let $M \in \mathcal{R}^{s\times s}$ be the matrix of change of basis from R_0 to R_0' such that $R_0 M = R_0'$. Since $N = R_0' L' = R_0 M L'$, it follows that $L = M L'$. This gives

$$L' J_v(x^*) R_0' = M^{-1} M L' J_v(x^*) R_0 M = M^{-1} L J_v(x^*) R_0 M.$$

□

Note that the total amounts T do not appear in the entries of $J_{F_T}(x^*)$.

In Example (1.3) we consider the following matrix R_0 and corresponding matrix L:

$$R_0 = \begin{pmatrix} -1 & 0 \\ 1 & 0 \\ 0 & 1 \\ 0 & -1 \end{pmatrix}, \qquad L = \begin{pmatrix} 1 & -1 & 0 \\ 0 & -1 & 1 \end{pmatrix}.$$

By using the parametrization of the positive steady states in (1.8), the Jacobian matrix of f and v evaluated at a steady state x^* are

$$J_f(x^*) = \begin{pmatrix} -\kappa_1 & \frac{\kappa_3 x_4}{x_2} & \kappa_2 x_2 & 0 \\ \kappa_1 & -\frac{\kappa_3 x_4}{x_2} & -\kappa_2 x_2 & 0 \\ 0 & -\frac{\kappa_3 x_4}{x_2} & -\kappa_2 x_2 & \kappa_3 \\ 0 & \frac{\kappa_3 x_4}{x_2} & \kappa_2 x_2 & -\kappa_3 \end{pmatrix}.$$

Using L and R_0 above, we will be interested in the eigenvalues of the following matrix

$$Q_{\kappa,x_2,x_4} = \begin{pmatrix} -\kappa_1 - \frac{\kappa_3 x_4}{x_2} & -\kappa_2 x_2 \\ -\frac{\kappa_3 x_4}{x_2} & -\kappa_2 x_2 - \kappa_3 \end{pmatrix}. \tag{2.5}$$

By studying the eigenvalues of Q_{κ,x_2,x_4} for *all* values of the reaction rate constants κ and $x_2, x_4 > 0$, we are considering the eigenvalues of all possible positive steady states of the network. In the notation of (1.9), we have $\xi = (x_2, x_4)$.

For an arbitrary reaction network, given a parametrization as in (1.9) with indeterminate ξ, using matrices R_0, L as introduced now, we obtain a family of matrices $Q_{\kappa,\xi}$ whose entries are rational function in κ, ξ.

2.2 Contributions in Bistability and Oscillations in Chemical Reaction Networks

Now we give an overview of our contributions regarding the presence of bistability and oscillations in Chemical Reaction Networks.

2.2.1 Algorithm for detecting bistability

This work corresponds to paper I in the last section of this thesis, currently under revision. The main contribution here is to give an algorithm for exploring the presence of bistability in a Chemical Reaction Network.

In our setting the steady states will be given by an algebraic parametrization of the positive steady state variety. In practice, under mass-action kinetics, the entries of ϕ are rational functions in ξ. Strategies for finding positive parametrizations are reviewed in [11].

When the positive steady state variety can be parametrized, we study the eigenvalues of $J_f(\phi(\xi))$ restricted to a stoichiometric compatibility class. We proved that when all but the last Hurwitz determinants are positive and it is possible to parametrize the solutions of all but one entries of $F_T(x)$ with a univariate function $\varphi(z) : \mathcal{E} \to \mathbb{R}^n_{>0}$, then there are as many positive steady states as positive roots of a rational function. Furthermore, their stability depends on the derivative of said function. We state now formally these results

Proposition 8. *Fix $W \in \mathbb{R}^{d\times n}, T \in \mathbb{R}^d$ and F_T as in Eq. (1.7). Assume there exist an open interval $\mathcal{E} \subseteq \mathbb{R}$, a differentiable function $\varphi\colon \mathcal{E} \to \mathbb{R}^n_{>0}$, and indices i, j such that $\varphi_i'(z) \neq 0$ and $F_{T,\ell}(\varphi(z)) = 0$ for all $\ell \neq j$. Then, for any solution z^* to*

$$F_{T,j}(\varphi(z)) = 0, \qquad z \in \mathcal{E}, \tag{2.6}$$

$x^* = \varphi(z^*)$ is a positive solution to $F_T(x) = 0$ and further

$$\det(J_{F_T}(x^*)) = \frac{(-1)^{i+j}}{\varphi_i'(z^*)}(F_{T,j} \circ \varphi)'(z^*) \det(J_{F_T}(x^*)_{J,I}),$$

where $I = \{1, \dots, n\} \setminus \{i\}$ and $J = \{1, \dots, n\} \setminus \{j\}$.

With this proposition (corresponding to Proposition 2 in Paper I) we proved the theorem below associated with detecting bistability. This result corresponds to Theorem 1 in Paper I at the end of this Thesis.

Theorem 16. *Let $T, \mathcal{E}, \varphi, i, j, I, J$ be as in Proposition 8. Assume that*

- *the sign of $\frac{1}{\varphi_i'(z)} \det(J_{F_T}(\varphi(z))_{J,I})$ is independent of $z \in \mathcal{E}$ and is nonzero, and*
- *the first $s-1$ Hurwitz determinants of $q_{x^*}(\lambda)$ are positive for all positive steady states x^*.*

If $z_1 < \cdots < z_\ell$ are the positive solutions to Eq. (2.6) and all are simple, then either $\varphi(z_1), \varphi(z_3), \dots$ are exponentially stable and $\varphi(z_2), \varphi(z_4), \dots$ are unstable, or the other way around. Specifically, $\varphi(z_1)$ is exponentially stable if and only if

$$\frac{(-1)^{s+i+j}}{\varphi_i'(z_1)}(F_{T,j} \circ \varphi)'(z_1) \det(J_{F_T}(\varphi(z_1))_{J,I}) > 0.$$

This theorem allows us to propose a method for detecting bistability in Chemical Reaction Networks, that is summarized in Figure 2.4. The method was successfully applied in the networks in Figure 2.5 coming from cell signalling. Each item in this figure shows the original network, where Theorem 16 could not be applied, and the reduced network that satisfy the hypotheses of Theorem 16, which we used to ensure the presence of bistability.

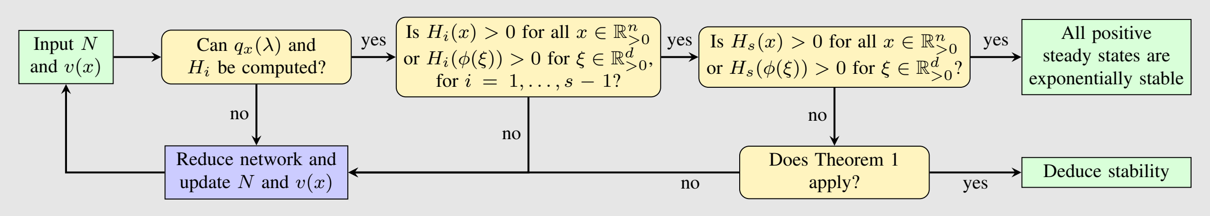


Figure 2.4: Flow chart depicting method for detecting bistability.

As an example to illustrate the use of the theorem, consider the network on the left of item (a) in Figure 2.5. This network is the combination of two one-site modification cycles where the same kinase E activates the phosphorylation process and two different phosphates F_1 and F_2 catalyze the dephosphorylation process:

$$\mathrm{S_0 + E} \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} \mathrm{S_0E} \xrightarrow{\kappa_3} \mathrm{S_1 + E} \qquad \mathrm{S_1 + E} \underset{\kappa_5}{\overset{\kappa_4}{\rightleftharpoons}} \mathrm{S_1E} \xrightarrow{\kappa_6} \mathrm{S_2 + E}$$

$$\mathrm{S_1 + F_1} \underset{\kappa_8}{\overset{\kappa_7}{\rightleftharpoons}} \mathrm{S_1F_1} \xrightarrow{\kappa_9} \mathrm{S_0 + F_1} \qquad \mathrm{S_2 + F_2} \underset{\kappa_{11}}{\overset{\kappa_{10}}{\rightleftharpoons}} \mathrm{S_2F_2} \xrightarrow{\kappa_{12}} \mathrm{S_1 + F_2}\,.$$

The species are renamed as $S_0 = X_1, S_1 = X_2, S_2 = X_3, E = X_4, F_1 = X_5, F_2 = X_6, S_0E = X_7, S_1E = X_8, S_1F_1 = X_9, S_2F_2 = X_{10}$ and their concentrations are denoted in lower case letters.

For this network it is necessary to compute 6 Hurwitz determinants, which could be effectively computed and whose signs were analyzed up to H_4. They have positive coefficients. However, the analysis of the sign of H_5 was interrupted as it was taking a long time to finish and it was not possible to store the polynomial in the expanded format in a regular PC. To compute and study this determinant more effectively, we use a monomial positive parametrization ϕ of the steady state variety, given by

$$x_1 = \frac{(\kappa_2+\kappa_3)(\kappa_5+\kappa_6)\kappa_7\kappa_9\kappa_{10}\kappa_{12}}{\kappa_1\kappa_3\kappa_4\kappa_6(\kappa_8+\kappa_9)(\kappa_{11}+\kappa_{12})}\frac{x_3x_5x_6}{x_4^2} \qquad x_8 = \frac{\kappa_{10}\kappa_{12}}{\kappa_6(\kappa_{11}+\kappa_{12})}x_3x_6$$

$$x_2 = \frac{(\kappa_5+\kappa_6)\kappa_{10}\kappa_{12}}{\kappa_4\kappa_6(\kappa_{11}+\kappa_{12})}\frac{x_3x_6}{x_4} \qquad x_9 = \frac{(\kappa_5+\kappa_6)\kappa_7\kappa_{10}\kappa_{12}}{\kappa_4\kappa_6(\kappa_8+\kappa_9)(\kappa_{11}+\kappa_{12})}\frac{x_3x_5x_6}{x_4}$$

$$x_7 = \frac{(\kappa_5+\kappa_6)\kappa_7\kappa_9\kappa_{10}\kappa_{12}}{\kappa_3\kappa_4\kappa_6(\kappa_8+\kappa_9)(\kappa_{11}+\kappa_{12})}\frac{x_3x_5x_6}{x_4} \qquad x_{10} = \frac{\kappa_{10}}{\kappa_{11}+\kappa_{12}}x_3x_6.$$

Using this parametrization, it was possible to compute $H_5(\phi)$. However, the sign of this function remains unclear since it has coefficients with different signs.

We consider next the reduced network obtained by first removing all the reverse reactions and then the intermediates S_1F_1 and S_2F_2. When removing these intermediates the reactions $\mathrm{S_1 + F_1 \longrightarrow S_1F_1 \longrightarrow S_0 + F_1}$ and $\mathrm{S_2 + F_2 \longrightarrow S_2F_2 \longrightarrow S_1 + F_2}$ become $\mathrm{S_1 + F_1 \longrightarrow S_0 + F_1}$ and $\mathrm{S_2 + F_2 \longrightarrow S_1 + F_2}$ respectively. The reduced network is depicted on the right side of Figure 2.5. The species are now renamed as $S_0 = X_1, S_1 = X_2, S_2 = X_3, E = X_4, F_1 = X_5, F_2 = X_6, S_0E = X_7, S_1E = X_8$, and their concentrations are denoted in lower case letters. For this reduced network it was necessary to compute 4 Hurwitz determinants. In this case H_1, H_2 and H_3 are positive. However the sign of H_4 is unclear even after evaluating in a positive parametrization of the steady state variety ϕ. In this situation we explore the possibility of applying Theorem 16 to deduce bistability.

The conservation laws of the system are

$$x_1 + x_2 + x_3 + x_7 + x_8 = T_1, \quad x_4 + x_7 + x_8 = T_2, \quad x_5 = T_3 \quad \text{and } x_6 = T_4.$$

Taking the indices i_1, i_2, i_3, i_4 as $1, 4, 5, 6$ respectively, we construct F_T. The solutions of $F_{T,\ell} = 0$ for $\ell = 2, 3, 4, 5, 6$ are written in terms of $z = x_2$ as

$$\varphi(z) = \left(\frac{\tau_2\tau_5T_3(\tau_3z+\tau_4)z}{\tau_1\tau_4(\tau_2T_2-\tau_5T_3z)}, z, \frac{\tau_3\tau_4z(\tau_2T_2-\tau_5T_3z)}{\tau_2\tau_6T_4(\tau_3z+\tau_4)}, \frac{\tau_4(\tau_2T_2-\tau_5T_3z)}{\tau_2(\tau_3z+\tau_4)}, T_3, T_4, \frac{\tau_5T_3z}{\tau_2}, \frac{\tau_3(\tau_2T_2-\tau_5T_3z)z}{\tau_2(\tau_3z+\tau_4)}\right),$$

for every $z \in \mathcal{E}$, where $\mathcal{E} = \left(0, \frac{T_2\tau_2}{T_3\tau_5}\right)$.

Note that $\varphi_2(z) = z$ and $\varphi_2'(z) = 1 \neq 0$. This means that the positive steady states in the stoichiometric compatibility class defined by T are in one to one correspondence with the positive roots of $F_{T,1}(\varphi(z))$ in $\mathcal{E}$. This rational function, presented below, has as numerator a polynomial of degree 3.

$$\begin{aligned} F_{T,1}(\varphi(z)) = & \frac{1}{T_4\tau_1\tau_2\tau_4\tau_6(T_3\tau_5z-T_2\tau_2)(\tau_3z+\tau_4)} \left(-T_3\tau_3\tau_5(T_3\tau_1\tau_4^2\tau_5 - T_4\tau_1\tau_2\tau_4\tau_6 + T_4\tau_2^2\tau_3\tau_6)z^3 - \right. \\ & \tau_4(T_1T_3T_4\tau_1\tau_2\tau_3\tau_5\tau_6 - T_2T_3T_4\tau_1\tau_2\tau_3\tau_5\tau_6 - T_3^2T_4\tau_1\tau_4\tau_5^2\tau_6 - 2T_2T_3\tau_1\tau_2\tau_3\tau_4\tau_5 + T_2T_4\tau_1\tau_2^2\tau_3\tau_6 - T_3T_4\tau_1\tau_2\tau_4\tau_5\tau_6 \\ & + 2T_3T_4\tau_2^2\tau_3\tau_5\tau_6)z^2 + \tau_2\tau_4(T_1T_2T_4\tau_1\tau_2\tau_3\tau_6 - T_1T_3T_4\tau_1\tau_4\tau_5\tau_6 - T_2^2T_4\tau_1\tau_2\tau_3\tau_6 - T_2T_3T_4\tau_1\tau_4\tau_5\tau_6 \\ & \left. - T_2^2\tau_1\tau_2\tau_3\tau_4 - T_2T_4\tau_1\tau_2\tau_4\tau_6 - T_3T_4\tau_2\tau_4\tau_5\tau_6)z + T_1T_2T_4\tau_1\tau_2^2\tau_4^2\tau_6\right). \end{aligned}$$

We have already shown that the second hypothesis of Theorem 16 holds. For the first hypothesis, a straightforward computation shows that

$$\det(J_{F_T}(\varphi(z))_{J,I}) = \tau_1\tau_4\tau_6T_4(\tau_5T_3z - \tau_2T_2),$$

which is negative for every $z \in \mathcal{E}$. We further have $s = 4$, $i = 2$, $j = 1$, and the independent term of the numerator of $F_{T,1}(\varphi(z))$ is negative, meaning that $(F_{T,j} \circ \varphi)'(z_1) > 0$. This gives that the sign of

$$\frac{(-1)^{s+i+j}}{\varphi_i'(z_1)}(F_{T,j} \circ \varphi)'(z_1) \det(J_{F_T}(\varphi(z_1))_{J,I})$$

is $(-1)^{4+1+2}(+1)(-1) = 1$ positive. Using Theorem 16, we conclude that for every set of parameters such that $F_{T,1}(\varphi(z))$ has three roots $z_1 < z_2 < z_3$ in $\mathcal{E}$, the steady states $\varphi(z_1), \varphi(z_3)$ are exponentially stable and $\varphi(z_2)$ is unstable.

	Network	Reduced network		Network	Reduced network
(a)	$S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$	$S_0 + E \rightarrow S_0E \rightarrow S_1 + E$	**(d)**	$S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$	$S_0 + E \rightarrow S_0E \rightarrow S_1 + E$
	$S_1 + E \rightleftharpoons S_1E \rightarrow S_2 + E$	$S_1 + E \rightarrow S_1E \rightarrow S_2 + E$		$S_1 + E \rightleftharpoons S_1E \rightarrow S_2 + E$	$S_1 + E \rightarrow S_2 + E$
	$S_1 + F_1 \rightleftharpoons S_1F_1 \rightarrow S_0 + F_1$	$S_1 + F_1 \rightarrow S_0 + F_1$		$S_2 + F \rightleftharpoons S_2F \rightarrow S_1 + F$	$S_2 + F \rightarrow S_1 + F$
	$S_2 + F_2 \rightleftharpoons S_2F_2 \rightarrow S_1 + F_2$	$S_2 + F_2 \rightarrow S_1 + F_2$		$S_1 + F \rightleftharpoons S_1F \rightarrow S_0 + F$	$S_1 + F \rightarrow S_0 + F$
(b)	$S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$	$S_0 + E \rightarrow S_1 + E$	**(e)**	$S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$	$S_0 + E \rightarrow S_1 + E$
	$S_1 + F \rightleftharpoons S_1F \rightarrow S_0 + F$	$S_1 + F \rightarrow S_0 + F$		$S_1 + F \rightleftharpoons S_1F \rightarrow S_0 + F$	$S_1 + F \rightarrow S_1F \rightarrow S_0 + F$
	$P_0 + S_1 \rightleftharpoons P_0S_1 \rightarrow P_1 + S_1$	$P_0 + S_1 \rightarrow P_0S_1 \rightarrow P_1 + S_1$		$P_0 + E \rightleftharpoons P_0E \rightarrow P_1 + E$	$P_0 + E \rightarrow P_0E \rightarrow P_1 + E$
	$P_1 + F \rightleftharpoons P_1F \rightarrow P_0 + F$	$P_1 + F \rightarrow P_1F \rightarrow P_0 + F$		$P_1 + F \rightleftharpoons P_1F \rightarrow P_0 + F$	$P_1 + F \rightarrow P_0 + F$
(c)	$S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$	$S_0 + E \rightarrow S_1 + E$	**(f)**	$S_0 + E_1 \rightleftharpoons S_0E_1 \rightarrow S_1 + E_1$	$S_0 + E_1 \rightarrow E_1S_0 \rightarrow S_1 + E_1$
	$S_1 + F_1 \rightleftharpoons S_1F_1 \rightarrow S_0 + F_1$	$S_1 + F_1 \rightarrow S_1F_1 \rightarrow S_0 + F_1$		$S_0 + E_2 \rightleftharpoons S_0E_2 \rightarrow S_1 + E_2$	$S_0 + E_2 \rightarrow S_1 + E_2$
	$P_0 + S_1 \rightleftharpoons P_0S_1 \rightarrow P_1 + S_1$	$P_0 + S_1 \rightarrow P_1 + S_1$		$E_1 \rightleftharpoons E_2$	$E_2 + S_0 \rightarrow E_1S_0$
	$P_0 + E \rightleftharpoons P_0E \rightarrow P_1 + E$	$P_0 + E \rightarrow P_0E \rightarrow P_1 + E$		$S_0E_1 \rightleftharpoons S_0E_2$	$E_1 \rightarrow E_2$
	$P_1 + F_2 \rightleftharpoons P_1F_2 \rightarrow P_0 + F_2$	$P_1 + F_2 \rightarrow P_0 + F_2$		$S_1 \rightarrow S_0$	$S_1 \rightarrow S_0$

Figure 2.5: Multistationary networks and reductions to explore bistability.

All that is left is to show that the reduced network admits three positive steady states in some stoichiometric compatibility class for some choice of τ, or what is the same, that $F_{T,1}(\varphi(z))$ admits three roots in $\mathcal{E}$. To this end, we apply the method from [11], which consists of finding values for the rate constants and concentration variables such that $\det(J_{F_T}(\phi))$, where ϕ is a parametrization of the steady states, is negative. We have

$$\phi(x_3, x_4, x_5, x_6) = \left(\frac{\tau_5\tau_6 x_3 x_5 x_6}{\tau_1\tau_3 x_4^2}, \frac{\tau_6 x_3 x_6}{\tau_3 x_4}, x_3, x_4 x, x_5, x_6, \frac{\tau_5\tau_6 x_3 x_5 x_6}{\tau_2\tau_3 x_4}, \frac{\tau_6 x_3 x_6}{\tau_4} \right) \qquad \text{and}$$

$$\det(J_{F_T}(\phi)) = \tau_1\tau_2\tau_6^2 x_3 x_6^2 - \tau_1\tau_4\tau_5\tau_6 x_3 x_5 x_6 + 2\frac{\tau_2\tau_5\tau_6^2 x_3 x_5 x_6^2}{x_4} + \frac{\tau_4\tau_5^2\tau_6^2 x_3 x_5^2 x_6^2}{\tau_3 x_4^2}$$
$$+ \tau_1\tau_2\tau_3\tau_6 x_4^2 x_6 + \tau_1\tau_4\tau_5\tau_6 x_4 x_5 x_6 + \tau_1\tau_2\tau_3\tau_4 x_4^2 + \tau_1\tau_2\tau_4\tau_6 x_4 x_6 + \tau_2\tau_4\tau_5\tau_6 x_5 x_6.$$

By letting $\tau_i = 1$, for $i = 1, \ldots, 6$ and $x_3 = 100, x_4 = 10, x_5 = 10, x_6 = 1$, this determinant is -280, which is negative. This implies that the stoichiometric compatibility class containing $\phi(100, 10, 10, 1)$ has more than one positive steady state. Specifically, this class corresponds to $T_1 = 320, T_2 = 210, T_3 = 10, T_4 = 1$. Either by solving the steady state equations or finding the roots of $F_{T,1}(\varphi(z))$ for this choice of parameters, we confirm that the system has three positive steady states.

Therefore, the reduced network is bistable for all choice of parameter values for which there are three positive steady states, and the original network admits bistability in some region of the parameter space.

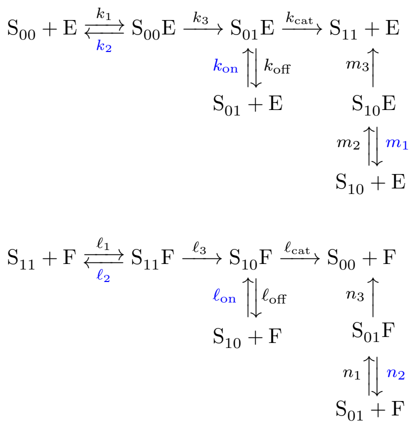


Figure 2.6: ERK regulation network.

2.2.2 Bistability and oscillations in a model of ERK regulation

This is a joint project with Anne Shiu and Nida Obatake from Texas A&M and with Xiaoxian Tang from Beihang University. In this project we study the presence of bistability and oscillations in the network in Figure 2.6. This network comprises extracellular signal-regulated kinase (ERK) regulation by dual-site phosphorylation by the kinase MEK (denoted by E) and dephosphorylation by the phosphatase MKP3 (F) [52]. This network, which we call the ERK network, has an important role in regulating many cellular activities, with dysregulation implicated in many cancers [55]. Accordingly, an important problem is to understand the dynamical properties of the ERK network, with the goal of predicting effects arising from mutations or drug treatments [26]. It had been proven by Rubinstein, Mattingly, Berezhkovskii, and Shvartsman [52] that this network exhibits bistability and oscillations for some choice of rate constants. However, when the reaction rates k_{cat} and ℓ_{cat} are much larger than k_{off} and ℓ_{off}, then the behaviour of the network tends to the fully processive network (obtained from the figure by removing all the vertical reactions) that does not have the capacity for multistationarity. In this project we studied how bistability and oscillations are preserved or lost under structural modifications of the network. The aim with this work was to give an insight on how bistability arises from the fully processive network to the full ERK regulation network.

We found that bistability only arises when the reaction rates k_{on} or ℓ_{on} are positive. If both of these reactions are removed from the network, then multistationarity is lost, and thus bistability. Opposite to this, the presence of oscillations is more robust under modifications. We found oscillations in the full network, an irreversible version of the network (obtained by removing all the reactions with blue labels on Figure 2.6) and in a reduced model depicted in Figure 2.7. To prove that oscillations arise in the different

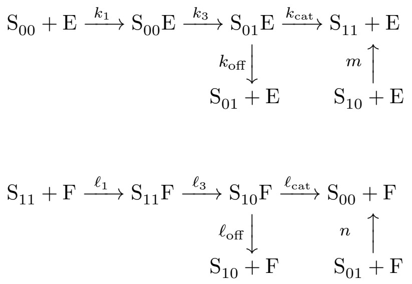


Figure 2.7: Reduced model of ERK regulation obtained by removing reverse reactions and intermediate species.

models, we used algebraic parametrizations of the steady state varieties, the Hurwitz determinants associated to the characteristic polynomials of the Jacobian associated with each network and the Newton polytopes and outer normal cones arising from the Hurwitz determinants.

The use of the outer normal cones and the Newton polytope allowed us to compute effectively parameter values and positive steady states where a Hopf bifurcation appeared and generated oscillatory behaviour in the different systems.

2.2.3 Hopf bifurcations in MAPK cascade

Mitogen-activated protein kinase (MAPK) cascades represent a step of chemical signal transduction in cellular systems. They are involved in various positive and negative feedback loops regulating intracellular processes and, even though they are interconnected with other networks, the study of the cascade by itself has proven to be of great importance [31, 34, 42, 51].

Consider the Huang-Ferrell model on the left part of Figure 2.9. The building blocks of this network are Michaelis-Menten mechanisms in each layer. The labelled curved arrows linking a substrate and its phosphorylation, represent each catalysis as presented in Figure 2.8. Additionally, the product of each layer, acts as an enzyme for the next layer.

This cascade has three layers. In [39] it was shown that actually having a negative feedback as depicted on the right side of Figure 2.9 produced oscillatory behaviour. Additionally, in [42] it was proven that actually bistability arises only in the network circled in the right side of Figure 2.9, and it was believed that this bistability was the cause for the oscillatory behaviour in the MAPK cascade as presented on the left side. In this work we proved that actually the network depicted in Figure 2.10 has oscillatory behaviour arising from a Hopf bifurcation. It is known that this network is monostationary; however the stability of the steady states had not been stablished. Using Propositions 4 and 5 we actually found a set of parameters and a steady state such that the network admits Hopf bifurcations. This precludes the possibility of the steady states being asymptotically stable. We now expand the details of the procedure. The network in Figure 2.10 represents the following set of reactions.

$$\begin{aligned} \mathrm{A}+\mathrm{E} &\rightleftharpoons \mathrm{AE} \longrightarrow \mathrm{A_p} \\ \mathrm{A_p}+\mathrm{F_1} &\rightleftharpoons \mathrm{A_p} \longrightarrow \mathrm{A}+\mathrm{F_1} \\ \mathrm{B}+\mathrm{A_p} &\rightleftharpoons \mathrm{BA_p} \longrightarrow \mathrm{B_p}+\mathrm{A_p} \\ \mathrm{B_p}+\mathrm{F_2} &\rightleftharpoons \mathrm{B_pF_2} \longrightarrow \mathrm{B}+\mathrm{F_2}. \end{aligned}$$

We consider the reduced network obtained by removing the reverse reactions and the intermediate $\mathrm{B_pF_2}$ as explained in Section 1.4.1, obtaining the network

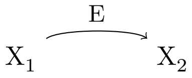
$$\begin{aligned} \mathrm{A}+\mathrm{E} &\xrightarrow{\kappa_1} \mathrm{AE} \xrightarrow{\kappa_2} \mathrm{A_p} \\ \mathrm{A_p}+\mathrm{F_1} &\xrightarrow{\kappa_3} \mathrm{A_p} \xrightarrow{\kappa_4} \mathrm{A}+\mathrm{F_1} \\ \mathrm{B}+\mathrm{A_p} &\xrightarrow{\kappa_5} \mathrm{BA_p} \xrightarrow{\kappa_6} \mathrm{B_p}+\mathrm{A_p} \\ \mathrm{B_p}+\mathrm{F_2} &\xrightarrow{\kappa_7} \mathrm{B}+\mathrm{F_2}. \end{aligned}$$

This network has 10 species, its stoichiometric subspace has dimension 5, and the positive steady state variety can be parametrized as

$$\varphi = \left(\frac{\kappa_3\kappa_6 x_4 x_7 x_6}{\kappa_4 x_3 \kappa_1 x_5}, \frac{\kappa_6 x_4 x_7}{\kappa_4 x_3}, x_3, x_4, x_5, x_6, x_7, \frac{\kappa_3\kappa_6 x_4 x_7 x_6}{\kappa_4 x_3 \kappa_2}, \frac{\kappa_3\kappa_6 x_4 x_7 x_6}{\kappa_4 x_3 \kappa_7}, \frac{\kappa_6 x_4 x_7}{\kappa_5} \right).$$

The characteristic polynomial p associated with the Jacobian of the rate function evaluated at $\varphi(x)$, has degree 5. For studying the sign of the real part of p, we use the Hurwitz criterion 15. For this polynomial we found that all the Hurwitz determinants were rational functions with positive coefficients, except for the second to last determinant, H_4. This determinant has monomials with positive and negative signs. We then used the Newton Polytope and Propositions 5 and 4 to find values for the reaction rate constants and a steady state where $H_4(\varphi) = 0$, and a Hopf bifurcation arises. The values that we found for the reaction rate constants are are follows,

$$\begin{aligned} \kappa_1 =& 1, \kappa_3 = 1, k_2 = 0.0002212391389, \kappa_4 = 2.613954709 * 10^{10}, \\ \kappa_5 =& 1, \kappa_6 = 7.283594782 * 10^{-6}, k_7 = 0.0002212391389, \end{aligned}$$



represents $\mathrm{X_1} + \mathrm{E} \rightleftharpoons \mathrm{X_1E} \longrightarrow \mathrm{X_2} + \mathrm{E}$

Figure 2.8: Two species $\mathrm{X_1}$ and $\mathrm{X_2}$ linked by a curved arrow with label E, denote the Michaelis-Menten mechanism where the transformation of $\mathrm{X_1}$ into $\mathrm{X_2}$ is catalysed by the enzyme E, as depicted on the right.

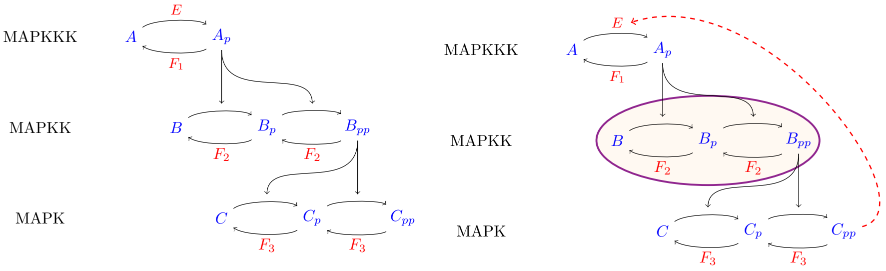


Figure 2.9: on the left we have MAPK cascade. Huang, Ferrell model. On the right a MAPK cascade. Huang, Ferrell model with negative feedback. The circled network is bistable.

and the steady state is

$$[A] = 3.694678854 \times 10^{-8}, [A_p] = 1.768358432 \times 10^{-11}, [B] = 0.00001575714096, [B_p] = 1, [E] = 0.0004786230419,$$
$$[F_1] = 1, [F_2] = 1, [AE] = 7.992972856 \times 10^{-8}, [A_pF_1] = 7.992972856 \times 10^{-8}, [BA_p] = 7.283594782 \times 10^{-6},$$

where $[X]$ denotes the concentration of species X. So far, with this set of parameters, we found an approximation to a Hopf bifurcation for the system. However, we have not been able to numerically visualize the oscillations. We believe that this is due to a numerical issue as the parameters are either too big or too small. This is still work in progress and we will explore different methods for finding a better approximation of a Hopf bifurcation.

2.2.4 Characteristic polynomial in Chemical Reaction Networks

Consider the chemical reaction network $\mathcal{N}_1$ given by

$$\alpha_1 X_1 + \alpha_2 X_2 + \cdots + \alpha_t X_t \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} X_n \xrightarrow{\kappa_3} \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_{n-1} X_{n-1}. \tag{2.7}$$

In this network the set of species $\{X_1, \dots, X_n\}$ is ordered in such a way that the first t species form the reactant and the last species, X_n, is an intermediate.

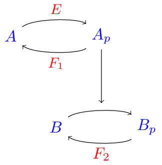


Figure 2.10: MAPK cascade.

Consider now the network $\mathcal{N}_2$ given by

$$\alpha_1 X_1 + \alpha_2 X_2 + \cdots + \alpha_t X_t \xrightarrow{\tau_1} X_n \xrightarrow{\tau_2} \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_{n-1} X_{n-1}. \tag{2.8}$$

$\mathcal{N}_2$ is a subnetwork of $\mathcal{N}_1$ with the same stochiometric subspace, this implies that $\mathcal{N}_1$ has at least as many steady states as $\mathcal{N}_2$ as mentioned in Section 1.4.1. In this particular case, the parameters can be chosen in such a way that the positive steady states are actually the same. With this in mind we now pursue the following question: given κ_1, κ_2 and κ_3 is it possible to find values for τ_1 and τ_2, such that both networks have the same characteristic polynomial arising from the Jacobian of their rate function? Furthermore, given a chemical reaction network $\mathcal{N}$ that has $\mathcal{N}_1$ as a subnetwork, is it possible to find a set of parameters for $\mathcal{G}$ such that the characteristic polynomials of $\mathcal{N}$ and the reaction obtained by removing the same reverse reaction in $\mathcal{N}_1$, are equal? These questions are directed to explore whether removing the reverse reaction with label κ_2 could change the stability properties of the steady states.

Note that if it is possible to find parameters that preserve both characteristic polynomials, the stability of the steady states will be the same for both networks. This means that we are comparing the families of networks given by varying parameters, in particular explore whether the stability properties of the network and possible behaviours are the same.

Networks $\mathcal{N}_1$ and $\mathcal{N}_2$

In this section we prove that, under certain condition, the first question above has an affirmative answer. Specifically, we prove the following theorem.

Theorem 17. *Consider the networks $\mathcal{N}_1$ and $\mathcal{N}_2$ as in (2.7) and (2.8), and let f_1 and f_2 be their rate functions. Then, given positive parameters κ_1, κ_2 and κ_3, it is possible to find values τ_1 and τ_2 for $\mathcal{N}_2$ such that the characteristic polynomial of J_{f_1} and J_{f_2} are equal.*

Before proving the theorem, we find the system of ODEs modelling the dynamics of each network and compute the corresponding characteristic polynomials. Under mass action kinetics the system of ODEs associated with $\mathcal{N}_1$ is given by

$$\begin{aligned}
\dot{x_i} &= -\alpha_i \kappa_1 x_1^{\alpha_1} x_2^{\alpha_2} \cdots x_t^{\alpha_t} + \alpha_i \kappa_2 x_n + \beta_i \kappa_3 x_n && \text{for } i = 1, \ldots, t \\
\dot{x_j} &= \beta_j \kappa_3 x_j && \text{for } j = t+1, \ldots, n-1 \\
\dot{x_n} &= \kappa_1 x_1^{\alpha_1} x_2^{\alpha_2} \cdots x_t^{\alpha_t} - \kappa_2 x_n - \kappa_3 x_n.
\end{aligned}$$

The steady state equations can be written as

$$\begin{pmatrix} 0 \\ \vdots \\ 0 \end{pmatrix} = f_1(x) = \begin{pmatrix} -\alpha_1 & \alpha_1 & \beta_1 \\ -\alpha_2 & \alpha_2 & \beta_2 \\ \vdots & \vdots & \vdots \\ -\alpha_t & \alpha_t & \beta_t \\ 0 & 0 & \beta_{t+1} \\ \vdots & \vdots & \vdots \\ 0 & 0 & \beta_{n-1} \\ 1 & -1 & -1 \end{pmatrix} \begin{pmatrix} \kappa_1 x_1^{\alpha_1} x_2^{\alpha_2} \cdots x_t^{\alpha_t} \\ \kappa_2 x_n \\ \kappa_3 x_n \end{pmatrix},$$

and the stochiometric matrix has rank at most 2. With these steady state equations, the positive steady state variety can be parametrized as

$$\varphi(x_1, x_2, \ldots, x_{n-1}) = \left(x_1, x_2, \ldots, x_{n-1}, \frac{\kappa_1}{\kappa_2 + \kappa_3} x_1^{\alpha_1} x_2^{\alpha_2} \cdots x_t^{\alpha_t} \right)$$

and the stability of a positive steady state $x^* = \varphi(x_1, x_2, \ldots, x_{n-1})$ is given by the roots of the characteristic polynomial of $J_f(x^*)$.

If we denote by $\mathtt{G}_1$ the gradient of $\kappa_1 x_1^{\alpha_1} x_2^{\alpha_2} \cdots x_t^{\alpha_t}$ with respect to the variables $x_1, \ldots, x_{n-1}$, we can write $J_f(x)$ as

$$J_1 = \begin{pmatrix} -\alpha_1 \mathtt{G}_1 & \alpha_1 \kappa_2 + \beta_1 \kappa_3 \\ -\alpha_2 \mathtt{G}_1 & \alpha_2 \kappa_2 + \beta_2 \kappa_3 \\ \vdots & \vdots \\ -\alpha_t \mathtt{G}_1 & \alpha_t \kappa_2 + \beta_t \kappa_3 \\ 0 & \beta_{t+1} \kappa_3 \\ \vdots & \vdots \\ 0 & \beta_{n-1} \kappa_3 \\ \mathtt{G}_1 & -\kappa_2 - \kappa_3 \end{pmatrix},$$

taking into account that $\mathtt{G}_1$ is a row vector of length $n-1$ where the last $n-1-t$ entries are zero.

In order to compute the characteristic polynomial of J_1, it is necessary to evaluate the Jacobian in the steady state. In this case, after evaluating J_1 in the parametrization, we obtain the same matrix, because the variable x_n does not appear in J_1. Therefore, the characteristic polynomial of J_1 is

$$p_1(\lambda) = \lambda^n + c_1 \lambda^{n-1} + c_2 \lambda^{n-2} \tag{2.9}$$

where

$$c_1 = \kappa_2 + \kappa_3 + \kappa_1 \sum_{i=1}^{t} \alpha_i^2 x_1^{\alpha_1} \cdots x_i^{\alpha_i - 1} \cdots x_t^{\alpha_t} \qquad \text{and} \qquad c_2 = \kappa_1 \kappa_3 \sum_{i=1}^{t} (\alpha_i - \beta_i) \alpha_i x_1^{\alpha_1} \cdots x_i^{\alpha_i - 1} \cdots x_t^{\alpha_t}.$$

Recall that c_1 is the sum of the diagonal of J_1 and c_2 corresponds to the sum of all the principal minors of J_1 of size 2×2. Since the rank of the stoichiometric matrix is at most 2, all the minors of size greater or equal than 3 are zero, which implies that the coefficients of λ^ℓ are equal to zero for $\ell > n-2$ (see [48]).

Following the same procedure to compute the characteristic polynomial associated with $\mathcal{N}_2$, we have the steady state equations given by

$$\begin{pmatrix} 0 \\ \vdots \\ 0 \end{pmatrix} = \begin{pmatrix} -\alpha_1 & \beta_1 \\ -\alpha_2 & \beta_2 \\ \vdots & \vdots \\ -\alpha_t & \beta_t \\ 0 & \beta_{t+1} \\ \vdots & \vdots \\ 0 & \beta_{n-1} \\ 1 & -1 \end{pmatrix} \begin{pmatrix} \tau_1 x_1^{\alpha_1} x_2^{\alpha_2} \cdots x_t^{\alpha_t} \\ \tau_2 x_n \end{pmatrix}.$$

The Jacobian J_2 can be written as

$$J_2 = \begin{pmatrix} -\alpha_1 \mathtt{G}_2 & \beta_1 \tau_2 \\ -\alpha_2 \mathtt{G}_2 & \beta_2 \tau_2 \\ \vdots & \vdots \\ -\alpha_t \mathtt{G}_2 & \beta_t \tau_2 \\ 0 & \beta_{t+1} \tau_2 \\ \vdots & \vdots \\ 0 & \beta_{n-1} \tau_2 \\ \mathtt{G}_2 & -\tau_2 \end{pmatrix},$$

where $\mathtt{G}_2$ is the gradient of $\tau_1 x_1^{\alpha_1} x_2^{\alpha_2} \cdots x_t^{\alpha_t}$ with respect to $x_1, \dots, x_{n-1}$. The characteristic polynomial is

$$(\lambda) = \lambda^n + d_1 \lambda^{n-1} + d_2 \lambda^{n-2} \tag{2.10}$$

where

$$d_1 = \tau_2 + \tau_1 \sum_{i=1}^{t} \alpha_i^2 x_1^{\alpha_1} \cdots x_i^{\alpha_i - 1} \cdots x_t^{\alpha_t} \qquad \text{and} \qquad d_2 = \tau_1 \tau_2 \sum_{i=1}^{t} (\alpha_i - \beta_i) \alpha_i x_1^{\alpha_1} \cdots x_i^{\alpha_i - 1} \cdots x_t^{\alpha_t}.$$

With these computations, we proceed to prove the theorem.

Proof. **Case 1: $\alpha_i = \beta_i$ for every $i = 1, \dots, t$.** If $\alpha_i = \beta_i$ for every $i = 1, \dots, t$ the coefficients c_2 and d_2, of p_1 and p_2 respectively, are equal to zero, therefore it is only necessary to study the coefficients c_1 and d_1. Consider the functions

$$\Phi_\kappa : \mathbb{R}^3_{>0} \times \mathbb{R}^{n-1}_{>0} \longrightarrow \mathbb{R}_{>0} : (\kappa_1, \kappa_2, \kappa_3, x_1, \dots, x_{n-1}) \mapsto c_1$$

and

$$\Phi_\tau : \mathbb{R}^2_{>0} \times \mathbb{R}^{n-1}_{>0} \longrightarrow \mathbb{R}_{>0} : (\tau_1, \tau_2, x_1, \dots, x_{n-1}) \mapsto d_1.$$

If we show that $Im(\Phi_\kappa) = Im(\Phi_\tau)$, we can conclude that given a set of parameters τ_1, τ_2 for $\mathcal{N}_2$ it is possible to find parameters $\kappa_1, \kappa_2, \kappa_3$ for $\mathcal{N}_1$ such that $c_1 = d_1$. In this case we can prove that these functions are actually surjective: Let $a \in \mathbb{R}_{>0}$ and consider the vector $(\kappa_1, \kappa_2, \kappa_3, \frac{1}{s}, \frac{1}{s}, \dots, \frac{1}{s})$. We have that

$$\begin{aligned}\Phi_\kappa\left(\kappa_1, \kappa_2, \kappa_3, \frac{1}{s}, \frac{1}{s}, \dots, \frac{1}{s}\right) =& \kappa_2 + \kappa_3 + \kappa_1 \sum_{i=1}^{t} \alpha_i^2 \left(\frac{1}{s}\right)^{\alpha_1} \cdots \left(\frac{1}{s}\right)^{\alpha_i - 1} \cdots \left(\frac{1}{s}\right)^{\alpha_t} \\ =& \kappa_2 + \kappa_3 + \kappa_1 \sum_{i=1}^{t} \alpha_i^2 \left(\frac{1}{s}\right)^{\alpha_1 + \cdots + \alpha_t - 1} \\ =& \kappa_2 + \kappa_3 + \kappa_1 \left(\frac{1}{s}\right)^{\alpha_1 + \cdots + \alpha_t - 1} \sum_{i=1}^{t} \alpha_i^2.\end{aligned}$$

If $\alpha_1 + \cdots + \alpha_t = 1$, then

$$\Phi_\kappa\left(\kappa_1, \kappa_2, \kappa_3, \frac{1}{s}, \frac{1}{s}, \dots, \frac{1}{s}\right) = \kappa_2 + \kappa_3 + \kappa_1 \sum_{i=1}^{t} \alpha_i^2$$

and taking $\kappa_2 = \kappa_3 = \frac{a}{3}$ and $\kappa_1 = \frac{a}{3\sum_{i=1}^{t} \alpha_i^2}$ we found an element in $\mathbb{R}^3_{>0} \times \mathbb{R}^{n-1}_{>0}$ such that $\Phi_\kappa\left(\kappa_1, \kappa_2, \kappa_3, \frac{1}{s}, \frac{1}{s}, \dots, \frac{1}{s}\right) = a$.

If $\alpha_1 + \cdots + \alpha_t > 1$, then we can take $\kappa_1 = 1$ and $\kappa_2 = \kappa_3 = \frac{a}{3}$ and find c such that

$$\left(\frac{1}{c}\right)^{\alpha_1 + \cdots + \alpha_t - 1} \sum_{i=1}^{t} \alpha_i^2 = \frac{1}{3},$$

which gives us an element in $\mathbb{R}^3_{>0} \times \mathbb{R}^{n-1}_{>0}$ such that $\Phi_\kappa\left(\kappa_1, \kappa_2, \kappa_3, \frac{1}{s}, \frac{1}{s}, \dots, \frac{1}{s}\right) = a$.

With an analogous procedure it is possible to show that the function Φ_τ is surjective over $\mathbb{R}_{>0}$. This implies that $Im(\Phi_\kappa) = Im(\Phi_\tau)$ and it is possible to find parameters for $\mathcal{N}_1$ and $\mathcal{N}_2$ such that $p_1 = P_2$.

Case 2: $\alpha_i \neq \beta_i$ for some $i = 1, \dots, t$. If $\alpha_i \neq \beta_i$ for some $i = 1, \dots, t$, the coefficients c_2 and d_2 of p_1 and p_2 respectively, are different from zero. We define the functions

$$\Phi_\kappa : \mathbb{R}^3_{>0} \times \mathbb{R}^{n-1}_{>0} \longrightarrow \mathbb{R}^2_{>0} : (\kappa_1, \kappa_2, \kappa_3, x_1, \dots, x_{n-1}) \mapsto (c_1, c_2)$$

and

$$\Phi_\tau : \mathbb{R}^2_{>0} \times \mathbb{R}^{n-1}_{>0} \longrightarrow \mathbb{R}^2_{>0} : (\tau_1, \tau_2, x_1, \dots, x_{n-1}) \mapsto (d_1, d_2).$$

Our goal is to prove that $Im(\Phi_\kappa) \subset Im(\Phi_\tau)$. Consider an element $(a, b) \in Im(\Phi_\kappa)$. If $(a, b) \in Im(\Phi_\kappa)$ there exist $(\kappa_1, \kappa_2, \kappa_3, x_1 \dots, x_{n-1}) \in \mathbb{R}^3_{>0} \times \mathbb{R}^{n-1}_{>0}$ such that

$$\Phi_\kappa(\kappa_1, \kappa_2, \kappa_3, x_1 \dots, x_{n-1}) = (a, b)$$

, in particular, this implies that $a > 0$ for every element in $Im(\Phi_\kappa)$. Now, note that from the definition of b we have that

$$\kappa_3 = \frac{b}{\kappa_1 \sum_{i=1}^{t} (\alpha_i - \beta_i)\alpha_i x_1^{\alpha_1} \cdots x_i^{\alpha_i - 1} \cdots x_t^{\alpha_t}}.$$

Replacing the value of κ_3 in the definition of a we get the following identity

$$\kappa_1^2 \left(\sum_{i=1}^{t} \alpha_i^2 x_1^{\alpha_1} \cdots x_i^{\alpha_i - 1} \cdots x_t^{\alpha_t} \right) \left(\sum_{i=1}^{t} (\alpha_i - \beta_i)\alpha_i x_1^{\alpha_1} \cdots x_i^{\alpha_i - 1} \cdots x_t^{\alpha_t} \right) - \kappa_1(a - \kappa_2) \left(\sum_{i=1}^{t} (\alpha_i - \beta_i)\alpha_i x_1^{\alpha_1} \cdots x_i^{\alpha_i - 1} \cdots x_t^{\alpha_t} \right) + b = 0.$$

To simplify the notation we will factor $X := x_1^{\alpha_1 - 1} \cdots x_i^{\alpha_i - 1} \cdots x_t^{\alpha_t - 1}$ from the sums and we will denote the monomial $x_1 \cdots x_{i-1} x_{i+1} \cdots x_t$ by $\overline{x_i}$, so we can write the expression above as

$$\kappa_1^2 X^2 \left(\sum_{i=1}^{t} \alpha_i^2 \overline{x_i} \right) \left(\sum_{i=1}^{t} (\alpha_i - \beta_i)\alpha_i \overline{x_i} \right) - \kappa_1(a - \kappa_2) X \left(\sum_{i=1}^{t} (\alpha_i - \beta_i)\alpha_i \overline{x_i} \right) + b = 0.$$

Since $\kappa_1 > 0$ is a component of the preimage of (a, b), this specific value of κ_1 is a positive root of the quadratic equation in κ_1 given by this identity. In particular this means that

$$(a - \kappa_2)^2 X^2 \left(\sum_{i=1}^{t} (\alpha_i - \beta_i)\alpha_i \overline{x_i} \right)^2 - 4bX^2 \left(\sum_{i=1}^{t} \alpha_i^2 \overline{x_i} \right) \left(\sum_{i=1}^{t} (\alpha_i - \beta_i)\alpha_i \overline{x_i} \right) \geq 0$$

and, given that $a^2 > (a - \kappa_2)^2$, we have

$$a^2 X^2 \left(\sum_{i=1}^{t} (\alpha_i - \beta_i)\alpha_i \overline{x_i} \right)^2 - 4bX^2 \left(\sum_{i=1}^{t} \alpha_i^2 \overline{x_i} \right) \left(\sum_{i=1}^{t} (\alpha_i - \beta_i)\alpha_i \overline{x_i} \right) \geq 0.$$

This is precisely the discriminant of the quadratic equation on τ_1

$$\tau_1^2 X^2 \left(\sum_{i=1}^{t} \alpha_i^2 \overline{x_i} \right) \left(\sum_{i=1}^{t} (\alpha_i - \beta_i)\alpha_i \overline{x_i} \right) - \tau_1 a X \left(\sum_{i=1}^{t} (\alpha_i - \beta_i)\alpha_i \overline{x_i} \right) + b = 0. \tag{2.11}$$

Hence, this equation has real solutions. If we prove that one of these solutions is positive, taking τ_1 as this root, and taking

$$\tau_2 = \frac{b}{\tau_1 X \sum_{i=1}^{t}(\alpha_i - \beta_i)\alpha_i \overline{x_i}}$$

we find an element $(\tau_1, \tau_2, x_1, \dots, x_{n-1}) \in \mathbb{R}^2_{>0} \times \mathbb{R}^{n-1}_{>0}$ such that $\Phi_\tau(\tau_1, \tau_2, x_1, \dots, x_{n-1}) = (a, b)$.

In order to prove that (2.11) has a positive root, notice that b and $\sum_{i=1}^{t}(\alpha_i - \beta_i)\alpha_i \overline{x_i}$ have the same sign, this means that the signs of the coefficients of (2.11) alternate. If both roots were negative, all the coefficients of the polynomial would be positive, which contradicts the definition of b.

We have proven that $Im(\Phi_\kappa) \subset Im(\Phi_\tau)$. Which completes the proof of the theorem.

□

Network $\mathcal{N}_1$ as a subnetwork of a bigger network $\mathcal{N}$

We consider the network $\mathcal{N}_1$ as a subnetwork of another network $\mathcal{N}$. In particular, we assume that $\mathcal{N}_1$ is one linkage class of $\mathcal{N}$. Removing the reaction labelled with κ_2 in $\mathcal{N}_1$, produces a subnetwork $\mathcal{N}'$ of $\mathcal{N}$, that has $\mathcal{N}_2$ as one of its linkage classes. The goal now is to study whether, given a set of parameters for $\mathcal{N}$, it is possible to find a set of parameters for $\mathcal{N}'$ such that the characteristic polynomials coming from their Jacobian, are the same. With this in mind, we proved the following theorem.

Theorem 18. *Let the networks $\mathcal{N}_1$ and $\mathcal{N}_2$ be the reactions (2.7) and (2.8). Let $\mathcal{N}$ be a network that has $\mathcal{N}_1$ as a linkage class, that is $\mathcal{N} = \mathcal{N}_1 \cup G$ for some reaction network G, and consider $\mathcal{N}'$ as the subnetwork of $\mathcal{N}$ defined as $\mathcal{N}' = \mathcal{N}_2 \cup G$. If the reactant of $\mathcal{N}_1$ and G do not have any species in common, then given a set of positive reaction rate constants for $\mathcal{N}$, there exists a set of positive values for the reaction rate constants of $\mathcal{N}'$ such that the characteristic polynomials associated with both networks are equal.*

Proof. Assume that the set of species is ordered in a way such that the first s species and X_n are the species in the reactant of $\mathcal{N}_1$. This means that the species $X_{s+1}, \dots, X_{n-1}$ might be part of complexes in G. The rate function of $\mathcal{N}$ is given by

$$f(x_1, \dots, x_n) = \left(\begin{array}{ccc|c} -\alpha_1 & \alpha_1 & \beta_1 & \\ \vdots & \vdots & \vdots & \mathbf{0} \\ -\alpha_s & \alpha_s & \beta_s & \\ \hline 0 & 0 & \beta_{s+1} & \\ \vdots & \vdots & \vdots & N_G \\ 0 & 0 & \beta_{n-1} & \\ \hline 1 & -1 & -1 & 0 \ \cdots \ 0 \end{array}\right) \begin{pmatrix} \kappa_1 x_1^{\alpha_1} x_2^{\alpha_2} \cdots x_s^{\alpha_s} \\ \kappa_2 x_n \\ \kappa_3 x_n \\ v_G \end{pmatrix}$$

where v_G is the vector of kinetics for reactions in G and N_G are the rows of the stoichiometric matrix of G. The equation for the concentration of X_n is

$$\dot{x_n} = \kappa_1 x_1^{\alpha_1} \cdots x_s^{\alpha_s} - (\kappa_2 + \kappa_3)x_n,$$

so the positive steady state variety is contained in the variety parametrized as

$$x_n = \frac{\kappa_1}{(\kappa_2 + \kappa_3)} x_1^{\alpha_1} \cdots x_s^{\alpha_s}.$$

Note that under these conditions, the monomials in v_G do not involve any of the variables $x_1, \dots, x_s$.

To simplify the notation on the computation of the Jacobian, we denote by $\mathtt{G}_1$ the gradient of $\kappa_1 x_1^{\alpha_1} x_2^{\alpha_2} \cdots x_s^{\alpha_t}$ with respect to the variables $x_1, \dots, x_t$. We have that J_f is equal to

$$J_f = \left(\begin{array}{c|c|c} -\alpha_1 \mathtt{G}_1 & & -\alpha_1\kappa_2 + \beta_1\kappa_3 \\ \vdots & \mathbf{0} & \vdots \\ -\alpha_t \mathtt{G}_1 & & -\alpha_t\kappa_2 + \beta_1\kappa_3 \\ \hline 0 & & \beta_{t+1}\kappa_3 \\ \vdots & N_G J_{v_G} & \vdots \\ 0 & & \beta_n\kappa_3 \\ \hline \mathtt{G}_1 & 0\cdots0 & -\kappa_2 - \kappa_3 \end{array} \right).$$

Reordering of the rows and columns we get the following block matrix

$$J_f = \left(\begin{array}{cc|c} -\alpha_1 \mathtt{G}_1 & -\alpha_1\kappa_2 + \beta_1\kappa_3 & \\ \vdots & \vdots & \mathbf{0} \\ -\alpha_t \mathtt{G}_1 & -\alpha_t\kappa_2 + \beta_1\kappa_3 & \\ \mathtt{G}_1 & -\kappa_2 - \kappa_3 & \\ \hline 0 & \beta_{t+1}\kappa_3 & \\ \vdots & \vdots & N_G J_{v_G} \\ 0 & \beta_n\kappa_3 & \end{array} \right).$$

This is a block lower-triangular matrix, and so is $\lambda I - J_f$, which implies that the characteristic polynomial of J_f is equal to de product of the characteristic polynomial of the blocks in the diagonal. The characteristic polynomial associated with $\mathcal{N}_1$ is given by the characteristic polynomial of the matrix

$$\left(\begin{array}{cc|c}
-\alpha_1 \mathtt{G}_1 & -\alpha_1\kappa_2+\beta_1\kappa_3 & \\
\vdots & \vdots & \mathbf{0} \\
-\alpha_t \mathtt{G}_1 & -\alpha_t\kappa_2+\beta_1\kappa_3 & \\
\mathtt{G}_1 & -\kappa_2-\kappa_3 & \\
\hline
0 & \beta_{t+1}\kappa_3 & 0\cdots 0 \\
\vdots & \vdots & \vdots \\
0 & \beta_n\kappa_3 & 0\cdots 0
\end{array}\right).$$

This is also a block lower-triangular matrix that has the characteristic polynomial of the first block as a factor. This factor determines the coefficients of the characteristic polynomial, this means that this factor is equal to the polynomial $p_1(\lambda) = \lambda^2 + c_1\lambda + c_2$ in (2.9) defined before for $\mathcal{N}_1$. We have proven that there exist a set of parameters τ_1, τ_2 such that the characteristic polynomial of $\mathcal{N}_2$ has the same coefficients of the characteristic polynomial of $\mathcal{N}_2$, so using these parameters for $\mathcal{N}_2$ and the same parameters for G we have that the characteristic polynomials of $\mathcal{N}_1 \cup G$ and $\mathcal{N}_2 \cup G$ have the same coefficients. □

Now, if we consider the case where the reactant of $\mathcal{N}_1$ and G have species in common, we do not get a block matrix when computing the associated Jacobian. However, for the case where the species in the reactant of $\mathcal{N}_1$ appear only in the products of G, it was possible for us to give an insight on whether it was possible to find parameters that preserve the characteristic polynomial. Namely, consider an order of the set of species such that the first s species are in $\mathcal{N}_1$, additionally, from this s species, the first t appear only in the reactant of $\mathcal{N}_1$. This implies that the stoichiometric coefficient of the variables $x_1, \ldots, x_t$ is equal to 0 in G. In this case, the rate function is

$$f(x_1,\ldots,x_n) = \left(\begin{array}{ccc|c}
-\alpha_1 & \alpha_1 & \beta_1 & \\
\vdots & \vdots & \vdots & \mathbf{0} \\
-\alpha_s & \alpha_s & \beta_s & \\
\hline
-\alpha_{s+1} & \alpha_{s+1} & \beta_{s+1} & \\
\vdots & \vdots & \vdots & N_G[s+1,\ldots,t] \\
-\alpha_t & \alpha_t & \beta_t & \\
\hline
0 & 0 & \beta_{t+1} & \\
\vdots & \vdots & \vdots & N_G[t+1,\ldots,n] \\
0 & 0 & \beta_{n-1} & \\
\hline
1 & -1 & -1 & 0 \quad \cdots \quad 0
\end{array}\right)
\begin{pmatrix}
\kappa_1 x_1^{\alpha_1} x_2^{\alpha_2} \cdots x_t^{\alpha_t} \\
\kappa_2 x_n \\
\kappa_3 x_n \\
v_G
\end{pmatrix}$$

Where v_G is the vector of kinetics for reactions in G and $N_G[t+1,\ldots,s]$ and $N_G[s+1,\ldots,n]$ are the rows of the stoichiometric matrix of G. Note that the vector v_G does not depend on the variables $x_1,\ldots,x_t$. Denoting by $\mathtt{G}_1$ the gradient of $\kappa_1 x_1^{\alpha_1} x_2^{\alpha_2} \cdots x_s^{\alpha_t}$

with respect to the variables $x_1, \dots, x_s$, and by $\mathtt{G}_2$ the gradient of $\kappa_1 x_1^{\alpha_1} x_2^{\alpha_2} \cdots x_t^{\alpha_t}$ with respect to the variables $x_{s+1}, \dots, x_t$, we can write the Jacobian of f as follows.

$$J_f = \left(\begin{array}{c|c|c|c}
-\alpha_1\mathtt{G}_1 & -\alpha_1\mathtt{G}_2 & & \alpha_1\kappa_2+\beta_1\kappa_3 \\
\vdots & \vdots & \mathbf{0} & \vdots \\
-\alpha_s\mathtt{G}_1 & -\alpha_s\mathtt{G}_2 & & \alpha_s\kappa_2+\beta_s\kappa_3 \\ \hline
-\alpha_{s+1}\mathtt{G}_1 & -\alpha_{s+1}\mathtt{G}_2 & & \alpha_{s+1}\kappa_2+\beta_{s+1}\kappa_3 \\
\vdots & \vdots & N_G[s+1,\dots,t]J_{v_G} & \vdots \\
-\alpha_t\mathtt{G}_1 & -\alpha_t\mathtt{G}_2 & & \alpha_t\kappa_2+\beta_t\kappa_3 \\ \hline
0 & 0 & & \beta_{t+1}\kappa_3 \\
\vdots & \vdots & N_G[t+1,\dots,n]J_{v_G} & \vdots \\
0 & 0 & & \beta_{n-1}\kappa_3 \\ \hline
\mathtt{G}_1 & \mathtt{G}_2 & \mathbf{0} & -\kappa_2-\kappa_3
\end{array}\right).$$

As before, this matrix has the same characteristic polynomial as the following matrix obtained by reordering rows and columns

$$\left(\begin{array}{ccc|c}
-\alpha_1\mathtt{G}_1 & -\alpha_1\mathtt{G}_2 & \alpha_1\kappa_2+\beta_1\kappa_3 & \\
\vdots & \vdots & \vdots & \mathbf{0} \\
-\alpha_s\mathtt{G}_1 & -\alpha_s\mathtt{G}_2 & \alpha_s\kappa_2+\beta_s\kappa_3 & \\
-\alpha_{s+1}\mathtt{G}_1 & \alpha_{s+1}\kappa_2+\beta_{s+1}\kappa_3 & -\alpha_{s+1}\mathtt{G}_2 & \\
\vdots & \vdots & \vdots & N_G[s+1,\dots,t]J_{v_G} \\
-\alpha_t\mathtt{G}_1 & \alpha_t\kappa_2+\beta_t\kappa_3 & -\alpha_t\mathtt{G}_2 & \\
\mathtt{G}_1 & \mathtt{G}_2 & -\kappa_2-\kappa_3 & \mathbf{0} \\ \hline
0 & 0 & \beta_{t+1}\kappa_3 & \\
\vdots & \vdots & \vdots & N_G[t+1,\dots,n]J_{v_G} \\
0 & 0 & \beta_{n-1}\kappa_3 &
\end{array}\right).$$

This is a block matrix of the form $\left(\begin{array}{c|c} A & B \\ \hline C & D \end{array}\right)$ where the blocks are defined by the straight lines. Note that A is the matrix associated to the factor of the characteristic polynomial of $\mathcal{N}_1$ that determines its coefficients, that is $\det(\lambda I - A) = \lambda^2 + c_1\lambda + c_2$. To compute the characteristic polynomial associated with $\mathcal{N}_1 \cup G$ we have to compute the determinant of the matrix $\left(\begin{array}{c|c} \lambda I - A & B \\ \hline C & \lambda I - D \end{array}\right)$, which is equal to $\det(\lambda I - A)\det(\lambda I - D - C(\lambda I - A)^{-1}B)$. This could give us conditions for finding matching parameters in some cases. However, this is a work in progress and we will pursue some results on this conditions in the future.

3

Absolute Concentration Robustness

3.1 What is Absolute Concentration Robustness?

In this chapter we focus on Absolute Concentration Robustness (ACR). This property has been studied extensively as it identifies species whose concentration is robust to changes on other concentrations in a system. We start with the definition of ACR and then we discuss previous results and summarize our contributions.

Definition 30. Let $\mathcal{N}$ be a Chemical Reaction Network with fixed kinetics and assume that $\mathcal{N}$ has positive steady states. The network $\mathcal{N}$ has ACR in the species X_i if there exists $c^* \in \mathbb{R}_{>0}$ such that for every positive steady state, the concentration of X_i is equal to c^*.

Having ACR means that the concentration of X_i does not depend on the total amounts of the system, that is, it does not depend on the stoichiometric compatibility class. We consider systems with mass action kinetics. In this case, ACR in a species X_i means that the positive steady state variety is constant in the i-th variable.

As an example consider the network

$$\begin{aligned} \mathrm{X}_1 + \mathrm{X}_2 &\xrightarrow{\kappa_1} 2\,\mathrm{X}_2 \\ \mathrm{X}_2 &\xrightarrow{\kappa_2} \mathrm{X}_1 \end{aligned} \tag{3.1}$$

The steady state equations are

$$\begin{aligned} 0 &= -\kappa_1 x_1 x_2 + \kappa_2 x_2 \\ 0 &= \kappa_1 x_1 x_2 - \kappa_2 x_2, \end{aligned}$$

and its positive steady state variety can be parametrized as

$$\varphi(x_2) = \left(\frac{\kappa_1}{\kappa_2}, x_2\right).$$

From this parametrization we see that the concentration of X_1 at steady state is the same for every stoichiometric compatibility class. This implies, that the network has ACR in X_1.

Detecting whether a network has ACR is not an easy task. However, some structural conditions relying on the deficiency of the network have been shown to ensure the presence of ACR. We now expand on these results and discuss other approaches coming from computational algebraic geometry.

We start with a theorem by Shinar and Feinberg for precluding ACR in networks with deficiency zero[56].

Theorem 19 (Shinar and Feinberg)**.** *Consider a Chemical Reaction Network with mass-action kinetics in which the underlying reaction network is weakly reversible and has a deficiency of zero. Then, no matter what values the rate constants take, there is no species with absolute concentration robustness.*

This result precludes the presence of ACR in any network with deficiency zero whose positive steady state variety is nonempty. For networks with deficiency one, Shinar and Feinberg also proved the following result [56].

Theorem 20 (Shinar and Feinberg)**.** *Consider a Chemical Reaction Network with mass-action kinetics, that admits a positive steady state and for which the underlying reaction network has a deficiency of one. If, in the network, there are distinct non-terminal complexes that differ only in species X_i, then the system has absolute concentration robustness in X_i.*

This last result is one of the most relevant sufficient conditions for ACR. It appears in [38] as a consequence of what they call *Type 1 complex-linear invariants* and it is the main tool on the computational approach for detecting ACR in module-based combinatorial networks presented in [40]. Theorem 20 is a consequence of a more general result.

Theorem 21. *Let $\mathcal{N}$ be a Chemical Reaction Network with mass action kinetics, that admits a positive steady state x^* and for which the underlying reaction network has a deficiency of one. If c, c' are non-terminal complexes, then each positive equilibrium x^{**} of the system satisfies the equation*

$$(c' - c) \cdot \ln\left(\frac{x^{**}}{x^*}\right) = 0,$$

where $\frac{x^{**}}{x^*}$ denotes the vector $\left(\frac{x_1^{**}}{x_1^*}, \dots, \frac{x_n^{**}}{x_n^*}\right)$ and the natural logarithm of the vector corresponds to applying logarithm component-wise.

With this theorem, if two non-terminal complexes only differ in one species $\mathrm{X_i}$, then all the entries of $c' - c$ are zero, except for the i-th entry. This implies that the inner product $(c' - c) \cdot \ln\left(\frac{x^{**}}{x^*}\right) = 0$, becomes

$$(c_i' - c_i)\left(\ln(x_i^{**}) - \ln(x_i^*)\right) = 0.$$

Since $c_i' - c_i \neq 0$, we have that $\ln(x_i^{**}) - \ln(x_i^*) = 0$ and using the injectivity of the logarithm function, we conclude that $x_i^{**} = x_i^*$. This computation is valid for any pair of positive steady states; therefore, the network has ACR in $\mathrm{X_i}$ and Theorem 20 is proved.

ACR is also studied from a purely algebraic point of view in the PhD thesis of Mercedes Pérez Millán [43]. One part of the analysis is based on the radical of the saturation of the steady state ideal with the monomial $x_1 \cdots x_n$; if a univariate polynomial with a unique positive root is contained in this saturation ideal, then the network has ACR. Another part of the analysis consists in defining Complex Absolute Concentration Robustness (CACR) and studying its relation with ACR. Namely, a network with n species has CACR in the species $\mathrm{X_i}$ if there is a constant $c^* \in \mathbb{C} \setminus \{0\}$ such that for every solution $x^* \in \mathbb{C}^n$ of the steady state equations, the value of x_i^* is equal to c^*. If in this definition, the value c^* is real and positive, then the network also has ACR in $\mathrm{X_i}$.

Finally, we mention the work by Tonello and Johnston [60] where a more general type of robustness is considered. In their work, network translations are used to compute robust ratios. In this setting Theorem 20 is also a consequence of a robust ratio between two non-terminal complexes that differ only in one species. In addition to detecting ACR, with the tools proposed in [60], it is possible to find the value of the concentration of the species that exhibits ACR.

In the rest of this chapter, we present our results regarding two questions:

1. Is it possible to characterize ACR for small networks? In particular, for networks with 2 reactions?
2. Is ACR preserved under some structural changes of the network?

In the first section of this chapter we explore question 1. This work was initiated during a research visit to Anne Shiu at Texas A&M and is a joint work with her and Nicolette Meshkat at Santa Clara University. In the second section we study question 2, and we consider removal and addition of intermediates, and removal and addition of inflow-outflow reactions.

3.2 ACR in networks with two reactions

In this section we explore the detection of ACR in networks with n species, two reactions and $n-1$ conservation laws using similar techniques as the ones in [37] for detecting multistationarity. These techniques rely on a graphic representation of chemical reaction networks and the relation between them and the positive steady states.

We will deduce algebraic conditions for ACR, that will be translated to the graphic setting. Additionally, we study the stability properties of the steady states arising from these networks.

3.2.1 ACR in networks with two reactions, n species and $n-1$ conservation laws

Consider a network of the form

$$\begin{aligned} \alpha_{11}\mathrm{X}_1 + \alpha_{21}\mathrm{X}_2 + \cdots + \alpha_{\mathrm{n}1}\mathrm{X}_\mathrm{n} &\xrightarrow{\kappa_1} \beta_{11}\mathrm{X}_1 + \beta_{21}\mathrm{X}_2 + \cdots + \beta_{\mathrm{n}1}\mathrm{X}_\mathrm{n} \\ \alpha_{12}\mathrm{X}_1 + \alpha_{22}\mathrm{X}_2 + \cdots + \alpha_{\mathrm{n}2}\mathrm{X}_\mathrm{n} &\xrightarrow{\kappa_2} \beta_{12}\mathrm{X}_1 + \beta_{22}\mathrm{X}_2 + \cdots + \beta_{\mathrm{n}2}\mathrm{X}_\mathrm{n} \end{aligned} \tag{3.2}$$

with $n \geq 2$. With mass action kinetics, the dynamics of the network are modelled by the system of ODEs

$$\dot{x_i} = \kappa_1(\beta_{i1} - \alpha_{i1})x^{\alpha_1} + \kappa_2(\beta_{i2} - \alpha_{i2})x^{\alpha_2} \quad \text{for} \quad i = 1, \ldots, n$$

where $\alpha_1 = (\alpha_{11}, \alpha_{21}, \ldots, \alpha_{n1})$, $\alpha_2 = (\alpha_{12}, \alpha_{22}, \ldots, \alpha_{n2})$ and $x^{\alpha_j} = x_1^{\alpha_{1j}} x_2^{\alpha_{2j}} \cdots x_n^{\alpha_{nj}}$ for $j = 1, 2$.

We now make some remarks regarding the existence of positive steady states and conservation laws.

alg 1. If $(\beta_{i1} - \alpha_{i1}) = (\beta_{i2} - \alpha_{i2}) = 0$ for some i, then $\dot{x_i} = 0$ and the concentration of x_i is constant through time. In this case, the species $\mathrm{X_i}$ does not have ACR as its concentration is equal to its initial amount and, clearly, depends on the stoichiometric compatibility class.

alg 2. If for some i, $(\beta_{i1} - \alpha_{i1}) = 0$ and $(\beta_{i2} - \alpha_{i2}) \neq 0$ or the other way around, then the differential equation for x_i is

$$\dot{x_i} = \kappa_2(\beta_{i2} - \alpha_{i2})x^{\alpha_2} \quad \text{or} \quad \dot{x_i} = \kappa_1(\beta_{i1} - \alpha_{i1})x^{\alpha_1}$$

respectively. This means that there are only boundary steady states.

alg 3. If $\beta_{i1} - \alpha_{i1}$ and $\beta_{i2} - \alpha_{i2}$ have the same sign for some i, then the equation $\dot{x_i} = 0$ does not have positive solutions and the system only has boundary steady states.

alg 4. If the network has $n-1$ conservation laws, then there is a matrix W, of size $(n-1) \times n$ that is row reduced and such that the equations given by $Wx = T$ define the stoichiometric compatibility class determined by the total amounts in the vector $T = (T_1, \ldots, T_{n-1})$. Since the rank of this matrix is $n-1$ and it is row reduced, the identity matrix of size $n-1$, I_{n-i}, is a submatrix of W. Let j be the index of the column of W that is not part of the I_{n-1}, and let $\{i_1, \ldots, i_{n-1}\}$ be the index corresponding to the first nonzero entry of the k-th row of W. The conservation laws can be written as

$$w_{ki_k}x_{i_k} + w_{kj}x_j = T_k$$

for $k = 1, \ldots, n-1$. That is,

$$w_{ki_k}\dot{x}_{i_k} + w_{kj}\dot{x}_j = 0.$$

If we define $w_k = \frac{-w_{kj}}{w_{ki_k}}$ then $\dot{x_{i_k}} = w_k\dot{x_j}$.

From now on we assume that the situations mentioned on remarks alg 1, alg 2 and alg 3 above do not hold. That is, we assume that $\beta_{i1} \neq \alpha_{i1}$ and $\beta_{i2} \neq \alpha_{i2}$ for every $i = 1, \ldots, n$ and that $\beta_{i1} - \alpha_{i1}$ and $\beta_{i2} - \alpha_{i2}$ have different signs. This assumption is a necessary condition for the existence of positive steady states.

Under these assumptions, we prove the following theorem.

Theorem 22. *Let $\mathcal{N}$ be a network with n species and 2 reactions as in (3.2). Assume that $\mathcal{N}$ has $n-1$ conservation laws and that the positive steady state variety is nonempty. Then the species X_{i_0} has ACR if and only if, $\alpha_{i1} = \alpha_{i2}$ for every $i \neq i_0$ and $\alpha_{i_01} \neq \alpha_{i_02}$.*

Proof. We start by proving that if $\alpha_{i1} = \alpha_{i2}$ for every $i \neq i_0$ and $\alpha_{i_01} \neq \alpha_{i_02}$, then the species X_{i_0} has ACR. Under this hypothesis the steady state equations are

$$0 = \kappa_1(\beta_{i1} - \alpha_{i1})x^{\alpha_1} + \kappa_2(\beta_{i2} - \alpha_{i2})x^{\alpha_1 + (\alpha_{i_02} - \alpha_{i_01})e_{i_0}} \quad \text{for} \quad i = 1, \ldots, n$$

where e_{i_0} is the canonical vector with zero in all but the i_0-th entry. Factoring x^{α_1}, the steady state equations are given by

$$0 = x^{\alpha_1}\left[\kappa_1(\beta_{i1} - \alpha_{i1}) + \kappa_2(\beta_{i2} - \alpha_{i2})x^{(\alpha_{i_02} - \alpha_{i_01})e_{i_0}}\right]$$

and the positive steady states are determined by the solutions of the factor on the right. Since the network has $n-1$ conservation laws, the positive steady state variety is determined only by one equation. We take the i_0-th equation, and after finding its solutions, the positive steady states can be parametrized as

$$\phi(x_1, \ldots, x_{i_0-1}, x_{i_0+1}, \ldots, x_{n-1}) = (x_1, \ldots, x_{i_0-1}, M, x_{i_0+1}, \ldots, x_{n-1}) \tag{3.3}$$

with $M = \left(\frac{-\kappa_1(\beta_{i_01} - \alpha_{i_01})}{\kappa_2(\beta_{i_02} - \alpha_{i_02})}\right)^{\frac{1}{\alpha_{i_02} - \alpha_{i_01}}} > 0$ as the positive steady state variety is nonempty (recall remarks alg 1, alg 2 and alg 3). From this parametrization we conclude that the network has ACR in X_{i_0}.

Now we prove that if the network has ACR in X_{i_0}, then $\alpha_{i1} = \alpha_{i2}$ for every $i \neq i_0$ and $\alpha_{i_0 1} \neq \alpha_{i_0 2}$. Let

$$0 = \kappa_1(\beta_{j1} - \alpha_{j1})x^{\alpha_1} + \kappa_2(\beta_{j2} - \alpha_{j2})x^{\alpha_2}$$

be the steady state equation that determines the positive steady state variety. The positive steady state equation can be written as

$$0 = x^{\alpha_1}\left[\kappa_1(\beta_{j1} - \alpha_{j1}) + \kappa_2(\beta_{j2} - \alpha_{j2})x^{\alpha_2 - \alpha_1}\right]$$

and the positive steady states satisfy

$$x^{\alpha_2 - \alpha_1} = \frac{-\kappa_1(\beta_{j1} - \alpha_{j1})}{\kappa_2(\beta_{j2} - \alpha_{j2})}.$$

If $i_0 \notin \mathrm{Supp}(\alpha_2 - \alpha_1)$, then the positive steady states do not depend on x_{i_0} and this variable can take any positive value, that is, X_{i_0} does not have ACR. On the other hand, if $i_0 \in \mathrm{Supp}(\alpha_2 - \alpha_1)$ but $\mathrm{Supp}(\alpha_2 - \alpha_1) \neq \{i_0\}$, then there is another k such that $\alpha_{k1} \neq \alpha_{k2}$. Thus, it is possible to express x_{i_0} in terms of x_k and it is possible to find two positive steady states with different values of x_{i_0}, which contradicts the assumption of ACR. Therefore, $\alpha_{i1} = \alpha_{i2}$ for every $i \neq i_0$ and $\alpha_{i_0 1} \neq \alpha_{i_0 2}$, which is what we wanted to prove. □

So far, these conditions are related to the structural conditions in Theorems 20 and 19. Note that, if the network (3.2) has 3 or 4 complexes, there are 1 or 2 linkage classes respectively and, since the dimension of the stoichiometric subspace is always 1, the deficiency of the network is 1. In this case, using Theorem 20 we obtain the same sufficient condition for ACR. However, for these small networks, this condition is also necessary. If the network has 2 complexes, then the network corresponds to only one reversible reaction, its deficiency is equal to zero and Theorem 19 guarantees that there is no ACR.

Graphic representation of a network

We represent network 3.2 graphically with two arrows in $\mathbb{R}^n$ going from $(\alpha_{11}, \alpha_{21}, \ldots, \alpha_{n1})$ to $(\beta_{11}, \beta_{21}, \ldots, \beta_{n1})$ and from $(\alpha_{12}, \alpha_{22}, \ldots, \alpha_{n2})$ to $(\beta_{12}, \beta_{22}, \ldots, \beta_{n2})$. For example, the graphic representation of network (3.1) is presented in Figure 3.1 With this representation we explore graphic conditions for the network to have ACR in one species. First note that remarks alg 1-3, regarding the existence of positive steady states, and the condition alg 4, regarding conservation laws, are equivalent to some conditions on the graphic representation.

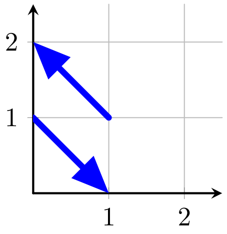


Figure 3.1: Graphic representation of network (3.1)

gr 1. The condition $(\beta_{i1} - \alpha_{i1}) = (\beta_{i2} - \alpha_{i2}) = 0$ for some i means that the projection of both arrows on the i-th axis is equal to two points. As mentioned before, this implies that $\dot{x_i} = 0$ and the concentration of this species depends on the stoichiometric compatibility class.

gr 2. The condition $(\beta_{i1} - \alpha_{i1}) = 0$ and $(\beta_{i2} - \alpha_{i2}) \neq 0$, or the other way around, means that the projection of the arrows on the i-th coordinate axis is equal to one point and one arrow. In this case the network does not have positive steady states.

gr 3. The condition $(\beta_{i1} - \alpha_{i1})$ and $(\beta_{i2} - \alpha_{i2})$ having the same sign is equivalent to the projections of both arrows on the i-th coordinate axis being arrows pointing in the same direction.

gr 4. The condition $\dot{x_{i_k}} = w_k \dot{x_j}$, is equivalent to

$$\kappa_1(\beta_{i_k 1} - \alpha_{i_k 1})x^{\alpha_1} + \kappa_2(\beta_{i_k 2} - \alpha_{i_k 2})x^{\alpha_2} = \kappa_1 w_k(\beta_{j1} - \alpha_{j1})x^{\alpha_1} + \kappa_2 w_k(\beta_{j2} - \alpha_{j2})x^{\alpha_2}.$$

This means that $(\beta_{i_k 1} - \alpha_{i_k 1}) = w_k(\beta_{j1} - \alpha_{j1})$ and $(\beta_{i_k 2} - \alpha_{i_k 2}) = w_k(\beta_{j2} - \alpha_{j2})$, and in the graphic representation this corresponds to the condition that the projection of the arrows on the i_k-th coordinate axis is equal to two arrows pointing in opposite directions and satisfying $\frac{\beta_{i_k 1} - \alpha_{i_k 1}}{\beta_{i_k 2} - \alpha_{i_k 2}} = \frac{\beta_{j1} - \alpha_{j1}}{\beta_{j2} - \alpha_{j2}}$.

Under this setting we can detect ACR in the species X_{i_0} graphically as follows.

Theorem 23. *Let $\mathcal{N}$ be a network with n species and 2 reactions as in (3.2). Assume that $\mathcal{N}$ has $n - 1$ conservation laws and that the positive steady state variety is nonempty. Then the species X_{i_0} has ACR if and only if, in the graphic representation of the network, the projection of the arrows on the i-th coordinate axis corresponds to one of the options in Figure 3.2 for $i \neq i_0$ and the projection on the i_0-th axis corresponds to one case in Figure 3.3.*

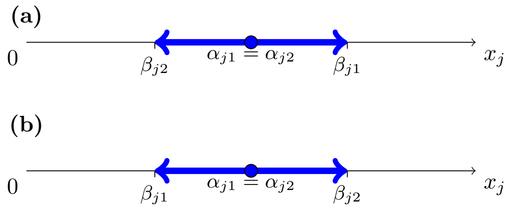


Figure 3.2: Projection of the arrows arising from the graphic representation of $\mathcal{N}$ on the i-th axis.

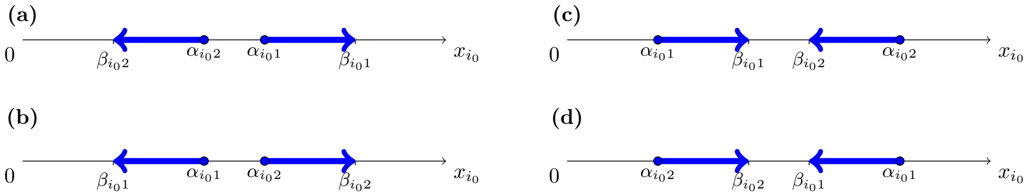


Figure 3.3: Projection of the arrows arising from the graphic representation of $\mathcal{N}$ on the i_0-th axis.

ACR in networks with two variables and two reactions

To have a better understanding of the graphic representation and Theorems 22 and 23, we present the particular case of networks with two species and two reactions:

$$\begin{aligned} \alpha_{11}\mathrm{X}_1 + \alpha_{21}\mathrm{X}_2 &\xrightarrow{\kappa_1} \beta_{11}\mathrm{X}_1 + \beta_{21}\mathrm{X}_2 \\ \alpha_{12}\mathrm{X}_1 + \alpha_{22}\mathrm{X}_2 &\xrightarrow{\kappa_2} \beta_{12}\mathrm{X}_1 + \beta_{22}\mathrm{X}_2 \end{aligned} \tag{3.4}$$

In this case the graphic representation corresponds to two arrows in the plane and conditions **gr 1-3** refer to the slope of the arrows and their direction. In particular **gr 1.** and **gr 2.** correspond to one of the arrows (or both) being parallel to one of the axis; and **gr 4.** means that both arrows have the same slope. Theorem 23 becomes

Theorem 24. *Network 3.4 has ACR in X_1 if, and only if, the graphic representation of the network is as in Figure 3.4. Similarly, the network has ACR in X_2 if, and only its graphic representation is as in Figure 3.5.*

3.2.2 Stability

After characterizing the presence of ACR in a network $\mathcal{N}$ in terms of its graphic representation, we proceed to link said representation with the stability of the steady

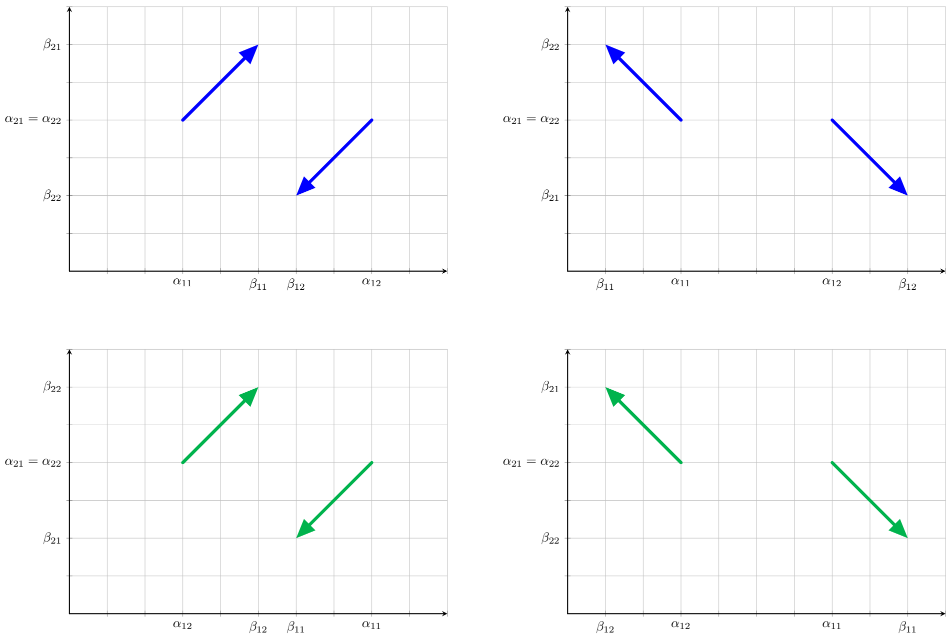


Figure 3.4: Networks with ACR in X_1. The networks on the left column have exponentially stable steady states and the networks on the right column have unstable steady states.

states in each stoichiometric compatibility class.

Theorem 25. *Let $\mathcal{N}$ be a chemical reaction network with n species and two reactions as in (3.2). Assume that the network has $n-1$ conservation laws and that it has ACR in species X_{i_0}. Then a steady state in a stoichiometric compatibility class is exponentially stable if and only if, the projection on the i_0-th axis is as in options (c) and (d) from Figure 3.3, and it is unstable if, and only if, the projection on the i_0-th axis is as in options (a) and (b) from Figure 3.3.*

Proof. The stability proof relies on the Hurwitz criterion and on the Center Manifold Theorem as seen in Chapter 2. We start by computing the Jacobian J_f and then we evaluate at a parameterization ϕ. Recall from remark **alg 4**. above, that the existence of $n-1$ conservation laws means that there is an index j such that the for every i_k with $k = 1, \ldots, n-1$, $\dot{x_{i_k}} = \omega_k \dot{x_j}$. This implies that the entries of the rate function f correspond to the j-the entry times the associated scalar ω_{i_k}. Additionally, the assumption of ACR in X_{i_0} means that $\alpha_{i1} = \alpha_{i2}$ for every $i \neq i_0$. We can write then the rate function f as

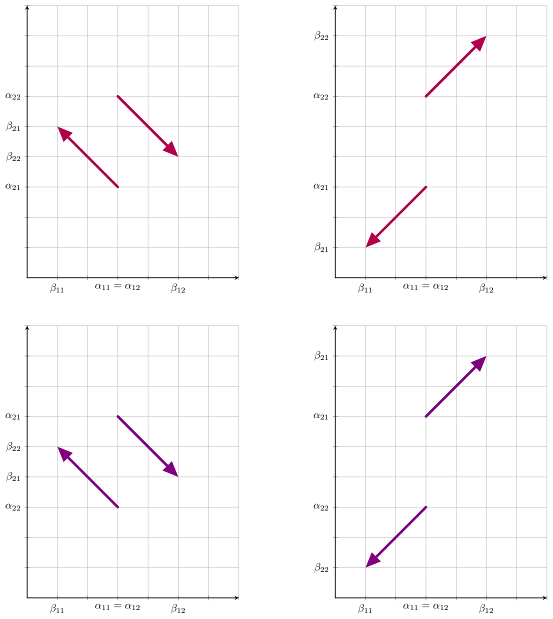


Figure 3.5: Networks with ACR in X_2. The networks on the left have exponentially stable steady states and the networks on the right have unstable steady states.

follows,

$$f = \begin{bmatrix} \omega_1 x^{\alpha_1}\left[\kappa_1(\beta_{j1}-\alpha_{j1}) + \kappa_2(\beta_{j2}-\alpha_{j2})x^{(\alpha_{i_0 2}-\alpha_{i_0 1})e_{i_0}}\right] \\ \vdots \\ \omega_{j-1} x^{\alpha_1}\left[\kappa_1(\beta_{j1}-\alpha_{j1}) + \kappa_2(\beta_{j2}-\alpha_{j2})x^{(\alpha_{i_0 2}-\alpha_{i_0 1})e_{i_0}}\right] \\ x^{\alpha_1}\left[\kappa_1(\beta_{j1}-\alpha_{j1}) + \kappa_2(\beta_{j2}-\alpha_{j2})x^{(\alpha_{i_0 2}-\alpha_{i_0 1})e_{i_0}}\right] \\ \omega_{j+1} x^{\alpha_1}\left[\kappa_1(\beta_{j1}-\alpha_{j1}) + \kappa_2(\beta_{j2}-\alpha_{j2})x^{(\alpha_{i_0 2}-\alpha_{i_0 1})e_{i_0}}\right] \\ \vdots \\ \omega_n x^{\alpha_1}\left[\kappa_1(\beta_{j1}-\alpha_{j1}) + \kappa_2(\beta_{j2}-\alpha_{j2})x^{(\alpha_{i_0 2}-\alpha_{i_0 1})e_{i_0}}\right] \end{bmatrix}.$$

Its Jacobian J_f is a matrix with rank 1 whose characteristic polynomial is

$$p_{J_f}(\lambda) = \lambda^{n-1}\left(\lambda - tr(J_f)\right),$$

where $tr(J_f)$ denotes the trace of F_j. Now, we take the parameterization ϕ given by equation (3.3) and study p_{J_f} after evaluating at ϕ. First note that $tr(J_f) = \langle w, \nabla f_j \rangle$, where ∇f_j is the gradient of the j-th component of f, and $w = (w_1, \ldots, w_{j-1}, 1, w_{j+1}, \ldots, w_n)$. The i-th entry of ∇f_j is equal to

$$\begin{aligned}(\nabla f_j)_i =& \kappa_1(\beta_{j1}-\alpha_{j1})\alpha_{i1}x^{\alpha_1 - e_i} + \kappa_2(\beta_{j2}-\alpha_{j2})\alpha_{i2}x^{\alpha_2 - e_i} \\ =& x^{\alpha_1 - e_i}\left[\kappa_1(\beta_{j1}-\alpha_{j1})\alpha_{i1} + \kappa_2(\beta_{j2}-\alpha_{j2})\alpha_{i2}x_{i_0}^{\alpha_{i_0 2}-\alpha_{i_0 1}}\right]\end{aligned}$$

and evaluating at ϕ we have

$$\begin{aligned}(\nabla f_j(\phi))_i =& x^{\alpha_1 - e_i}\left[\kappa_1(\beta_{j1}-\alpha_{j1})\alpha_{i1} + \kappa_2(\beta_{j2}-\alpha_{j2})\alpha_{i2}\left(\left(\frac{-\kappa_1(\beta_{j1}-\alpha_{j1})}{\kappa_2(\beta_{j2}-\alpha_{j2})}\right)^{\frac{1}{\alpha_{i_0 2}-\alpha_{i_0 1}}}\right)^{\alpha_{i_0 2}-\alpha_{i_0 1}}\right] \\ =& x^{\alpha_1 - e_i}\left[\kappa_1(\beta_{j1}-\alpha_{j1})\alpha_{i1} - \kappa_1(\beta_{j1}-\alpha_{j1})\alpha_{i2}\right]\end{aligned}$$

and note that the last expression is equal to zero for every $i \neq i_0$ because $\alpha_{i1} = \alpha_{i2}$. If $i = i_0$, then $(\nabla f_j(\phi))_{i_0} = x^{\alpha_1 - e_{i_0}} k_1(\beta_{j1}-\alpha_{j1})(\alpha_{i_0 1}-\alpha_{i_0 2})$. Thus,

$$tr(J_f) = \langle w, \nabla f_j \rangle = x^{\alpha_1 - e_{i_0}} k_1 w_{i_0}(\beta_{j1}-\alpha_{j1})(\alpha_{i_0 1}-\alpha_{i_0 2}) = x^{\alpha_1 - e_{i_0}} k_1(\beta_{i_0 1}-\alpha_{i_0 1})(\alpha_{i_0 1}-\alpha_{i_0 2})$$

and the sign of this term determines the stability of a steady state. If $tr(J_f) > 0$, then the steady state is unstable and if $tr(J_f) < 0$, then the steady state is exponentially stable. We conclude that if $(\beta_{i_0 1} - \alpha_{i_0 1})$ and $(\alpha_{i_0 1} - \alpha_{i_0 2})$ have the same sign (or equivalently the projection on the i_0-h axis is as in (a) and (b) in Figure 3.3), then the steady state is unstable. Similarly, if $(\beta_{i_0 1} - \alpha_{i_0 1})$ and $(\alpha_{i_0 1} - \alpha_{i_0 2})$ have opposite signs (or equivalently the projection on the i_0-h axis is as in (c) and (c) in Figure 3.3), then the steady state is exponentially stable. □

Using the previous theorem we can asses the stability of a steady state by looking at its graphic representation. In the particular case of two variables, the result states that the networks with ACR and exponentially stable steady states have a graphic representation as in the left columns of Figures 3.4 and 3.5, and the networks with unstable steady states have a graphic representation as in the right column of said figures.

Remark: We have completely determined which networks with two reactions, two variables and one conservation law have ACR. Note that when the graphical representation is used, it is easy to see that ACR is preserved under equal translations of both reactions, under horizontal or vertical translation of only one of the reactions (depending on which variable has ACR) and scaling of the reactions.

3.3 Preservation and loss of ACR

The main goal of this section is to give an overview of how ACR is preserved when performing structural modifications of the network.

The modifications that we consider here are removal and addition of intermediates, removal and addition of inflow and outflow reactions and removal and addition of reactions that preserve the stoichiometric subspace. These modifications have been explained in detail in the framework 1.

3.3.1 Removal and addition of intermediates

First we prove that ACR in one species is inherited by the networks obtained when removing one intermediate, for the reaction rate constants that satisfy the realization conditions mentioned in Section 1.4

Theorem 26. *Let $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a reaction network and $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$ be the network obtained by removing one intermediate Y. G has ACR in a species X_i, that is not an intermediate, for some reaction rate constants κ, if and only if G^* has ACR in X_i for any matching rate constants $\psi(\kappa)$.*

This results follows from Theorem 7, because for matching reaction rate constants the steady state equations of the species different from Y are the same in both cases, after evaluating the equations of the extension model in the solutions of the equation $\dot{y} = 0$. As an example consider the network

$$\begin{array}{r} \mathrm{X}_1 + \mathrm{X}_2 \longrightarrow \mathrm{Y}_1 \longrightarrow 2\,\mathrm{X}_2 \\ \mathrm{X}_2 \longrightarrow \mathrm{X}_1\,. \end{array} \tag{3.5}$$

If we remove the intermediate Y_1, we are left with the network (3.1). Since network (3.1) has ACR in X_1, the previous theorem guarantees that the network with the intermediate also has ACR in X_1.

Using the theorem on the networks obtained by adding one intermediate at a time, we can guarantee that networks such as

$$\begin{array}{r} \mathrm{X}_1 + \mathrm{X}_2 \longrightarrow \mathrm{Y}_1 \longrightarrow \mathrm{Y}_2 \longrightarrow 2\,\mathrm{X}_2 \\ \mathrm{X}_2 \rightleftharpoons \mathrm{Y}_3 \longrightarrow \mathrm{X}_1 \end{array} \tag{3.6}$$

or

$$\begin{array}{r} \mathrm{X}_1 + \mathrm{X}_2 \longrightarrow 2\,\mathrm{X}_2 \rightleftharpoons \mathrm{Y}_1 \\ \mathrm{X}_2 \rightleftharpoons \mathrm{Y}_2 \longrightarrow \mathrm{X}_1 \end{array} \tag{3.7}$$

also have ACR in X_1.

3.3.2 Removal and addition of inflow and outflow reactions

We now consider the addition of inflow and outflow reactions on a network with ACR in a species X_i. The first result relates to the addition of inflow and outflow reactions for the species with ACR and the second result analyses the addition of inflow and outflow reactions of a species in a conservation law.

Theorem 27. *Let $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a reaction network with mass action kinetics and let $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$ be the network obtained by adding the reactions* $0 \underset{\kappa_{out}}{\overset{\kappa_{in}}{\rightleftarrows}} X_i$.

1. *If G has ACR in the species X_i, then then G^* has a positive steady state if the quotient $\frac{\kappa_{in}}{\kappa_{out}}$ is equal to the steady state concentration of X_i in G.*
2. *If X_i is in the support of some conservation law for G, then G^* has ACR on X_i.*

Proof. Let x^* be a positive steady state of G. By definition $f_G(x^*) = 0$, where f_G is the rate function associated with G. Note that the rate function of G^*, f_{G^*}, only differs from f_G in the i-th entry, and additionally $f_{G^*,i} = f_{G,i} + \kappa_{in} - \kappa_{out} x_i$.

1. Note that $f_{G^*,i}(x^*) = \kappa_{in} - \kappa_{out} x_i^*$, and this monomial vanishes if $x_i^* = \frac{\kappa_{in}}{\kappa_{out}}$. As a result, every positive steady state of G is a positive steady state for G^* if $x_i^* = \frac{\kappa_{in}}{\kappa_{out}}$, and the positive steady state variety associated with G^* is nonempty.
2. Let $\sum_{j=1}^{n} \omega_j \dot{x}_j = 0$ be a conservation law for G such that $\omega_i \neq 0$. This conservation law implies that $\sum_{j=1}^{n} \omega_j f_{G,j} = 0$ and, adding $\kappa_{in} - \kappa_{out} x_i$ to both sides of the equation, we have that $\sum_{j=1}^{n} \omega_j f_{G^*,j} = \kappa_{in} - \kappa_{out} x_i$. This guarantees that the monomial $\kappa_{in} - \kappa_{out} x_i$ is contained in the steady state ideal; thus, the steady state variety is constant in x_i and X_i has ACR.

□

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Papers

I

Detecting parameter regions for bistability in reaction networks

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Detecting parameter regions for bistability in reaction networks

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Abstract

Deciding whether and where a system of parametrized ordinary differential equations displays bistability, that is, has at least two asymptotically stable steady states for some choice of parameters, is a hard problem. For systems modeling biochemical reaction networks, we introduce a procedure to determine, exclusively via symbolic computations, the stability of the steady states for unspecified parameter values. In particular, our approach fully determines the stability type of all steady states of a broad class of networks. To this end, we combine the Hurwitz criterion, reduction of the steady state equations to one univariate equation, and structural reductions of the reaction network. Using our method, we prove that bistability occurs in open regions in parameter space for many relevant motifs in cell signaling.

1 Introduction

Bistability, that is, the existence of at least two stable steady states in a dynamical system, has been linked to switch-like behavior in biological networks and cellular decision making and it has been observed experimentally in a variety of systems [25, 32, 33]. However, proving the existence of bistability in a parameter-dependent mathematical model is in general hard.

We focus on (bio)chemical reaction networks with associated kinetics, giving rise to systems of Ordinary Differential Equations (ODEs) that model the change in the concentration of the species of the network over time. These systems come equipped with unknown parameters and, ideally, one wishes to determine properties of the family of ODEs for varying parameter values. Here we are concerned with stability of the steady states, and focus on the following three questions: (1) if the network admits only one steady state for all parameter choices, is it asymptotically stable? (2) can parameter values be chosen such that the system is bistable? (3) does it hold that for any choice of parameters yielding at least three steady states, two of them are asymptotically stable?

As the parameters are regarded unknown, explicit expressions for the steady states are rarely available. Problem (1) has been shown to be tractable for certain classes of networks. For example, the only steady state of complex balanced networks admits a Lyapunov function making it asymptotically stable [18, 27]. The use of Lyapunov functions and the theory of monotone systems has been employed more broadly to other classes of networks [15, 1, 3]. Finally, algebraic criteria as the Hurwitz criterion or the study of P-matrices also provide asymptotic stability of steady states, often in combination with algebraic parametrizations of the steady state variety. In [9] the Hurwitz criterion is analyzed using graphical methods.

Problem (2) is much harder and typically tackled by first deciding whether the network admits multiple steady states using one of the many available methods [30], and then numerically computing the steady states and their stability for a suitable choice of parameter values. Rigorous proofs of bistability require advanced analytical arguments such as bifurcation theory and geometric singular perturbation theory, as employed in [26, 20] for futile cycles. The use of the Hurwitz criterion to prove bistability is anecdotal, as rarely explicit descriptions of the steady states can be found. Problem (3) has been addressed for small systems using case-by-case approaches, but no systematic strategy has been proposed.

We devise a flow chart to solve the problems (1)-(3) using computer-based proofs relying only on symbolic operations. This is achieved by combining three key ingredients. First, we apply the Hurwitz criterion [5, 2] on the characteristic polynomial of the Jacobian of the ODE system evaluated at a parametrization of the steady states. Second, we observe that when all but the last Hurwitz determinants are positive (meaning that instabilities only arise via an eigenvalue with positive real part), and further the solutions to the steady state equations are in one to one correspondence with the zeros of a univariate function, then the stability of the steady states is completely determined and question (3) can be answered (Theorem 2). We claim that the hypotheses of Theorem 2 often hold when the network is small in terms of number of variables and reactions. Third, if Theorem 2 does not apply, we reduce the network to a smaller one for which Theorem 2 applies. Afterwards, we infer stability properties of the steady states of the original network in an open parameter region. To this end, many reduction techniques have been proposed [4, 23], but often removal of reactions [29] and of intermediates [23] suffice for (bio)chemical networks.

Even though our approach demands heavy symbolic computations, we illustrate how problem (3) can successfully be tackled for small networks, and further, we prove the existence of bistability in open regions of the parameter space for several relevant cell signaling motifs. In particular, for many networks we provide the first proof of bistability in an open region of the parameter space, with the exception of the double phosphorylation cycle, whose bistability was proven in [26].

2 Mathematical framework

We use the following notation: J_f is the Jacobian matrix of a function f. We denote by $V^\perp$ the orthogonal complement of a vector space V. For $A \in \mathbb{R}^{n\times n}$ and $I, J \subseteq \{1, \dots, n\}$, we let $A_{I,J}$ be the submatrix of A with rows (resp. columns) with indices in I (resp. J).

Finally, we denote by $p_A(\lambda)$ the characteristic polynomial of A.

2.1 Reaction networks

We consider reaction networks over a set of *species* $\mathcal{S} = \{X_1, \ldots, X_n\}$ given by a collection of reactions

$$r_j \colon \sum\nolimits_{i=1}^n \alpha_{ij} X_i \longrightarrow \sum\nolimits_{i=1}^n \beta_{ij} X_i \quad \text{for } j = 1, \ldots, m \tag{1}$$

with $\alpha_{ij} \neq \beta_{ij}$ for at least one index i. Let x_i denote the concentration of X_i. Given a differentiable *kinetics* $v \colon \mathbb{R}^n_{\geq 0} \to \mathbb{R}^m_{\geq 0}$, the dynamics of the concentrations of the species in the network over time t are modeled by means of a system of autonomous ODEs,

$$\tfrac{dx}{dt} = Nv(x), \qquad x = (x_1, \ldots, x_n) \in \mathbb{R}^n_{\geq 0}, \tag{2}$$

where $N \in \mathbb{R}^{n \times m}$ is the *stoichiometric matrix* with j^{th} column $(\beta_{1j} - \alpha_{1j}, \ldots, \beta_{nj} - \alpha_{nj})$. We write $f(x) := Nv(x)$. With *mass-action* kinetics, we have $v_j(x) = \kappa_j x_1^{\alpha_{1j}} \cdots x_n^{\alpha_{nj}}$ where $\kappa_j > 0$ is a *rate constant*, shown often as a label of the reaction.

Under mild conditions, satisfied by common kinetics including mass-action, $\mathbb{R}^n_{\geq 0}$ and $\mathbb{R}^n_{>0}$ are forward invariant by Eq. (2), [37]. Furthermore, any trajectory of Eq. (2) is confined to a so-called *stoichiometric compatibility class* $(x_0 + S) \cap \mathbb{R}^n_{\geq 0}$ with $x_0 \in \mathbb{R}^n_{\geq 0}$, where S is the column span of N and called the *stoichiometric subspace* [19]. The set $(x_0 + S) \cap \mathbb{R}^n_{\geq 0}$ is the solution set of the equations $Wx = T$ with $W \in \mathbb{R}^{d \times n}$ any matrix whose rows form a basis of $S^\perp$ and $T = Wx_0 \in \mathbb{R}^d$. These equations are called *conservation laws* and the defined stoichiometric compatibility class is denoted by $\mathcal{P}_T$.

2.2 Steady states

The steady states (or equilibria) of the network are the non-negative solutions to $Nv(x) = 0$. The positive steady states, that is, the solutions in $\mathbb{R}^n_{>0}$, define the *positive steady state variety* V^+. The existence of d linearly independent conservation laws implies that d steady state equations are redundant. We let $s = \dim(S)$, $d = n - s$, and $W \in \mathbb{R}^{d \times n}$ be row reduced with $i_1, \ldots, i_d$ the indices of the first non-zero coordinate of each row. For $T \in \mathbb{R}^d$, define

$$F_T(x)_i = \begin{cases} f_i(x) & i \notin \{i_1, \ldots, i_d\} \\ (Wx - T)_i & i \in \{i_1, \ldots, i_d\}, \end{cases} \tag{3}$$

which arises after replacing redundant equations in $Nv(x) = 0$ with $Wx = 0$. Hence, the solutions to $F_T(x) = 0$ are the steady states in $\mathcal{P}_T$ [10, 38]. A steady state x^* is *non-degenerate* if $\ker(J_f(x^*)) \cap S = \{0\}$, or equivalently, if $\det(J_{F_T}(x^*)) \neq 0$ [38]. Observe that $J_{F_T}(x^*)$ is independent of T.

As an example, consider the following reaction network

$$\mathrm{X}_1 \xrightarrow{\kappa_1} \mathrm{X}_2 \qquad \mathrm{X}_2 + \mathrm{X}_3 \xrightarrow{\kappa_2} \mathrm{X}_1 + \mathrm{X}_4 \qquad \mathrm{X}_4 \xrightarrow{\kappa_3} \mathrm{X}_3. \tag{4}$$

This is a simplified model of a two-component system, consisting of a histidine kinase HK and a response regulator RR [10]. Both occur unphosphorylated (X_1, X_3) and phosphorylated (X_2, X_4). With mass-action kinetics, the ODE system is

$$\begin{aligned} \tfrac{dx_1}{dt} &= -\kappa_1 x_1 + \kappa_2 x_2 x_3 & \qquad \tfrac{dx_3}{dt} &= -\kappa_2 x_2 x_3 + \kappa_3 x_4 \\ \tfrac{dx_2}{dt} &= \kappa_1 x_1 - \kappa_2 x_2 x_3 & \qquad \tfrac{dx_4}{dt} &= \kappa_2 x_2 x_3 - \kappa_3 x_4, \end{aligned}$$

and we consider $\mathcal{P}_T$ defined by $x_1 + x_2 = T_1$ and $x_3 + x_4 = T_2$. With this choice, $F_T(x)$ is

$$\big(x_1 + x_2 - T_1, \kappa_1 x_1 - \kappa_2 x_2 x_3, x_3 + x_4 - T_2, \kappa_2 x_2 x_3 - \kappa_3 x_4\big).$$

In this example, the positive steady states are the positive solutions to the equations $\frac{dx_2}{dt} = \frac{dx_4}{dt} = 0$, which when solved for x_1 and x_3 lead to the following parametrization of V^+:

$$\phi(x_2, x_4) = \left(\tfrac{\kappa_3 x_4}{\kappa_1}, x_2, \tfrac{\kappa_3 x_4}{\kappa_2 x_2}, x_4\right), \qquad (x_2, x_4) \in \mathbb{R}^2_{>0}. \tag{5}$$

In general, we refer to a *positive parametrization* as any bijective map of the form

$$\phi\colon \mathbb{R}^d_{>0} \to V^+ \qquad \text{with} \quad \xi \mapsto \phi(\xi). \tag{6}$$

In practice, under mass-action kinetics, the entries of ϕ are rational functions in ξ. Strategies for finding positive parametrizations are reviewed in [10].

2.3 Multistationarity and bistability

A network is *multistationary* if it has at least two positive steady states in some $\mathcal{P}_T$, that is, $F_T(x) = 0$ has at least two positive solutions for some $T \in \mathbb{R}^d$. A *monostationary* network has exactly one positive steady state in each $\mathcal{P}_T$. Under some conditions, if the sign of $\det(J_{F_T}(x^*))$ is $(-1)^s$ for all positive steady states x^*, then the network is monostationary; if the sign is $(-1)^{s+1}$ for some x^*, then it is multistationary [10]. Using a positive parametrization, this result yields inequalities in the rate constants that guarantee or preclude multistationarity [11, 10].

Given $\frac{dx}{dt} = f(x)$, a steady state x^* is *stable* if for each $\epsilon > 0$ small enough, there exists $\delta > 0$ such that solutions starting within distance δ of x^*, remain within distance ϵ. If additionally δ can be chosen such that solutions tend to x^* as time increases, x^* is *asymptotically stable*. If x^* is not stable, then it is *unstable*. If all eigenvalues of $J_f(x^*)$ have negative real part, then x^* is *exponentially stable*. Exponential stability implies asymptotic stability (§2.7-2.8 in [35]). If one eigenvalue has positive real part, then x^* is unstable. For further discussions on stability we refer to [35].

The stability of a steady state is studied relatively to $\mathcal{P}_T$. A network that admits two asymptotically stable positive steady states in some $\mathcal{P}_T$ is called *bistable*. Detecting multistationarity and bistability is challenging already for medium sized networks. To overcome computational difficulties one may employ structural modifications of the network, such as removal of intermediates or reactions. Specifically, given two networks $\mathcal{G}$ and $\mathcal{G}'$, $\mathcal{G}'$ is a *subnetwork* of $\mathcal{G}$ if it arises after removing reactions of $\mathcal{G}$ [29]. An *intermediate* Y is a species that appears only in the form Y at either side of a reaction, and is both a product and a reactant in the network. Removal of intermediates leads to a new network after collapsing into one reaction all paths of reactions from and to non-intermediates and through intermediates [23]. For example, the species $\mathrm{S_0E}$ in $\mathrm{S_0} + \mathrm{E} \rightleftharpoons \mathrm{S_0E} \longrightarrow \mathrm{S_1} + \mathrm{E}$ is an intermediate. Its removal yields the reaction $\mathrm{S_0} + \mathrm{E} \longrightarrow \mathrm{S_1} + \mathrm{E}$.

Assume mass-action kinetics and that $\mathcal{G}'$ either is a subnetwork of $\mathcal{G}$ such that both stoichiometric subspaces agree, or arises from $\mathcal{G}$ by removal of intermediates and satisfies a technical condition on the rate constants [23, 36]. If $\mathcal{G}'$ has ℓ_1 exponentially stable and ℓ_2 non-degenerate unstable steady states in some $\mathcal{P}_T$ for some rate constants κ, then $\mathcal{G}$ has at least ℓ_1 exponentially stable and ℓ_2 non-degenerate unstable steady states in some $\mathcal{P}_T$ for some rate constants $\widetilde{\kappa}$. If $\mathcal{G}'$ is a subnetwork of $\mathcal{G}$, then $\widetilde{\kappa}$ agrees with κ for the common reactions and is small enough for the reactions that only are in $\mathcal{G}$. A more intricate but explicit description of the rate constants $\widetilde{\kappa}$ exists also for the removal of intermediates [23].

2.4 The Jacobian matrix of reaction networks

In the context of reaction networks, we determine stability based on the eigenvalues of the Jacobian of the restriction of Eq. (2) to $\mathcal{P}_T$. To this end, we consider the projection of $J_f(x)$ onto the stoichiometric subspace S by writing the ODE system in local coordinates of $\mathcal{P}_T$. Let $R_0 \in \mathbb{R}^{n\times s}$ be a matrix whose columns form a basis of S and $L \in \mathbb{R}^{s\times m}$ such that $N = R_0 L$. Then the projection of $J_f(x)$ onto S is $LJ_v(x)R_0$.

Proposition 1. *The characteristic polynomials* $p_{J_f(x)}$ *and* $p_{LJ_v(x)R_0}$ *satisfy*

$$p_{J_f(x)}(\lambda) = \lambda^{n-s} p_{LJ_v(x)R_0}(\lambda).$$

Further, the independent term of $p_{LJ_v(x)R_0}(\lambda)$ *is* $(-1)^s \det(J_{F_T}(x))$*, with* F_T *as in Eq.* (3) *for any choice of* W*.*

The proof of Proposition 3 can be found in A. According to Proposition 3, the s eigenvalues of $Q_x := LJ_v(x)R_0$ are the eigenvalues of $J_f(x)$ once zero counted with multiplicity d is disregarded. In order to study the (sign of the real part of the) spectrum of the matrices Q_x when x is a positive steady state, we use a positive parametrization. We denote the characteristic polynomial $p_{Q_x}(\lambda)$ by $q_x(\lambda)$.

For example, for the network in Eq. (4), we consider the matrices $R_0, L, J_v(x)$ respectively to be

$$\begin{bmatrix} -1 & 0 \\ 1 & 0 \\ 0 & 1 \\ 0 & -1 \end{bmatrix}, \begin{bmatrix} 1 & -1 & 0 \\ 0 & -1 & 1 \end{bmatrix}, \begin{bmatrix} \kappa_1 & 0 & 0 & 0 \\ 0 & \kappa_2 x_3 & \kappa_2 x_2 & 0 \\ 0 & 0 & 0 & \kappa_3 \end{bmatrix},$$

and we are interested in the eigenvalues of the matrix

$$Q_x = LJ_v(x)R_0 = \begin{bmatrix} -\kappa_1 - \kappa_2 x_3 & -\kappa_2 x_2 \\ -k_2 x_3 & -\kappa_2 x_2 - \kappa_3 \end{bmatrix} \tag{7}$$

evaluated at a steady state $x^* = \phi(x_2, x_4)$. Thus, by analyzing the eigenvalues of $Q_{\phi(x_2,x_4)}$ for *all* values of κ and $x_2, x_4 > 0$, we study the stability of all positive steady states.

We conclude this part with a key technical result (proven in A) on the determinant of $J_f(x^*)$ in the particular case where system $F_T(x) = 0$ is reduced to one univariate equation.

Proposition 2. *Fix* $W \in \mathbb{R}^{d\times n}, T \in \mathbb{R}^d$ *and* F_T *as in Eq. (3). Assume there exist an open interval* $\mathcal{E} \subseteq \mathbb{R}$*, a differentiable function* $\varphi\colon \mathcal{E} \to \mathbb{R}^n_{>0}$*, and indices* i, j *such that* $\varphi_i'(z) \neq 0$ *and* $F_{T,\ell}(\varphi(z)) = 0$ *for all* $\ell \neq j$*. Then, for any solution* z^* *to*

$$F_{T,j}(\varphi(z)) = 0, \qquad z \in \mathcal{E}, \tag{8}$$

$x^* = \varphi(z^*)$ *is a positive solution to* $F_T(x) = 0$ *and further*

$$\det(J_{F_T}(x^*)) = \frac{(-1)^{i+j}}{\varphi_i'(z^*)}(F_{T,j}\circ\varphi)'(z^*)\det(J_{F_T}(x^*)_{J,I}),$$

where $I = \{1,\dots,n\}\setminus\{i\}$ *and* $J = \{1,\dots,n\}\setminus\{j\}$*.*

In practice, $\varphi_i(z) = z$, $\mathcal{E} \subseteq \mathbb{R}_{>0}$, and the solutions to $F_T(x) = 0$ are in one to one correspondence with the solutions to Eq. (9). In this case, given the positive solutions $z_1 < \dots < z_\ell$ of Eq. (9), the sign of the derivative of $F_{T,j}(\varphi(z))$ evaluated at $z_1, \dots, z_\ell$ alternates if all the steady states are non-degenerate. If additionally the sign of $\frac{1}{\varphi_i'(z)}\det(J_{F_T}(\varphi(z))_{J,I})$ is independent of the choice of z, then the sign of $\det(J_{F_T}(\varphi(z_\ell)))$ depends only on the sign of the derivative of $(F_{T,j}\circ\varphi)$ at z_ℓ. We will exploit this fact below.

2.5 Algebraic criteria for stability

We present now the Hurwitz criterion [5, 2], which determines whether all the roots of a polynomial have negative real part.

Criterion 1 (Hurwitz). *Let* $p(x) = a_s x^s + \ldots + a_0$ *be a real polynomial with* $a_s > 0$ *and* $a_0 \neq 0$*. The Hurwitz matrix* $H = (h_{ij})$ *associated with* p *has entries* $h_{i,j} = a_{s-2i+j}$ *for* $i, j = 1, \dots, s$*, by letting* $a_k = 0$ *if* $k \notin \{0, \dots, s\}$*:*

$$H = \begin{bmatrix} a_{s-1} & a_s & 0 & 0 & \cdots & 0 \\ a_{s-3} & a_{s-2} & a_{s-1} & a_s & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & a_{6-s} & \cdots & a_2 \\ 0 & 0 & 0 & 0 & \cdots & a_0 \end{bmatrix} \in \mathbb{R}^{s\times s}.$$

The i^{th} *Hurwitz determinant is* $H_i = \det(H_{I,I})$*,* $I = \{1, \dots, i\}$*. Then, every root of* p *has negative real part if and only if* $H_i > 0$ *for all* $i = 1, \dots, s$*. If* $H_i < 0$ *for some* i*, then a root of* p *has positive real part.*

Note that $H_s = a_0 H_{s-1}$. Pairs of imaginary roots (leading to Hopf bifurcations) arise when at least one of the H_i vanish [39] (see [12] in the context of reaction networks).

For the polynomial $q_x(\lambda)$ or $q_{\phi(\xi)}(\lambda)$, the Hurwitz determinants are typically rational functions in x or ξ. In Example in Eq. (4), the polynomial $q_x(\lambda)$ for Q_x as in Eq. (7) is

$$\lambda^2 + (\kappa_2 x_2 + \kappa_2 x_3 + \kappa_1 + \kappa_3)\lambda + \kappa_1\kappa_2 x_2 + \kappa_2\kappa_3 x_3 + \kappa_1\kappa_3,$$

and the Hurwitz determinants are $H_1 = \kappa_2(x_2 + x_3) + \kappa_1 + \kappa_3$ and $H_2 = ((\kappa_1 x_2 + \kappa_3 x_3)\kappa_2 + \kappa_1\kappa_3)H_1$. Both determinants are polynomials in κ, x with positive coefficients and hence positive for all $\kappa, x \in \mathbb{R}^3_{>0}$. By the Hurwitz criterion any positive steady state is exponentially stable. This network has exactly one steady state in each $\mathcal{P}_T$ [10], and we now additionally conclude that *the only steady state is exponentially stable.*

Often for small networks, all but the last Hurwitz determinants are positive. Then, the stability of a steady state x^* is fully determined by the sign of H_s, which agrees with the sign of the independent term of $q_{x^*}(\lambda)$, which in turn is $(-1)^s \det(J_{F_T}(x^*))$ by Proposition 3. Together with Proposition 4 we obtain the following theorem, proved in Appendix A.

Theorem 1. *Let $T, \mathcal{E}, \varphi, i, j, I, J$ be as in Proposition 4. Assume that*

- *the sign of $\frac{1}{\varphi_i'(z)} \det(J_{F_T}(\varphi(z))_{J,I})$ is independent of $z \in \mathcal{E}$ and is nonzero, and*
- *the first $s-1$ Hurwitz determinants of $q_{x^*}(\lambda)$ are positive for all positive steady states x^*.*

If $z_1 < \cdots < z_\ell$ are the positive solutions to Eq. (9) *and all are simple, then either $\varphi(z_1), \varphi(z_3), \ldots$ are exponentially stable and $\varphi(z_2), \varphi(z_4), \ldots$ are unstable, or the other way around. Specifically, $\varphi(z_1)$ is exponentially stable if and only if*

$$\frac{(-1)^{s+i+j}}{\varphi_i'(z_1)} (F_{T,j} \circ \varphi)'(z_1) \det(J_{F_T}(\varphi(z_1))_{J,I}) > 0.$$

In practice, $F_{T,j}(\varphi(z)) = \frac{a(z)}{b(z)}$ is a rational function in z with $b(z) > 0$ in $\mathcal{E}$. Then the zeros of $F_{T,j}(\varphi(z))$ are the roots of $a(z)$, and the signs of $(F_{T,j} \circ \varphi)'(z^*)$ and $a'(z^*)$ agree for all $z^* \in \mathcal{E}$ such that $a(z^*) = 0$ (Lemma 1 in A).

We illustrate Theorem 2 with a **hybrid histidine kinase** network with mass-action kinetics [31], see Fig. 1. We rename the species as follows: X_1=$\mathrm{HK_{00}}$, X_2=$\mathrm{HK_{p0}}$, X_3=$\mathrm{HK_{0p}}$, X_4=$\mathrm{HK_{pp}}$, X_5=Htp and X_6=$\mathrm{Htp_p}$. The associated ODE system is

$$\begin{aligned}
\tfrac{dx_1}{dt} &= -\kappa_1 x_1 + \kappa_4 x_3 x_5 & \tfrac{dx_4}{dt} &= \kappa_3 x_3 - \kappa_5 x_4 x_5 \\
\tfrac{dx_2}{dt} &= \kappa_1 x_1 - \kappa_2 x_2 + \kappa_5 x_4 x_5 & \tfrac{dx_5}{dt} &= -\kappa_4 x_3 x_5 - \kappa_5 x_4 x_5 + \kappa_6 x_6 \\
\tfrac{dx_3}{dt} &= -\kappa_3 x_3 + \kappa_2 x_2 - \kappa_4 x_3 x_5 & \tfrac{dx_6}{dt} &= \kappa_4 x_3 x_5 - \kappa_6 x_6 + \kappa_5 x_4 x_5.
\end{aligned}$$

The conservation laws of the system are $T_1 = x_1 + x_2 + x_3 + x_4$, $T_2 = x_5 + x_6$. Hence

$$F_T(x) = \begin{bmatrix} x_1 + x_2 + x_3 + x_4 - T_1 \\ \kappa_5 x_4 x_5 + \kappa_1 x_1 - \kappa_2 x_2 \\ -\kappa_4 x_3 x_5 + \kappa_2 x_2 - \kappa_3 x_3 \\ -\kappa_5 x_4 x_5 + \kappa_3 x_3 \\ x_5 + x_6 - T_2 \\ \kappa_4 x_3 x_5 + \kappa_5 x_4 x_5 - \kappa_6 x_6 \end{bmatrix}.$$

Here $s = 4$. The existence of three positive steady states for this network was established in [31]. We compute $q_x(\lambda)$ and the Hurwitz determinants in Maple 2019 and obtain that all but the last are polynomials in x and κ with positive coefficients, hence positive when evaluated at a positive steady state (see Appendix C).

We proceed to decide whether Theorem 2 applies. In [31], it was shown that the assumptions of Proposition 4 hold with $i = j = 5$, with $z = x_5$ being the concentration of Htp, $F_{T,5}$ corresponding to the conservation law with T_2, and $\mathcal{E} = \mathbb{R}_{>0}$. That is, the

Hybrid histidine kinase

$\mathrm{HK_{00}} \rightarrow \mathrm{HK_{p0}} \rightarrow \mathrm{HK_{0p}} \rightarrow \mathrm{HK_{pp}}$ $\mathrm{Htp_p} \rightarrow \mathrm{Htp}$

$\mathrm{HK_{pp}} + \mathrm{Htp} \rightarrow \mathrm{HK_{p0}} + \mathrm{Htp_p}$ $\mathrm{HK_{0p}} + \mathrm{Htp} \rightarrow \mathrm{HK_{00}} + \mathrm{Htp_p}$

Two substrate enzyme catalysis

$\mathrm{E} + \mathrm{S_1} \rightleftharpoons \mathrm{ES_1}$ $\mathrm{E} + \mathrm{S_2} \rightleftharpoons \mathrm{ES_2}$ $\mathrm{ES_1S_2} \rightleftharpoons \mathrm{E} + \mathrm{P}$

$\mathrm{S_2} + \mathrm{ES_1} \rightleftharpoons \mathrm{ES_1S_2}$ $\mathrm{S_1} + \mathrm{ES_2} \rightleftharpoons \mathrm{ES_1S_2}$

Gene transcription network

$\mathrm{X_1} \rightarrow \mathrm{X_1} + \mathrm{P_1}$ $\mathrm{X_2} \rightarrow \mathrm{X_2} + \mathrm{P_2}$ $\mathrm{P_1} \rightarrow 0$ $\mathrm{P_2} \rightarrow 0$

$\mathrm{X_2} + \mathrm{P_1} \rightleftharpoons \mathrm{X_2P_1}$ $2\,\mathrm{P_2} \rightleftharpoons \mathrm{P_2P_2}$ $\mathrm{X_1} + \mathrm{P_2P_2} \rightleftharpoons \mathrm{X_1P_2P_2}$

Figure 1: Three networks where stability of steady states is fully determined.

solutions to the four steady state equations together with the conservation law associated with T_1 can be parametrized by a function φ that only depends on x_5. The denominator of $(F_{T,5} \circ \varphi)(z)$ is positive and its numerator is a polynomial of degree 3 in z, which can have $1, 2$ or 3 positive roots, depending on the choice of parameters. Additionally, $\det(J_{F_T}(\varphi(x_i))_{J,I})$ is a rational function with all coefficients positive. Thus, we are in the situation of Theorem 2. Since the independent term of the numerator of $(F_{T,5} \circ \varphi)(z)$ is negative, its first root has positive derivative. Further, the sign of $\frac{(-1)^{s+i+j}}{\varphi_5'(z)} = (-1)^{4+5+5}$ is $+1$, and hence, the sign of

$$\frac{(-1)^{s+i+j}}{\varphi_i'(z_1)} (F_{T,j} \circ \varphi)'(z_1) \det(J_{F_T}(\varphi(z_1))_{J,I})$$

is positive as well. We conclude, using Theorem 2, that whenever the network has three positive steady states coming from the roots $z_1 < z_2 < z_3$ of $(F_{T,5} \circ \varphi)(z)$, then the steady states $\varphi(z_1)$ and $\varphi(z_3)$ are exponentially stable and the steady state $\varphi(z_2)$ is unstable. We have shown that *this network displays bistability whenever there are three positive steady states*

3 Symbolic determination of stability

We now combine the ingredients introduced in the previous section into a strategy to determine the stability of positive steady states and, importantly, detect bistability, using (mainly) the Hurwitz criterion and Theorem 2. Given a reaction network with kinetics $v(x)$ the steps taken are depicted in Fig. 2. Specifically, we find $q_x(\lambda)$ and the Hurwitz determinants. If all determinants are positive, then the positive steady states are exponentially stable. If only the last Hurwitz determinant can be negative, then we attempt to apply Theorem 2. We only find a parametrization ϕ as in Eq. (6) when the sign of H_i is not determined for arbitrary positive x.

If some of the steps fail, then we consider reduced networks by removing either reactions that do not change the stoichiometric subspace, or intermediates. If stability is determined for a reduced network, then we conclude that the original network has at least the same number of positive steady states and stability properties as the reduced network in an open parameter region. In particular, if the reduced network has bistability, then so does the original network.

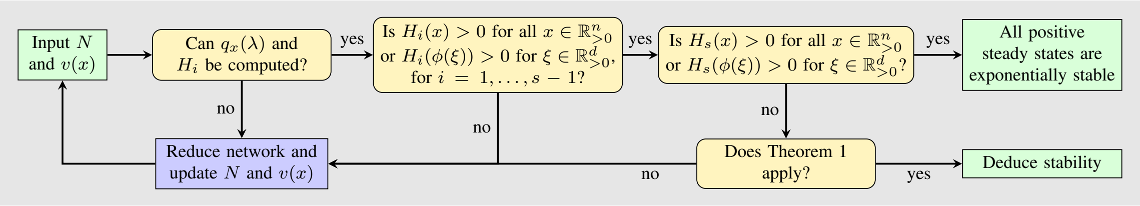

Figure 2: Flow chart of our approach to study the stability of steady states. Here ϕ is a positive parametrization.

We now use this approach on the remaining networks in Fig. 1. We consider a **two substrate enzyme catalysis** mechanism, comprising an enzyme E that binds two substrates, S_1 and S_2, and catalyzes the reversible conversion to P. Taken with mass-action kinetics this network has one positive steady state in each $\mathcal{P}_T$ for any κ [10]. All but the last of the four Hurwitz determinants are positive for $x \in \mathbb{R}^6_{>0}$. We find a positive parametrization ϕ by solving the steady state equations in the concentrations of $\mathrm{ES_1}$, $\mathrm{ES_2}$, $\mathrm{ES_1S_2}$ and P using the procedure in [22], see Appendix C. After evaluating at ϕ, H_4 becomes a rational function with only positive coefficients. Hence, all Hurwitz determinants are positive at a positive steady state, showing that *the only positive steady state is exponentially stable.*

Next, we consider the **gene transcription network** in Fig. 1 with mass-action kinetics. For any choice of rate constants there exist at least two positive steady states in some $\mathcal{P}_T$ [10]. The computation of the Hurwitz determinants for arbitrary $x \in \mathbb{R}^7_{>0}$ gives that only H_1, H_2 are positive, but after evaluating at a positive parametrization, all but the last Hurwitz determinants are positive. We proceed to verify the assumptions of Theorem 2, see Appendix C. We obtain that the maximum number of positive steady states in any $\mathcal{P}_T$ is 3, and that, *whenever the network has one positive steady state, then it is exponentially stable, and if it has three positive steady states, then two of them are exponentially stable and one is unstable.*

3.1 Bistability in cell signaling

After having illustrated our approach with selected examples, we now investigate relevant cell signaling motifs. All networks in Fig. 3 are known to be **monostationary** under mass-action [21]. All Hurwitz determinants of $q_x(\lambda)$ are positive for positive x, without the need of a positive parametrization, see Appendix C. Hence for any rate constants, each network in Fig. 3 *has exactly one positive steady state in each $\mathcal{P}_T$, which further is exponentially stable.*

In the examples so far, we have not employed network reduction techniques, because all steps of the method could be carried through and the stability of a steady state depended only on the sign of the determinant of the Jacobian. This scenario is quite restrictive, as it implies that instabilities arise from a unique eigenvalue with positive real part. The networks in Fig. 4 are all known to be **multistationary** with mass-action [21, 24]. Our method fails on the original networks: for network (c), the computation of the Hurwitz determinants was not possible in a regular PC due to lack of memory, and for the rest of the networks $H_i > 0$ for $i \neq s$ does not hold. However, Theorem 2 applies to the reduced networks in Fig. 4. In particular, all reduced networks in Fig. 4 display bistability whenever they have three positive steady states. Hence, after lifting stability to the original network, *for all networks in Fig. 4, there is an open parameter region where the network has two exponentially stable positive steady states.*

4 Computational challenges

In our context the Hurwitz determinants are symbolic and depend on κ and x or ξ. Their computation requires the storage of functions with many terms, which easily becomes unfeasible in a regular PC. For example, for network (6) in Fig. 3, H_4 and H_5 are polynomials in κ and x with respectively 1,732,192 and 37,609,352 monomials, before the evaluation at a parametrization.

For medium sized networks some tricks can be applied under mass-action. A first strategy is to parametrize the positive steady state variety using *convex parameters* introduced by Clarke [9, 17]. This conversion may reduce the number of parameters, mainly if the network has few reversible reactions.

The second strategy encodes a monomial $\eta\, x_1^{a_1} \cdots x_n^{a_n}$ as an $(n+1)$-tuple $(\eta, a_1, \dots, a_n)$, and exploits relations among the H_i obtained by expanding recursively along the last column, e.g. $H_3 = a_{s-3}H_2 - a_{s-1}(a_{s-4}H_1 + a_s a_{s-5})$ for a generic polynomial. Assume H_i is written as a sum of terms that can be computed. We gather the $(n+1)$-tuples of each term into a list L_1 and create a list L_2 of the exponents for which η is not a positive function. For each element in L_2, we consider the tuples in L_1 with that exponent, sum the coefficients and inspect the sign. If all coefficients are nonnegative, then so is H_i. This procedure requires substantially less memory, but it might take time as lists are long. With this strategy we have determined the sign of H_5 in networks (5) and (6) in Fig. 3.

In special situations, H_i can be computed for x, but evaluating at a parametrization and expanding the resulting polynomial to inspect its sign encounters memory issues.

(1)	$S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$ $S_1 + F \rightleftharpoons S_1F \rightarrow S_0 + F$	**(2)**	$S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$ $S_1 + E \rightleftharpoons S_1E \rightarrow S_0 + E$
(3)	$S_0 + E_1 \rightleftharpoons S_0E_1 \rightarrow S_1 + E_1$ $S_0 + E_2 \rightleftharpoons S_0E_2 \rightarrow S_1 + E_2$ $S_1 + F \rightleftharpoons S_1F \rightarrow S_0 + F$	**(4)**	$S_0 + E_1 \rightleftharpoons S_0E_1 \rightarrow S_1 + E_1$ $S_0 + E_2 \rightleftharpoons S_0E_2 \rightarrow S_1 + E_2$ $S_1 + F_1 \rightleftharpoons S_1F_1 \rightarrow S_0 + F_1$ $S_1 + F_2 \rightleftharpoons S_1F_2 \rightarrow S_0 + F_2$
(5)	$S_0 + E_1 \rightleftharpoons S_0E_1 \rightarrow S_1 + E_1$ $S_1 + E_2 \rightleftharpoons S_1E_2 \rightarrow S_2 + E_2$ $S_1 + F_1 \rightleftharpoons S_1F_1 \rightarrow S_0 + F_1$ $S_2 + F_2 \rightleftharpoons S_2F_2 \rightarrow S_1 + F_2$	**(6)**	$S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$ $P_0 + E \rightleftharpoons P_0E \rightarrow P_1 + E$ $S_1 + F_1 \rightleftharpoons S_1F_1 \rightarrow S_0 + F_1$ $P_1 + F_2 \rightleftharpoons P_1F_2 \rightarrow P_0 + F_2$

Figure 3: Monostationary networks. In all networks, the symbols E, F, S, P refer to kinases, phosphatases, and substrates respectively. Taken with mass-action kinetics, all networks admit exactly one positive steady state in $\mathcal{P}_T$, which further is exponentially stable. Networks (1)-(4) model the phosphorylation of one substrate via different mechanisms. Network (5) models a substrate with two phosphorylation sites, while network (6) models the phosphorylation of two different substrates.

This problem can be addressed if there exists a *monomial positive parametrization* of the form $\phi(\xi) = \alpha \circ \xi^B$ with $B \in \mathbb{Z}^{d\times n}$ such that $\phi(\xi)_i = \alpha_i(\xi^B)_i = \alpha_i \xi_1^{b_{1i}} \cdots \xi_d^{b_{di}}$, with α depending on the rate constants [34]. In this case, a monomial $\eta x_1^{a_1} \cdots x_n^{a_n}$ of H_i becomes $\eta \alpha_1^{a_1} \cdots \alpha_n^{a_n} \xi^{Ba}$. Hence, we first write all monomials as an $(n+1)$-tuple and record the evaluation with a new $(n+d+1)$-tuple $(\eta, a_1, \ldots, a_n, (Ba)_1, \ldots, (Ba)_d)$. We proceed as before to group the tuples and study the sign of the coefficients. With this approach, we computed the Hurwitz determinants of networks(a), (b), (d) and (e) in Fig. 4. However, H_{s-1} is not positive and Theorem 2 does not apply.

To verify that a polynomial with both positive and negative coefficients attains both signs, one can study the associated Newton polytope, as employed in the context of reaction networks in [10, 12] to cite a few. To assert that a polynomial only attains positive values despite having negative coefficients, one can employ techniques from sum-of-squares [7] and polynomial optimization via sums of nonnegative circuit polynomials [16, 28]. However, the size of the polynomials we encounter make these approaches challenging.

	Network	**Reduced network**		**Network**	**Reduced network**
(a)	$S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$	$S_0 + E \rightarrow S_0E \rightarrow S_1 + E$	**(d)**	$S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$	$S_0 + E \rightarrow S_0E \rightarrow S_1 + E$
	$S_1 + E \rightleftharpoons S_1E \rightarrow S_2 + E$	$S_1 + E \rightarrow S_1E \rightarrow S_2 + E$		$S_1 + E \rightleftharpoons S_1E \rightarrow S_2 + E$	$S_1 + E \rightarrow S_2 + E$
	$S_1 + F_1 \rightleftharpoons S_1F_1 \rightarrow S_0 + F_1$	$S_1 + F_1 \rightarrow S_0 + F_1$		$F + S_2 \rightleftharpoons S_2F \rightarrow F + S_1$	$F + S_2 \rightarrow F + S_1$
	$S_2 + F_2 \rightleftharpoons S_2F_2 \rightarrow S_1 + F_2$	$S_2 + F_2 \rightarrow S_1 + F_2$		$F + S_1 \rightleftharpoons S_1F \rightarrow F + S_0$	$F + S_1 \rightarrow F + S_0$
(b)	$S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$	$S_0 + E \rightarrow S_1 + E$	**(e)**	$S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$	$S_0 + E \rightarrow S_1 + E$
	$S_1 + F \rightleftharpoons S_1F \rightarrow S_0 + F$	$S_1 + F \rightarrow S_0 + F$		$S_1 + F \rightleftharpoons S_1F \rightarrow S_0 + F$	$S_1 + F \rightarrow S_1F \rightarrow S_0 + F$
	$P_0 + S_1 \rightleftharpoons P_0S_1 \rightarrow P_1 + S_1$	$P_0 + S_1 \rightarrow P_0S_1 \rightarrow P_1 + S_1$		$P_0 + E \rightleftharpoons P_0E \rightarrow P_1 + E$	$P_0 + E \rightarrow P_0E \rightarrow P_1 + E$
	$P_1 + F \rightleftharpoons P_1F \rightarrow P_0 + F$	$P_1 + F \rightarrow P_1F \rightarrow P_0 + F$		$P_1 + F \rightleftharpoons P_1F \rightarrow P_0 + F$	$P_1 + F \rightarrow P_0 + F$
(c)	$S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$	$S_0 + E \rightarrow S_1 + E$	**(f)**	$S_0 + E_1 \rightleftharpoons S_0E_1 \rightarrow S_1 + E_1$	$S_0 + E_1 \rightarrow E_1S_0 \rightarrow S_1 + E_1$
	$S_1 + F_1 \rightleftharpoons S_1F_1 \rightarrow S_0 + F_1$	$S_1 + F_1 \rightarrow S_1F_1 \rightarrow S_0 + F_1$		$S_0 + E_2 \rightleftharpoons S_0E_2 \rightarrow S_1 + E_2$	$S_0 + E_2 \rightarrow S_1 + E_2$
	$P_0 + S_1 \rightleftharpoons P_0S_1 \rightarrow P_1 + S_1$	$P_0 + S_1 \rightarrow P_1 + S_1$		$E_1 \rightleftharpoons E_2$	$E_2 + S_0 \rightarrow E_1S_0$
	$P_0 + E \rightleftharpoons P_0E \rightarrow P_1 + E$	$P_0 + E \rightarrow P_0E \rightarrow P_1 + E$		$S_0E_1 \rightleftharpoons S_0E_2$	$E_1 \rightarrow E_2$
	$P_1 + F_2 \rightleftharpoons P_1F_2 \rightarrow P_0 + F_2$	$P_1 + F_2 \rightarrow P_0 + F_2$		$S_1 \rightarrow S_0$	$S_1 \rightarrow S_0$

Figure 4: Multistationary networks and reductions to assert bistability. (a) E_1 and E_2 are two conformations of a kinase that catalyzes the phosphorylation of S_0 [24]. The reduced network is obtained by removing the intermediate E_2S_0 and all reverse reactions. (b) A cascade of two one-site modification cycles with the same phosphatase F. The reduced network is obtained by removing the intermediates S_0E and S_1F and all reverse reactions. (c) A cascade of one-site modification cycles where the same kinase E acts in both layers. The reduced network is obtained by removing the reverse reactions and intermediates S_0E, P_0S_1 and P_1F_2. (d) Distribute and sequential phosphorylation of a substrate. The reduced network is obtained by removing the intermediates S_1E, S_2F and S_1F and all reverse reactions. (e) Phosphorylation of two substrates by the same kinase and phosphatase. The reduced network is obtained by removing the intermediates S_0E and P_1F and all reverse reactions. (f) Phosphorylation of a substrate with two sites catalyzed by the same kinase and two different phosphatases. The reduced network is obtained by removing all reverse reactions and the intermediates S_1F_1 and S_2F_2.

5 Discussion

All the steps of our procedure to determine the stability are symbolic and therefore provide computer-assisted proofs for bistability. In the most favorable scenario where Theorem 2 applies, the number of unstable and exponentially stable steady states is completely determined, and question (3) in the Introduction is answered. In particular, if the reduced univariate equation has at least three solutions and the first steady state is exponentially stable, the parameter region of bistability agrees with the parameter region giving three positive steady states. Finding the latter poses a simpler (though still hard) challenge, which can be addressed using recent methods [10, 6].

Under mass-action kinetics, reduction of the steady state equations to one polynomial can in principle be achieved using Groebner bases and invoking the Shape Lemma [13]. However, positivity is not addressed and the interval $\mathcal{E}$ in Proposition 4 is rarely explicit. Reduction to one polynomial arises often after exploiting the inherent linearity the equations have [22].

In our procedure, the Hurwitz criterion can be replaced by other criteria of algebraic nature, namely the Liénard-Chipart criterion in [14] or checking whether the matrix Q_{x^*} is both a P-matrix and sign-symmetric. However, these criteria can only be used to assert exponential stability (see Appendix B, where these criteria are applied to the network in Eq. (4)).

We have illustrated with numerous realistic examples that our approach determines bistability after performing network reduction. To our knowledge, this is a new result for all networks in Fig. 4 but network (d). For this one, bistability was formally proven in [26] using methods from geometric singular perturbation theory and the accurate study of a reduced network. We see our approach as a big step towards the automatic detection of bistability in open parameter regions, which relies on purely algebraic manipulations instead of advanced analytic arguments. Although the approach is applicable to arbitrary ODE systems, the special structure of the systems arising from reaction networks, specifically linearity, the existence of conservation laws and reduction techniques, make the approach particularly suited for this scenario.

Methods. All computations were made in `Maple 2019`. We first compute the Hurwitz determinants of a generic degree n polynomial and then evaluate at the coefficients of $q_x(\lambda)$. We disregarded the Routh table from the package DynamicSystems for using more memory.

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Appendices

In the Appendix A we provide the proofs of the results in the main text, in Appendix B expand on the stability criteria, and finally in Appendix C provide the details of the examples.

A Proofs of the results in the main text

We prove here the results in the main text. For completeness, we include the notation and the statements here, sometimes in an expanded form.

A.1 Proof of Proposition 3

Consider a matrix $R_0 \in \mathbb{R}^{n\times s}$ whose columns form a basis of S. This basis gives the system of coordinates in S. Therefore, given coordinates $z = (z_1, \dots, z_s)$ in S, the vector $R_0 z$ is the vector of coordinates in the canonical basis of $\mathbb{R}^n$. Conversely, selecting a matrix $R_1 \in \mathbb{R}^{s\times n}$ such that $R_1 R_0 = I_{s\times s}$, we can write a vector $x \in S$ given in the canonical basis of $\mathbb{R}^n$, as a vector in local coordinates, by doing the product $R_1 x$.

Using these matrices, the ODE system restricted to $(x^*+S)\cap\mathbb{R}^n_{\geq 0}$ in local S coordinates is

$$\dot{z} = R_1 f(R_0 z + x^*)$$

after translating the steady state x^* to the origin. The Jacobian matrix associated with this system at 0 is $R_1 J_f(x^*) R_0$. The following proposition shows some basic properties of $R_1 J_f(x^*) R_0$.

Proposition 3. *Consider a reaction network with rate function* $f(x) = Nv(x)$. *Let* $R_0 \in \mathbb{R}^{n\times s}$ *and* $R_1 \in \mathbb{R}^{s\times n}$ *be matrices such that the columns of* R_0 *form a basis of the stoichiometric subspace* S, *and* $R_1R_0 = I_{s\times s}$. *The following statements hold:*

- $R_1J_f(x^*)R_0 = LJ_v(x^*)R_0$, *where* $L \in \mathbb{R}^{s\times m}$ *is the matrix such that* $N = R_0L$. *In particular,* $R_1J_f(x^*)R_0$ *does not depend on the choice of* R_1.
- *If* $R_0, R_0' \in \mathbb{R}^{n\times s}$ *are two matrices with column span* S, *and* L, L' *are as in (i) for* R_0, R_0' *respectively, then the matrices* $L'J_v(x^*)R_0'$ *and* $LJ_v(x^*)R_0$ *are similar.*
- *For a positive steady state* x^*, *the characteristic polynomials* $p_{J_f}(\lambda)$ *and* $p_{LJ_v(x^*)R_0}(\lambda)$ *satisfy* $p_{J_f}(\lambda) = \lambda^{n-s}p_{LJ_v(x^*)R_0}(\lambda)$ *for any choice of* R_0.
- *The independent term of* $p_{LJ_v(x^*)R_0}(\lambda)$ *(or the coefficient of degree* $n-s$ *of* $p_{J_f}(\lambda)$*) equals* $(-1)^s \det(J_{F_T}(x^*))$, *with* F_T *given in the main text, for any choice of row-reduced matrix of conservation laws* W.

Proof. (i) Since the columns of N belong to S, we can uniquely write $N = R_0L$ with $L \in \mathbb{R}^{s\times m}$. Given that $J_f(x) = NJ_v(x)$, we have

$$R_1J_f(x^*)R_0 = R_1NJ_v(x^*)R_0 = R_1R_0LJ_v(x^*)R_0 = LJ_v(x^*)R_0.$$

(ii) Let $M \in \mathbb{R}^{s\times s}$ be the matrix of change of basis from R_0 to R_0' such that $R_0M = R_0'$. From $N = R_0'L' = R_0L$, it follows that $R_0ML' = R_0L$ and thus $L' = M^{-1}L$. This gives

$$L'J_v(x^*)R_0' = M^{-1}LJ_v(x^*)R_0M,$$

which implies that $L'J_v(x^*)R_0'$ and $LJ_v(x^*)R_0$ are similar.

(iii) Extend the matrix R_0 to a square matrix $R \in \mathbb{R}^{n\times n}$ by adding columns such that R has full rank n. Then the eigenvalues of the matrices $Q = R^{-1}J_f(x^*)R$ and $J_f(x^*)$ coincide. We choose R_1 as the first s rows of $R^{-1} = \begin{pmatrix} R_1 \\ R_1' \end{pmatrix}$. Then $R_1R_0 = I_{s\times s}$ and $R_1'R_0 = 0$. Since $\operatorname{Im}(J_f(x^*)) \subseteq S$, the column span of $J_f(x^*)$ and R_0 agree, and hence $R_1'J_f(x^*) = 0$. Then, the matrix Q is for the form

$$Q = \begin{pmatrix} R_1 \\ R_1' \end{pmatrix} J_f(x^*) \begin{pmatrix} R_0 & R_0' \end{pmatrix} = \begin{pmatrix} R_1J_f(x^*) \\ 0 \end{pmatrix} \begin{pmatrix} R_0 & R_0' \end{pmatrix} = \begin{pmatrix} R_1J_f(x^*)R_0 & R_1J_f(x^*)R_0' \\ 0 & 0 \end{pmatrix}.$$

Clearly, the characteristic polynomial $p_Q(\lambda)$ is equal to $\lambda^{n-s}p_{R_1J_f(x^*)R_0}(\lambda)$. Using $R_1J_f(x^*)R_0 = LJ_v(x^*)R_0$, this concludes the proof of (iii).

(iv) was proven in [38], Proposition 5.3. □

A.2 Proof of Proposition 4

We now turn into the proof of Proposition 4, whose statement we recall here.

Proposition 4. *Let* $W \in \mathbb{R}^{d\times n}, T \in \mathbb{R}^d$ *be fixed and consider* F_T *as in the main text. Assume that there exist an open interval* $\mathcal{E} \subseteq \mathbb{R}$*, a differentiable function* $\varphi\colon \mathcal{E} \to \mathbb{R}^n_{>0}$*, and indices* i, j *such that* $F_{T,\ell}(\varphi(z)) = 0$ *for all* $\ell \neq j$ *and* $\varphi_i'(z) \neq 0$*. Then, the set of positive solutions of the system* $F_T(x) = 0$ *contains the solutions to*

$$F_{T,j}(\varphi(z)) = 0, \quad x_\ell = \varphi_\ell(z), \quad \ell = 1, \dots, n \text{ and } z \in E, \tag{9}$$

and for a positive steady state $x^* = \varphi(z)$ *it holds*

$$\det(J_{F_T}(x^*)) = \frac{(-1)^{i+j}}{\varphi_i'(z)} (F_{T,j} \circ \varphi)'(z) \det(J_{F_T}(x^*)_{J,I}),$$

where $I = \{1, \dots, n\} \setminus \{i\}$ *and* $J = \{1, \dots, n\} \setminus \{j\}$*.*

Proof. In order to prove this identity, we make first three observations that rely on the definition of φ and on the chain rule for multivariate functions:

(1) By hypothesis, $\varphi(z) = (\varphi_1(z), \dots, \varphi_n(z))$. Therefore, $\varphi'(z) = (\varphi_1'(z), \dots, \varphi_n'(z))$.

(2) Since $F_{T,\ell}(\varphi(z)) = 0$ for all $\ell \neq j$, we have that $(F_T \circ \varphi)(z)$ is a vector with zeros in every entry except for the j-th entry, which is equal to $(F_{T,j} \circ \varphi)(z)$. This implies that $(F_T \circ \varphi)'(z)$ is also a vector with zero in every entry except in the j-th, that is equal to $(F_{T,j} \circ \varphi)'(z)$.

(3) By the chain rule $(F_T \circ \varphi)'(z) = J_{F_T}(\varphi(z))\varphi'(z)$.

From observations (2) and (3) we have that

$$J_{F_T}(\varphi(z))\varphi'(z) = (0, \dots, 0, (F_{T,j} \circ \varphi)'(z), 0, \dots, 0)^{tr}, \tag{10}$$

which means that the linear combination of the columns of $J_{F_T}(\varphi(z))$ given by the entries of $\varphi'(z)$ is equal to the vector on the right side of the equation. Now, using observation (1), we compute $\det(J_{F_T}(x^*))$. Indeed, denoting by $J^\ell_{F_T}$ the ℓ-th column of J_{F_T}, (10) gives

$$\varphi_i'(z) J^i_{F_T}(\varphi(z)) = (0, \dots, 0, (F_{T,j} \circ \varphi)'(z), 0, \dots, 0)^{tr} - \sum_{k=1, k\neq i}^{n} \varphi_k'(z) J^k_{F_T}(\varphi(z)).$$

Let $\widehat{J}_{F_T}(x^*)$ be the matrix obtained by replacing the i-th column of $J_{F_T}(\varphi(z))$ by the vector

$$\left(0, \dots, 0, \frac{(F_{T,j} \circ \varphi)'(z)}{\varphi_i'(z)}, 0, \dots, 0\right)^{tr}.$$

Then, $\det(J_{F_T}(\varphi(z)))$ and $\det\left(\widehat{J}_{F_T}(\varphi(z))\right)$ agree. Now, expanding the determinant of $\widehat{J}_{F_T}(\varphi(z))$ along the i-th column gives

$$\det(J_{F_T}(x^*)) = \frac{(-1)^{i+j}}{\varphi_i'(z)} (F_{T,j} \circ \varphi)'(z) \det(J_{F_T}(x^*)_{J,I}).$$

This concludes the proof. □

A.3 Proof of Theorem 2 and Lemma 1

Theorem 2. *Consider a reaction network and fix* T*. Let* $\mathcal{E}, \varphi, i, j, I, J$ *be as in Proposition 4. Assume that*

[itemsep=0pt]

- *the sign of* $\frac{1}{\varphi_i'(z)}\det(J_{F_T}(\varphi(z))_{J,I})$ *is independent of* $z \in E$ *and is nonzero, and*
- *the first* $s-1$ *Hurwitz determinants of* $q_{x^*}(\lambda)$ *are positive for all positive steady states* x^**.*

If $z_1 < \cdots < z_\ell$ *are the positive solutions to the equation* $F_{T,j}(\varphi(z)) = 0$*, and all have multiplicity one, then either* $\varphi(z_1), \varphi(z_3), \ldots$ *are exponentially stable and* $\varphi(z_2), \varphi(z_4), \ldots$ *are unstable, or the other way around. Specifically,* $\varphi(z_1)$ *is exponentially stable if*

$$\frac{(-1)^{s+i+j}}{\varphi_i'(z_1)}(F_{T,j} \circ \varphi)'(z_1)\det(J_{F_T}(\varphi(z_1))_{J,I}) > 0,$$

and unstable if it is negative.

Proof. By the second hypothesis, the first $s-1$ Hurwitz determinants are positive, so the stability only depends on the sign of the last Hurwitz determinant, which as discussed in the main text, agrees with the sign of $(-1)^s\det(J_{F_T}(\varphi(z)))$. According to Proposition 4, it further coincides with the sign of $\frac{(-1)^{s+i+j}}{\varphi_i'(z)}(F_{T,j} \circ \varphi)'(z)\det(J_{F_T}(\varphi(z))_{J,I})$. Since $\det(J_{F_T}(\varphi(z))_{J,I})$ has a constant sign for every $z \in E$, the sign of the last Hurwitz determinant changes when the sign of $(F_{T,j} \circ \varphi)'(z)$ does, and this is the derivative of a univariate differentiable function whose real positive roots $z_1 < \cdots < z_\ell$ have multiplicity one and are ordered in an increasing way. Given that $(F_{T,j} \circ \varphi)$ is differentiable, the sign of its derivative evaluated at consecutive roots alternates, that is $(F_{T,j} \circ \varphi)(z_1)' > 0, (F_{T,j} \circ \varphi)(z_3)' > 0, \ldots$ and $(F_{T,j} \circ \varphi)(z_2)' < 0, (F_{T,j} \circ \varphi)(z_4)' < 0, \ldots$ or the other way around.

In our setting this means that, once the sign of $(F_{T,j} \circ \varphi)'(z_k)$ is multiplied by $(-1)^{s+i+j}$ and by the sign of $\frac{1}{\varphi_i'(z)}\det(J_{F_T}(\varphi(z))_{J,I})$, either $\varphi(z_1), \varphi(z_3), \ldots$ are exponentially stable and $\varphi(z_2), \varphi(z_4), \ldots$ are unstable, or the other way around. In particular, if the sign of $(F_{T,j} \circ \varphi)(z_1)'$ times the sign of $(-1)^{s+i+j}\frac{1}{\varphi_i'(z)}\det(J_{F_T}(\varphi(z))_{J,I})$ is positive, then $\varphi(z_1)$ is exponentially stable. ☐

Lemma 1. *Under the assumptions of Theorem 2, assume* $(F_{T,j} \circ \varphi)(z) = \frac{a(z)}{b(z)}$ *is a rational function in* z *such that* $b(z)$ *is positive in* $\mathcal{E}$*. Then the zeros of* $(F_{T,j} \circ \varphi)(z)$ *agree with the roots of* $a(z)$ *and the sign of* $(F_{T,j} \circ \varphi)'(z^*)$*, and* $a'(z^*)$ *agree for all* $z^* \in \mathcal{E}$ *such that* $a(z^*) = 0$*.*

Proof. The first part is straightforward, since the denominator of $(F_{T,j} \circ \varphi)(z)$ does not vanish in $\mathcal{E}$. For the second part, we have $F_{T,j}(\varphi(z^*))' = \frac{a'(z^*)b(z^*) - a(z^*)b'(z^*)}{b(z^*)^2} = \frac{a'(z^*)}{b(z^*)}$, and the conclusion follows from the fact that $b(z^*) > 0$. ☐

B Other algebraic criteria for stability

In this section we discuss two other criteria to decide the stability of the steady states.

Similarly to the Hurwitz criterion, the Liénard-Chipart criterion in [14], determines whether all the roots of a polynomial have negative real part, and requires a smaller amount of computations than the Hurwitz criterion. Before introducing the criterion, we need some ingredients.

Definition 1.

- *The Bezout matrix of two polynomials* $h(x) = h_n x^n + h_{n-1}x^{n-1} + \cdots + h_1 x + h_0$ *and* $g(x) = g_m x^m + g_{m-1}x^{m-1} + \cdots + g_1 x + g_0$ *with* $n \geq m$*, denoted by* $B_{h,g}$*, is defined as the representation matrix of the bilinear form given by*

$$B(h, g; x, y) = \frac{h(x)g(y) - h(y)g(x)}{x - y} = \sum_{i,j=0}^{n-1} b_{ik} x^i y^k,$$

  that is, $B_{h,g} := (b_{ik})$*. This is a symmetric matrix of size* $n \times n$*.*

- *A square matrix* $A \in \mathbb{R}^{n \times n}$ *is called a P-matrix if all its principals minors are positive, that is,* $\det(A_{I,I}) > 0$ *for every subset* $I \subseteq \{1, \ldots, n\}$*. If* A *is symmetric, this is equivalent to* A *being positive definite.*

We now present the first additional stability criterion.

Criterion 2 (Liénard-Chipart)**.** *All the roots of a polynomial* $p(x) = x^s + p_{s-1}x^{s-1} + \ldots + p_1 x + p_0$ *with* $p_i \in \mathbb{R}$ *and* $p_0 \neq 0$ *have negative real part if and only if, after writing* $p(x) = h(x^2) + xg(x^2)$*, the Bezout matrix* $B_{h,g}$ *of* h *and* g *is positive definite and* $p_i > 0$ *for* $i = 1, \ldots, s$*.*

In this criterion the polynomials h and g are associated with the even and odd parts of p respectively. Note that the degrees of h and g are at most $\lfloor \frac{s}{2} \rfloor$, therefore the size of $B_{h,g}$ is $\lfloor \frac{s}{2} \rfloor$. Additionally, since $B_{h,g}$ is symmetric, $B_{h,g}$ is positive definite if and only if it is a P-matrix.

Unlike the Hurwitz criterion, Liénard-Chipart does not give a result regarding instability. If $B_{h,g}$ is not a P-matrix, it is not possible to determine whether the eigenvalues that do not have negative real part, have positive or zero real part.

In order to apply the criterion to the running example in the main text (Eq. (4)), we write $q_x(\lambda)$ as $h(\lambda^2) + \lambda g(\lambda^2)$, with

$$h(\lambda) = \lambda + \kappa_1\kappa_2 x_2 + \kappa_2\kappa_3 x_3 + \kappa_1\kappa_3 \quad \text{and} \quad g(\lambda) = \kappa_2 x_2 + \kappa_2 x_3 + \kappa_1 + \kappa_3.$$

The Bezout matrix is then $B_{h,g} = (x_2 + x_3)\kappa_2 + \kappa_1 + \kappa_3$, which is clearly a P-matrix. By the Liénard-Chipart criterion, we conclude that all the roots of $q_x(\lambda)$ have negative real part, recovering thereby that the only positive steady state in each stoichiometric compatibility class is exponentially stable.

The Liénard-Chipart criterion is the most efficient if we consider the amount of determinants that have to be computed to reach a decision. Given that the Bezout matrix is symmetric, to check that is positive definite, it is only necessary to compute the principal minors and check whether they are positive. Thus the required amount of determinants is at most $\sum_{i=0}^{\lfloor\frac{n+1}{2}\rfloor}\binom{\lfloor\frac{n+1}{2}\rfloor}{i}$. Although this criteria computes the smallest amount of determinants, for some examples, the entries of the Bezout matrix are larger than the entries of the Jacobian. In those cases the memory of a regular PC is still not enough to store the computations.

We conclude the list of stability criteria of algebraic nature with one more criterion, which does not rely on the computation of the characteristic polynomial. For a square matrix A, we say that A is *sign symmetric* if $\det(A_{I,J})\det(A_{J,I}) \geq 0$ for every $I, J \subset \{1, \dots, n\}$ with the same cardinality.

Criterion 3 (P-matrices that are sign symmetric)**.** *If a square matrix A is both a P-matrix and sign symmetric, then all its eigenvalues have positive real part.*

With this criterion, proved in [8], if $-A$ is a P-matrix and sign-symmetric, then all its eigenvalues have negative real part. For reaction networks, we apply the criterion to the matrix $-Q_x$. In the running example in the main text, we compute the minors of size 1 and 2 of $A = -Q_x$. The minors of size 1 are the entries of the matrix, which are all positive. The only minor of size 2 is

$$\det(A) = \kappa_1\kappa_2x_2 + \kappa_2\kappa_3x_3 + \kappa_1\kappa_3.$$

All minors are polynomials that are positive for all $x \in \mathbb{R}^4_{>0}$ and positive κ. Therefore $-Q_x$ is a P-matrix and sign symmetric. Hence, with this new criterion we recover the conclusion that the only positive steady state is exponentially stable.

While the Hurwitz and Liénard-Chipart criteria are applied to the characteristic polynomial of $LJ_v(x^*)R_0$, which is independent of the choice of R_0, Criterion 3 is applied directly to the matrix $-LJ_v(x^*)R_0$. By Proposition 3, two different choices of R_0 give rise to two similar matrices. However, the properties of being P-matrix and sign symmetric are not preserved on similar matrices. As a small example consider

$$A = \begin{pmatrix} 2 & 1 \\ 3 & 4 \end{pmatrix} \qquad \text{and} \qquad B = \begin{pmatrix} -1 & -4 \\ 3 & 7 \end{pmatrix};$$

these matrices are similar through $M = \begin{pmatrix} 1 & 1 \\ 0 & 1 \end{pmatrix}$,but A is both a P-matrix and sign symmetric and B is neither.

A comparison of the amount of operations required for each stability criterion shows that deciding whether a matrix is a P-matrix and sign-symmetric requires the largest amount of operations. In this case, all $\sum_{i=1}^{s}\binom{s}{i}^2$ minors of the matrix must be computed, which requires the storage of a large amount of information given that the entries of the Jacobian are typically polynomials.

C Examples

In this section we provide extra details on the examples of the main text. For more details on the computations, you may request the Maple file to the authors.

C.1 Hybrid histidine kinase

We consider the following network with mass-action kinetics

$$\mathrm{HK}_{00} \xrightarrow{\kappa_1} \mathrm{HK}_{p0} \xrightarrow{\kappa_2} \mathrm{HK}_{0p} \xrightarrow{\kappa_3} \mathrm{HK}_{pp} \qquad \mathrm{HK}_{0p} + \mathrm{Htp} \xrightarrow{\kappa_4} \mathrm{HK}_{00} + \mathrm{Htp}_p$$

$$\mathrm{Htp}_p \xrightarrow{\kappa_6} \mathrm{Htp} \qquad \mathrm{HK}_{pp} + \mathrm{Htp} \xrightarrow{\kappa_5} \mathrm{HK}_{p0} + \mathrm{Htp}_p.$$

We rename the species as follows: X_1=HK_{00}, X_2=$\mathrm{HK}_{\mathrm{p0}}$, X_3=$\mathrm{HK}_{\mathrm{0p}}$, X_4=$\mathrm{HK}_{\mathrm{pp}}$, X_5=Htp and X_6=$\mathrm{Htp}_{\mathrm{p}}$. The concentration of the species X_i is denoted by x_i. With mass-action kinetics, the associated ODE system is

$$\begin{aligned}
\tfrac{dx_1}{dt} &= -\kappa_1 x_1 + \kappa_4 x_3 x_5 & \tfrac{dx_4}{dt} &= \kappa_3 x_3 - \kappa_5 x_4 x_5 \\
\tfrac{dx_2}{dt} &= \kappa_1 x_1 - \kappa_2 x_2 + \kappa_5 x_4 x_5 & \tfrac{dx_5}{dt} &= -\kappa_4 x_3 x_5 - \kappa_5 x_4 x_5 + \kappa_6 x_6 \\
\tfrac{dx_3}{dt} &= -\kappa_3 x_3 + \kappa_2 x_2 - \kappa_4 x_3 x_5 & \tfrac{dx_6}{dt} &= \kappa_4 x_3 x_5 - \kappa_6 x_6 + \kappa_5 x_4 x_5.
\end{aligned}$$

The conservation laws of the system are

$$T_1 = x_1 + x_2 + x_3 + x_4 \qquad T_2 = x_5 + x_6.$$

Hence

$$F_T(x) = \begin{pmatrix} x_1 + x_2 + x_3 + x_4 - T_1 \\ \kappa_5 x_4 x_5 + \kappa_1 x_1 - \kappa_2 x_2 \\ -\kappa_4 x_3 x_5 + \kappa_2 x_2 - \kappa_3 x_3 \\ -\kappa_5 x_4 x_5 + \kappa_3 x_3 \\ x_5 + x_6 - T_2 \\ \kappa_4 x_3 x_5 + \kappa_5 x_4 x_5 - \kappa_6 x_6 \end{pmatrix}.$$

Here $s = 4$. The existence of three positive steady states for this network was established in [31]. We compute $q_x(\lambda)$ and the Hurwitz determinants in Maple and obtain that all but the last are polynomials in x and κ with positive coefficients, hence positive when evaluated at a positive steady state.

We proceed to decide whether Theorem 2 applies. In [31], it was shown that the assumptions of Proposition 4 hold with $i = j = 5$, $\varphi_5(z) = z$ and $F_{T,5}$ corresponds to the conservation law with T_2. That is, the solutions to the four steady state equations together with the conservation law associated with T_1 can be parametrized by a function φ that only depends on $z = x_5$ and whose domain is $\mathcal{E} = \mathbb{R}_{>0}$. The denominator of $(F_{T,5} \circ \varphi)(z)$ is positive and its numerator is a polynomial of degree 3 in z, which can have $1, 2$ or 3 positive roots, depending on the choice of parameters. Additionally, $\det(J_{F_T}(\varphi(x_i))_{J,I})$ is a rational function with all coefficients positive. Thus, we are in the situation of Theorem 2 and can employ Lemma 1. Since the independent term of the numerator of $(F_{T,5} \circ \varphi)(z)$ is negative, its first root has positive derivative. Further, the sign of $\frac{(-1)^{s+i+j}}{\varphi_5'(z)} = (-1)^{4+5+5}$ is $+1$, and hence, the sign of

$$\frac{(-1)^{s+i+j}}{\varphi_i'(z_1)} (F_{T,j} \circ \varphi)'(z_1) \det(J_{F_T}(\varphi(z_1))_{J,I})$$

is positive as well. We conclude, using Theorem 2, that whenever the network has three positive steady states coming from the roots $z_1 < z_2 < z_3$ of $(F_{T,5} \circ \varphi)(z)$, then the steady states $\varphi(z_1)$ and $\varphi(z_3)$ are exponentially stable and the steady state $\varphi(z_2)$ is unstable. We have shown that this network displays bistability whenever there are three positive steady states.

C.2 Two substrate enzyme catalysis

We consider now the following network with mass-action kinetics

$$\mathrm{E}+\mathrm{S}_1 \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} \mathrm{ES}_1 \quad \mathrm{E}+\mathrm{S}_2 \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} \mathrm{ES}_2 \quad \mathrm{S}_2+\mathrm{ES}_1 \underset{\kappa_6}{\overset{\kappa_5}{\rightleftharpoons}} \mathrm{ES}_1\mathrm{S}_2 \quad \mathrm{ES}_1\mathrm{S}_2 \underset{\kappa_8}{\overset{\kappa_7}{\rightleftharpoons}} \mathrm{E}+\mathrm{P} \quad \mathrm{S}_1+\mathrm{ES}_2 \underset{\kappa_{10}}{\overset{\kappa_9}{\rightleftharpoons}} \mathrm{ES}_1\mathrm{S}_2.$$

This network consists of an enzyme E that binds two substrates, S_1 and S_2, in order to catalyze the reversible conversion to the product P. The binding is unordered. It was proven in [10] that this network has a unique steady state in each stoichiometric compatibility class for every set of reaction rate constants. We now prove that this steady state is exponentially stable. First, denote the species as X_1=E, X_2=S$_1$, X_3=ES$_1$, X_4=S$_2$, X_5=ES$_2$, X_6=ES$_1$S$_2$ and X_7=P. The concentration of species X_i is denoted by x_i. With this notation the ODE system is

$$\begin{aligned}
\tfrac{dx_1}{dt} &= -\kappa_1x_1x_2-\kappa_3x_1x_4-\kappa_{10}x_1x_7+\kappa_2x_3+\kappa_4x_5+\kappa_9x_6 & \tfrac{dx_5}{dt} &= \kappa_3x_1x_4-\kappa_8x_2x_5-\kappa_4x_5+\kappa_7x_6\\
\tfrac{dx_2}{dt} &= -\kappa_1x_1x_2-\kappa_8x_2x_5+\kappa_2x_3+\kappa_7x_6 & \tfrac{dx_6}{dt} &= \kappa_5x_3x_4+\kappa_8x_2x_5+\kappa_{10}x_1x_7\\
\tfrac{dx_3}{dt} &= \kappa_1x_1x_2-\kappa_5x_3x_4-\kappa_2x_3+\kappa_6x_6 & &\quad -\kappa_6x_6-\kappa_7x_6-\kappa_9x_6\\
\tfrac{dx_4}{dt} &= -\kappa_3x_1x_4-\kappa_5x_3x_4+\kappa_4x_5+\kappa_6x_6 & \tfrac{dx_7}{dt} &= -\kappa_{10}x_1x_7+\kappa_9x_6,
\end{aligned}$$

and the conservation laws are

$$x_1+x_5+x_5+x_6=T_1, \qquad x_2+x_3+x_6+x_7=T_2 \qquad \text{and} \qquad x_4+x_5+x_6+x_7=T_3.$$

With this choice of conservation laws we have

$$F_T(x)=\begin{pmatrix} x_1+x_5+x_5+x_6-T_1\\ x_2+x_3+x_6+x_7-T_2\\ \kappa_1x_1x_2-\kappa_5x_3x_4-\kappa_2x_3+\kappa_6x_6\\ x_4+x_5+x_6+x_7-T_3\\ \kappa_3x_1x_4-\kappa_8x_2x_5-\kappa_4x_5+\kappa_7x_6\\ \kappa_5x_3x_4+\kappa_8x_2x_5+\kappa_{10}x_1x_7-\kappa_6x_6-\kappa_7x_6-\kappa_9x_6\\ -\kappa_{10}x_1x_7+\kappa_9x_6 \end{pmatrix}.$$

Here $s=4$. We compute $q_x(\lambda)$ and the Hurwitz determinants in Maple, and find that all but the last have all coefficients positive, and thus are positive.

We find next a positive parametrization by solving the steady state equations in the variables x_3, x_5, x_6, x_7 following the methods proposed in [22, 10]:

$$\begin{aligned}
x_3 &= \frac{x_1x_2(\kappa_1\kappa_6\kappa_8x_2+\kappa_3\kappa_6\kappa_8x_4+\kappa_1\kappa_4\kappa_6+\kappa_1\kappa_4\kappa_7)}{\kappa_2\kappa_6\kappa_8x_2+\kappa_4\kappa_5\kappa_7x_4+\kappa_2\kappa_4\kappa_6+\kappa_2\kappa_4\kappa_7} & x_5 &= \frac{x_1x_4(\kappa_1\kappa_5\kappa_7x_2+\kappa_3\kappa_5\kappa_7x_4+\kappa_2\kappa_3\kappa_6+\kappa_2\kappa_3\kappa_7)}{\kappa_2\kappa_6\kappa_8x_2+\kappa_4\kappa_5\kappa_7x_4+\kappa_2\kappa_4\kappa_6+\kappa_2\kappa_4\kappa_7}\\
x_6 &= \frac{x_1x_2x_4(\kappa_1\kappa_5\kappa_8x_2+\kappa_3\kappa_5\kappa_8x_4+\kappa_1\kappa_4\kappa_5+\kappa_2\kappa_3\kappa_8)}{\kappa_2\kappa_6\kappa_8x_2+\kappa_4\kappa_5\kappa_7x_4+\kappa_2\kappa_4\kappa_6+\kappa_2\kappa_4\kappa_7} & x_7 &= \frac{x_2x_4\kappa_9(\kappa_1\kappa_5\kappa_8x_2+\kappa_3\kappa_5\kappa_8x_4+\kappa_1\kappa_4\kappa_5+\kappa_2\kappa_3\kappa_8)}{(\kappa_2\kappa_6\kappa_8x_2+\kappa_4\kappa_5\kappa_7x_4+\kappa_2\kappa_4\kappa_6+\kappa_2\kappa_4\kappa_7)\kappa_{10}}.
\end{aligned}$$

After evaluation of the independent term of $q_x(\lambda)$ at the parametrization, H_4 becomes positive. We conclude that for any choice of reaction rate constants, the network for two substrate enzyme catalysis has exactly one positive steady state in each stoichiometric compatibility class, which is exponentially stable.

C.3 Gene transcription network

We consider now the following gene transcription network shown in the main text:

$$\mathrm{X}_1 \xrightarrow{\kappa_1} \mathrm{X}_1 + \mathrm{P}_1 \qquad \mathrm{X}_2 \xrightarrow{\kappa_2} \mathrm{X}_2 + \mathrm{P}_2 \qquad\qquad \mathrm{P}_1 \xrightarrow{\kappa_3} 0 \qquad\qquad \mathrm{P}_2 \xrightarrow{\kappa_4} 0$$

$$\mathrm{X}_2 + \mathrm{P}_1 \underset{\kappa_6}{\overset{\kappa_5}{\rightleftharpoons}} \mathrm{X}_2\mathrm{P}_1 \qquad 2\,\mathrm{P}_2 \underset{\kappa_8}{\overset{\kappa_7}{\rightleftharpoons}} \mathrm{P}_2\mathrm{P}_2 \qquad \mathrm{X}_1 + \mathrm{P}_2\mathrm{P}_2 \underset{\kappa_{10}}{\overset{\kappa_9}{\rightleftharpoons}} \mathrm{X}_1\mathrm{P}_2\mathrm{P}_2.$$

We denote the species as $X_1 = \mathrm{X}_1$, $X_2 = \mathrm{X}_2$, $X_3 = \mathrm{P}_1$, $X_4 = \mathrm{P}_2$, $X_5 = \mathrm{X}_2\mathrm{P}_1$, $X_6 = \mathrm{P}_2\mathrm{P}_2$, and $X_7 = X_1P_2P_2$ and denote their concentrations with lower case letters. Additionally, we are under the assumption of mass-action kinetics. It was proven in [10] that for each set of positive reaction rate constants there is a stoichiometric compatibility class that contains at least two positive steady states. The ODE system is

$$\begin{aligned}
\tfrac{dx_1}{dt} &= -\kappa_9 x_1 x_6 + \kappa_{10} x_7 & \tfrac{dx_2}{dt} &= -\kappa_5 x_2 x_3 + \kappa_6 x_5\\
\tfrac{dx_3}{dt} &= -\kappa_5 x_2 x_3 + \kappa_1 x_1 - \kappa_3 x_3 + \kappa_6 x_5 & \tfrac{dx_4}{dt} &= -2\,\kappa_7 x_4^2 + \kappa_2 x_2 - \kappa_4 x_4 + 2\,\kappa_8 x_6\\
\tfrac{dx_5}{dt} &= \kappa_5 x_2 x_3 - \kappa_6 x_5 & \tfrac{dx_6}{dt} &= \kappa_7 x_4^2 - \kappa_9 x_1 x_6 - \kappa_8 x_6 + \kappa_{10} x_7\\
\tfrac{dx_7}{dt} &= \kappa_9 x_1 x_6 - \kappa_{10} x_7, & &
\end{aligned}$$

and the conservation laws are

$$x_1 + x_7 = T_1 \qquad \text{and} \qquad x_2 + x_5 = T_2.$$

These give rise to the function $F_T(x)$:

$$F_T(x) = \begin{pmatrix} x_1 + x_7 - T_1 \\ x_2 + x_5 - T_2 \\ -\kappa_5 x_2 x_3 + \kappa_1 x_1 - \kappa_3 x_3 + \kappa_6 x_5 \\ -2\kappa_7 x_4^2 + \kappa_2 x_2 - \kappa_4 x_4 + 2\kappa_8 x_6 \\ \kappa_5 x_2 x_3 - \kappa_6 x_5 \\ \kappa_7 x_4^2 - \kappa_9 x_1 x_6 - \kappa_8 x_6 + \kappa_{10} x_7 \\ \kappa_9 x_1 x_6 - \kappa_{10} x_7 \end{pmatrix}.$$

Here $s = 5$. We find $q_x(\lambda)$ and compute the 5 Hurwitz determinants for $x \in \mathbb{R}^7_{>0}$. We find that H_3, H_4, H_5 have coefficients of both signs. We proceed to find a parametrization by solving the steady state equations in $x_3, \ldots, x_7$, which gives:

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

After evaluating H_3, H_4 and H_5 in this parametrization, H_3 and H_4 become rational functions in x_1, x_2 and κ with all coefficients positive. Hence they are positive as well.

This means that the stability of the steady state is determined by the sign of H_5. We check whether we can apply Theorem 2. By solving $F_T(x) = 0$ in $x_2, \ldots, x_7$ after removal of the conservation law with total amount T_1, we obtain

$$\begin{aligned}
x_2 &= \frac{\kappa_3 \kappa_6 T_2}{\kappa_1 \kappa_5 x_1 + \kappa_3 \kappa_6}, \quad & x_3 &= \frac{\kappa_1 x_1}{\kappa_3}, & x_4 &= \frac{\kappa_2 \kappa_3 \kappa_6 T_2}{\left(\kappa_1 \kappa_5 x_1 + \kappa_3 \kappa_6\right) \kappa_4},\\
x_5 &= \frac{\kappa_1 \kappa_5 x_1 T_2}{\kappa_1 \kappa_5 x_1 + \kappa_3 \kappa_6}, \quad & x_6 &= \frac{\kappa_2^2 \kappa_3^2 \kappa_6^2 \kappa_7 T_2^2}{(\kappa_1 \kappa_5 x_1 + \kappa_3 \kappa_6)^2 \kappa_4^2 \kappa_8}, & x_7 &= \frac{\kappa_2^2 \kappa_3^2 \kappa_6^2 \kappa_7 \kappa_9 x_1 T_2^2}{\left(\kappa_1 \kappa_5 x_1 + \kappa_3 \kappa_6\right)^2 \kappa_4^2 \kappa_8 \kappa_{10}}.
\end{aligned}$$

These expressions define φ, with $z = x_1$, $\varphi_1(z) = z$ and $\mathcal{E} = \mathbb{R}_{>0}$. By inserting these expressions into the conservation law with T_1, we conclude that the solutions of $F_T(x) = 0$ are in one to one correspondence with the zeroes of the function

$$(F_{T,1} \circ \varphi)(z) = \frac{1}{(\kappa_1\kappa_5 z + \kappa_3\kappa_6)^2\kappa_4^2\kappa_8\kappa_{10}} \left[\kappa_1^2\kappa_4^2\kappa_5^2\kappa_8\kappa_{10}z^3 + (-T_1\kappa_1\kappa_5 + 2\kappa_3\kappa_6)\kappa_1\kappa_4^2\kappa_5\kappa_8\kappa_{10}z^2 + \right.$$
$$\left.(T_2^2\kappa_2^2\kappa_3^2\kappa_6^2\kappa_7\kappa_9 - 2T_1\kappa_1\kappa_3\kappa_4^2\kappa_5\kappa_6\kappa_8\kappa_{10} + \kappa_3^2\kappa_4^2\kappa_6^2\kappa_8\kappa_{10})z - T_1\kappa_3^2\kappa_4^2\kappa_6^2\kappa_8\kappa_{10}\right]$$

The numerator of this function has degree 3 in z, so using Lemma 1, the maximum number of positive steady states in each stoichiometric compatibility class is 3. We next verify that the first hypothesis in Theorem 2 is satisfied. Specifically,we compute

$$\frac{1}{\varphi_i'(z)} \det(J_{F_T}(\varphi(z))_{J,I}) = \det(J_{F_T}(\varphi(z))_{\{2,\ldots,7\},\{2,\ldots,7\}}) = -(\kappa_1\kappa_5 x_1 + \kappa_3\kappa_6)\kappa_4\kappa_8\kappa_{10} < 0.$$

Since the sign of this polynomial is constant in $\mathcal{E}$, we apply Theorem 2. We have that the sign of

$$\frac{(-1)^{s+1+1}}{\varphi_1'(z_1)}(F_{T,1} \circ \varphi)'(z_1) \det(J_{F_T}(\varphi(z_1))_{J,I})$$

is positive since $s + i + j = 7$ and the sign of $(F_{T,1} \circ \varphi)'(z_1)$ is positive as the independent term of the numerator of $(F_{T,1} \circ \varphi)(z_1)$ is negative. Therefore, the stability of the steady states alternate with z starting with an exponentially stable steady state. Specifically, if a stoichiometric compatibility class has one positive steady state, then it is exponentially stable. If it has three positive steady states, then two of them are exponentially stable and the other one is unstable. Bistability is guaranteed whenever the network has three positive steady states.

C.4 Monostationary networks from Fig. 3 in the main text

Networks (1) to (4) are straightforward to analyze, since all coefficients of the Hurwitz determinants in x and κ are positive; hence the Hurwitz determinants are positive for all $x \in \mathbb{R}^n_{>0}$ and $\kappa \in \mathbb{R}^m_{>0}$.

For networks (5) and (6) the computation was interrupted as it took long. In both networks $s = 6$, and the first four Hurwitz determinants could be computed. These determinants are polynomials in κ and x with positive coefficients, thus they are positive for every positive steady state. In order to compute H_5, we rely on an identity that holds for the Hurwitz determinants of a generic polynomial of degree 6. Namely, for a generic polynomial $h(t) = a_6t^6 + a_5t^5 + a_4t^4 + a_3t^3 + a_2t^2 + a_1t + a_0$, the fifth Hurwitz determinant can be written in terms of the previous ones as follows

$$H_5 = a_1H_4 + a_0(-a_0a_5^3 + a_1a_5H_2 - a_3H_3).$$

With this identity, we analyze the sign of the coefficients of H_5 by studying separately the coefficients of $A = a_0(-a_0a_5^3 + a_1a_5H_2 - a_3H_3)$ and $B = a_1H_4$ after substituting a_i for the coefficient of λ^i in $q_x(\lambda)$. First note that the coefficients of B are positive because both a_1 and H_4 are polynomials with positive coefficients. Now, to study the sign of A we identify each term $\overline{\kappa}_\alpha x^\alpha$ with a tuple of the form $(\overline{\kappa}_\alpha, \alpha)$, that is, each term corresponds to a tuple whose first entry is its coefficient and the other entries correspond to the powers of the variables $x_1, \ldots, x_{11}$ respectively. With this identification it is possible to store the polynomial using less memory and the sign of each coefficient can be explored by studying the first entry of each tuple. Note that each $\overline{\kappa}_\alpha$ is a polynomial in the rate constants κ; furthermore, after analyzing their sign we found that these polynomials have negative coefficients. This implies that if H_5 has terms with negative coefficients, they come from monomials in A. We use this to find the coefficients of H_5 by adding only monomials of B that have the same exponents to monomials in A. In networks (5) and (6) this meant that we were analyzing only 24196 and 27982 coefficients instead of 37319 and 36970 coefficients respectively. After these computations we found that H_5 is a polynomial with positive coefficients. Therefore, positive for every positive steady state.

Knowing this about H_5, we also conclude that $H_6 = a_0 H_5$ is a polynomial with positive coefficients and the unique steady state in each stoichiometric compatibility class is exponentially stable for every set of parameters. Note that in these computations we do not need to evaluate at a positive parametrization, meaning all Hurwitz determinants are positive for arbitrary positive κ and x.

C.5 Multistationary networks from Fig. 4 in the main text

We consider now the networks in Fig. 4 in the main text, which all are known to be multistationary We sketch here why the procedure fails for each network, and how it applies to the reduced network.

Network (a). This network is the combination of two one-site modification cycles where the same kinase E activates the phosphorylation process and two different phosphates F_1 and F_2 catalyze the dephosphorylation process:

$$\begin{array}{ll}
\mathrm{S_0 + E} \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} \mathrm{S_0E} \xrightarrow{\kappa_3} \mathrm{S_1 + E} & \qquad \mathrm{S_1 + E} \underset{\kappa_5}{\overset{\kappa_4}{\rightleftharpoons}} \mathrm{S_1E} \xrightarrow{\kappa_6} \mathrm{S_2 + E} \\
\mathrm{S_1 + F_1} \underset{\kappa_8}{\overset{\kappa_7}{\rightleftharpoons}} \mathrm{S_1F_1} \xrightarrow{\kappa_9} \mathrm{S_0 + F_1} & \qquad \mathrm{S_2 + F_2} \underset{\kappa_{11}}{\overset{\kappa_{10}}{\rightleftharpoons}} \mathrm{S_2F_2} \xrightarrow{\kappa_{12}} \mathrm{S_1 + F_2}\,.
\end{array}$$

The species are renamed as $S_0 = X_1, S_1 = X_2, S_2 = X_3, E = X_4, F_1 = X_5, F_2 = X_6, S_0E = X_7, S_1E = X_8, S_1F_1 = X_9, S_2F_2 = X_{10}$ and their concentrations are denoted in lower case letters.

Since the polynomial $q_x(\lambda)$ has degree 6, it is necessary to compute 6 Hurwitz determinants. These determinants were computed and their signs were analyzed up to H_4, and they have positive coefficients. However, the analysis of the sign of H_5 was interrupted as it was taking a long time to finish and it was not possible to store the polynomial in the expanded format in a regular PC. To compute and study this determinant more effectively, we use a monomial positive parametrization ϕ of the steady state variety, given by

$$x_1 = \frac{(\kappa_2+\kappa_3)(\kappa_5+\kappa_6)\kappa_7\kappa_9\kappa_{10}\kappa_{12}}{\kappa_1\kappa_3\kappa_4\kappa_6(\kappa_8+\kappa_9)(\kappa_{11}+\kappa_{12})}\frac{x_3x_5x_6}{x_4^2} \qquad x_8 = \frac{\kappa_{10}\kappa_{12}}{\kappa_6(\kappa_{11}+\kappa_{12})}x_3x_6$$

$$x_2 = \frac{(\kappa_5+\kappa_6)\kappa_{10}\kappa_{12}}{\kappa_4\kappa_6(\kappa_{11}+\kappa_{12})}\frac{x_3x_6}{x_4} \qquad x_9 = \frac{(\kappa_5+\kappa_6)\kappa_7\kappa_{10}\kappa_{12}}{\kappa_4\kappa_6(\kappa_8+\kappa_9)(\kappa_{11}+\kappa_{12})}\frac{x_3x_5x_6}{x_4}$$

$$x_7 = \frac{(\kappa_5+\kappa_6)\kappa_7\kappa_9\kappa_{10}\kappa_{12}}{\kappa_3\kappa_4\kappa_6(\kappa_8+\kappa_9)(\kappa_{11}+\kappa_{12})}\frac{x_3x_5x_6}{x_4} \qquad x_{10} = \frac{\kappa_{10}}{\kappa_{11}+\kappa_{12}}x_3x_6.$$

This parametrization, written with the notation of the manuscript, corresponds to

$$\alpha = \left(\frac{(\kappa_2+\kappa_3)(\kappa_5+\kappa_6)\kappa_7\kappa_9\kappa_{10}\kappa_{12}}{\kappa_1\kappa_3\kappa_4\kappa_6(\kappa_8+\kappa_9)(\kappa_{11}+\kappa_{12})}, \frac{(\kappa_5+\kappa_6)\kappa_{10}\kappa_{12}}{\kappa_4\kappa_6(\kappa_{11}+\kappa_{12})}, 1, 1, 1, 1, \frac{(\kappa_5+\kappa_6)\kappa_7\kappa_9\kappa_{10}\kappa_{12}}{\kappa_3\kappa_4\kappa_6(\kappa_8+\kappa_9)(\kappa_{11}+\kappa_{12})}, \right.$$
$$\left. \frac{\kappa_{10}\kappa_{12}}{\kappa_6(\kappa_{11}+\kappa_{12})}, \frac{(\kappa_5+\kappa_6)\kappa_7\kappa_{10}\kappa_{12}}{\kappa_4\kappa_6(\kappa_8+\kappa_9)(\kappa_{11}+\kappa_{12})}, \frac{\kappa_{10}}{\kappa_{11}+\kappa_{12}}\right),$$

$$B = \begin{bmatrix} 1 & 1 & 1 & 0 & 0 & 0 & 1 & 1 & 1 & 1 \\ -2 & -1 & 0 & 1 & 0 & 0 & -1 & 0 & -1 & 0 \\ 1 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 1 & 0 \\ 1 & 1 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 \end{bmatrix} \qquad \text{and} \qquad \xi = (x_3, x_4, x_5, x_6).$$

Using this parametrization and the identification of the monomials with tuples, it was possible to compute $H_5(\phi)$. However, the sign of this function remains unclear since it has coefficients with different signs.

We consider next the **reduced network** obtained by first removing all the reverse reactions and then the intermediates $\mathrm{S_1F_1}$ and $\mathrm{S_2F_2}$. When removing these intermediates the reactions $\mathrm{S_1 + F_1 \longrightarrow S_1F_1 \longrightarrow S_0 + F_1}$ and $\mathrm{S_2 + F_2 \longrightarrow S_2F_2 \longrightarrow S_1 + F_2}$ become $\mathrm{S_1 + F_1 \longrightarrow S_0 + F_1}$ and $\mathrm{S_2 + F_2 \longrightarrow S_1 + F_2}$ respectively. The reduced network is

$$\mathrm{S_0 + E} \xrightarrow{\tau_1} \mathrm{S_0E} \xrightarrow{\tau_2} \mathrm{S_1 + E} \qquad \mathrm{S_1 + E} \xrightarrow{\tau_3} \mathrm{S_1E} \xrightarrow{\tau_4} \mathrm{S_2 + E}$$
$$\mathrm{S_1 + F_1} \xrightarrow{\tau_5} \mathrm{S_0 + F_1} \qquad \mathrm{S_2 + F_2} \xrightarrow{\tau_6} \mathrm{S_1 + F_2}.$$

The species are now renamed as $S_0 = X_1, S_1 = X_2, S_2 = X_3, E = X_4, F_1 = X_5, F_2 = X_6, S_0E = X_7, S_1E = X_8$, and their concentrations are denoted in lower case letters. The polynomial $q_x(\lambda)$ associated with this network has degree 4 and, when computing the Hurwitz determinants we have that H_1, H_2 and H_3 are positive. However the sign of H_4 is unclear even after evaluating in a positive parametrization of the steady state variety ϕ. In this situation we explore the possibility of applying Theorem 2 to deduce bistability.

The conservation laws of the system are

$$x_1 + x_2 + x_3 + x_7 + x_8 = T_1, \quad x_4 + x_7 + x_8 = T_2, \quad x_5 = T_3 \quad \text{and } x_6 = T_4.$$

Taking the indices i_1, i_2, i_3, i_4 as $1, 4, 5, 6$ respectively, we construct F_T as in equation [3] in the manuscript. The solutions of $F_{T,\ell} = 0$ for $\ell = 2, 3, 4, 5, 6$ are written in terms of $z = x_2$ as

$$\varphi(z) = \left(\frac{\tau_2\tau_5T_3(\tau_3z+\tau_4)z}{\tau_1\tau_4(\tau_2T_2-\tau_5T_3z)}, z, \frac{\tau_3\tau_4z(\tau_2T_2-\tau_5T_3z)}{\tau_2\tau_6T_4(\tau_3z+\tau_4)}, \frac{\tau_4(\tau_2T_2-\tau_5T_3z)}{\tau_2(\tau_3z+\tau_4)}, T_3, T_4, \frac{\tau_5T_3z}{\tau_2}, \frac{\tau_3(\tau_2T_2-\tau_5T_3z)z}{\tau_2(\tau_3z+\tau_4)}\right),$$

for every $z \in \mathcal{E}$, where $\mathcal{E} = \left(0, \frac{T_2\tau_2}{T_3\tau_5}\right)$.

Note that $\varphi_2(z) = z$ and $\varphi_2'(z) = 1 \neq 0$. This means that the positive steady states in the stoichiometric compatibility class defined by T are in one to one correspondence with the positive roots of $F_{T,1}(\varphi(z))$ in $\mathcal{E}$. This rational function, presented below, has as numerator a polynomial of degree 3.

$$\begin{aligned}F_{T,1}(\varphi(z)) =& \frac{1}{T_4\tau_1\tau_2\tau_4\tau_6(T_3\tau_5 z - T_2\tau_2)(\tau_3 z + \tau_4)}\left(-T_3\tau_3\tau_5(T_3\tau_1\tau_4^2\tau_5 - T_4\tau_1\tau_2\tau_4\tau_6 + T_4\tau_2^2\tau_3\tau_6)z^3 - \right.\\ & \tau_4(T_1T_3T_4\tau_1\tau_2\tau_3\tau_5\tau_6 - T_2T_3T_4\tau_1\tau_2\tau_3\tau_5\tau_6 - T_3^2T_4\tau_1\tau_4\tau_5^2\tau_6 - 2T_2T_3\tau_1\tau_2\tau_3\tau_4\tau_5 + T_2T_4\tau_1\tau_2^2\tau_3\tau_6 - T_3T_4\tau_1\tau_2\tau_4\tau_5\tau_6\\ & + 2T_3T_4\tau_2^2\tau_3\tau_5\tau_6)z^2 + \tau_2\tau_4(T_1T_2T_4\tau_1\tau_2\tau_3\tau_6 - T_1T_3T_4\tau_1\tau_4\tau_5\tau_6 - T_2^2T_4\tau_1\tau_2\tau_3\tau_6 - T_2T_3T_4\tau_1\tau_4\tau_5\tau_6\\ & \left. - T_2^2\tau_1\tau_2\tau_3\tau_4 - T_2T_4\tau_1\tau_2\tau_4\tau_6 - T_3T_4\tau_2\tau_4\tau_5\tau_6)z + T_1T_2T_4\tau_1\tau_2^2\tau_4^2\tau_6\right).\end{aligned}$$

We have already shown that the second hypothesis of Theorem 2 holds. For the first hypothesis, a straightforward computation shows that

$$\det(J_{F_T}(\varphi(z))_{J,I}) = \tau_1\tau_4\tau_6T_4(\tau_5T_3z - \tau_2T_2),$$

which is negative for every $z \in \mathcal{E}$. We further have $s = 4$, $i = 2$, $j = 1$, and the independent term of the numerator of $F_{T,1}(\varphi(z))$ is negative, meaning that $(F_{T,j} \circ \varphi)'(z_1) > 0$. This gives that the sign of

$$\frac{(-1)^{s+i+j}}{\varphi_i'(z_1)}(F_{T,j} \circ \varphi)'(z_1)\det(J_{F_T}(\varphi(z_1))_{J,I})$$

is $(-1)^{4+1+2}(+1)(-1) = 1$ positive. Using Theorem 2, we conclude that for every set of parameters such that $F_{T,1}(\varphi(z))$ has three roots $z_1 < z_2 < z_3$ in $\mathcal{E}$, the steady states $\varphi(z_1), \varphi(z_3)$ are exponentially stable and $\varphi(z_2)$ is unstable.

All that is left is to show that the reduced network admits three positive steady states in some stoichiometric compatibility class for some choice of τ, or what is the same, that $F_{T,1}(\varphi(z))$ admits three roots in $\mathcal{E}$. To this end, we apply the method from [10], which consists of finding values for the rate constants and concentration variables such that $\det(J_{F_T}(\phi))$, where ϕ is a parametrization of the steady states, is negative. We have

$$\phi(x_3, x_4, x_5, x_6) = \left(\frac{\tau_5\tau_6x_3x_5x_6}{\tau_1\tau_3x_4^2}, \frac{\tau_6x_3x_6}{\tau_3x_4}, x_3, x_4x, x_5, x_6, \frac{\tau_5\tau_6x_3x_5x_6}{\tau_2\tau_3x_4}, \frac{\tau_6x_3x_6}{\tau_4}\right) \qquad \text{and}$$

$$\begin{aligned}\det(J_{F_T}(\phi)) =& \tau_1\tau_2\tau_6^2x_3x_6^2 - \tau_1\tau_4\tau_5\tau_6x_3x_5x_6 + 2\frac{\tau_2\tau_5\tau_6^2x_3x_5x_6^2}{x_4} + \frac{\tau_4\tau_5^2\tau_6^2x_3x_5^2x_6^2}{\tau_3x_4^2}\\ & + \tau_1\tau_2\tau_3\tau_6x_4^2x_6 + \tau_1\tau_4\tau_5\tau_6x_4x_5x_6 + \tau_1\tau_2\tau_3\tau_4x_4^2 + \tau_1\tau_2\tau_4\tau_6x_4x_6 + \tau_2\tau_4\tau_5\tau_6x_5x_6.\end{aligned}$$

By letting $\tau_i = 1$, for $i = 1, \ldots, 6$ and $x_3 = 100, x_4 = 10, x_5 = 10, x_6 = 1$, this determinant is -280, which is negative. This implies that the stoichiometric compatibility class containing $\phi(100, 10, 10, 1)$ has more than one positive steady state. Specifically, this class corresponds to $T_1 = 320, T_2 = 210, T_3 = 10, T_4 = 1$. Either by solving the steady state equations or finding the roots of $F_{T,1}(\varphi(z))$ for this choice of parameters, we confirm that the system has three positive steady states.

Therefore, the reduced network is bistable for all choice of parameter values for which there are three positive steady states, and the original network admits bistability in some region of the parameter space.

Network (b). This network is the combination of two one-site modification cycles in a cascade, where the same phosphatase F acts in both layers.

$$\begin{array}{ll}
\mathrm{S_0} + \mathrm{E} \xrightleftharpoons[\kappa_2]{\kappa_1} \mathrm{S_0E} \xrightarrow{\kappa_3} \mathrm{S_1} + \mathrm{E} & \qquad \mathrm{S_1} + \mathrm{F} \xrightleftharpoons[\kappa_5]{\kappa_4} \mathrm{S_1F} \xrightarrow{\kappa_6} \mathrm{S_0} + \mathrm{F} \\
\mathrm{P_0} + \mathrm{S_1} \xrightleftharpoons[\kappa_8]{\kappa_7} \mathrm{P_0S_1} \xrightarrow{\kappa_9} \mathrm{P_1} + \mathrm{S_1} & \qquad \mathrm{P_1} + \mathrm{F} \xrightleftharpoons[\kappa_{11}]{\kappa_{10}} \mathrm{P_1F} \xrightarrow{\kappa_{12}} \mathrm{P_0} + \mathrm{F}.
\end{array}$$

We rename the species as $E = X_1, F = X_2, S_0 = X_3, S_1 = X_4, P_0 = X_5, P_1 = X_6, S_0E = X_7, S_1F = X_8, P_0S_1 = X_9, P_1F = X_{10}$, and their concentrations are denoted by lower case letters. For this network the polynomial $q_x(\lambda)$ has degree 6; and after some computations it was possible to prove that H_1, H_2, H_3 are positive polynomials. However, the sign of H_4 is unclear and the direct computation of $H_4(\phi)$ was not feasible as the memory in a regular PC was not enough. The positive steady state variety has a monomial parametrization ϕ. We use the identification of monomials with tuples, to compute and analyze $H_4(\phi)$ more efficiently. With the notation of the manuscript ϕ corresponds to

$$\alpha = \left(\frac{\kappa_4\kappa_6\kappa_{10}\kappa_{12}(\kappa_2+\kappa_3)(\kappa_8+\kappa_9)}{\kappa_1\kappa_3\kappa_7\kappa_9(\kappa_5\kappa_{11}+\kappa_5\kappa_{12}+\kappa_6\kappa_{11}+\kappa_6\kappa_{12})}, 1, 1, \frac{(\kappa_8+\kappa_9)\kappa_{10}\kappa_{12}}{(\kappa_{11}+\kappa_{12})\kappa_7\kappa_9}, 1, 1, \right.$$
$$\left. \frac{\kappa_4\kappa_6\kappa_{10}\kappa_{12}(\kappa_8+\kappa_9)}{\kappa_3\kappa_7\kappa_9(\kappa_5\kappa_{11}+\kappa_5\kappa_{12}+\kappa_6\kappa_{11}+\kappa_6\kappa_{12})}, \frac{\kappa_4\kappa_{10}\kappa_{12}(\kappa_8+\kappa_9)}{\kappa_7\kappa_9(\kappa_5\kappa_{11}+\kappa_5\kappa_{12}+\kappa_6\kappa_{11}+\kappa_6\kappa_{12})}, \frac{\kappa_{10}\kappa_{12}}{\kappa_9(\kappa_{11}+\kappa_{12})}, \frac{\kappa_{10}}{\kappa_{11}+\kappa_{12}} \right),$$

$$B = \begin{bmatrix} 2 & 1 & 0 & 2 & 0 & 0 & 2 & 2 & 1 & 1 \\ -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & -1 & 1 & 0 & 1 & -1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 1 & 1 & 1 & 1 & 1 \end{bmatrix} \qquad \text{and} \qquad \xi = (x_2, x_3, x_5, x_6).$$

Using this identification it was possible to compute $H_4(\phi)$. However, its sign was still unclear as we encountered both positive and negative coefficients.

We then proceed to **reduce the network** by removing all reverse reactions and the intermediates $\mathrm{S_0E}$ and $\mathrm{S_1F}$. That is, the reactions $\mathrm{S_0} + \mathrm{E} \longrightarrow \mathrm{S_0E} \longrightarrow \mathrm{S_1} + \mathrm{E}$ and $\mathrm{S_1}+\mathrm{F} \longrightarrow \mathrm{S_1F} \longrightarrow \mathrm{S_0}+\mathrm{F}$ are transformed into $\mathrm{S_0}+\mathrm{E} \longrightarrow \mathrm{S_1}+\mathrm{E}$ and $\mathrm{S_1}+\mathrm{F} \xrightarrow{\tau_2} \mathrm{S_0}+\mathrm{F}$ respectively. We are left with the following reduced network

$$\begin{array}{ll}
\mathrm{S_0} + \mathrm{E} \xrightarrow{\tau_1} \mathrm{S_1} + \mathrm{E} & \qquad \mathrm{S_1} + \mathrm{F} \xrightarrow{\tau_2} \mathrm{S_0} + \mathrm{F} \\
\mathrm{P_0} + \mathrm{S_1} \xrightarrow{\tau_3} \mathrm{P_0S_1} \xrightarrow{\tau_4} \mathrm{P_1} + \mathrm{S_1} & \qquad \mathrm{P_1} + \mathrm{F} \xrightarrow{\tau_5} \mathrm{P_1F} \xrightarrow{\tau_6} \mathrm{P_0} + \mathrm{F}.
\end{array}$$

In this network we rename the species as $E = X_1, F = X_2, S_0 = X_3, S_1 = X_4, P_0 = X_5, P_1 = X_6, P_0S_1 = X_9, P_1F = X_{10}$. Their concentrations are denoted with lower case letters. The polynomial $q_x(\lambda)$ has degree 4 and, after computing the Hurwitz determinants, we have that H_1, H_2, H_3 are positive. Therefore, the stability of the positive steady states depends on the sign of $H_4(\phi)$. In this situation we explore the possibility of applying Theorem 2 to ensure bistability.

The conservation laws of the system are

$$x_1 = T_1, \quad x_5 + x_6 + x_9 + x_{10} = T_2, \quad x_3 + x_4 + x_9 = T_3, \quad x_2 + x_{10} = T_4.$$

Taking the indices $i_1 = 1, i_2 = 2, i_3 = 3, i_4 = 5$ we define F_T as in equation [3] in the manuscript. Furthermore, the solutions of $F_{T,\ell} = 0$ for $\ell \neq 5$ can be positively parametrized in terms of $z = x_6$ as

$$\begin{aligned}\varphi(z) = \Bigg(T_1, \frac{\tau_6 T_4}{\tau_5 z + \tau_6},\ \frac{T_4\tau_2\tau_6((T_3\tau_4 - T_4\tau_6)\tau_5 z + T_3\tau_4\tau_6)}{\tau_4(T_1\tau_1\tau_5 z + T_1\tau_1\tau_6 + T_4\tau_2\tau_6)(\tau_5 z + \tau_6)}, \frac{T_1\tau_1((T_3\tau_4 - T_4\tau_6)\tau_5 z + T_3\tau_4\tau_6)}{\tau_4(T_1\tau_1\tau_5 z + T_1\tau_1\tau_6 + T_4\tau_2\tau_6)},\\ \frac{\tau_5\tau_6 T_4\tau_4(T_1\tau_1\tau_5 z + T_1\tau_1\tau_6 + T_4\tau_2\tau_6)z}{T_1\tau_1\tau_3((T_3\tau_4 - T_4\tau_6)\tau_5 z + T_3\tau_4\tau_6)(\tau_5 z + \tau_6)}, z, \frac{\tau_5\tau_6 T_4 z}{\tau_4(\tau_5 z + \tau_6)}, \frac{T_4\tau_5 z}{\tau_5 z + \tau_6}\Bigg)\end{aligned}$$

for every $z \in \mathcal{E}$, where $\mathcal{E} = \mathbb{R}_{>0}$ if $T_3\tau_4 - T_4\tau_6 > 0$ or $\mathcal{E} = \left(0, \frac{T_3\tau_4\tau_6}{-\tau_5(T_3\tau_4 - T_4\tau_6)}\right)$ if $T_3\tau_4 - T_4\tau_6 \leq 0$. Note that the positive steady states in the stoichiometric compatibility class defined by T are in one to one correspondence with the zeros of $F_{T,5}(\varphi(z))$, below, contained in $\mathcal{E}$.

$$\begin{aligned}F_{T,5}(\varphi(z)) =& \frac{1}{T_1\tau_1\tau_3\tau_4(T_3\tau_4\tau_5 z - T_4\tau_5\tau_6 z + T_3\tau_4\tau_6)(\tau_5 z + \tau_6)}\left[T_1\tau_1\tau_3\tau_4\tau_5^2(T_3\tau_4 - T_4\tau_6)z^3 - T_1\tau_1\tau_5(T_2T_3\tau_3\tau_4^2\tau_5\right.\\ &- T_2T_4\tau_3\tau_4\tau_5\tau_6 - T_3T_4\tau_3\tau_4^2\tau_5 - T_3T_4\tau_3\tau_4\tau_5\tau_6 + T_4^2\tau_3\tau_4\tau_5\tau_6 + T_4^2\tau_3\tau_5\tau_6^2 - 2T_3\tau_3\tau_4^2\tau_6 + T_4\tau_3\tau_4\tau_6^2\\ &- T_4\tau_4^2\tau_5\tau_6)z^2 - \tau_4\tau_6(2T_1T_2T_3\tau_1\tau_3\tau_4\tau_5 - T_1T_2T_4\tau_1\tau_3\tau_5\tau_6 - T_1T_3T_4\tau_1\tau_3\tau_4\tau_5\\ &\left.- T_1T_3T_4\tau_1\tau_3\tau_5\tau_6 - T_1T_3\tau_1\tau_3\tau_4\tau_6 - T_1T_4\tau_1\tau_4\tau_5\tau_6 - T_4^2\tau_2\tau_4\tau_5\tau_6)z - T_1T_2T_3\tau_1\tau_3\tau_4^2\tau_6^2\right].\end{aligned}$$

The numerator of this univariate rational function has degree 3 and the denominator is positive in $\mathcal{E}$. Additionally, $\varphi_6(z) = z$ and $\varphi_6'(z) = 1 \neq 0$. With this parametrization where $i = 6, j = 5$, we have all the elements required in the statement of Theorem 2. We also know from the analysis of the Hurwitz determinants that the second hypothesis of Theorem 2 holds. It remains to see that the first hypothesis of the theorem holds, but this is verified with a simple computation: The polynomial

$$\det(J_{F_T}(\varphi(z))_{J,I}) = -\tau_3 T_1\tau_1((T_3\tau_4 - T_4\tau_6)\tau_5 z + T_3\tau_4\tau_6)$$

is negative for every $z \in \mathcal{E}$. Using Theorem 2, and the fact that the independent term of $F_{T,5}(\varphi(z))$ is negative, we conclude that for every set of parameters such that $F_{T,5}(\varphi(z))$ has 3 positive roots $z_1 < z_2 < z_3$ in $\mathcal{E}$, the sign of

$$\frac{(-1)^{s+i+j}}{\varphi_i'(z_1)}(F_{T,j} \circ \varphi)'(z_1)\det(J_{F_T}(\varphi(z_1))_{J,I})$$

is $(-1)^{6+5+4}(1)(-1) = 1$ positive and thus $\varphi(z_1), \varphi(z_3)$ are exponentially stable and $\varphi(z_2)$ is unstable.

It remains to see that there is a set of parameters such that the network admits three positive steady states. To do so, we apply the method from [10] and find a set of parameters and a steady state such that $\det(J_{F_T}(\phi)) < 0$ given a parametrization ϕ. We have

$$\phi(x_2, x_3, x_5, x_6) = \left(\frac{\tau_2\tau_5 x_2^2 x_6}{\tau_1\tau_3 x_3 x_5}, x_2, x_3, \frac{\tau_5 x_2 x_6}{\tau_3 x_5}, x_6, \frac{\tau_5 x_2 x_6}{\tau_4}, \frac{\tau_5 x_2 x_6}{\tau_6}\right) \qquad \text{and}$$

$$\begin{aligned}\det(J_{F_T}(\phi)) = \frac{1}{\tau_3 x_3 x_5^2}\Big(&\tau_2\tau_5 x_2^2(\tau_3\tau_4\tau_5 x_2 x_3 x_5 x_6 - \tau_3\tau_4\tau_5 x_3 x_5^2 x_6 + \tau_3\tau_4\tau_5 x_3 x_5 x_6^2 + \tau_3\tau_5\tau_6 x_2 x_3 x_5 x_6\\ &+ \tau_3\tau_5\tau_6 x_2 x_5^2 x_6 + \tau_4\tau_5^2 x_2^2 x_6^2 + \tau_4\tau_5^2 x_2 x_6^3 + \tau_5^2\tau_6 x_2^2 x_6^2 + \tau_3\tau_4\tau_6 x_3 x_5^2 + \tau_3\tau_4\tau_6 x_3 x_5 x_6\\ &+ \tau_4\tau_5\tau_6 x_2 x_5 x_6 + \tau_4\tau_5\tau_6 x_2 x_6^2)\Big).\end{aligned}$$

Taking $\tau_i = 1$ for $i = 1, \ldots, 6$ and $x_2 = 1, x_3 = 10, x_5 = 20, x_6 = 10$ this determinant is -4500. This implies that the stoichiometric compatibility class containing $\phi(1, 10, 20, 10)$

has more than one positive steady state. The total amounts defining it are $T_1 = \frac{1}{20}, T_2 = 50, T_3 = \frac{41}{2}, T_4 = 11$. Using these parameters and solving either $F_T(x) = 0$ or finding the roots of $F_{T,5}(\varphi)$ we verify that this stoichiometric compatibility class has in fact three positive steady states.

We conclude that the reduced network is bistable for every set of parameters for which there are three positive steady states, and the original network admits bistability in some region of the parameter space.

Network (c). This network is the combination of two one-site modification cycles in a cascade, where the same kinase E acts in both layers:

$$\begin{aligned}
&\mathrm{S_0 + E} \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} \mathrm{S_0E} \xrightarrow{\kappa_3} \mathrm{S_1 + E}\\
&\mathrm{S_1 + F_1} \underset{\kappa_5}{\overset{\kappa_4}{\rightleftharpoons}} \mathrm{S_1F_1} \xrightarrow{\kappa_6} \mathrm{S_0 + F_1}\\
&\mathrm{P_0 + S_1} \underset{\kappa_8}{\overset{\kappa_7}{\rightleftharpoons}} \mathrm{P_0S_1} \xrightarrow{\kappa_9} \mathrm{P_1 + S_1}\\
&\mathrm{P_0 + E} \underset{\kappa_{14}}{\overset{\kappa_{13}}{\rightleftharpoons}} \mathrm{P_0E} \xrightarrow{\kappa_{15}} \mathrm{P_1 + E}\\
&\mathrm{P_1 + F_2} \underset{\kappa_{11}}{\overset{\kappa_{10}}{\rightleftharpoons}} \mathrm{P_1F_2} \xrightarrow{\kappa_{12}} \mathrm{P_0 + F_2}.
\end{aligned}$$

The species are renamed as $S_0 = X_1, S_1 = X_2, P_0 = X_3, P_1 = X_4, E = X_5, F_1 = X_6, F_2 = X_7, S_0E = X_8, S_1F_1 = X_9, P_0S_1 = X_{10}, P_1F_2 = X_{11}, P_0E = X_{12}$ and their concentrations are denoted with lower case letters. For this network, the polynomial $q_x(\lambda)$ has degree 7 and the determinants H_1, H_2, H_3 are positive polynomials. However, the computations of the other determinants was not possible as there was not enough memory to store the computations in a regular PC. In this case, we could not parametrize the positive steady state variety by monomials; therefore, it is not possible to use the identification between monomials and tuples to analyze the sign of the remaining determinants.

We proceed directly to **reduce the network** by removing all the reverse reactions first, and then the intermediates $\mathrm{S_0E}$,$\mathrm{P_0S_1}$ and $\mathrm{P_1F_2}$. That is, the reactions $\mathrm{S_0 + E} \longrightarrow \mathrm{S_0E} \longrightarrow \mathrm{S_1 + E}$, $\mathrm{P_0 + S_1} \longrightarrow \mathrm{P_0S_1} \longrightarrow \mathrm{P_1 + S_1}$ and $\mathrm{P_1 + F_2} \longrightarrow \mathrm{P_1F_2} \longrightarrow \mathrm{P_0 + F_2}$ are transformed into $\mathrm{S_0 + E} \longrightarrow \mathrm{S_1 + E}$, $\mathrm{P_0 + S_1} \longrightarrow \mathrm{P_1 + S_1}$ and $\mathrm{P_1 + F_2} \longrightarrow \mathrm{P_0 + F_2}$ respectively. We are left with the following network

$$\begin{aligned}
&\mathrm{S_0 + E} \xrightarrow{\tau_1} \mathrm{S_1 + E}\\
&\mathrm{S_1 + F_1} \xrightarrow{\tau_2} \mathrm{S_1F_1} \xrightarrow{\tau_3} \mathrm{S_0 + F_1}\\
&\mathrm{P_0 + S_1} \xrightarrow{\tau_4} \mathrm{P_1 + S_1}\\
&\mathrm{P_0 + E} \xrightarrow{\tau_6} \mathrm{P_0E} \xrightarrow{\tau_7} \mathrm{P_1 + E}\\
&\mathrm{P_1 + F_2} \xrightarrow{\tau_5} \mathrm{P_0 + F_2}.
\end{aligned}$$

The species are renamed as $S_0 = X_1, S_1 = X_2, P_0 = X_3, P_1 = X_4, E = X_5, F_1 = X_6, F_2 = X_7, S_1F_1 = X_9, P_0E = X_{12}$ and their concentrations are denoted by lower case letters. The polynomial $q_x(\lambda)$ associated to this network has degree 4 and H_1, H_2, H_3 are positive after evaluating in the following positive parametrization ϕ of the steady state variety:

$$\phi(x_1, x_2, x_3, x_6, x_7) = \left(x_1, x_2, x_3, \frac{x_2x_3(\tau_1\tau_4x_1 + \tau_2\tau_6x_6)}{\tau_1\tau_5x_1x_7}, \frac{\tau_2x_2x_6}{\tau_1x_1}, x_6, x_7, \frac{\tau_2x_2x_6}{\tau_3}, \frac{\tau_6x_3\tau_2x_2x_6}{\tau_1x_1\tau_7} \right).$$

However, the sign of H_4 is unclear. We now explore the use of Theorem 2 to ensure bistability. The conservation laws of the system are

$$x_1 + x_2 + x_9 = T_1, \quad x_3 + x_4 + x_{12} = T_2, \quad x_5 + x_{12} = T_3, \quad x_6 + x_9 = T_4, \quad x_7 = T_5.$$

Taking $i_1 = 1, i_2 = 3, i_3 = 5, i_4 = 6, i_5 = 7$ we define $F_T(x)$ as in equation [3] of the manuscript. Additionally, the solutions of $F_{T,\ell}(x) = 0$ for $\ell \neq 6$ can be parametrized in terms of $z = x_3$ as

$$\varphi(z) = \Big(\frac{b_1(z)\tau_3}{(\tau_3\tau_6 z + T_3\tau_1\tau_7 + \tau_3\tau_7)\tau_4 z}, \frac{b_2(z)}{\tau_4 z(\tau_6 z + \tau_7)}, z, \frac{(-\tau_6 z^2 + (T_2\tau_6 - T_3\tau_6 - \tau_7)z + T_2\tau_7)}{\tau_6 z + \tau_7}, \frac{T_3\tau_7}{\tau_6 z + \tau_7},$$
$$\frac{b_1(z)T_3\tau_1\tau_3\tau_7}{\tau_2(\tau_3\tau_6 z + T_3\tau_1\tau_7 + \tau_3\tau_7)b_2(z)}, T_5, \frac{b_1(z)T_3\tau_1\tau_7}{\tau_4 z(\tau_6 z + \tau_7)(\tau_3\tau_6 z + T_3\tau_1\tau_7 + \tau_3\tau_7)}, \frac{\tau_6 T_3 z}{\tau_6 z + \tau_7}\Big),$$

where

$$b_1(z) = (T_1\tau_4 + T_5\tau_5)\tau_6 z^2 + (-T_2T_5\tau_5\tau_6 + T_3T_5\tau_5\tau_6 + T_1\tau_4\tau_7 + T_3\tau_6\tau_7 + T_5\tau_5\tau_7)z - T_2T_5\tau_5\tau_7,$$
$$b_2(z) = -T_5\tau_5\tau_6 z^2 + (T_2T_5\tau_5\tau_6 - T_3T_5\tau_5\tau_6 - T_3\tau_6\tau_7 - T_5\tau_5\tau_7)z + T_2T_5\tau_5\tau_7.$$

The parametrization is positive if and only if $b_1(z), b_2(z) > 0$. This happens for every $z \in \mathcal{E}$, where $\mathcal{E} = (\beta_1, \beta_2)$ with β_1 and β_2 the (only) positive roots of the polynomials b_1 and b_2 respectively.

The steady states in each stoichiometric compatibility class are in one to one correspondence with the roots of $F_{T,6}(\varphi(z))$ in $\mathcal{E}$. The numerator of this function is a polynomial of degree 5 and the denominator is positive in $\mathcal{E}$. Additionally, $\varphi_3(z) = z$ and $\varphi_3'(z) = 1 \neq 0$. With this parametrization and taking $i = 3, j = 6, s = 4$, we have all the elements in the statement of Theorem 2. From the computations before, we know that the first three Hurwitz determinants are positive and thus, the second hypothesis of the theorem holds. We only need to verify the first hypothesis of the theorem, but this is done by computing the required determinant

$$\det(J_F(\varphi(z))_{J,I}) = \frac{-\tau_2(\tau_3\tau_6 z + T_3\tau_1\tau_7 + \tau_3\tau_7)\, b_2(z)}{\tau_6 z + \tau_7},$$

which is negative for every $z \in \mathcal{E}$. We are then in the setting of Theorem 2. We need to decide the sign of $(F_{T,j} \circ \varphi)'(z_1)$ at the first root of the numerator of $F_{T,j} \circ \varphi$. Indirect evaluation of $F_{T,j} \circ \varphi$ at β_1, by isolating T_2 from $b_2(z) = 0$ and substitution into $F_{T,j} \circ \varphi$, shows that $F_{T,j} \circ \varphi$ is negative at β_1. Hence the derivative at z_1 is positive. We conclude that for every set of parameters such that $F_{T,6}(\varphi(z))$ has more than two positive roots $z_1, z_2, \ldots$, the sign of

$$\frac{(-1)^{s+i+j}}{\varphi_i'(z_1)} (F_{T,j} \circ \varphi)'(z_1) \det(J_{F_T}(\varphi(z_1))_{J,I})$$

is $(-1)^{3+6+4}(1)(-1) = 1$ positive. Therefore, the steady states $\varphi(z_1), \varphi(z_3), \ldots$ are exponentially stable and $\varphi(z_2), \ldots$ are unstable.

It remains to see that there is a set of parameters such that the stoichiometric compatibility class in fact contains more than two positive steady states. This is done with the method presented in [10], that relies on the sign of $\det(J_F(\phi))$, with ϕ as above. According to the method, we have to find a set of parameters and a steady state such that

$\det(J_F(\phi))$ is negative. The stoichiometric compatibility class containing these steady states, contains at leas another one. In this network

$$
\begin{aligned}
\det(J_F(\phi)) =& -\tau_1\tau_2^2\tau_4\tau_6x_1^2x_2^2x_3x_6 + \tau_1\tau_2\tau_3\tau_5\tau_6x_1^2x_3x_6x_7 + \tau_1\tau_2\tau_3\tau_4\tau_7x_1^2x_2x_6 + \tau_1\tau_2\tau_3\tau_5\tau_7x_1^2x_6x_7 \\
&+ \tau_1\tau_2^2\tau_4\tau_6x_1x_2^3x_3x_6 + \tau_1\tau_2^2\tau_4\tau_7x_1x_2^3x_6 + \tau_1\tau_2^2\tau_4\tau_6x_1x_2^2x_3x_6^2 + \tau_1\tau_2^2\tau_5\tau_6x_1x_2^2x_3x_6x_7 \\
&+ \tau_1\tau_2^2\tau_4\tau_7x_1x_2^2x_6^2 + \tau_1\tau_2^2\tau_5\tau_7x_1x_2^2x_6x_7 + \tau_1\tau_2\tau_3\tau_4\tau_6x_1x_2^2x_3x_6 + \tau_1\tau_2\tau_3\tau_4\tau_7x_1x_2^2x_6 \\
&+ \tau_1\tau_2^2\tau_5\tau_6x_1x_2x_3x_6^2x_7 + \tau_1\tau_2\tau_3\tau_5\tau_6x_1x_2x_3x_6x_7 + \tau_2^2\tau_5(\tau_1\tau_7 + \tau_3\tau_6)x_1x_2x_7x_6^2 \\
&+ \tau_1\tau_2\tau_3\tau_5\tau_7x_1x_2x_6x_7 + \tau_2^2\tau_3\tau_6\tau_7x_1x_2x_6^2 + \tau_2^3\tau_5\tau_6x_2^3x_6^2x_7 + x_2^2\tau_5x_7\tau_2^3\tau_6x_6^3 \\
&+ \tau_2^3\tau_6\tau_7x_2^3x_6^2 + \tau_2^3\tau_6\tau_7x_2^2x_6^3 + \tau_2^2\tau_3\tau_5\tau_6x_2^2x_6^2x_7 + \tau_2^2\tau_3\tau_6\tau_7x_2^2x_6^2.
\end{aligned}
$$

Taking $\tau_i = 1$ for $i = 1, \dots, 7$ and $x_1 = 40, x_2 = 10, x_3 = 1, x_6 = 1, x_7 = 1$, this determinant is -32000. This implies that the stoichiometric compatibility class containing $\phi(x)$ has more than one positive steady state. The total amounts defining the stoichiometric compatibility class are $T_1 = 60, T_2 = \frac{23}{2}, T_3 = \frac{1}{2}, T_4 = 11, T_5 = 1$. Using these parameters and solving either $F_T(x) = 0$ or $F_{T,6}(\varphi(z)) = 0$ we verify that this stoichiometric compatibility class has three positive steady states as desired.

We conclude that the reduced network is bistable for every set of parameters for which there are three positive steady states, and the original network admits bistability in some region of the parameter space.

Network (d). In this network a kinase E and a phosphatase F act on a substrate S_0 and the two sites of its phosphorylation S_1 and S_2.

$$
\mathrm{E} + \mathrm{S}_0 \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} \mathrm{S}_0\mathrm{E} \xrightarrow{\kappa_3} \mathrm{E} + \mathrm{S}_1 \underset{\kappa_8}{\overset{\kappa_7}{\rightleftharpoons}} \mathrm{S}_1\mathrm{E} \xrightarrow{\kappa_9} \mathrm{S}_2 + \mathrm{E}
$$

$$
\mathrm{F} + \mathrm{S}_2 \underset{\kappa_{11}}{\overset{\kappa_{10}}{\rightleftharpoons}} \mathrm{S}_2\mathrm{F} \xrightarrow{\kappa_{12}} \mathrm{F} + \mathrm{S}_1 \underset{\kappa_5}{\overset{\kappa_4}{\rightleftharpoons}} \mathrm{S}_1\mathrm{F} \xrightarrow{\kappa_6} \mathrm{F} + \mathrm{S}_0
$$

The species are renamed as $E = X_1, F = X_2, S_0 = X_3, S_1 = X_4, S_2 = X_5, S_0E = X_6, S_1E = X_7, S_2F = X_8, S_1F = X_9$ and their concentrations are denoted with lower case letters. In this network the polynomial $q_x(\lambda)$ has degree 6 and, the first three Hurwitz determinants are polynomials with positive coefficients; therefore, they are positive for every positive steady state. Regarding the determinants H_4 and H_5, the sign of H_4 is unclear and the analysis of the sign of H_5 was stopped as the computations could not be stored in a regular PC. In this case, the positive steady state variety can be parametrized by monomials as follows

$$
\begin{aligned}
x_3 &= \frac{(\kappa_2+\kappa_3)\kappa_4\kappa_6\kappa_{10}\kappa_{12}(\kappa_8+\kappa_9)}{\kappa_1\kappa_3\kappa_7\kappa_9(\kappa_5+\kappa_6)(\kappa_{11}+\kappa_{12})}\frac{x_2^2x_5}{x_1^2} & x_7 &= \frac{\kappa_{10}\kappa_{12}}{\kappa_9(\kappa_{11}+\kappa_{12})}x_2x_5 \\
x_4 &= \frac{(\kappa_8+\kappa_9)\kappa_{10}\kappa_{12}}{\kappa_7\kappa_9(\kappa_{11}+\kappa_{12})}\frac{x_2x_5}{x_1} & x_8 &= \frac{\kappa_{10}}{\kappa_{11}+\kappa_{12}}x_2x_5 \\
x_6 &= \frac{\kappa_4\kappa_6\kappa_{10}\kappa_{12}(\kappa_8+\kappa_9)}{\kappa_3\kappa_7\kappa_9(\kappa_5+\kappa_6)(\kappa_{11}+\kappa_{12})}\frac{x_2^2x_5}{x_1} & x_9 &= \frac{\kappa_4\kappa_{10}\kappa_{12}(\kappa_8+\kappa_9)}{\kappa_7\kappa_9(\kappa_5+\kappa_6)(\kappa_{11}+\kappa_{12})}\frac{x_2^2x_5}{x_1}.
\end{aligned}
$$

With the notation of the manuscript, this parametrization ϕ corresponds to

$$
\begin{aligned}
\alpha = \Bigg(&1, 1, \frac{(\kappa_2+\kappa_3)\kappa_4\kappa_6\kappa_{10}\kappa_{12}(\kappa_8+\kappa_9)}{\kappa_1\kappa_3\kappa_7\kappa_9(\kappa_5+\kappa_6)(\kappa_{11}+\kappa_{12})}, \frac{(\kappa_8+\kappa_9)\kappa_{10}\kappa_{12}}{\kappa_7\kappa_9(\kappa_{11}+\kappa_{12})}, 1, \frac{\kappa_4\kappa_6\kappa_{10}\kappa_{12}(\kappa_8+\kappa_9)}{\kappa_3\kappa_7\kappa_9(\kappa_5+\kappa_6)(\kappa_{11}+\kappa_{12})}, \\
&\frac{\kappa_{10}\kappa_{12}}{\kappa_9(\kappa_{11}+\kappa_{12})}, \frac{\kappa_{10}}{\kappa_{11}+\kappa_{12}}, \frac{\kappa_4\kappa_{10}\kappa_{12}(\kappa_8+\kappa_9)}{\kappa_7\kappa_9(\kappa_5\kappa_{11}+\kappa_5\kappa_{12}+\kappa_6\kappa_{11}+\kappa_6\kappa_{12})}\Bigg),
\end{aligned}
$$

$$B = \begin{bmatrix} 1 & 0 & -2 & -1 & 0 & -1 & 0 & 0 & -1 \\ 0 & 1 & 2 & 1 & 0 & 2 & 1 & 1 & 2 \\ 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \end{bmatrix} \qquad \text{and} \qquad \xi = (x_1, x_2, x_5).$$

Using the identification between monomials and tuples, it was possible to compute both $H_4(\phi)$ and $H_5(\phi)$ and, after studying their sign, we found that $H_4(\phi)$ is a positive polynomial, but the sign of $H_5(\phi)$ was not clear.

We then proceed to **reduce the network** by removing all the reverse reactions and the intermediates $\mathrm{S_1E}$, $\mathrm{S_2F}$ and $\mathrm{S_1F}$. That is, the reactions $\mathrm{S_1 + E \longrightarrow S_1E \longrightarrow S_2 + E}$, $\mathrm{S_2 + F \longrightarrow S_2F \longrightarrow S_1 + F}$ and $\mathrm{S_1 + F \longrightarrow S_1F \longrightarrow S_0 + F}$ become $\mathrm{S_1 + E \longrightarrow S_2 + E}$, $\mathrm{S_2 + F \longrightarrow S_1 + F}$ and $\mathrm{S_1 + F \longrightarrow S_0 + F}$ respectively. The reduced network is

$$\begin{gathered} \mathrm{S_0 + E} \xrightarrow{\tau_1} \mathrm{ES_0} \xrightarrow{\tau_2} \mathrm{S_1 + E} \xrightarrow{\tau_3} \mathrm{S_2 + E} \\ \mathrm{S_2 + F} \xrightarrow{\tau_4} \mathrm{S_1 + F} \xrightarrow{\tau_5} \mathrm{S_0 + F}. \end{gathered}$$

We rename the species as $E = X_1, F = X_2, S_0 = X_3, S_1 = X_4, S_2 = X_5, S_0E = X_6$ and their concentrations are denoted with lower case letters. The polynomial $q_x(\lambda)$ has degree 3 and, after computing the Hurwitz determinants, it was possible to prove that H_1 and H_2 are polynomials with positive coefficients and, thus positive for every positive steady state. However, the sign of H_3 was unclear even after evaluating at the parametrization ϕ of the positive steady state variety below;

$$\phi(x_1, x_2, x_3) = \left(x_1, x_2, x_3, \frac{\tau_1 x_1 x_3}{\tau_5 x_2}, \frac{\tau_3 x_1^2 \tau_1 x_3}{\tau_4 \tau_5 x_2^2}, \frac{\tau_1 x_1 x_3}{\tau_2} \right).$$

We now check if it is possible to apply Theorem 2 to deduce bistability. The conservation laws of the system are

$$x_1 + x_6 = T_1, \quad x_2 = T_2 \quad \text{and} \quad x_3 + x_4 + x_5 + x_6 = T_3.$$

Taking $i_1 = 1, i_2 = 2, i_3 = 3$ we define $F_T(x)$ as in equation [3] of the manuscript. The solutions of $F_{T,\ell}(x) = 0$ for $\ell = 2, \ldots, 6$ can be parametrized in terms of $z = x_1$ as

$$\begin{aligned} \varphi(z) = \Bigg(z, T_2, & \frac{T_2^2 T_3 \tau_2 \tau_4 \tau_5}{\tau_1\tau_2\tau_3 z^2 + T_2\tau_1\tau_4(T_2\tau_5 + \tau_2)z + T_2^2\tau_2\tau_4\tau_5}, \frac{T_2 T_3 \tau_1 \tau_2 \tau_4 z}{\tau_1\tau_2\tau_3 z^2 + T_2\tau_1\tau_4(T_2\tau_5 + \tau_2)z + T_2^2\tau_2\tau_4\tau_5}, \\ & \frac{T_3 \tau_1 \tau_2 \tau_3 z^2}{\tau_1\tau_2\tau_3 z^2 + T_2\tau_1\tau_4(T_2\tau_5 + \tau_2)z + T_2^2\tau_2\tau_4\tau_5}, \frac{T_2^2 T_3 \tau_1 \tau_4 \tau_5 z}{\tau_1\tau_2\tau_3 z^2 + T_2\tau_1\tau_4(T_2\tau_5 + \tau_2)z + T_2^2\tau_2\tau_4\tau_5} \Bigg). \end{aligned}$$

This parametrization is positive for every $z \in \mathcal{E} = \mathbb{R}_{>0}$ and the positive steady states in the stoichiometric compatibility class are in one to one correspondence with the positive roots of $F_{T,1}(\varphi(z))$. This is a rational function whose numerator is a polynomial of degree 3 and positive denominator:

$$\begin{aligned} F_{T,1}(\varphi(z)) = \frac{1}{\tau_1\tau_2\tau_3 z^2 + T_2\tau_1\tau_4(T_2\tau_5 + \tau_2)z + T_2^2\tau_2\tau_4\tau_5} & \left[\tau_1\tau_2\tau_3 z^3 + (T_2^2\tau_1\tau_4\tau_5 - T_1\tau_1\tau_2\tau_3 + T_2\tau_1\tau_2\tau_4)z^2 + \right. \\ & \left. (-T_1T_2^2\tau_1\tau_4\tau_5 + T_2^2T_3\tau_1\tau_4\tau_5 - T_1T_2\tau_1\tau_2\tau_4 + T_2^2\tau_2\tau_4\tau_5)z - T_1T_2^2\tau_2\tau_4\tau_5 \right]. \end{aligned}$$

Therefore, there are at most three positive steady states in each stoichiometric compatibility class. Additionally $\varphi_1(z) = z$ and $\varphi_1'(z) = 1 \neq 0$. With this parametrization, where, $i = j = 1$ and $s = 3$, we have all the elements in the statement of Theorem 2. From the analysis of the Hurwitz determinants we know that the second hypothesis of the theorem holds. It remains to see whether the first hypothesis also holds. The determinant that we have to study is

$$\det(J_F(\varphi(z))_{J,I}) = -T_2^2\tau_1\tau_4\tau_5 z - \tau_1\tau_2\tau_3 z^2 - T_2\tau_1\tau_2\tau_4 z - T_2^2\tau_2\tau_4\tau_5,$$

which is negative for every $z \in \mathbb{R}_{>0}$. We are in the setting of Theorem 2 and we conclude that for every set of parameters such that $F_{T,1}(\varphi(z))$ has three positive roots $z_1 < z_2 < z_3$, the sign of $(F_{T,j} \circ \varphi)'(z_1)$ is positive as the independent term of $(F_{T,j} \circ \varphi)(z)$ is negative. Furthermore, the sign of

$$\frac{(-1)^{s+i+j}}{\varphi_i'(z_1)}(F_{T,j} \circ \varphi)'(z_1)\det(J_{F_T}(\varphi(z_1))_{J,I})$$

is equal to $(-1)^{1+1+3}(1)(-1) = 1$, which is positive. This implies that the steady states $\varphi(z_1), \varphi(z_3)$ are exponentially stable and $\varphi(z_2)$ is unstable.

We now verify that there exist a set of parameters such that the network has three positive steady states. In order to do so, we use the method from [10] and find values for the parameters and a steady state such that $\det(J_{F_T}(\phi))$ is negative. In this case

$$\det(J_{F_T}(\phi)) = -\tau_1^2\tau_3 x_1^2 x_3 + \tau_1\tau_4\tau_5 x_1 x_2^2 + \tau_1\tau_4\tau_5 x_2^2 x_3 + \tau_1\tau_2\tau_3 x_1^2 + \tau_1\tau_2\tau_4 x_1 x_2 + \tau_2\tau_4\tau_5 x_2^2.$$

Taking $\tau_i = 1$ for $i = 1, \ldots, 5$ and $x_1 = 5, x_2 = 1, x_3 = 5$ this determinant is equal to -84. This means that the stoichiometric compatibility class containing $\phi(x)$ has more than one positive steady state. The total amounts defining it are $T_1 = 30, T_2 = 1, T_3 = 180$. Using these parameters and solving either $F_T(x) = 0$ or $F_{T,1}(\varphi(z)) = 0$ we prove that there are three positive steady states in his stoichiometric compatibility class, as desired.

We conclude that the reduced network is bistable for every set of parameters for which there are three positive steady states, and the original network admits bistability in some region of the parameter space.

Network (e). In this network two substrates $\mathrm{S_0}$ and $\mathrm{P_0}$ are phosphorylated by the same kinase E, and dephosphorylated by the same phosphatase F.

$$\begin{gathered}
\mathrm{S_0 + E} \underset{\kappa_2}{\overset{\kappa_1}{\rightleftarrows}} \mathrm{S_0E} \xrightarrow{\kappa_3} \mathrm{S_1 + E} \\
\mathrm{S_1 + F} \underset{\kappa_5}{\overset{\kappa_4}{\rightleftarrows}} \mathrm{S_1F} \xrightarrow{\kappa_6} \mathrm{S_0 + F} \\
\mathrm{P_0 + E} \underset{\kappa_8}{\overset{\kappa_7}{\rightleftarrows}} \mathrm{P_0E} \xrightarrow{\kappa_9} \mathrm{P_1 + E} \\
\mathrm{P_1 + F} \underset{\kappa_{11}}{\overset{\kappa_{10}}{\rightleftarrows}} \mathrm{P_1F} \xrightarrow{\kappa_{12}} \mathrm{P_0 + F.}
\end{gathered}$$

We rename the species as $E = X_1, F = X_2, S_0 = X_3, S_1 = X_4, P_0 = X_5, P_1 = X_6, S_0E = X_7, S_1F = X_8, P_0E = X_9, P_1F = X_{10}$, and their concentrations are denoted by lower case letters. For this network the polynomial $q_x(\lambda)$ has degree 6 and from the 6 Hurwitz determinants, the first three are positive for every positive steady state. The sign of H_4 is unclear and the analysis of the sign of H_5 was interrupted as the expanded computations could not be stored in a regular PC.

The positive steady state variety associated with this network can be parametrized by monomials as follows

$$\begin{aligned} x_3 &= \frac{\kappa_4\kappa_6(\kappa_2+\kappa_3)}{\kappa_1\kappa_3(\kappa_5+\kappa_6)}\frac{x_2x_4}{x_1} & x_8 &= \frac{\kappa_{10}\kappa_{12}}{\kappa_9(\kappa_{11}+\kappa_{12})}x_2x_6 \\ x_5 &= \frac{(\kappa_8+\kappa_9)\kappa_{10}\kappa_{12}}{(\kappa_{11}+\kappa_{12})\kappa_7\kappa_9}\frac{x_2x_6}{x_1} & x_9 &= \frac{\kappa_4}{\kappa_5+\kappa_6}x_2x_4 \\ x_7 &= \frac{\kappa_4\kappa_6}{\kappa_3(\kappa_5+\kappa_6)}x_2x_4 & x_{10} &= \frac{\kappa_{10}}{\kappa_{11}+\kappa_{12}}x_2x_6. \end{aligned}$$

With the notation of the manuscript, this parametrization ϕ corresponds to

$$\alpha = \left(1, 1, \frac{\kappa_4\kappa_6(\kappa_2+\kappa_3)}{\kappa_1\kappa_3(\kappa_5+\kappa_6)}, 1, \frac{(\kappa_8+\kappa_9)\kappa_{10}\kappa_{12}}{(\kappa_{11}+\kappa_{12})\kappa_7\kappa_9}, 1, \frac{\kappa_4\kappa_6}{\kappa_3(\kappa_5+\kappa_6)}, \frac{\kappa_{10}\kappa_{12}}{\kappa_9(\kappa_{11}+\kappa_{12})}, \frac{\kappa_4}{\kappa_5+\kappa_6}, \frac{\kappa_{10}}{\kappa_{11}+\kappa_{12}}\right),$$

$$B = \begin{bmatrix} 1 & 0 & -1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 & 1 & 0 & 1 & 1 & 1 & 1 \\ 0 & 0 & 1 & 1 & 0 & 0 & 1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 0 & 1 & 0 & 1 \end{bmatrix} \quad \text{and} \quad \xi = (x_1, x_2, x_4, x_6).$$

Using the identification between monomials and tuples it was possible for us to compute $H_4(\phi)$ and $H_5(\phi)$ and, after studying the signs of their coefficients, we have that $H_4(\phi)$ is positive but the sign of $H_5(\phi)$ is still unclear.

We proceed to **reduce the network** by removing all the reverse reactions and the intermediates $\mathrm{S_0E}$ and $\mathrm{P_1F}$. That is, the reactions $\mathrm{S_0 + E \longrightarrow S_0E \longrightarrow S_1 + E}$ and $\mathrm{P_1 + F \longrightarrow P_1F \longrightarrow P_0 + F}$ become $\mathrm{S_0 + E \longrightarrow S_1 + E}$ and $\mathrm{P_1 + F \longrightarrow P_0 + F}$ respectively. The reduced network is

$$\begin{aligned} &\mathrm{S_0 + E} \xrightarrow{\tau_1} \mathrm{S_1 + E} \\ &\mathrm{S_1 + F} \xrightarrow{\tau_2} \mathrm{S_1F} \xrightarrow{\kappa_3} \mathrm{S_0 + F} \\ &\mathrm{P_0 + E} \xrightarrow{\tau_4} \mathrm{P_0E} \xrightarrow{\kappa_5} \mathrm{P_1 + E} \\ &\mathrm{P_1 + F} \xrightarrow{\tau_6} \mathrm{P_0 + F}. \end{aligned}$$

The species are renamed as $E = X_1, F = X_2, S_0 = X_3, S_1 = X_4, P_0 = X_5, P_1 = X_6, S_1F = X_8, P_0E = X_9$ and their concentrations are denoted by lower case letters. The polynomial $q_x(\lambda)$ associated with this network has degree 4. The Hurwitz determinants H_1, H_2 and H_3 are positive for every positive steady state. However, the sign of H_4 is unclear even after evaluating at the parametrization ϕ below:

$$\phi(x_1, x_2, x_4, x_6) = \left(x_1, x_2, \frac{\tau_2 x_2 x_4}{\tau_1 x_1}, x_4, \frac{\tau_6 x_2 x_6}{\tau_4 x_1}, x_6, \frac{\tau_6 x_2 x_6}{\tau_5}, \frac{\tau_2 x_2 x_4}{\tau_3}\right).$$

We explore the possibility of using Theorem 2 to ensure bistability.

The conservation laws of the system are

$$x_1 + x_8 = T_1, \quad x_2 + x_9 = T_2, \quad x_3 + x_4 + x_9 = T_3 \quad \text{and} \quad x_5 + x_6 + x_8 = T_4.$$

Taking $i_1 = 1, i_2 = 2, i_3 = 3, i_4 = 5$ we define $F_T(x)$ as in equation [3] in the manuscript. Furthermore, the solutions of $F_{T,\ell}(x) = 0$ for $\ell \neq 2$ can be positively parametrized in terms of $z = x_5$ as

$$\varphi(z) = \left(\frac{T_1\tau_5}{\tau_4 z + \tau_5}, \frac{-T_1\tau_4\tau_5 z}{\tau_6\, b_1(z)}, \frac{(\tau_4 z + \tau_5)T_3\tau_2\tau_3\tau_4 z}{b_2(z)}, \frac{-\tau_1 T_3\tau_3\tau_6\, b_1(z)}{b_2(z)}, z, \frac{-b_1(z)}{\tau_4 z + \tau_5}, \frac{\tau_4 T_1 z}{\tau_4 z + \tau_5}, \frac{T_3 T_1\tau_1\tau_2\tau_4\tau_5 z}{b_2(z)} \right)$$

where

$$\begin{aligned} b_1(z) &:= \tau_4 z^2 + ((T_1 - T_4)\tau_4 + \tau_5)z - T_4\tau_5, \\ b_2(z) &:= (-\tau_1\tau_3\tau_4\tau_6 + \tau_2\tau_3\tau_4^2)z^2 + (T_1\tau_1\tau_2\tau_4\tau_5 - T_1\tau_1\tau_3\tau_4\tau_6 + T_4\tau_1\tau_3\tau_4\tau_6 - \tau_1\tau_3\tau_5\tau_6 + \tau_2\tau_3\tau_4\tau_5)z \\ &\quad + T_4\tau_1\tau_3\tau_5\tau_6. \end{aligned}$$

Here $\mathcal{E} = (0, \beta_1)$ where β_1 is the positive root of $b_1(z)$, such that $b_1(z) < 0$ and $b_2(z) > 0$ in $\mathcal{E}$.

The positive steady states in the stoichiometric compatibility class are in one to one correspondence with the roots of $F_{T,2}(\varphi(z))$. This is a rational function whose numerator is a polynomial of degree four with positive independent term and the denominator is positive in $\mathcal{E}$. Additionally, $\varphi_5(z) = z$ and $\varphi_5'(z) = 1 \neq 0$ and taking $i = 5, j = 2$ we have all the elements in the statement of Theorem 2. From the analysis of the Hurwitz determinants we know that the second hypothesis in the theorem holds. We now compute the relevant determinant to verify the first hypothesis:

$$\det(J_F(\varphi(z))_{J,I}) = \frac{-T_1\tau_5\, b_2(z)}{\tau_4 z + \tau_5}.$$

This determinant is negative for every $z \in \mathcal{E}$. We are then in the setting of Theorem 2. The sign of $(F_{T,2} \circ \varphi)'(z_1)$, for z_1 the first root of $(F_{T,2} \circ \varphi)(z_1)$, is positive as the independent term of $(F_{T,2} \circ \varphi)(z)$ is negative. Hence, the sign of

$$\frac{(-1)^{s+i+j}}{\varphi_i'(z_1)} (F_{T,2} \circ \varphi)'(z_1) \det(J_{F_T}(\varphi(z_1))_{J,I})$$

is $(-1)^{5+2+4}(1)(-1) = 1$, which is positive. Thus, we conclude that the steady states $\varphi(z_1), \varphi(z_3)$ are exponentially stable and $\varphi(z_2), \ldots$ are unstable.

It is left to see that there exists a set of parameters such that the stoichiometric compatibility class has three positive steady states. In order to do so, we use the method from [10] and search for parameters and values of the concentration variables such that $\det(J_F(\phi))$ is negative:

$$\begin{aligned} \det(J_F(\phi)) =& \tau_1\tau_2\tau_4\tau_6 x_1^2 x_2^2 + \tau_1\tau_2\tau_4\tau_6 x_1^2 x_2 x_4 + (\tau_2\tau_5 + \tau_3\tau_6)\tau_1\tau_4 x_1^2 x_2 + \tau_1\tau_2\tau_4\tau_5 x_1^2 x_4 + \tau_1\tau_3\tau_4\tau_5 x_1^2 \\ &+ (\tau_1\tau_5 + \tau_3\tau_4)\tau_2\tau_6 x_1 x_2^2 + \tau_1\tau_2\tau_5\tau_6 x_1 x_2 x_4 + (\tau_1\tau_6 + \tau_2\tau_4)\tau_3\tau_5 x_1 x_2 + \tau_1\tau_2\tau_6^2 x_2^3 x_6 + \tau_2\tau_3\tau_5\tau_6 x_2^2 \\ &- \left(\frac{\tau_2}{\tau_1} - \frac{\tau_6}{\tau_4} \right) \tau_1\tau_2\tau_4\tau_6 x_6 x_4 x_2^2 + \tau_1\tau_3\tau_6^2 x_6 x_2^2 + \frac{\tau_2\tau_3\tau_6^2 x_2^3 x_6}{x_1}. \end{aligned}$$

Taking $\tau_3 = 1, \tau_5 = 1, \tau_1 = 1, \tau_4 = 1, \tau_2 = 2, \tau_6 = 1$ and $x_1 = 1, x_2 = 1, x_4 = 9, x_6 = 9$ the value of the determinant is -48. This implies that the stoichiometric compatibility class containing $\phi(1, 1, 9, 9)$ has more than one positive steady states and, solving either $F_T(x) = 0$ or $F_{T,2}(\varphi(z)) = 0$ we verify that it has three positive steady states.

We conclude that the reduced network is bistable for every set of parameters for which there are three positive steady states, and the original network admits bistability in some region of the parameter space.

Network (f). E corresponds to a kinase that exists in two conformations: E_1 (relaxed state) and E_2 (tensed state). Each conformation acts as a kinase for a common substrate S_0. We denote by S_1 the phosphorylated form of the substrate. We assume that the intermediate kinase-substrate complexes, E_1S_0 and E_2S_0, also undergo conformational change.

$$\begin{aligned}
&\mathrm{E_1} + \mathrm{S_0} \xrightleftharpoons[\kappa_2]{\kappa_1} \mathrm{E_1S_0} \xrightarrow{\kappa_3} \mathrm{E_1} + \mathrm{S_1}\\
&\mathrm{E_2} + \mathrm{S_0} \xrightleftharpoons[\kappa_5]{\kappa_4} \mathrm{E_2S_0} \xrightarrow{\kappa_6} \mathrm{E_2} + \mathrm{S_1}\\
&\mathrm{E_1} \xrightleftharpoons[\kappa_9]{\kappa_8} \mathrm{E_2}\\
&\mathrm{E_1S_0} \xrightleftharpoons[\kappa_{11}]{\kappa_{10}} \mathrm{E_2S_0}\\
&\mathrm{S_1} \xrightarrow{\kappa_7} \mathrm{S_0}.
\end{aligned}$$

The species are renamed as $E_1 = X_1, E_2 = X_2, E_1S_0 = X_3, E_2S_0 = X_4, S_0 = X_5, S_1 = X_6$ and their concentrations are denoted by lower case letters. The polynomial $q_x(\lambda)$ associated with this network has degree 4. After computing and evaluating the Hurwitz determinants at a positive parametrization ϕ of the positive steady state variety, the sign of H_3 and H_4 is unclear. Since the hypotheses of Theorem 2 do not hold, we **reduce the network** by removing the intermediate $\mathrm{E_2S_0}$ and all the reverse reactions. When removing $\mathrm{E_2S_0}$ the reactions $\mathrm{E_2}+\mathrm{S_0} \xrightleftharpoons{} \mathrm{E_2S_0} \longrightarrow \mathrm{E_2}+\mathrm{S_1}$ become $\mathrm{E_2}+\mathrm{S_0} \longrightarrow \mathrm{E_2}+\mathrm{S_1}$ and the path $\mathrm{E_2}+\mathrm{S_0} \xrightleftharpoons{} \mathrm{E_2S_0} \xrightleftharpoons{} \mathrm{E_1S_0}$ is collapsed to $\mathrm{E_2}+\mathrm{S_0} \longrightarrow \mathrm{E_1S_0}$. After removing the reverse reactions the reduced network is

$$\begin{aligned}
&\mathrm{E_1} + \mathrm{S_0} \xrightarrow{\tau_1} \mathrm{E_1S_0} \xrightarrow{\tau_2} \mathrm{E_1} + \mathrm{S_1}\\
&\mathrm{E_2} + \mathrm{S_0} \xrightarrow{\tau_3} \mathrm{E_2} + \mathrm{S_1}\\
&\mathrm{E_2} + \mathrm{S_0} \xrightarrow{\tau_6} \mathrm{E_1S_0}\\
&\mathrm{E_1} \xrightarrow{\tau_5} \mathrm{E_2}\\
&\mathrm{S_1} \xrightarrow{\tau_4} \mathrm{S_0}.
\end{aligned}$$

The species are renamed as $E_1 = X_1, E_2 = X_2, E_1S_0 = X_3, S_0 = X_5, S_1 = X_6$ and their concentrations are denoted by lower case letters. We have $s = 3$. For this reduced network, $q_x(\lambda)$ has degree 3 and the determinants H_1, H_2 are positive for every positive steady state. This means that the stability of steady states depends on the sign of H_3. We explore the possibility of applying Theorem 2 to ensure bistability. The conservation laws of the network are

$$x_1 + x_2 + x_3 = T_1 \quad \text{and} \quad x_3 + x_5 + x_6 = T_2.$$

Taking $i_1 = 1$ and $i_2 = 3$ we define $F_T(x)$ as in equation [3] from the manuscript. The solutions of $F_{T,\ell}(x) = 0$ for $\ell \neq 3$ can be parametrized in terms of $z = x_5$ as

$$\varphi(z) = \left(\frac{T_1\tau_2\tau_6 z}{b(z)}, \frac{T_1\tau_2\tau_5}{b(z)}, \frac{T_1\tau_6 z(\tau_1 z + \tau_5)}{b(z)}, z, \frac{T_1\tau_2 z(\tau_1\tau_6 z + \tau_3\tau_5 + \tau_5\tau_6)}{\tau_4\, b(z)} \right),$$

with $b(z) = \tau_1\tau_6 z^2 + (\tau_2 + \tau_5)\tau_6 z + \tau_2\tau_5$, and this parametrization is positive for every $z \in \mathcal{E} = \mathbb{R}_{>0}$. Additionally $\varphi_4(z) = z$ and $\varphi_4'(z) = 1 \neq 0$. With this parametrization, the positive steady states in one stoichiometric compatibility class are in one to one correspondence with the positive roots of $F_{T,3}(\varphi(x)) = 0$, that is a rational function whose numerator is a polynomial of degree 3 and has positive denominator:

$$F_{T,3}(\varphi(x)) = \frac{1}{(\tau_1\tau_6 z^2 + \tau_2\tau_6 z + \tau_5\tau_6 z + \tau_2\tau_5)\tau_4} \left[\tau_1\tau_4\tau_6 z^3 + (T_1\tau_1\tau_2\tau_6 + T_1\tau_1\tau_4\tau_6 - T_2\tau_1\tau_4\tau_6 + \tau_2\tau_4\tau_6 + \tau_4\tau_5\tau_6)z^2 + \right. \\ \left. (T_1\tau_2\tau_3\tau_5 + T_1\tau_2\tau_5\tau_6 + T_1\tau_4\tau_5\tau_6 - T_2\tau_2\tau_4\tau_6 - T_2\tau_4\tau_5\tau_6 + \tau_2\tau_4\tau_5)z - T_2\tau_2\tau_4\tau_5\right].$$

Here $i = 4, j = 3$. After studying the Hurwitz determinants we know that the second hypothesis of Theorem 2 holds. We now check the sign of $\det(J_F(\varphi(z)_{J,I})$ on the first hypothesis of Theorem 2:

$$\det(J_F(\varphi(z)_{J,I}) = \tau_4\, b(z),$$

which is positive for every $z \in \mathcal{E}$. We are now in the setting of Theorem 2. The sign of $(F_{T,3} \circ \varphi)'(z_1)$, for z_1 the first positive root of $F_{T,3}(\varphi(z))$, is positive as the independent term of $(F_{T,3} \circ \varphi)(z)$ is negative. Furthermore, the sign of

$$\frac{(-1)^{s+i+j}}{\varphi_i'(z_1)}(F_{T,3} \circ \varphi)'(z_1)\det(J_{F_T}(\varphi(z_1))_{J,I})$$

is $(-1)^{3+3+4}(1)(1) = 1$ positive, and the steady states $\varphi(z_1), \varphi(z_3), \ldots$ are exponentially stable and $\varphi(z_2), \ldots$ are unstable.

It remains to see that there exists a set of parameters such that the stoichiometric compatibility class has three positive steady states. In order to do this we use the method proposed in [10] and find values for the parameters and concentration variables such that $\det(J_F(\phi))$ is negative for the parametrization ϕ given as:

$$\phi(x_2, x_5) = \left(\frac{\tau_6 x_2 x_5}{\tau_5}, x_2, \frac{\tau_6 x_2 x_5(\tau_1 x_5 + \tau_5)}{\tau_2\tau_5}, x_5, \frac{x_2 x_5(\tau_1\tau_6 x_5 + \tau_3\tau_5 + \tau_5\tau_6)}{\tau_4\tau_5}\right).$$

We obtain

$$\det(J_F(\phi)) = \tau_1(\tau_2\tau_6 - \tau_3\tau_5 + \tau_4\tau_6)\tau_6 x_2 x_5^2 + 2(\tau_2 + \tau_4)\tau_1\tau_5\tau_6 x_2 x_5 + (\tau_2\tau_3 + \tau_2\tau_6 + \tau_4\tau_6)\tau_5^2 x_2 \\ + \tau_1\tau_4\tau_5\tau_6 x_5^2 + (\tau_2 + \tau_5)\tau_4\tau_5\tau_6 x_5 + \tau_2\tau_4\tau_5^2.$$

Taking $\tau_i = 1$ for $i = 1, \ldots, 6$ and $x_2 = 2, x_5 = 6$ the value of the determinant is -35. This means that the stoichiometric compatibility class containing $\phi(x)$ has three positive steady states. The total amounts defining this class are $T_1 = 98, T_2 = 222$ and solving $F_T(x) = 0$ or $F_{T,3}(\varphi(z)) = 0$ we verify that there are three positive steady states.

We conclude that the reduced network is bistable for every set of parameters for which there are three positive steady states, and the original network admits bistability in some region of the parameter space.

II

Oscillations and bistability in a model of ERK regulation

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Oscillations and bistability in a model of ERK regulation

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Abstract

This work concerns the question of how two important dynamical properties, oscillations and bistability, emerge in an important biological signaling network. Specifically, we consider a model for dual-site phosphorylation and dephosphorylation of extracellular signal-regulated kinase (ERK). We prove that oscillations persist even as the model is greatly simplified (reactions are made irreversible and intermediates are removed). Bistability, however, is much less robust – this property is lost when intermediates are removed or even when all reactions are made irreversible. Moreover, bistability is characterized by the presence of two reversible, catalytic reactions: as other reactions are made irreversible, bistability persists as long as one or both of the specified reactions is preserved. Finally, we investigate the maximum number of steady states, aided by a network's "mixed volume" (a concept from convex geometry). Taken together, our results shed light on the question of how oscillations and bistability emerge from a limiting network of the ERK network – namely, the fully processive dual-site network – which is known to be globally stable and therefore lack both oscillations and bistability. Our proofs are enabled by a Hopf bifurcation criterion due to Yang, analyses of Newton polytopes arising from Hurwitz determinants, and recent characterizations of multistationarity for networks having a steady-state parametrization.

Keywords: chemical reaction network, Hopf bifurcation, oscillation, bistable, Newton polytope, mixed volume

1 Introduction

In recent years, significant attention has been devoted to the question of how bistability and oscillations emerge in biological networks involving multisite phosphorylation (Conradi & Shiu 2018). Such networks are of great biological importance (Cohen 2000). The one we consider is the network, depicted in Figure 1, comprising extracellular signal-regulated kinase (ERK) regulation by dual-site phosphorylation by the kinase MEK (denoted by E) and dephosphorylation by the phosphatase MKP3 (F) (Rubinstein et al. 2016). This network, which we call the ERK network, has an important role in regulating many cellular activities, with dysregulation implicated in many cancers (Shaul & Seger 2007). Accordingly, an important problem is to understand the dynamical properties of the ERK network, with the goal of predicting effects arising from mutations or drug treatments (Futran et al. 2013).

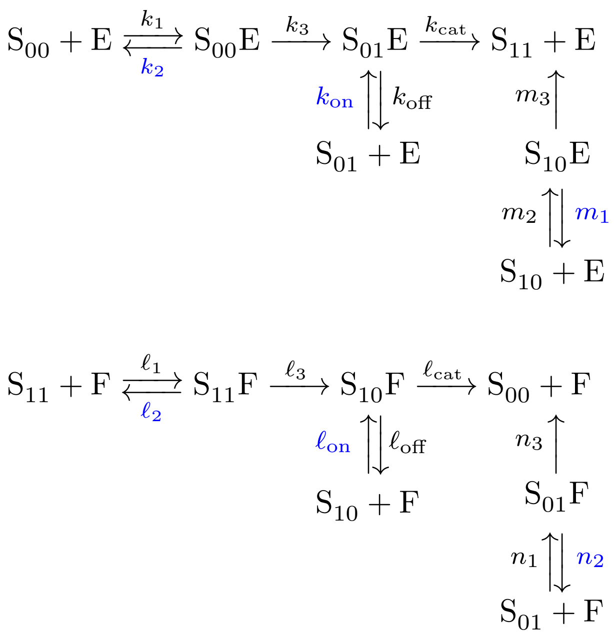

Figure 1: The (full) ERK network, from Rubinstein et al. (2016), with notation of Dickenstein et al. (2019). Each S_{ij} denotes an ERK phosphoform, with subscripts indicating at which of two sites phosphate groups are attached. The *fully processive* network is obtained by deleting all vertical reactions (those labeled by $k_{on}, k_{off}, m_1, m_2, m_3, \ell_{on}, \ell_{off}, n_1, n_2, n_3$). We also consider irreversible versions of the ERK network obtained by deleting some of the reactions labeled $k_2, k_{on}, m_1, l_2, \ell_{on}, n_2$ (in blue). In particular, deleting all six of those reactions yields the fully irreversible ERK network.

The ERK network was shown by Rubinstein, Mattingly, Berezhkovskii & Shvartsman (2016) to be bistable and exhibit oscillations (for some choices of rate constants). Rubinstein *et al.* also observed that the ERK network "limits" to a network without bistability or oscillations. Namely, when the rate constants k_{cat} and ℓ_{cat} are much larger than k_{off} and ℓ_{off}, respectively, this yields the "fully processive" network obtained by deleting all vertical arrows in Figure 1, which is globally convergent to a unique steady state (Conradi & Shiu 2015, Eithun & Shiu 2017, Rao 2017). Accordingly, Rubinstein *et al.* asked, How do bistability and oscillations in the ERK network emerge from the processive limit? This question was subsequently articulated as follows by Conradi & Shiu (2018):

Question 1.1. *When the processivity levels $p_k := k_{cat}/(k_{cat} + k_{off})$ and $p_\ell := \ell_{cat}/(\ell_{cat} + \ell_{off})$ are arbitrarily close to 1, is the ERK network still bistable and oscillatory?*

One of our main contributions is to lay foundation toward answering Question 1.1. Specifically, we answer a related question, *How do bistability and oscillations emerge from simpler versions of the ERK network?* Our main results, summarized in Table 1, are that oscillations are surprisingly robust to operations that simplify the network, while bistability is lost more easily. Specifically, oscillations persist even as reactions are made irreversible and intermediates are removed (see Section 4.1), while bistability is lost more quickly, when only a few reactions are made irreversible (Section 4.2). Taken together, our results form a case study for the problem of model choice – an investigation into the simplifications of a model that preserve important dynamical properties.

ERK network	Oscillations?	Bistability?	Maximum # steady states	Maximum # over $\mathbb{C}$
Full	Yes*	Yes*	Conjecture: 3	7
Irreversible	Yes**	If $k_{\text{on}} > 0$ or $\ell_{\text{on}} > 0$	1	5*
Reduced	Yes	No	1	3

Table 1: Summary of results. Yes* indicates results of Rubinstein et al. (2016). Yes** indicates that the fully irreversible ERK network exhibits oscillations (see Figure 3), and 5* indicates that 5 is the maximum number of complex-number steady states for the network obtained from the full ERK network by setting $k_{\text{on}} = 0$. For details on results, see Propositions 4.1, 4.5, and 5.9, and Theorem 4.6.

Our focus here – on determining which operations on the ERK network preserve oscillations and bistability – is similar in spirit to the recent approach proposed by Sadeghimanesh & Feliu (2018). Indeed, there has been significant interest in understanding which operations on networks preserve oscillations (Banaji 2018), bistability (Banaji & Pantea 2018, Feliu & Wiuf 2013, Joshi & Shiu 2013), and other properties (Gross et al. 2018).

A related topic – mentioned earlier – is the question of how dynamical properties arise in phosphorylation systems. Several works have examined this problem at the level of parameters, focusing on the question of which rate constants and/or initial conditions give rise to oscillations (Conradi et al. 2019) or bistability (Conradi et al. 2017, 2018). Our perspective is slightly different; instead of allowing parameter values to change, we modify the network itself. Accordingly, our work is similar in spirit to recent investigations into minimal oscillatory or bistable networks (Banaji 2018, Banaji & Pantea 2018, Hadač et al. 2017, Hernansaiz-Ballesteros et al. 2018, Joshi & Shiu 2017).

A key tool we use is a parametrization of the steady states. Such parametrizations have been shown in recent years to be indispensable for analyzing multistationarity (multiple steady states, which are necessary for bistability) and oscillations (Giaroli et al. 2018, Johnston et al. 2019, Thomson & Gunawardena 2009). Indeed, here we build on previous results (Conradi et al. 2017, 2019, Dickenstein et al. 2019).

Specifically, following the work of Conradi, Mincheva & Shiu (2019), we investigate oscillations by employing a steady-state parametrization together with a criterion proposed by Yang (2002) that characterizes Hopf bifurcations in terms of determinants of Hurwitz matrices. Using this approach, Conradi et al. (2019) showed that the Hopf bifurcations of a mixed-mechanism phosphorylation network lie on a hypersurface defined by the vanishing of a single Hurwitz determinant. For our ERK networks, however, the problem does not reduce to the analysis of a single polynomial, and the size of these polynomials makes the system difficult to solve. To this end, we introduce an algorithm for analyzing these polynomials, through their Newton polytopes, by using techniques from polyhedral geometry. Using this algorithm, we succeed in finding, for the reduced ERK network, a Hopf bifurcation giving rise to oscillations.

Finally, we investigate the precise number of steady states in ERK networks. For general networks, much has been done for determining which networks admit multiple steady states – see e.g., the articles of Conradi, Feliu, Mincheva & Wiuf (2017), Craciun & Feinberg (2006), Dickenstein, Pérez Millán, Shiu & Tang (2019), Feinberg (1980), Giaroli, Bihan & Dickenstein (2018), Joshi & Shiu (2015), and Müller, Feliu, Regensburger, Conradi, Shiu & Dickenstein (2016) – but there are few techniques for determining a network's maximum number of steady states. To this end, we introduce two related measures of a network, the maximum number of complex-number steady states and the "mixed volume". In general, the mixed volume is an upper bound on the number of complex-number steady states, but we show that these numbers are equal for ERK networks (Section 5).

The outline of our work is as follows. Section 2 contains background on chemical reaction systems, steady-state parametrizations, and Hopf bifurcations. We present steady-state parametrizations for the ERK network and the reduced ERK network in Section 3. Section 4 contains our main results on oscillations and bistability. Section 5 investigates the number of steady states and the relationship to mixed volumes. We end with a Discussion in Section 6.

2 Background

Here we introduce chemical reaction systems (Section 2.1), their steady-state parametrizations (Section 2.2), and Hopf bifurcations (Section 2.3).

2.1 Chemical reaction systems

Following Dickenstein et al. (2019), our notation closely matches that of Conradi et al. (2017). A reaction network G (or *network* for short) comprises a set of s species $\{X_1, X_2, \ldots, X_s\}$ and a set of m reactions:

$$\alpha_{1j}X_1 + \alpha_{2j}X_2 + \cdots + \alpha_{sj}X_s \ \to \ \beta_{1j}X_1 + \beta_{2j}X_2 + \cdots + \beta_{sj}X_s \ , \qquad \text{for } j = 1, 2, \ldots, m \ ,$$

where each α_{ij} and β_{ij} is a non-negative integer. The stoichiometric matrix of G, denoted by N, is the $s \times m$ matrix with (i, j)-entry equal to $\beta_{ij} - \alpha_{ij}$. Let $d = s - \text{rank}(N)$. The stoichiometric subspace, denoted by S, is the image of N. A conservation-law matrix of G, denoted by W, is a row-reduced $d \times s$-matrix whose rows form a basis of the orthogonal complement of S. If there exists a choice of W for which every entry is nonnegative and each column contains at least one nonzero entry (equivalently, each species occurs in at least one nonnegative conservation law), then G is conservative.

We denote the concentrations of the species $X_1, X_2, \ldots, X_s$ by $x_1, x_2, \ldots, x_s$, respectively. These concentrations, under the assumption of *mass-action kinetics*, evolve in time according to the following system of ODEs:

$$\dot{x} \ = \ f(x) \ := \ N \cdot \begin{pmatrix} \kappa_1 \, x_1^{\alpha_{11}} x_2^{\alpha_{21}} \cdots x_s^{\alpha_{s1}} \\ \kappa_2 \, x_1^{\alpha_{12}} x_2^{\alpha_{22}} \cdots x_s^{\alpha_{s2}} \\ \vdots \\ \kappa_m \, x_1^{\alpha_{1m}} x_2^{\alpha_{2m}} \cdots x_s^{\alpha_{sm}} \end{pmatrix} , \tag{1}$$

where $x = (x_1, x_2, \ldots, x_s)$, and each $\kappa_j \in \mathbb{R}_{>0}$ is called a reaction rate constant. By considering the rate constants as a vector of parameters $\kappa = (\kappa_1, \kappa_2, \ldots, \kappa_m)$, we have polynomials $f_{\kappa,i} \in \mathbb{Q}[\kappa, x]$, for $i = 1, 2, \ldots, s$. For ease of notation, we often write f_i rather than $f_{\kappa,i}$.

A trajectory $x(t)$ beginning at a positive vector $x(0) = x^0 \in \mathbb{R}^s_{>0}$ remains, for all positive time, in the following stoichiometric compatibility class with respect to the total-constant vector $c := Wx^0 \in \mathbb{R}^d$:

$$\mathcal{S}_c \ := \ \{x \in \mathbb{R}^s_{\geq 0} \mid Wx = c\} \ . \tag{2}$$

A steady state of (1) is a positive concentration vector $x^* \in \mathbb{R}^s_{\geq 0}$ at which the right-hand sides of the ODEs (1) vanish: $f(x^*) = 0$. We distinguish between positive steady states $x^* \in \mathbb{R}^s_{>0}$ and boundary steady states $x^* \in \mathbb{R}^s_{\geq 0} \backslash \mathbb{R}^s_{>0}$. Also, a steady state x^* is nondegenerate if $\mathrm{Im}\left(\mathrm{Jac}(f)(x^*)|_S\right)$ is the stoichiometric subspace S. (Here, $\mathrm{Jac}(f)(x^*)$ is the Jacobian matrix of f, with respect to x, at x^*.) A nondegenerate steady state is exponentially stable if each of the $\sigma := \dim(S)$ nonzero eigenvalues of $\mathrm{Jac}(f)(x^*)$ has negative real part.

A network is multistationary if there exists a positive rate-constant vector $\kappa \in \mathbb{R}^m_{>0}$ such that there exists two or more positive steady states of (1) in some stoichiometric compatibility class (2). Similarly, a network is bistable if there exists a positive rate-constant vector $\kappa \in \mathbb{R}^m_{>0}$ such that there exists two or more *exponentially stable* positive steady states of (1) in some stoichiometric compatibility class (2). Thus, every bistable network is multistationary. A network is monostationary[1] if, for every choice of positive rate constants, there is exactly one positive steady state in every stoichiometric compatibility class.

To analyze steady states within a stoichiometric compatibility class, we will use conservation laws in place of linearly dependent steady-state equations, as follows. Let $I = \{i_1 < i_2 < \cdots < i_d\}$ denote the indices of the first nonzero coordinate of the rows of conservation-law matrix W. Consider the function $f_{c,\kappa} : \mathbb{R}^s_{\geq 0} \to \mathbb{R}^s$ defined by

$$f_{c,\kappa,i} = f_{c,\kappa}(x)_i := \begin{cases} f_i(x) & \text{if } i \notin I, \\ (Wx - c)_k & \text{if } i \ = \ i_k \in I. \end{cases} \tag{3}$$

We call system (3), the system augmented by conservation laws. By construction, positive roots of the system of polynomial equations $f_{c,\kappa} = 0$ are precisely the positive steady states of (1) in the stoichiometric compatibility class (2) defined by the total-constant vector c.

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[1] Some authors define *monostationary* to be non-multistationary; the two definitions are equivalent for the ERK networks in this work.

2.2 Steady-state parametrizations

Here we introduce steady-state parametrizations (Definition 2.2) and recall from (Dickenstein et al. 2019) how to use them to determine whether a network is multistationary (Proposition 2.4). Later we will see how to use parametrizations to detect Hopf bifurcations (Proposition 4.1).

Definition 2.1. Let G be a network with m reactions and s species, and let $\dot{x} = f(x)$ denote the resulting mass-action system. Denote by W a $d \times s$ row-reduced conservation-law matrix and by I the set of indices of the first nonzero coordinates of its rows. Enumerate the complement of I as follows: $[s] \setminus I = \{j_1 < j_2 < \cdots < j_{s-d}\}$. A set of effective parameters for G is formed by polynomials $\bar{a}_1(\kappa), \bar{a}_2(\kappa), \ldots, \bar{a}_{\bar{m}}(\kappa) \in \mathbb{Q}(\kappa)$ for which the following hold:

(i) $\bar{a}_i(\kappa^*)$ is defined and, moreover, $\bar{a}_i(\kappa^*) > 0$ for every $i = 1, 2, \ldots, \bar{m}$ and for all $\kappa^* \in \mathbb{R}^m_{>0}$,

(ii) the reparametrization map below is surjective:

$$\begin{array}{rccl} \bar{a} & : & \mathbb{R}^m_{>0} & \rightarrow & \mathbb{R}^{\bar{m}}_{>0} \\ & & \kappa & \mapsto & (\bar{a}_1(\kappa), \bar{a}_2(\kappa), \ldots, \bar{a}_{\bar{m}}(\kappa)) \ , \end{array} \tag{4}$$

(iii) there exists an $(s-d) \times (s-d)$ matrix $M(\kappa)$ with entries in $\mathbb{Q}(\kappa) := \mathbb{Q}(\kappa_1, \kappa_2, \ldots, \kappa_m)$ such that:

  (a) for all $\kappa^* \in \mathbb{R}^m_{>0}$, the matrix $M(\kappa^*)$ is defined and, moreover, $\det M(\kappa^*) > 0$, and

  (b) letting $(\bar{h}_{j_\ell})$ denote the functions obtained from (f_{j_ℓ}) as follows:

$$(\bar{h}_{j_1}, \bar{h}_{j_2}, \ldots, \bar{h}_{j_{s-d}})^\top \quad := \quad M(\kappa)\ (f_{j_1}, f_{j_2}, \ldots, f_{j_{s-d}})^\top \ , \tag{5}$$

  every nonconstant coefficient in every $\bar{h}_{j_\ell}$ is equal to a rational-number multiple of some $\bar{a}_i(\kappa)$.

Given such a set of effective parameters, we consider for $\ell = 1, 2, \ldots, s-d$, polynomials $h_{j_\ell} = h_{j_\ell}(a; x) \in \mathbb{Q}[a_1, a_2, \ldots, a_{\bar{m}}][x]$ (here, the a_i's are indeterminates) such that:

$$\bar{h}_{j_\ell} \ = \ h_{j_\ell}|_{a_1 = \bar{a}_1(\kappa),\ \ldots\ ,\ a_{\bar{m}} = \bar{a}_{\bar{m}}(\kappa)} \ . \tag{6}$$

For $i = 1, 2, \ldots, s$ and any choice of $c \in \mathbb{R}^d_{>0}$ and $a \in \mathbb{R}^{\bar{m}}_{>0}$, set

$$h_{c,a}(x)_i \ := \ \begin{cases} h_i(a; x) & \text{if } i \notin I \\ (Wx - c)_k & \text{if } i = i_k \in I. \end{cases} \tag{7}$$

We call the function $h_{c,a} : \mathbb{R}^s_{>0} \to \mathbb{R}^s$ an effective steady-state function of G.

The "steady-state parametrizations" that we will use in this work belong to a subclass of the ones introduced by Dickenstein et al. (2019). Thus, for simplicity, Definition 2.2 below is more restrictive than Definition 3.6 of (Dickenstein et al. 2019). Specifically, our parametrizations have the form $\phi(\hat{a}; x)$, while those of (Dickenstein et al. 2019) are of the form $\phi(\hat{a}; \hat{x})$.

Definition 2.2. Let G be a network with m reactions, s species, and conservation-law matrix W. Let $f_{c,\kappa}$ arise from G and W as in (3). Suppose that $h_{c,a}$ is an effective steady-state function of G, as in (7), arising from a matrix $M(\kappa)$, as in (5), a reparametrization map $\bar{a}$, as in (4), and polynomials h_{j_ℓ}'s as in (6). The positive steady states of G admit a positive parametrization with respect to $h_{c,a}$ if there exists a function $\phi : \mathbb{R}^{\hat{m}}_{>0} \times \mathbb{R}^{s}_{>0} \to \mathbb{R}^{\bar{m}}_{>0} \times \mathbb{R}^{s}_{>0}$, for some $\hat{m} \leq \bar{m}$, which we denote by $(\hat{a}; x) \mapsto \phi(\hat{a}; x)$, such that:

(i) $\phi(\hat{a}; x)$ extends the vector $(\hat{a}; x)$. More precisely, there exists a natural projection $\pi : \mathbb{R}^{\bar{m}}_{>0} \times \mathbb{R}^{s}_{>0} \to \mathbb{R}^{\hat{m}}_{>0} \times \mathbb{R}^{s}_{>0}$ such that $\pi \circ \phi$ is equal to the identity map.

(ii) Consider any $(a; x) \in \mathbb{R}^{\bar{m}}_{>0} \times \mathbb{R}^{s}_{>0}$. Then, the equality $h_i(a; x) = 0$ holds for every $i \notin I$ if and only if there exists $\hat{a}^* \in \mathbb{R}^{\hat{m}}_{>0}$ such that $(a; x) = \phi(\hat{a}^*; x)$.

We call ϕ a positive parametrization or a steady-state parametrization.

Definition 2.3. Under the notation and hypotheses of Definition 2.2, assume that the steady states of G admit a positive parametrization with respect to $h_{c,a}$. For such a positive parametrization ϕ, the critical function $C : \mathbb{R}^{\hat{m}}_{>0} \times \mathbb{R}^{s}_{>0} \to \mathbb{R}$ is given by:

$$C(\hat{a}; x) \quad = \quad (\det \mathrm{Jac}\ h_{c,a})\,|_{(a;x)=\phi(\hat{a};x)} \ ,$$

where $\mathrm{Jac}(h_{c,a})$ denotes the Jacobian matrix of $h_{c,a}$ with respect to x.

The following result is a specialization[2] of Theorem 3.12 of (Dickenstein et al. 2019):

Proposition 2.4. *Under the notation and hypotheses of Definitions 2.1–2.3, assume also that G is a conservative network without boundary steady states in any compatibility class. Let N denote the stoichiometric matrix of G.*

(A) **Multistationarity.** *G is multistationary if there exists $(\hat{a}^*; x^*) \in \mathbb{R}^{\hat{m}}_{>0} \times \mathbb{R}^{s}_{>0}$ such that*

$$\mathrm{sign}(C(\hat{a}^*; x^*)) \ = \ (-1)^{\mathrm{rank}(N)+1} \ .$$

(B) **Monostationarity.** *G is monostationary if for all $(\hat{a}; x) \in \mathbb{R}^{\hat{m}}_{>0} \times \mathbb{R}^{s}_{>0}$,*

$$\mathrm{sign}(C(\hat{a}; x)) \ = \ (-1)^{\mathrm{rank}(N)} \ .$$

2.3 Hopf bifurcations

A simple Hopf bifurcation is a bifurcation in which a single complex-conjugate pair of eigenvalues of the Jacobian matrix crosses the imaginary axis, while all other eigenvalues remain with negative real parts. Such a bifurcation, if it is supercritical, generates nearby oscillations or periodic orbits (Liu 1994).

To detect simple Hopf bifurcations, we will use a criterion of Yang that characterizes Hopf bifurcations in terms of Hurwitz-matrix determinants (Proposition 2.6).

---

[2] As noted earlier, here we consider parametrizations of the form $\phi(\hat{a}; x)$, while Dickenstein et al. (2019) allowed those of the form $\phi(\hat{a}; \hat{x})$. Also, “conservative” in Proposition 2.4 can be generalized to “dissipative” (Dickenstein et al. 2019).

Definition 2.5. The i-th Hurwitz matrix of a univariate polynomial $p(\lambda) = b_0\lambda^n + b_1\lambda^{n-1} + \cdots + b_n$ is the following $i \times i$ matrix:

$$H_i \;=\; \begin{pmatrix} b_1 & b_0 & 0 & 0 & 0 & \cdots & 0 \\ b_3 & b_2 & b_1 & b_0 & 0 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & & \vdots \\ b_{2i-1} & b_{2i-2} & b_{2i-3} & b_{2i-4} & b_{2i-5} & \cdots & b_i \end{pmatrix} ,$$

in which the (k,l)-th entry is b_{2k-l} as long as $n \geq 2k - l \geq 0$, and 0 otherwise.

Consider an ODE system parametrized by $\mu \in \mathbb{R}$:

$$\dot{x} \;=\; g_\mu(x) \;,$$

where $x \in \mathbb{R}^n$, and $g_\mu(x)$ varies smoothly in μ and x. Assume that $x^0 \in \mathbb{R}^n$ is a steady state of the system defined by μ_0, that is, $g_{\mu_0}(x^0) = 0$. Assume, furthermore, that we have a smooth curve of steady states:

$$\mu \;\mapsto\; x(\mu) \tag{8}$$

(that is, $g_\mu\left(x(\mu)\right) = 0$ for all μ) and that $x(\mu_0) = x^0$. Denote the characteristic polynomial of the Jacobian matrix of g_μ, evaluated at $x(\mu)$, as follows:

$$p_\mu(\lambda) \;:=\; \det\left(\lambda I - \text{Jac }g_\mu\right)|_{x=x(\mu)} \;=\; \lambda^n + b_1(\mu)\lambda^{n-1} + \cdots + b_n(\mu) \;,$$

and, for $i = 1, \ldots, n$, define $H_i(\mu)$ to be the i-th Hurwitz matrix of $p_\mu(\lambda)$.

Proposition 2.6 (Yang's criterion (Yang 2002))**.** *Assume the above setup. Then, there is a simple Hopf bifurcation at x_0 with respect to μ if and only if the following hold:*

(i) $b_n(\mu_0) > 0$,

(ii) $\det H_1(\mu_0) > 0$, $\det H_2(\mu_0) > 0$, $\ldots$, $\det H_{n-2}(\mu_0) > 0$, *and*

(iii) $\det H_{n-1}(\mu_0) = 0$ *and* $\frac{d(\det H_{n-1}(\mu))}{d\mu}|_{\mu=\mu_0} \neq 0$.

2.4 Using parametrizations to detect Hopf bifurcations

Here we prove a new result on how to use steady-state parametrizations to detect Hopf bifurcations (Theorem 2.8). The result, which uses Yang's criterion, is a straightforward generalization of the approach used by Conradi et al. (2019). We include it here to use later in Section 4, and we also expect it to be useful in future work.

Lemma 2.7. *Let G be a network with s species, m reactions, and d conservation laws. Denote the ODEs by $\dot{x} = f(x)$, as in (1). Assume that the positive steady states of G admit a positive parametrization ϕ with respect to an effective steady-state function for which the reparametrization map (4) is just the identity map. In other words, the effective parameters*

$\bar{a}_i$ are the original rate constants κ_i, and so we write $\phi : \mathbb{R}^{\hat{m}}_{>0} \times \mathbb{R}^s_{>0} \to \mathbb{R}^m_{>0} \times \mathbb{R}^s_{>0}$ as $(\hat{\kappa}; x) \mapsto \phi(\hat{\kappa}; x)$. Assume moreover that each coordinate of ϕ_i is a rational function: $\phi_i(\hat{\kappa}; x) \in \mathbb{Q}(\hat{\kappa}; x)$ for $i = 1, 2, \ldots, m + s$. Then the following is a univariate, degree-$(s - d)$ polynomial in λ, with coefficients in $\mathbb{Q}(\hat{\kappa}; x)$:

$$q(\lambda) \ := \ \frac{1}{\lambda^d} \ \det\left(\lambda I - \text{Jac}\ f\right)|_{(\kappa;x) \ = \ \phi(\hat{\kappa};x)} \ . \tag{9}$$

Proof. This result is straightforward from the fact that the characteristic polynomial of Jac(f) is a polynomial of degree s and has zero as a root with multiplicity d (because of the d conservation laws). ☐

Theorem 2.8 (Hopf-bifurcation criterion)**.** *Assume the hypotheses of Lemma 2.7. Let $\mathfrak{h}_i$ (for $i = 1, 2, \ldots, s - d$) be the determinant of the i-th Hurwitz matrix of $q(\lambda)$ in (9). Let κ_j be one of the rate constants in the vector $\hat{\kappa}$. Then the following are equivalent:*

1. *there exists a rate-constant vector $\kappa^* \in \mathbb{R}^m_{>0}$ such that the resulting system (1) exhibits a simple Hopf bifurcation with respect to κ_j at some $x^* \in \mathbb{R}^s_{>0}$, and*
2. *there exist $\hat{\kappa}^* \in \mathbb{R}^{\hat{m}}_{>0}$ and $x^* \in \mathbb{R}^s_{>0}$ such that*
    - *(i) the constant term of the polynomial $q(\lambda)$, when evaluated at $(\hat{\kappa}; x) = (\hat{\kappa}^*; x^*)$, is positive,*
    - *(ii) $\mathfrak{h}_1(\hat{\kappa}^*; x^*) > 0$, $\mathfrak{h}_2(\hat{\kappa}^*; x^*) > 0$, $\ldots$, $\mathfrak{h}_{s-d-2}(\hat{\kappa}^*; x^*) > 0$, and*
    - *(iii) $\mathfrak{h}_{s-d-1}(\hat{\kappa}^*; x^*) = 0$ and $\frac{\partial \mathfrak{h}_{s-d-1}}{\partial \kappa_j}|_{(\hat{\kappa};x)=(\hat{\kappa}^*;x^*)} \neq 0$.*

Moreover, given $\hat{\kappa}^*$ and x^* as in (2), a simple Hopf bifurcation with respect to κ_j occurs at x^* when the vector of rate constants is taken to be $\kappa^* := \widetilde{\pi}(\phi(\hat{\kappa}^*; x^*))$. Here, $\widetilde{\pi} : \mathbb{R}^m_{>0} \times \mathbb{R}^s_{>0} \to \mathbb{R}^m_{>0}$ is the natural projection.

Proof. Due to the d conservation laws, we apply Yang's criterion (Proposition 2.6) to:

$$\frac{1}{\lambda^d} \det(\lambda I - \text{Jac}\ f)|_{x=x^*,\ \kappa_i=\kappa_i^* \ \textit{for all}\ i \neq j} \ .$$

Now our result follows directly from Proposition 2.6 and Definition 2.2. ☐

Remark 2.9. Theorem 2.8 easily generalizes beyond parametrizations of the form $\phi(\hat{\kappa}; x)$ to those of the form $\phi(\hat{\kappa}; \hat{x})$ or $\phi(\kappa; \hat{x})$. Indeed, one of the form $\phi(\kappa; \hat{x})$ was used by Conradi et al. (2019) to establish Hopf bifurcations in a mixed-mechanism phosphorylation system.

3 ERK networks and steady-state parametrizations

Here we introduce steady-state parametrizations for the full ERK network and also irreducible and reduced versions of the network (Propositions 3.1 and 3.3).

x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}	x_{11}	x_{12}
S_{00}	E	F	$S_{11}F$	$S_{10}F$	$S_{01}F$	$S_{01}E$	$S_{10}E$	S_{01}	S_{10}	$S_{00}E$	S_{11}

Table 2: Assignment of variables to species for the ERK network in Figure 1.

3.1 The (full) ERK network

For the full ERK network shown earlier in Figure 1, we let $x_1, x_2, \ldots, x_{12}$ denote the concentrations of the species in the order given in Table 2. The resulting ODE system (1) is as follows:

$$\begin{aligned}
\dot{x_1} &= -k_1x_1x_2 + k_2x_{11} + \ell_{\text{cat}}x_5 + n_3x_6 \\
\dot{x_2} &= -k_1x_1x_2 - k_{\text{on}}x_2x_9 - m_2x_2x_{10} + k_2x_{11} + k_{\text{cat}}x_7 + k_{\text{off}}x_7 + m_1x_8 + m_3x_8 \\
\dot{x_3} &= -\ell_1x_3x_{12} - \ell_{\text{on}}x_3x_{10} - n_1x_3x_9 + \ell_2x_4 + \ell_{\text{cat}}x_5 + \ell_{\text{off}}x_5 + n_2x_6 + n_3x_6 \\
\dot{x_4} &= \ell_1x_3x_{12} - \ell_2x_4 - \ell_3x_4 \\
\dot{x_5} &= \ell_{\text{on}}x_3x_{10} + \ell_3x_4 - \ell_{\text{cat}}x_5 - \ell_{\text{off}}x_5 \\
\dot{x_6} &= n_1x_3x_9 - n_2x_6 - n_3x_6 \\
\dot{x_7} &= k_{\text{on}}x_2x_9 + k_3x_{11} - k_{\text{cat}}x_7 - k_{\text{off}}x_7 \\
\dot{x_8} &= m_2x_2x_{10} - m_1x_8 - m_3x_8 \\
\dot{x_9} &= -k_{\text{on}}x_2x_9 - n_1x_3x_9 + k_{\text{off}}x_7 + n_2x_6 \\
\dot{x_{10}} &= -\ell_{\text{on}}x_3x_{10} - m_2x_2x_{10} + \ell_{\text{off}}x_5 + m_1x_8 \\
\dot{x_{11}} &= k_1x_1x_2 - k_2x_{11} - k_3x_{11} \\
\dot{x_{12}} &= -\ell_1x_3x_{12} + k_{\text{cat}}x_7 + \ell_2x_4 + m_3x_8
\end{aligned} \tag{10}$$

There are 18 rate constants k_i, ℓ_i, m_i, n_i. The 3 conservation laws correspond to the total amounts of substrate S, kinase E, and phosphatase F, respectively:

$$\begin{aligned}
x_1 + x_4 + x_5 + x_6 + x_7 + x_8 + x_9 + x_{10} + x_{11} + x_{12} &= \mathrm{S}_{\text{tot}} =: c_1 \\
x_2 + x_7 + x_8 + x_{11} &= \mathrm{E}_{\text{tot}} =: c_2 \\
x_3 + x_4 + x_5 + x_6 &= \mathrm{F}_{\text{tot}} =: c_3.
\end{aligned} \tag{11}$$

A steady-state parametrization for the full ERK network was given by Examples 3.1 and 3.7 in the article of Dickenstein et al. (2019). That parametrization, however, can not specialize to accommodate irreversible versions of the network (in the effective parameters given by Dickenstein et al. (2019), two of the denominators are k_{on} and ℓ_{on}, so we can not set those rate constants to 0). So, in the next subsection, we give an alternate steady-state parametrization that, although quite similar to the one of Dickenstein et al. (2019), specializes when considering irreversible versions of the network (see Proposition 3.1).

3.2 Irreversible versions of the ERK network

Here we consider networks obtained from the full ERK network (Figure 1) by making some reversible reactions irreversible. Specifically, we delete one or more of the reactions marked in blue in Figure 1. Our motivation for removing those specific reactions (the ones with rate constants $k_2, k_{\text{on}}, m_1, \ell_2, \ell_{\text{on}}, n_2$) rather than any of their opposite reactions is to preserve the main reaction pathways (from S_{00} to S_{11}, as well as S_{10} to S_{11}, S_{11} to S_{00}, and S_{01} to S_{00}). At the same time, we do not remove the reactions for k_{off} or ℓ_{off}, so that we can still pursue Question 1.1 (which involves k_{off} and ℓ_{off}) in a model with fewer reactions. We instead allow the removal of reactions k_{on} and ℓ_{on}.

Proposition 3.1 (Steady-state parametrization for full and irreversible ERK networks)**.** *Let $\mathcal{N}$ be the full ERK network or any network obtained from the full ERK network by deleting one or more the reactions corresponding to rate constants $k_2, k_{\text{on}}, m_1, \ell_2, \ell_{\text{on}}, n_2$ (marked in blue in Figure 1). Let $\mathbb{1}_{k_2}$ denote the indicator function that is 1 if the reaction labeled by k_2 is in $\mathcal{N}$ and 0 otherwise; analogously, we also define $\mathbb{1}_{k_{\text{on}}}$, $\mathbb{1}_{m_1}$, $\mathbb{1}_{\ell_2}$, $\mathbb{1}_{\ell_{\text{on}}}$, and $\mathbb{1}_{n_2}$. Also, define $\hat{a} := (a_2, a_4, a_6, a_8)$ if $\mathcal{N}$ contains the reactions labeled by k_{on} and ℓ_{on}, and $\hat{a} := (a_2, a_4, a_6)$ if $\mathcal{N}$ contains the reaction ℓ_{on} but not k_{on}, and $\hat{a} := (a_2, a_4, a_8)$ if $\mathcal{N}$ contains k_{on} but not ℓ_{on}, and $\hat{a} := (a_2, a_4)$ if $\mathcal{N}$ contains neither k_{on} nor ℓ_{on}. Then $\mathcal{N}$ admits an effective steady-state function $h_{c,a} : \mathbb{R}^{12}_{>0} \to \mathbb{R}^{12}$ given by:*

$$\begin{aligned}
h_{c,a,1} &= x_1 + x_4 + x_5 + x_6 + x_7 + x_8 + x_9 + x_{10} + x_{11} + x_{12} - c_1 \ , \\
h_{c,a,2} &= x_2 + x_7 + x_8 + x_{11} - c_2 \ , \\
h_{c,a,3} &= x_3 + x_4 + x_5 + x_6 - c_3 \ , \\
h_{c,a,4} &= a_{12} x_3 x_{12} - x_4 \ , \\
h_{c,a,5} &= a_3 x_4 - x_5 - a_2 x_8 \ , \\
h_{c,a,6} &= a_{13} x_3 x_9 - x_6 \ , \\
h_{c,a,7} &= a_5 x_{11} - a_4 x_6 - x_7 \ , \\
h_{c,a,8} &= a_{11} x_2 x_{10} - x_8 \ , \\
h_{c,a,9} &= a_9 x_7 - \mathbb{1}_{k_{\text{on}}} a_8 x_2 x_9 - x_6 \ , \\
h_{c,a,10} &= a_7 x_5 - \mathbb{1}_{\ell_{\text{on}}} a_6 x_3 x_{10} - x_8 \ , \\
h_{c,a,11} &= a_{10} x_1 x_2 - x_{11} \ , \\
h_{c,a,12} &= x_7 - a_1 x_5 \ .
\end{aligned} \tag{12}$$

Moreover, with respect to this effective steady-state function, the positive steady states of $\mathcal{N}$ admit the following positive parametrization:

$$\begin{aligned}
\phi : \mathbb{R}^{2+\mathbb{1}_{k_{\text{on}}}+\mathbb{1}_{\ell_{\text{on}}}+12}_{>0} &\to \mathbb{R}^{13+12}_{>0} \\
(\hat{a};\ x_1, x_2, \ldots, x_{12}) &\mapsto (a_1, a_2, \ldots, a_{13},\ x_1, x_2, \ldots, x_{12}) \ ,
\end{aligned}$$

given by

$$\begin{array}{lll}
a_1 := \dfrac{x_7}{x_5} & a_3 := \dfrac{a_2 x_8 + x_5}{x_4} & a_5 := \dfrac{a_4 x_6 + x_7}{x_{11}} \\
a_7 := \dfrac{\mathbb{1}_{\ell_{\text{on}}} a_6 x_3 x_{10} + x_8}{x_5} & a_9 := \dfrac{\mathbb{1}_{k_{\text{on}}} a_8 x_2 x_9 + x_6}{x_7} & a_{10} := \dfrac{x_{11}}{x_1 x_2} \\
a_{11} := \dfrac{x_8}{x_2 x_{10}} & a_{12} := \dfrac{x_4}{x_3 x_{12}} & a_{13} := \dfrac{x_6}{x_3 x_9} \ .
\end{array} \tag{13}$$

Proof. We will show that the map $\bar{a} : \mathbb{R}_{>0}^{12+\mathbb{1}_{k_2}+\mathbb{1}_{k_{\text{on}}}+\mathbb{1}_{m_1}+\mathbb{1}_{\ell_2}+\mathbb{1}_{\ell_{\text{on}}}+\mathbb{1}_{n_2}} \to \mathbb{R}_{>0}^{11+\mathbb{1}_{k_{\text{on}}}+\mathbb{1}_{\ell_{\text{on}}}}$, defined as follows, is a reparametrization map as in (4):

$$\begin{array}{lllll} \bar{a}_1 = \frac{\ell_{cat}}{k_{cat}}, & \bar{a}_2 = \frac{m_3}{\ell_{\text{cat}}}, & \bar{a}_3 = \frac{\ell_3}{\ell_{\text{cat}}}, & \bar{a}_4 = \frac{n_3}{k_{\text{cat}}}, & \bar{a}_5 = \frac{k_3}{k_{\text{cat}}}, \\ \bar{a}_6 = \frac{\mathbb{1}_{\ell_{\text{on}}}\ell_{\text{on}}}{m_3}, & \bar{a}_7 = \frac{\ell_{\text{off}}}{m_3}, & \bar{a}_8 = \frac{\mathbb{1}_{k_{\text{on}}}k_{\text{on}}}{n_3}, & \bar{a}_9 = \frac{k_{\text{off}}}{n_3}, & \bar{a}_{10} = \frac{k_1}{\mathbb{1}_{k_2}k_2+k_3}, \\ \bar{a}_{11} = \frac{m_2}{\mathbb{1}_{m_1}m_1+m_3}, & \bar{a}_{12} = \frac{\ell_1}{\mathbb{1}_{\ell_2}\ell_2+\ell_3}, & \bar{a}_{13} = \frac{n_1}{\mathbb{1}_{n_2}n_2+n_3}. & & \end{array} \tag{14}$$

In particular, we remove the effective parameter $\bar{a}_6$ (respectively, $\bar{a}_8$) if $\mathbb{1}_{\ell_{\text{on}}} = 0$ (respectively, $\mathbb{1}_{k_{\text{on}}} = 0$). Notice that each $\bar{a}_i$ (if it is not removed) is defined and positive for all $\kappa = (k_1, \ldots, n_3) \in \mathbb{R}_{>0}^{12+\mathbb{1}_{k_2}+\mathbb{1}_{k_{\text{on}}}+\mathbb{1}_{m_1}+\mathbb{1}_{\ell_2}+\mathbb{1}_{\ell_{\text{on}}}+\mathbb{1}_{n_2}}$.

We must show that the map $\bar{a}$ is surjective. Indeed, given $a \in \mathbb{R}_{>0}^{11+\mathbb{1}_{k_{\text{on}}}+\mathbb{1}_{\ell_{\text{on}}}}$, it is easy to check that a is the image under $\bar{a}$ of the vector obtained by removing every 0 coordinate from the following vector:

$$\begin{aligned} &(k_1, k_2, k_3, k_{\text{cat}}, k_{\text{on}}, k_{\text{off}}, \ell_1, \ell_2, \ell_3, \ell_{\text{cat}}, \ell_{\text{on}}, \ell_{\text{off}}, m_1, m_2, m_3, n_1, n_2, n_3) \;= \\ &((\mathbb{1}_{k_2} + a_5)a_{10}, \mathbb{1}_{k_2}, a_5, 1, \mathbb{1}_{k_{\text{on}}}a_4a_8, a_4a_9, (\mathbb{1}_{\ell_2} + a_1a_3)a_{12}, \mathbb{1}_{\ell_2}, a_1a_3, a_1, \mathbb{1}_{\ell_{\text{on}}}a_1a_2a_6, a_1a_2a_7, \mathbb{1}_{m_1}, \\ &\quad (\mathbb{1}_{m_1} + a_1a_2)a_{11}, a_1a_2, (\mathbb{1}_{n_2} + a_4)a_{13}, \mathbb{1}_{n_2}, a_4)\ . \end{aligned}$$

With an eye toward applying Definition 2.1, consider the following 9×9 matrix:

$$M(\kappa) := \begin{pmatrix} \frac{1}{\mathbb{1}_{\ell_2}\ell_2+\ell_3} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \frac{1}{\ell_{\text{cat}}} & 0 & 0 & \frac{1}{\ell_{\text{cat}}} & 0 & \frac{1}{\ell_{\text{cat}}} & 0 & 0 \\ 0 & 0 & \frac{1}{\mathbb{1}_{n_2}n_2+n_3} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \frac{1}{k_{\text{cat}}} & \frac{1}{k_{\text{cat}}} & 0 & \frac{1}{k_{\text{cat}}} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{\mathbb{1}_{m_1}m_1+m_3} & 0 & 0 & 0 & 0 \\ 0 & 0 & \frac{1}{n_3} & 0 & 0 & \frac{1}{n_3} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{m_3} & 0 & \frac{1}{m_3} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{1}{\mathbb{1}_{k_2}k_2+k_3} & 0 \\ \frac{1}{k_{\text{cat}}} & \frac{1}{k_{\text{cat}}} & 0 & 0 & \frac{1}{k_{\text{cat}}} & 0 & \frac{1}{k_{\text{cat}}} & 0 & \frac{1}{k_{\text{cat}}} \end{pmatrix}. \tag{15}$$

It is straightforward to check that $\det M(\kappa)$ is the product of all diagonal terms, and hence is positive for all $\kappa \in \mathbb{R}_{>0}^{12+\mathbb{1}_{k_2}+\mathbb{1}_{k_{\text{on}}}+\mathbb{1}_{m_1}+\mathbb{1}_{\ell_2}+\mathbb{1}_{\ell_{\text{on}}}+\mathbb{1}_{n_2}}$.

The mass-action ODEs of $\mathcal{N}$ are obtained from those (10) of the full ERK network by replacing the rate constants $k_2, k_{\text{on}}, m_1, \ell_2, \ell_{\text{on}}, n_2$, respectively, by $\mathbb{1}_{k_2}k_2$, $\mathbb{1}_{k_{\text{on}}}k_{\text{on}}$, $\mathbb{1}_{m_1}m_1$, $\mathbb{1}_{\ell_2}\ell_2$, $\mathbb{1}_{\ell_{\text{on}}}\ell_{\text{on}}$, and $\mathbb{1}_{n_2}n_2$, respectively. To the right-hand sides of these ODEs, we apply the recipe given in equations (5)–(7), using the effective parameters $\bar{a}_i$ in (14), the matrix $M(\kappa)$ in (15), and the conservation-law matrix W arising from the conservation laws (11). It is straightforward to check that the result is the function $h_{c,a}(x)$ given in (12).

Observe that, for the non-conservation-law equations $h_{c,a,4}, \ldots, h_{c,a,12}$ in (12), each non-constant coefficient is, up to sign, one of the a_i's. Hence, the $\bar{a}_i$'s in (14) are effective parameters, and the function in (12) is an effective steady-state function. Finally, the fact that ϕ is a positive parametrization with respect to (12) (as in Definition 2.2) follows directly from comparing equations (12) and (13). $\square$

Remark 3.2 (Multistationarity depends on only k_{on} and ℓ_{on})**.** Proposition 3.1 considers any network obtained by deleting any (or none) of the six reactions labeled by k_2, k_{on}, m_1, ℓ_2, ℓ_{on}, n_2. Nonetheless, the resulting steady-state parametrization (13) depends on k_{on} and ℓ_{on} but not any of the other rate constants. Thus, multistationarity for these irreversible networks depends only on whether the network contains k_{on} and ℓ_{on} (see Theorem 4.6).

3.3 The reduced ERK network

In the previous subsection, we consider irreversible versions of the ERK network. Now we further reduce the network by additionally removing some "intermediate complexes" (namely, $S_{10}E$ and $S_{01}F$). These operations yield the reduced ERK network in Figure 2. Note that in the process of removing intermediates, the reactions m_2 and m_3 (similarly, n_1 and n_3) are collapsed into a single reaction labeled m (respectively, n). A biological motivation for collapsing these reactions is the fact that intermediates are usually short-lived, so the simpler model may approximate the dynamics well.

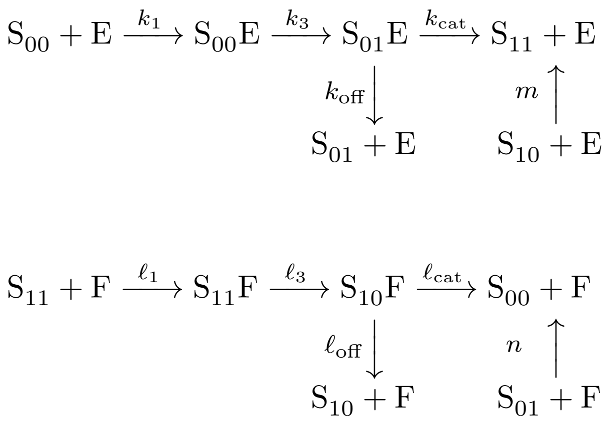

Figure 2: Reduced ERK network.

Our notion of removing intermediates matches that of Feliu & Wiuf (2013), who initiated the recent interest in the question of when dynamical properties are preserved when intermediates are added or removed (e.g., $S_{10} + E \leftrightarrows S_{10}E \to S_{11} + E$ versus $S_{10} \to S_{11}$). Our work, therefore, fits into this circle of ideas (Cappelletti & Wiuf 2017, Marcondes de Freitas et al. 2017, Sadeghimanesh & Feliu 2018).

x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}
S_{00}	E	$S_{00}E$	$S_{01}E$	S_{11}	S_{01}	S_{10}	F	$S_{11}F$	$S_{10}F$

Table 3: Assignment of variables to species for the reduced ERK network in Figure 2. (Many of the variables that are also in the full ERK, in Table 2, have been relabeled.)

In the reduced ERK network, the remaining 10 rate constants are as follows: $k_1, k_3, k_{\text{cat}}, k_{\text{off}}, m, \ell_1, \ell_3, \ell_{\text{cat}}, \ell_{\text{off}}, n$. Letting $x_1, x_2, \ldots, x_{10}$ denote the species concentrations in the order given in Table 3, the resulting mass-action kinetics ODEs are as follows:

$$\begin{aligned}
\dot{x_1} &= -k_1x_1x_2 + nx_6x_8 + \ell_{\text{cat}}x_{10} &&=: f_1\\
\dot{x_2} &= -k_1x_1x_2 + k_{\text{cat}}x_4 + k_{\text{off}}x_4 &&=: f_2\\
\dot{x_3} &= k_1x_1x_2 - k_3x_3 &&=: f_3\\
\dot{x_4} &= k_3x_3 - k_{\text{cat}}x_4 - k_{\text{off}}x_4 &&=: f_4\\
\dot{x_5} &= mx_2x_7 - \ell_1x_5x_8 + k_{\text{cat}}x_4 &&=: f_5\\
\dot{x_6} &= -nx_6x_8 + k_{\text{off}}x_4 &&=: f_6\\
\dot{x_7} &= -mx_2x_7 + \ell_{\text{off}}x_{10} &&=: f_7\\
\dot{x_8} &= -\ell_1x_5x_8 + \ell_{\text{off}}x_{10} + \ell_{\text{cat}}x_{10} &&=: f_8\\
\dot{x_9} &= \ell_1x_5x_8 - \ell_3x_9 &&=: f_9\\
\dot{x_{10}} &= -\ell_{\text{off}}x_{10} + \ell_3x_9 - \ell_{\text{cat}}x_{10} &&=: f_{10}.
\end{aligned} \tag{16}$$

The 3 conservation equations are:

$$\begin{aligned}
x_1 + x_3 + x_4 + x_5 + x_6 + x_7 + x_9 + x_{10} &= S_{\text{tot}} &&=: c_1\\
x_2 + x_3 + x_4 &= E_{\text{tot}} &&=: c_2\\
x_8 + x_9 + x_{10} &= F_{\text{tot}} &&=: c_3.
\end{aligned} \tag{17}$$

Proposition 3.3 (Steady-state parametrization for reduced ERK network)**.** *The reduced ERK network (Figure 2) admits an effective steady-state function $h_{c,a} : \mathbb{R}^{10}_{>0} \to \mathbb{R}^{10}$ given by:*

$$\begin{aligned}
&h_{c,a,1} = x_1 + x_3 + x_4 + x_5 + x_6 + x_7 + x_9 + x_{10} - c_1, && h_{c,a,2} = x_2 + x_3 + x_4 - c_2,\\
&h_{c,a,3} = -(k_{\text{cat}} + k_{\text{off}})\ell_{\text{cat}}x_{10} + k_1k_{\text{cat}}x_1x_2, && h_{c,a,4} = k_3x_3 - (k_{\text{cat}} + k_{\text{off}})x_4,\\
&h_{c,a,5} = \ell_{\text{off}}x_{10} - mx_2x_7, && h_{c,a,6} = \ell_1x_5x_8 - (\ell_{\text{cat}} + \ell_{\text{off}})x_{10},\\
&h_{c,a,7} = \ell_3x_9 - (\ell_{\text{cat}} + \ell_{\text{off}})x_{10}, && h_{c,a,8} = x_8 + x_9 + x_{10} - c_3,\\
&h_{c,a,9} = k_{\text{cat}}x_4 - \ell_{\text{cat}}x_{10}, && h_{c,a,10} = k_{\text{off}}\ell_{\text{cat}}x_{10} - k_{\text{cat}}nx_6x_8.
\end{aligned} \tag{18}$$

Moreover, with respect to this effective steady-state function, the positive steady states admit the following positive parametrization:

$$\begin{aligned}
\phi : \mathbb{R}^{3+10}_{>0} &\to \mathbb{R}^{10+10}_{>0}\\
(k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}},\ x_1, x_2, \ldots, x_{10}) &\mapsto (k_1, k_3, k_{\text{cat}}, k_{\text{off}}, m, \ell_1, \ell_3, \ell_{\text{cat}}, \ell_{\text{off}}, n,\ x_1, x_2, \ldots, x_{10})\ ,
\end{aligned} \tag{19}$$

given by

$$\begin{aligned}
k_1 &:= \frac{(k_{\text{cat}} + k_{\text{off}})x_4}{x_1x_2} & k_3 &:= \frac{(k_{\text{cat}} + k_{\text{off}})x_4}{x_3} & m &:= \frac{\ell_{\text{off}}x_{10}}{x_2x_7} & \ell_1 &:= \frac{\ell_{\text{off}}x_{10} + k_{\text{cat}}x_4}{x_5x_8}\\
\ell_3 &:= \frac{\ell_{\text{off}}x_{10} + k_{\text{cat}}x_4}{x_9} & \ell_{\text{cat}} &:= \frac{k_{\text{cat}}x_4}{x_{10}} & n &:= \frac{k_{\text{off}}x_4}{x_6x_8}.
\end{aligned} \tag{20}$$

In particular, the image of ϕ *is the following set of pairs of positive steady states and rate constants:*

$$\{(k^*; x^*) \in \mathbb{R}^{10+10}_{>0} \mid x^* \text{ is a steady state of } (16) \text{ when } k = k^*\} \ .$$

Here, k *denotes the vector* $(k_1, k_3, k_{\text{cat}}, k_{\text{off}}, m, \ell_1, \ell_3, \ell_{\text{cat}}, \ell_{\text{off}}, n)$*.*

Proof. Let W denote the conservation-law matrix arising from the conservation laws (17) for the reduced ERK network. Then $I = \{1, 2, 8\}$ is the set of indices of the first nonzero coordinates of the rows of W. We take $\mathbb{Q}(k_{\text{cat}}, k_{\text{off}})$-linear combinations of the f_i's in (16), where $i \notin I$, to obtain the following binomials in the x_i's:

$$\begin{aligned}
h_3 &:= (k_{\text{cat}} + k_{\text{off}})(f_5 + f_7 + f_9 + f_{10}) + k_{\text{cat}}(f_3 + f_4) &&= -(k_{\text{cat}} + k_{\text{off}})\ell_{\text{cat}} x_{10} + k_1 k_{\text{cat}} x_1 x_2 \\
h_4 &:= f_4 &&= k_3 x_3 - (k_{\text{cat}} + k_{\text{off}}) x_4 \\
h_5 &:= f_7 &&= \ell_{\text{off}} x_{10} - m x_2 x_7 \\
h_6 &:= f_9 + f_{10} &&= \ell_1 x_5 x_8 - (\ell_{\text{cat}} + \ell_{\text{off}}) x_{10} \\
h_7 &:= f_{10} &&= \ell_3 x_9 - (\ell_{\text{cat}} + \ell_{\text{off}}) x_{10} \\
h_9 &:= f_5 + f_7 + f_9 + f_{10} &&= k_{\text{cat}} x_4 - \ell_{\text{cat}} x_{10} \\
h_{10} &:= k_{\text{cat}} f_6 - k_{\text{off}}(f_5 + f_7 + f_9 + f_{10}) &&= k_{\text{off}} \ell_{\text{cat}} x_{10} - k_{\text{cat}} n x_6 x_8 .
\end{aligned}$$

Consider the (above) linear transformation from f_i to h_i ($i \notin I$). Let M denote the corresponding matrix representation (M plays the role of the matrix denoted by $M(\kappa)$ in Definition 2.1). It is straightforward to check that $\det M = k_{\text{cat}}^2$, which is positive when $k_{\text{cat}} > 0$.

Consider the reparametrization map $\bar{a} : \mathbb{R}^{10} \to \mathbb{R}^{10}$ defined by the identity map (and so is surjective). Then $\bar{a}$, together with the conservation-law matrix W and the matrix M, yield (as in Definition 2.1[3]) the effective steady-state function $h_{c,a}(x)$ given in (18).

To show that ϕ is a positive steady-state parametrization with respect to (18), as in Definition 2.2, it suffices to show the following claim:

Claim: For every $(k^*; x^*) \in \mathbb{R}^{10+10}_{>0}$, the steady-state condition holds – namely, $h_i(k^*; x^*) = 0$ for all $i \in \{3, 4, 5, 6, 7, 9, 10\}$ – if and only if $\phi(k^*_{\text{cat}}, k^*_{\text{off}}, \ell^*_{\text{off}}; x^*) = (k^*; x^*)$.

For the "⇒" direction, assume $h_i(k^*; x^*) = 0$ for all i. Then $h_9(k^*; x^*) = 0$ implies that

$$\ell^*_{\text{cat}} \ = \ \frac{k^*_{\text{cat}} x^*_4}{x^*_{10}} \ . \tag{21}$$

In other words, λ_{cat} – when evaluated at $(k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}}; x) = (k^*_{\text{cat}}, k^*_{\text{off}}, \ell^*_{\text{off}}; x^*)$ – equals ℓ^*_{cat}. Next, the equality $h_3(k^*; x^*) = 0$ implies that

$$k^*_1 \ = \ \frac{(k^*_{\text{cat}} + k^*_{\text{off}})\ell^*_{\text{cat}} x^*_{10}}{k^*_{\text{cat}} x^*_1 x^*_2} \ = \ \frac{(k^*_{\text{cat}} + k^*_{\text{off}}) x^*_4}{x^*_1 x^*_2} \ , \tag{22}$$

---

[3] In this case, Definition 2.1(iii)(b) requires every nonconstant coefficient in the effective steady-state function (18) to be a rational-number multiple of one of the rate constants. However, for the non-conservation-law equations in (18), many of the non-constant coefficients – such as $k_{\text{off}} \ell_{\text{cat}}$ – are not rational-number multiples of one of the rate constants. Nonetheless, these coefficients are all polynomials in the rate constants, and the relevant results by Dickenstein et al. (2019) hold in that generality.

where the final equality follows from equation (21). Thus, the expression for k_1 given after (20) – when evaluated at $(k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}}; x) = (k^*_{\text{cat}}, k^*_{\text{off}}, \ell^*_{\text{off}}; x^*)$ – equals k^*_1.

Similarly, the equality $h_4(k^*; x^*) = 0$ (respectively, $h_5(k^*; x^*) = 0$, $h_6(k^*; x^*) = 0$, $h_7(k^*; x^*) = 0$, or $h_{10}(k^*; x^*) = 0$) implies that κ_3 (respectively, m, ℓ_1, ℓ_3, or n) – when evaluated at $(k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}}; x) = (k^*_{\text{cat}}, k^*_{\text{off}}, \ell^*_{\text{off}}; x^*)$ – equals k^*_3 (respectively, m^*, ℓ^*_1, ℓ^*_3, or n^*). Thus, $\phi(k^*_{\text{cat}}, k^*_{\text{off}}, \ell^*_{\text{off}}; x^*) = (k^*; x^*)$.

The "$\Leftarrow$" direction is similar. Assume $\phi(k^*_{\text{cat}}, k^*_{\text{off}}, \ell^*_{\text{off}}; x^*) = (k^*; x^*)$. That is, the expressions for k_1, k_3, m, ℓ_1, ℓ_3, ℓ_{cat}, and n evaluate to, respectively, k^*_1, k^*_3, m^*, ℓ^*_1, ℓ^*_3, ℓ^*_{cat}, and n^*, when $(k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}}; x) = (k^*_{\text{cat}}, k^*_{\text{off}}, \ell^*_{\text{off}}; x^*)$. In particular, equation (21) holds, and so $h_9(k^*; x^*) = 0$. Similarly, $h_i(k^*; x^*) = 0$ for all other i (here we also use equation (21)). $\square$

Remark 3.4. The proof of Proposition 3.3 proceeds by performing linear operations on the steady-state polynomials to yield binomials g_i, and then solving for one k_j from each binomial to obtain the parametrization (19). This is similar in spirit to – but more general than – the approach prescribed in (Dickenstein et al. 2019, §4) for "linearly binomial" networks. Also, our linear operations were found "by hand", and so an interesting future direction is to develop efficient and systematic approaches to finding such operations leading to binomials.

Remark 3.5. The proof of Proposition 3.3 shows that the "steady-state ideal" (the ideal generated by the right-hand sides of the ODEs) of the reduced ERK network is generated by the binomials g_i. This network, therefore has, "toric steady states" (Pérez Millán et al. 2012). In contrast, the steady-state ideal of the full ERK network is not a binomial ideal (it is straightforward to check this computationally, e.g., using the `Binomials` package in `Macaulay2` (Grayson & Stillman n.d.)). As for the irreversible versions of the ERK network, when the reactions with rate constants k_{on} and ℓ_{on} are deleted, we see from (12) that the steady-state ideal becomes binomial. Hence, irreversible ERK networks that are missing both k_{on} and ℓ_{on} are "linearly binomial" as defined by Dickenstein et al. (2019).

Remark 3.6. All networks considered in this section are conservative, which can be seen from the conservation laws (11) for the full and irreversible ERK networks, and (17) for the reduced ERK network. Also for these networks, there are no boundary steady states in any compatibility class (it is straightforward to check this using results from (Angeli et al. 2007) or (Shiu & Sturmfels 2010)).

4 Main Results

Each ERK network we investigated admits oscillations via a Hopf bifurcation (Section 4.1). Bistability, however, is more subtle (Section 4.2).

4.1 Oscillations

The full ERK system (Figure 1) exhibits oscillations for some values of the rate constants (Rubinstein et al. 2016). We now investigate oscillations in the fully irreversible and reduced ERK networks.

4.1.1 Fully irreversible ERK network

As shown in Figure 3, the fully irreversible ERK network admits oscillations. That figure was generated using the following rate constants:

$$\begin{aligned}(k_1, k_3, k_{\text{cat}}, k_{\text{off}}, \ell_1, \ell_3, \ell_{\text{cat}}, \ell_{\text{off}}, m_2, m_3, n_1, n_3) \;=\; &(5241, 5314.5, 1291, 76.203, 64.271, \\ &\;44.965, 924970, 27238, 2.76250 \times 10^6, \\ &\;2.0451, 2.1496 \times 10^6, 1.3334)\;. \end{aligned} \tag{23}$$

These rate constants (23) come from the ones that Rubinstein *et al.* showed generate oscillations for the full ERK network (Rubinstein et al. 2016, Table 2) (we simply ignore their rate constants for the six deleted reactions). The approximate initial species concentrations used to generate Figure 3 are as follows (see supplementary file `ERK-Matcont.txt`):

$$\begin{aligned}(x_1, x_2, \ldots, x_{12}) \;\approx\; &(1.215 \times 10^{-5},\; 4.722 \times 10^{-5},\; 8.777 \times 10^{-4},\; 1.396 \times 10^{-3}, \\ &\;6.590 \times 10^{-8},\; 2.698 \times 10^{-3},\; 2.873 \times 10^{-4},\; 1.150 \times 10^{-3}, \\ &\;3.072 \times 10^{-3},\; 2.262 \times 10^{-6},\; 0.042, \quad 0.849)\;. \end{aligned} \tag{24}$$

In Figure 3, we notice some peculiarities in the graphs $x_i(t)$ of the species concentrations. The species concentrations x_1 and x_2 (corresponding to S_{00} and E, respectively) peak dramatically, while x_3 and x_6 (F and S_{01}F) stabilize momentarily at each peak. Also, each of $x_1, x_2, x_3, x_4, x_5, x_{10}, x_{11}$ deplete for some time in each period, whereas x_{12} (S_{11}) never depletes. Finally, the graphs of the pairs x_1 and x_2 are qualitatively similar, and also the pair x_3 and x_6, the pair x_4 and x_5, and the pair x_{10} and x_{11}.

Going beyond the fully irreversible ERK network, all other irreversible ERK networks – those obtained from the full ERK network by deleting one or more the reactions $k_2, k_{\text{on}}, m_1, \ell_2, \ell_{\text{on}}, n_2$ – also admit oscillations. This claim follows from Proposition 4.1 in the article by Banaji (2018), which "lifts" oscillations when one or more reactions are made reversible.

4.1.2 Reduced ERK network

We saw in the previous subsection that the fully irreversible ERK network exhibits oscillations. We now show that a simpler network - the reduced ERK network - also undergoes oscillations via a Hopf bifurcation. These oscillations are shown in Figure 4, and the rate constants that yield the corresponding Hopf bifurcation are specified in Theorem 4.3.

Compared to the oscillations for the irreversible ERK network (Figure 3), the oscillations in the reduced ERK network (Figure 4) are more uniform. Also, the period of oscillation is much shorter, and the amplitudes for species x_3, x_8, and x_{10} are small (this may be due to the choice of rate constants). Finally, three of the six species shown do not deplete completely, whereas nearly all the species of the fully irreversible ERK do deplete in each period.

We discovered oscillations by finding a Hopf bifurcation. How we found this bifurcation – via the Hopf-bifurcation criterion in Section 2.4 – is the focus of the rest of this subsection.

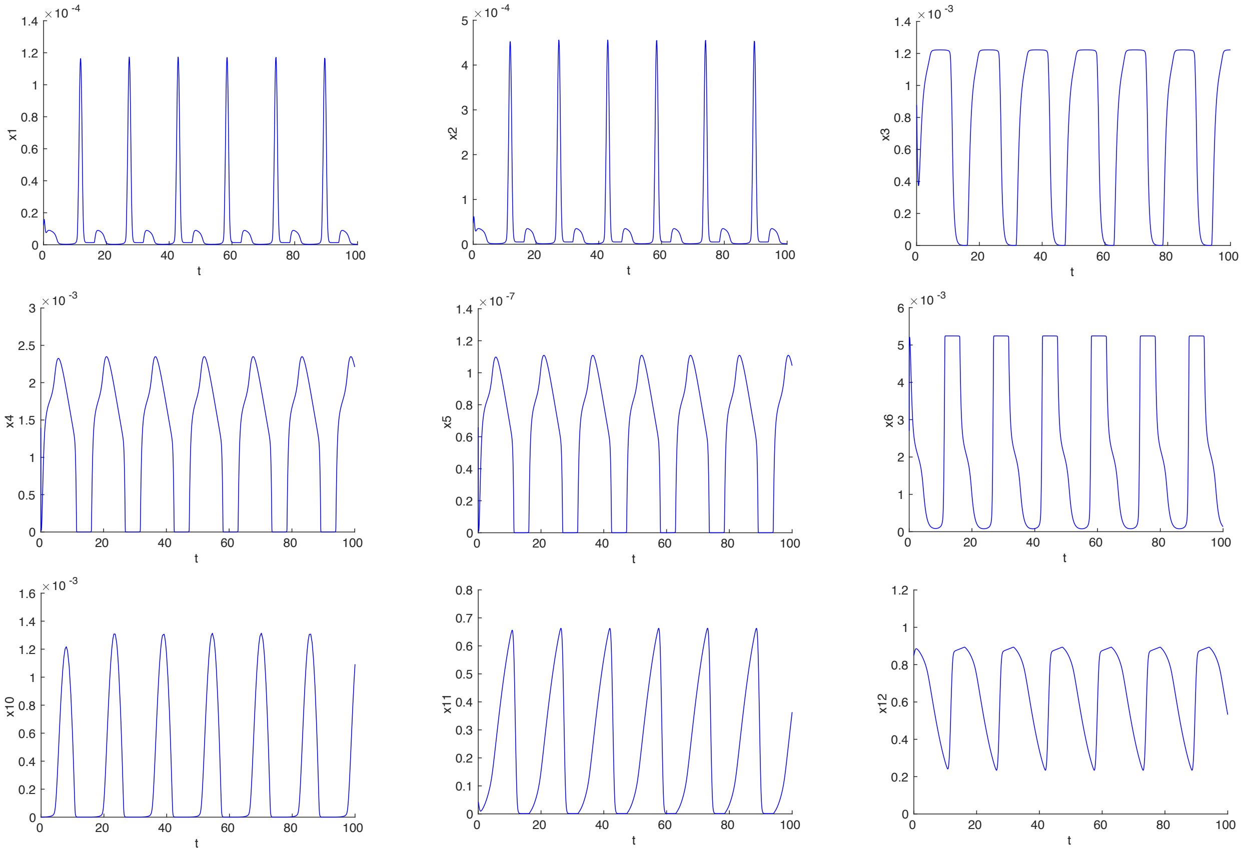

Figure 3: The fully irreversible ERK network undergoes oscillations when the rate constants are as in (23) and the initial species concentrations are as in (24). Displayed in this figure are all species concentrations, except x_7, x_8, and x_9. This figure was generated using `MATCONT`, a numerical bifurcation package (Dhooge et al. 2004). For details, see the supplementary file `ERK-Matcont.txt`.

Proposition 4.1 (Hopf criterion for reduced ERK)**.** *Consider the reduced ERK network, and let the polynomials f_i denote the right-hand sides of the resulting ODEs, as in (16). Let $\hat{\kappa} := (k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}})$ and $x := (x_1, x_2, \ldots, x_{10})$, and let ϕ be the steady-state parametrization (19). Then the following is a univariate, degree-7 polynomial in λ, with coefficients in $\mathbb{Q}(x)[\hat{\kappa}]$:*

$$q(\lambda) \;:=\; \frac{1}{\lambda^3}\ \det\left(\lambda I - \text{Jac}(f)\right)|_{(\kappa;x)=\phi(\hat{\kappa};x)}\ . \tag{25}$$

Now let $\mathfrak{h}_i$, for $i = 4, 5, 6$, denote the determinant of the i-th Hurwitz matrix of the polynomial $q(\lambda)$ in (25). Then the following are equivalent:

(1) there exists a rate-constant vector $\kappa^* \in \mathbb{R}^{10}_{>0}$ such that the resulting system (16) exhibits a simple Hopf bifurcation, with respect to k_{cat}, at some $x^* \in \mathbb{R}^{10}_{>0}$, and

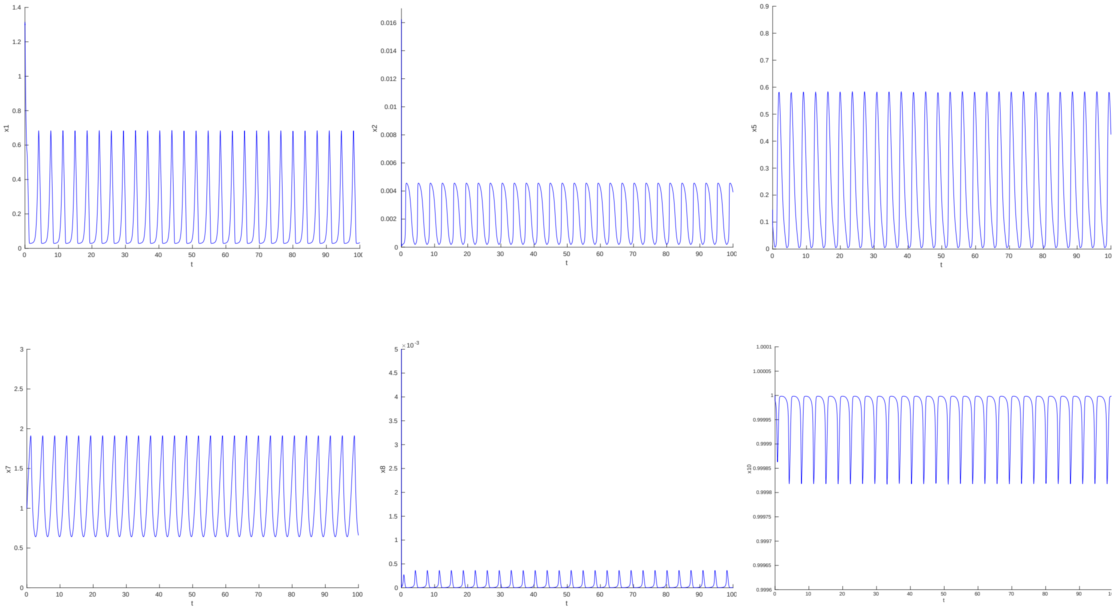

Figure 4: The reduced ERK network exhibits oscillations when the rate constants are approximately those in Theorem 4.3 and the initial species concentrations are close to the Hopf bifurcation. Details are in the supplementary file `ERK-Matcont.txt`. This figure, generated using `MATCONT`, displays all species concentrations, except x_3, x_4, x_6, and x_9.

(2) there exist $x^* \in \mathbb{R}^{10}_{>0}$ *and* $\hat{\kappa}^* \in \mathbb{R}^3_{>0}$ *such that*

$$\mathfrak{h}_4(\hat{\kappa}^*; x^*) > 0 \ , \ \mathfrak{h}_5(\hat{\kappa}^*; x^*) > 0 \ , \ \mathfrak{h}_6(\hat{\kappa}^*; x^*) = 0 \ , \ \text{and} \qquad (26)$$
$$\frac{\partial}{\partial k_{\text{cat}}} \mathfrak{h}_6(\hat{\kappa}; x)|_{(\hat{\kappa}; x) = (\hat{\kappa}^*; x^*)} \neq 0 \ .$$

Moreover, given $\hat{\kappa}^*$ *and* x^* *as in (2), a simple Hopf bifurcation with respect to* k_{cat} *occurs at* x^* *when the rate constants are taken to be* $\kappa^* := \widetilde{\pi}(\phi(\hat{\kappa}^*; x^*))$. *Here,* $\widetilde{\pi} : \mathbb{R}^{10}_{>0} \times \mathbb{R}^{10}_{>0} \to \mathbb{R}^{10}_{>0}$ *is the natural projection to the first 10 coordinates.*

Proof. The fact that $q(\lambda)$ is a degree-7 polynomial follows from Lemma 2.7, and the fact that its coefficients are in $\mathbb{Q}(x)[\hat{\kappa}]$ follows from inspecting equations (16) and (19). The rest of the result will follow immediately from Theorem 2.8 and Proposition 3.3, once we prove that $\mathfrak{h}_1$, $\mathfrak{h}_2$, $\mathfrak{h}_3$, and the constant term of $q(\lambda)$ are all positive when evaluated at any $(\hat{\kappa}; x) \in \mathbb{R}^3_{>0} \times \mathbb{R}^{10}_{>0}$. Indeed, this is shown in the supplementary file `reducedERK-hopf.mw`.

(In fact, even before substituting the parametrization $(\kappa; x) = \phi(\hat{\kappa}; x)$, the corresponding Hurwitz determinants are already positive polynomials.) □

Remark 4.2. Note that k_{cat} is the only free parameter, so it is the natural bifurcation parameter.

We now prove that the reduced ERK network gives rise to a Hopf bifurcation.

Theorem 4.3 (Hopf bifurcation in reduced ERK)**.** *The reduced ERK network exhibits a simple Hopf bifurcation with respect to the bifurcation parameter k_{cat} at the following point:*

$$x^* \approx (0.05952457867,\ 0.002204614024,\ 1,\ 1,\ 0.1518056972,\ 1,\ 1,\ 0.00001239529511,\ 1,\ 1)\ ,$$

when the rate constants are as follows:

$$\begin{aligned}(k_1^*, k_3^*, k_{\text{cat}}^*, k_{\text{off}}^*, m^*, \ell_1^*, \ell_3^*, \ell_{\text{cat}}^*, \ell_{\text{off}}^*, n^*) \ \approx\ & (5.562806640 \times 10^6,\ 730,\ 729,\ 1,\ 453.5941390,\\ & 3.879519315 \times 10^8,\ 730,\ 729,\ 1,\ 80675.77183)\end{aligned}$$

Here, ϕ is the parametrization (19), and $\widetilde{\pi}$ is the projection to the first 10 coordinates.

Proof. By Proposition 4.1, we need only show that the inequalities and equality in (26) are satisfied at $x = x^*$ (with x^* given in the statement of the theorem) and $\hat{\kappa} = \hat{\kappa}^* = (9, 1, 1)$. These are verified in the supplementary file `reducedERK-hopf.mw`. □

Remark 4.4. The Hopf bifurcation given in Theorem 4.3 was found by analyzing the Newton polytopes of $\mathfrak{h}_4$, $\mathfrak{h}_5$, and $\mathfrak{h}_6$. The theory behind this approach is presented in Appendix B, and the steps we took to find the Hopf bifurcation are listed in Appendix C. We include these appendices for readers who wish to apply similar approaches to other systems.

4.2 Bistability

Although the full ERK network is bistable (Rubinstein et al. 2016), we now prove that the reduced ERK network is not bistable (Proposition 4.5). As for irreversible ERK networks, some of them are bistable, and we show that bistability is controlled by the two reactions k_{on} and ℓ_{on} (Theorem 4.6).

Proposition 4.5. *The reduced ERK network is not multistationary, and hence not bistable.*

Proof. Let $\mathcal{N}$ denote the reduced ERK network. By definition and Proposition 3.3, we obtain the following critical function for $\mathcal{N}$:

$$C(\hat{a}; x) \quad = \quad (\det \text{Jac}\ h_{c,a})\,|_{(a;x)=\phi(\hat{a};x)}\ , \tag{27}$$

where $\hat{a} = (k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}})$, the function $h_{c,a}$ is as in (18), and $\phi(\hat{a}; x)$ is as in (19).

This critical function $C(\hat{a}; x)$ (see the supplementary file `reducedERK-noMSS.mw`) is a rational function, where the denominator is the following monomial: $x_1 x_2 x_3 x_5 x_6 x_7 x_8 x_9$.

The numerator of $C(\hat{a}; x)$ is the following polynomial, which is negative when evaluated at any $(\hat{a}; x) \in \mathbb{R}^3_{>0} \times \mathbb{R}^{10}_{>0}$:

$$\begin{aligned} &- k_{\text{cat}}^3 (k_{\text{cat}} + k_{\text{off}})^2 x_4^3 (k_{\text{cat}} x_4 + \ell_{\text{off}} x_{10})^2 \ell_{\text{off}} k_{\text{off}} (x_1 x_2 x_8 + x_1 x_3 x_8 + x_1 x_4 x_8 + x_{10} x_2 x_5 \\ &\quad + x_{10} x_2 x_6 + x_{10} x_2 x_8 + x_2 x_3 x_8 + x_2 x_4 x_8 + x_2 x_5 x_8 + x_2 x_5 x_9 + x_2 x_6 x_8 + x_2 x_6 x_9 \\ &\quad + x_2 x_7 x_8 + x_2 x_8 x_9 + x_3 x_7 x_8 + x_4 x_7 x_8) \ . \end{aligned}$$

Thus, the following holds for all $(\hat{a}; x) \in \mathbb{R}^3_{>0} \times \mathbb{R}^{10}_{>0}$:

$$\text{sign}(C(\hat{a}; x)) = -1 = (-1)^{\text{rank}(N)} \ ,$$

where the final equality uses the fact that the stoichiometric matrix N has rank $10 - 3 = 7$.

So, by Proposition 2.4 and the fact that $\mathcal{N}$ is conservative with no boundary steady states in any stoichiometric compatibility class (Remark 3.6), $\mathcal{N}$ is monostationary. Thus, $\mathcal{N}$ is *not* multistationary and so, by definition, is *not* bistable. □

Although the reduced ERK network is not bistable (Proposition 4.5), the next result shows that irreversible versions of the full ERK network are bistable, as long as one of the reactions labeled by k_{on} and ℓ_{on} is present. That is, this result tells us which reactions can be safely deleted (in contrast to standard results concerning reactions that can be added, e.g., the articles of Banaji (2018) and Banaji & Pantea (2018)) while preserving bistability. (In the next section, we investigate the precise number of steady states of ERK networks; see Proposition 5.9).

Theorem 4.6 (Bistability in irreversible ERK networks)**.** *Consider any network $\mathcal{N}$ obtained from the full ERK network by deleting one or more of the reactions corresponding to rate constants $k_2, k_{\text{on}}, m_1, \ell_2, \ell_{\text{on}}, n_2$ (blue in Figure 1). Then the following are equivalent:*

(1) $\mathcal{N}$ is multistationary,

(2) $\mathcal{N}$ is bistable, and

(3) $\mathcal{N}$ contains at least one of the reactions labeled by k_{on} and ℓ_{on}.

Proof. By definition, every bistable network is multistationary, so (2) ⇒ (1). We therefore need only show (1) ⇒ (3) ⇒ (2). (All computations below are found in our supplementary file `irreversibleERK.mw`).

For (1) ⇒ (3), we will prove ¬(3) ⇒ ¬(1): Assume that $\mathcal{N}$ contains neither the reaction labeled by k_{on} nor the reaction ℓ_{on}. Our proof here is analogous to that of Proposition 4.5. By Proposition 3.1, we obtain a critical function, $C(\hat{a}; x)$, for $\mathcal{N}$ of the form (27), where now $h_{c,a}$ is as in (12) (with $\mathbb{1}_{k_{\text{on}}} = \mathbb{1}_{\ell_{\text{on}}} = 0$) and $\phi(\hat{a}; x)$ is as in (13) (with $\hat{a} = (a_2, a_4)$).

Here, $\det \text{Jac}(h_{c,a})$ is a rational function with denominator equal to $k_{\text{off}} x_2 (n_2 + n_3) \ell_{\text{cat}} l_3 k_3 m_3$, which is always positive. The numerator is a polynomial of degree 5 in the variables x_2, x_3, and x_9 with coefficients that are always negative (see the supplementary file). The critical function $C(\hat{a}; x)$ is obtained by substituting the positive parametrization into $\det \text{Jac}(h_{c,a})$. Hence, for all $(\hat{a}; x) \in \mathbb{R}^2_{>0} \times \mathbb{R}^{12}_{>0}$, the equality $\text{sign}(C(\hat{a}; x)) = -1 = (-1)^{\text{rank}(N)}$

holds, because the stoichiometric matrix N has rank $12-3=9$. So, by Proposition 2.4 (recall from Remark 3.6 that $\mathcal{N}$ is conservative with no boundary steady states in any stoichiometric compatibility class), $\mathcal{N}$ is *not* multistationary.

Now we show (3) $\Rightarrow$ (2), that is, if $\mathcal{N}$ contains at least one of the reactions labeled by k_{on} and ℓ_{on} then $\mathcal{N}$ is bistable. By symmetry (from exchanging in the network E, S_{00}, and S_{01} with, respectively, F, S_{11}, and S_{10}), we may assume that $\mathcal{N}$ contains k_{on}.

Consider the network $\mathcal{N}'$ obtained from the full ERK network by deleting all reactions marked in blue in Figure 1, except for k_{on} (equivalently, we set $k_2 = m_1 = \ell_2 = \ell_{\text{on}} = n_2 = 0$). We will show that the following total constants and rate constants yield bistability:

$$\begin{aligned} (c_1,\ c_2,\ c_3) \ &= \ (46, 13, 13)\ , \quad \text{and} \\ (k_1, k_3, k_{\text{cat}}, k_{\text{on}}, k_{\text{off}}, \ell_1, \ell_3, \ell_{\text{cat}}, \ell_{\text{off}}, m_2, m_3, n_1, n_3) \ &= \\ (2, 1.1, 1, 5, 15, 2, 1.1, 1, 10, 20, 10, 20, 10)\ . \end{aligned} \tag{28}$$

Among the resulting three steady states (see the supplementary file), one of them is approximately:

$$\begin{array}{llllll} (20.72107755, & 0.2956877203, & 3.248789181, & 7.821850626, & 0.7821850626, & 1.147175131, \\ 0.7821850626, & 0.7821850626, & 0.1765542587, & 1.322653950, & 11.13994215, & 1.324191138)\ . \end{array}$$

At the above steady state, the Jacobian matrix (of the system obtained from (10) by making the substitutions (28) and $k_2 = m_1 = \ell_2 = \ell_{\text{on}} = n_2 = 0$) has three zero eigenvalues (due to the three conservation laws). For the remaining eigenvalues, the real parts are approximately:

$$\begin{array}{lll} -76.0913958200572, & -70.7106617930401\ , & -16.3022723748274, \\ -10.9324829878475, & -10.9324829878475, & -8.81318904794782\ , \\ -4.88866989801728, & -4.88866989801728\ , & -0.0545784672515179\ , \end{array}$$

Thus, the nonzero eigenvalues have strictly negative real part, so the steady state is exponentially stable.

Another steady state is approximately

$$\begin{array}{llllll} (0.1782157709, & 8.088440520, & 0.2275355904, & 11.45336411, & 1.145336411, & 0.1737638914, \\ 1.145336411, & 1.145336411, & 0.3818389270, & 0.07080081803, & 2.620886659, & 27.68512059)\ . \end{array}$$

At this steady state, the real part of the eigenvalues of the Jacobian matrix of the system are, in addition to the three zero eigenvalues, approximately as follows:

$$\begin{array}{lll} -163.308657649675, & -68.5596972162577\ , & -57.0205793889569\ , \\ -16.4435472947534, & -12.1029003142539, & -9.27515541335710, \\ -9.27515541335710, & -3.08709626767693\ , & -0.209550347944487. \end{array}$$

This steady state is also exponentially stable. (A third steady state, not shown, is unstable.) Hence, $\mathcal{N}'$ is bistable. Finally, as $\mathcal{N}'$ is a subnetwork obtained from $\mathcal{N}$ by making some reversible reactions irreversible, then by (Joshi & Shiu 2013, Theorem 3.1), bistability "lifts" from $\mathcal{N}'$ to $\mathcal{N}$. Thus, $\mathcal{N}$ is bistable. $\square$

We obtain the following consequence of Theorem 4.6.

Corollary 4.7. *The fully irreversible ERK network is monostationary.*

Proof. The fully irreversible ERK network contains neither the reaction labeled by k_{on} nor the one labeled ℓ_{on}, so Theorem 4.6 implies that the network is not multistationary. Thus, by a standard application of Brouwer's fixed-point theorem, together with the fact that the network is conservative and has no boundary steady states in any stoichiometric compatibility class (cf. Remark 3.9 in the article by Pérez Millán et al. (2012)), there is – for every choice of positive rate constants – exactly one positive steady state in every stoichiometric compatibility class. □

5 Maximum number of steady states

In the previous section, we saw that the full ERK network and some irreversible ERK networks (those with k_{on} or ℓ_{on}) are bistable, admitting two stable steady states in a stoichiometric compatibility class. The question arises, Do these networks admit three or more such steady states? We suspect not (Conjecture 5.10).

As a step toward resolving this problem, here we investigate the maximum number of positive steady states in ERK networks, together with some related measures we introduce, the maximum number of (non-boundary) complex-number steady states and the "mixed volume". The mixed volume is always an upper bound on the number of complex steady states (Proposition 5.8), but we show these numbers are equal for ERK networks (Proposition 5.9).

5.1 Background and new definitions

Here we recall a network's maximum number of positive steady states (Joshi & Shiu 2017), and then extend the definition to allow for complex-number steady states.

Definition 5.1. A network admits k positive steady states (for some $k \in \mathbb{Z}_{\geq 0}$) if there exists a choice of positive rate constants so that the resulting mass-action system (1) has exactly k positive steady states in some stoichiometric compatibility class (2).

Joshi & Shiu (2017) allowed $k = \infty$ when there are infinitely many steady states in a stoichiometric compatibility class. Here, however, we do not allow $k = \infty$ so that we consider isolated roots only (as in Proposition 5.5 below).

Definition 5.2. Let G be a network with s species, m reactions, and a $d \times s$ conservation-law matrix W, which results in the system augmented by conservation laws $f_{c,\kappa}$, as in (3). The network G admits k steady states over $\mathbb{C}^*$ if there exists a choice of positive rate constants $\kappa \in \mathbb{R}^m_{>0}$ and a total-constant vector $c \in \mathbb{R}^d$ such that the system $f_{c,\kappa} = 0$ has exactly k solutions in $(\mathbb{C}^*)^s = (\mathbb{C} \setminus \{0\})^s$.

It is straightforward to check that Definition 5.2 does not depend on the choice of W.

Definition 5.3. The maximum number of positive steady states (respectively, maximum number of steady states over $\mathbb{C}^*$) of a network G is the maximum value of k for which G admits k positive steady states (respectively, k steady states over $\mathbb{C}^*$).

Next we recall, from convex geometry, the concept of mixed volume, which we will apply to reaction networks. For background on convex and polyhedral geometry (such as polytopes and Minkowski sums), we direct the reader to the book of Ziegler (1995). In particular, for a polynomial $f = b_1 x^{\sigma_1} + b_2 x^{\sigma_2} + \cdots + b_\ell x^{\sigma_\ell} \in \mathbb{R}[x_1, x_2, \ldots, x_s]$, where the exponent vectors $\sigma_i \in \mathbb{Z}^s$ are distinct and $b_i \neq 0$ for all i, the Newton polytope of f is the convex hull of its exponent vectors: $\mathrm{Newt}(f) := \mathrm{conv}\{\sigma_1,\ \sigma_2,\ \ldots\ ,\ \sigma_\ell\} \ \subseteq\ \mathbb{R}^s$.

Definition 5.4. Let $P_1, P_2, \ldots, P_s \subseteq \mathbb{R}^s$ be polytopes. The volume of the Minkowski sum $\lambda_1 P_1 + \lambda_2 P_2 + \ldots + \lambda_s P_s$ is a homogeneous polynomial of degree s in nonnegative variables $\lambda_1, \lambda_2, \ldots, \lambda_s$. In this polynomial, the coefficient of $\lambda_1 \lambda_2 \cdots \lambda_s$, denoted by $\mathrm{Vol}(P_1, P_2, \ldots, P_s)$, is the mixed volume of $P_1, P_2, \ldots, P_s$.

The mixed volume counts the number of solutions in $(\mathbb{C}^*)^s$ of a generic polynomial system.

Proposition 5.5 (Bernshtein's theorem (Bernshtein 1975))**.** *Consider s real polynomials $g_1, g_2, \ldots, g_s \in \mathbb{R}[x_1, x_2, \ldots, x_s]$. Then the number of isolated solutions in $(\mathbb{C}^*)^s$, counted with multiplicity, of the system $g_1(x) = g_2(x) = \cdots = g_s(x) = 0$ is at most* $\mathrm{Vol}(\mathrm{Newt}(g_1), \ldots, \mathrm{Newt}(g_s))$.

Definition 5.6. Let G be a network with s species, m reactions, and a $d \times s$ conservation-law matrix W, which results in the system augmented by conservation laws $f_{c,\kappa}$, as in (3). Let $c^* \in \mathbb{R}^d_{\neq 0}$, and let $\kappa^* \in \mathbb{R}^m_{>0}$ be generic. Let $P_1, P_2, \ldots, P_s \subset \mathbb{R}^s$ be the Newton polytopes of $f_{c^*,\kappa^*,1}, f_{c^*,\kappa^*,2}, \ldots, f_{c^*,\kappa^*,s}$, respectively. The mixed volume of G (with respect to W) is the mixed volume of $P_1, P_2, \ldots, P_s$.

A closely related definition is introduced and analyzed by Gross & Hill (2019).

Remark 5.7. The mixed volume (Definition 5.6) is well defined. Indeed, it is straightforward to check that the exponents appearing in f_{c^*,κ^*} are the same as long as $c^* \in \mathbb{R}^d_{\neq 0}$ and κ^* is chosen generically (so that no coefficients of f_{c^*,κ^*} vanish, or equivalently certain linear combinations of the κ_j's do not vanish).

5.2 Results

Every positive steady state is a steady state over $\mathbb{C}^*$. Also, the mixed volume pertains to polynomial systems with the same supports (i.e., the exponents that appear in each polynomial) as the augmented system $f_{c,\kappa} = 0$ (but without constraining the coefficients to come from a reaction network). We obtain, therefore, the bounds in the following result:

Proposition 5.8. *For every network, the following inequalities hold among the maximum number of positive steady states, the maximum number of steady states over $\mathbb{C}^*$, and the mixed volume of the network (with respect to any conservation-law matrix):*

$$\text{max \# of positive steady states} \ \leq\ \text{max \# of steady states over } \mathbb{C}^* \ \leq\ \text{mixed volume}\ .$$

Proof. This result follows from Proposition 5.5 and Definitions 5.1–5.3. □

We investigate the numbers in Proposition 5.8 for ERK networks in the following result.

Proposition 5.9. *Consider four ERK networks: the full ERK network, the full ERK network with the reaction k_{on} removed, the fully irreversible network, and the reduced network. For these networks, the following numbers (or bounds on them) are given in Table 4: the maximum number of positive steady states, the maximum number of steady states over $\mathbb{C}^*$, and the mixed volume of the network (with respect to the consveration laws* (11) *or* (17)*).*

ERK network	Max # positive steady states	Max # over $\mathbb{C}^*$	Mixed volume
Full	≥ 3	7	7
Full with $k_{\text{on}} = 0$	≥ 3	5	5
Fully irreversible	1	3	3
Reduced	1	3	3

Table 4: Results on ERK networks.

Proof. The results on the mixed volume were computed using the `PHCpack` (Gross et al. 2013) package in `Macaulay2` (Grayson & Stillman n.d.). See the supplementary file `ERK-mixedVol.m2`.

The mixed volume is an upper bound on the maximum number of steady states over $\mathbb{C}^*$ (Proposition 5.8), so we need only show that each network admits the number shown in Table 4 for steady states over $\mathbb{C}^*$.

The full ERK network admits 7 steady states over $\mathbb{C}^*$ (including 3 positive steady states) (Dickenstein et al. 2019, Example 3.18). Next, we consider the remaining three networks (see the supplementary file `ERK-MaxComplexNumber.nb`).

For the full ERK network with $k_{on} = 0$, when $(c_1, c_2, c_3) = (1, 2, 3)$ and $(k_1, k_2, k_3, k_{\text{cat}}, k_{\text{on}}, k_{\text{off}}, \ell_1, \ell_2, \ell_3, \ell_{\text{cat}}, \ell_{\text{on}}, \ell_{\text{off}}, m_1, m_2, m_3, n_1, n_2, n_3) = (3, 25, 1, 5, 0, 6, 5, 23, 11, 13, 43, 41, 12, 7, 8, 12, 31, 21)$, we obtain 5 steady states over $\mathbb{C}^*$, three real and one complex-conjugate pair, which are approximately as follows:

```
(21.7475, 1.97705,          2.40601, 2.64849,          0.760404, 0.564871,        -24.1306, -0.973762,
-2.51373, -0.28488,         -7.81077, -18.495),
(5.4105 + 14.8132 i,        0.491864 + 1.34665 i,      1.97942 - 3.45492 i,       1.66315 - 1.90055 i,
0.189178 + 0.517943 i,      0.140532 + 0.384758 i,     -5.88178 - 12.7049 i,      1.00714 + 0.997852 i,
1.13283 + 0.533085 i,       0.470121 + 0.662785 i,     -9.72843 - 0.81303 i,      -0.749157 - 12.0899 i),
(5.4105 - 14.8132 i,        0.491864 - 1.34665 i,      1.97942 + 3.45492 i,       1.66315 + 1.90055 i,
0.189178 - 0.517943 i,      0.140532 - 0.384758 i,     -5.88178 + 12.7049 i,      1.00714 - 0.997852 i,
1.13283 - 0.533085 i,       0.470121 - 0.662785 i,     -9.72843 + 0.81303 i,      -0.749157 + 12.0899 i)
(9.63546, 0.875951,         -0.488295, 0.0430355,      0.336904, 0.250272,        -8.02311, 2.36979,
0.45764, 0.173889,          -10.4083, 0.123488),       and
( 0.163415, 0.0148559,      0.00111949, 0.00756688,    0.00571382, 0.00424455,    1.82061, 2.98247,
0.00616705, 0.00175686,     0.777908, 0.0172524).
```

For the fully irreversible ERK network, when $(c_1, c_2, c_3) = (1, 2, 3)$ and $(k_1, k_3, k_{\text{cat}}, k_{\text{off}}, \ell_1, \ell_3, \ell_{\text{cat}}, \ell_{\text{off}}, m_2, m_3, n_1, n_3) = (3, 1, 5, 6, 5, 11, 13, 41, 7, 8, 12, 21)$, there are 3 steady states over $\mathbb{C}^*$, all real, with approximate values:

(14.199, 1.29082, 2.5444, 2.43721, 0.496468, 0.368805, -16.0342, -0.302478,
-2.13373, -0.181355, -0.295181, -17.7264),
(0.490202, 0.0445638, 0.0878422, 0.0841415, 0.0171399, 0.0127325, 1.37739, 2.88599,
0.00772073, 0.0728849, 0.118631, 0.0641415), and
(1.9419, 0.176536, 0.34798, 0.33332, 0.0678986, 0.050439, -0.466416, 2.54834,
0.0346375, -0.852654, -1.38782, 0.287758).

For the reduced ERK network, let $(c_1, c_2, c_3) = (1, 2, 3)$ and $(k_1, k_3, k_{\text{cat}}, k_{\text{off}}, m, n, \ell_1, \ell_3, \ell_{\text{cat}}, \ell_{\text{off}}) = (3, 4, 1, 5, 6, 8, 7, 11, 12, 5)$. We obtain 3 steady states over $\mathbb{C}^*$, all real, which are approximately:

(-0.843105, -37.1185, 23.4711, 15.6474, -9.92245, -30.6429, -0.0292745, -0.319149,
2.0152, 1.30395),
(0.314129, 1.4361, 0.338341, 0.22556, 0.015463, 0.0477534, 0.0109073, 2.95215,
0.0290494, 0.0187967), and
(-2.47545, -0.954967, 1.77298, 1.18199, 0.087009, 0.268704, -0.0859532, 2.74928,
0.152226, 0.0984989).

Finally, we examine the maximum number of positive steady states. We already saw that the fully irreversible and reduced networks are monostationary (Corollary 4.7 and Proposition 4.5, respectively). For the "partially irreversible" network, we saw in the proof of Theorem 4.6 that it admits 3 positive steady states. As for the full network, as noted above, 3 positive steady states were shown in (Dickenstein et al. 2019, Example 3.18). ☐

Table 4 suggests that the mixed volume is a measure of the complexity of a network. The full ERK network is multistationary, and its mixed volume is 7. The mixed volume drops to 5 when $k_{\text{on}} = 0$. When the network is further simplified to the fully irreversible, or even to the reduced ERK network, the mixed volume becomes 3, and bistability is lost as well.

Finally, we conjecture that the bounds in Table 4 are strict, and ask about stability.

Conjecture 5.10. *For the full ERK network and the full ERK network with $k_{\text{on}} = 0$, the maximum number of positive (respectively, positive stable) steady states is 3 (respectively, 2).*

6 Discussion

Phosphorylation plays a key role in cellular signaling networks, such as *mitogen-activated protein kinase (MAPK) cascades*, which enable cells to make decisions (to differentiate, proliferate, die, and so on) (Chang & Karin 2001). This decision-making role of MAPK cascades suggests that they exhibit switch-like behavior, i.e., bistability. Indeed, bistability in such cascades has been seen in experiments (Bagowski & Ferrell 2001, Bhalla et al. 2002). Oscillations also have been observed (Hilioti et al. 2008, Hu et al. 2013), hinting at a role in timekeeping. Indeed, multisite phosphorylation is the main mechanism for establishing the 24-hour period in eukaryotic circadian clocks (Ode & Ueda 2017, Virshup & Forger 2009).

These experimental findings motivated the questions we pursued. Specifically, we investigated robustness of oscillations and bistability in models of ERK regulation by dual-site phosphorylation. Bistability, we found, is quickly lost when reactions are made irreversible. Indeed, bistability is characterized by the presence of two specific reactions. Oscillations, in contrast, persist even as the network is greatly simplified. Indeed, we discovered oscillations in the reduced ERK network. Moreover, this network has the same number of reactions (ten) as the mixed-mechanism network which Suwanmajo and Krishnan surmised "could be the simplest enzymatic modification scheme that can intrinsically exhibit oscillation" (Suwanmajo & Krishnan 2015, §3.1). Our reduced ERK network, therefore, may also be such a minimal oscillatory network.

Returning to our bistability criterion (Theorem 4.6), recall that this result elucidates which reactions can be safely *deleted* while preserving bistability – in contrast to standard results concerning reactions that can be *added* (Banaji & Pantea 2018, Feliu & Wiuf 2013, Joshi & Shiu 2013). We desire more results of this type, so we comment on how we proved our result. The key was the special form of the steady-state parametrization. In particular, following the article by Dickenstein et al. (2019), our parametrizations allow both species concentrations and rate constants to be solved (at steady state) in terms of other variables. Additionally, a single parametrizations specialized (by setting rates to zero for deleted reactions) to obtain parametrizations for a whole family of networks. Together, these properties gave us access to new information on how bistability is controlled. We are interested, therefore, in the following question: *Which networks admit a steady-state parametrization that specializes for irreversible versions of the network?*

Our results on oscillations were enabled by new mathematical approaches to find Hopf bifurcations. Specifically, building on the article by Conradi et al. (2019), we gave a Hopf-bifurcation criterion for networks admitting a steady-state parametrization. Additionally, we successfully applied this criterion to the reduced ERK network by analyzing the Newton polytopes of certain Hurwitz determinants. We expect these techniques to apply to more networks.

Finally, our work generated a number of open questions. First, what are the mixed volumes of irreversible versions of the ERK network (beyond those shown in Table 4)? In particular, is there a mixed-volume analogue of our bistability criterion, which is in terms of the reactions k_{on} and ℓ_{on}? And, what is the maximum number of (stable) steady states in the full ERK network (Conjecture 5.10)? Progress toward these questions will yield further insight into robustness of bistability and oscillations in biological signaling networks.

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A Files in the Supporting Information

Table 5 lists the files in the Supporting Information, and the result/proof each file supports. All files can be found at the online repository: `https://github.com/neeedz/ERK`.

Name	File type	Result
`ERK-Matcont.txt`	text file with `MATCONT` instructions	Figures 3 and 4
`irreversibleERK.mw`	`Maple`	Theorem 4.6
`reducedERK-noMSS.mw`	`Maple`	Proposition 4.5
`reducedERK-hopf.mw`	`Maple`	Theorem 4.3
`reducedERK-cones.sws`	`Sage`	Theorem 4.3
`ERK-mixedVol.m2`	`PHCPack`	Proposition 5.9
`ERK-MaxComplexNumber.nb`	`Mathematica`	Proposition 5.9

Table 5: Supporting Information files and the results they support.

B Newton-polytope method

Here we show how analyzing the Newton polytopes of two polynomials can reveal whether there is a positive point at which one polynomial is positive and simultaneously the other is zero (Proposition B.2 and Algorithm 1). In Appendix C, we show how we used this approach, which we call the Newton-polytope method, to find a Hopf bifurcation leading to oscillations in the reduced ERK network (in Theorem 4.3).

Notation B.1. Consider a polynomial $f = b_1 x^{\sigma_1} + b_2 x^{\sigma_2} + \cdots + b_\ell x^{\sigma_\ell} \in \mathbb{R}[x_1, x_2, \ldots, x_s]$, where the exponent vectors $\sigma_i \in \mathbb{Z}^s_{\geq 0}$ are distinct and $b_i \neq 0$ for all i. A vertex σ_i of $\mathrm{Newt}(f)$, the Newton polytope of f, is a positive vertex (respectively, negative vertex) if the corresponding monomial of f is positive, i.e., $b_i > 0$ (respectively, $b_i < 0$). Also, $N_f(\sigma)$ denotes the outer normal cone of the vertex σ of $\mathrm{Newt}(f)$, i.e., the cone generated by the outer normal vectors to all supporting hyperplanes of $\mathrm{Newt}(f)$ containing the vertex σ. Finally, for a cone C, let $\mathrm{int}(C)$ denote the relative interior of the cone.

For an extensive discussion on polytopes and normal cones, see the book of Ziegler (1995).

Proposition B.2. *Let $f, g \in \mathbb{R}[x_1, x_2, \ldots x_s]$. Assume that α is a positive vertex of* $\mathrm{Newt}(f)$*, β_+ is a positive vertex of* $\mathrm{Newt}(g)$*, and β_- is a negative vertex of* $\mathrm{Newt}(g)$*. Then, if* $\mathrm{int}(N_f(\alpha)) \cap \mathrm{int}(N_g(\beta_+))$ *and* $\mathrm{int}(N_f(\alpha)) \cap \mathrm{int}(N_g(\beta_-))$ *are both nonempty, then there exists $x^* \in \mathbb{R}^s_{>0}$ such that $f(x^*) > 0$ and $g(x^*) = 0$.*

To prove Proposition B.2 we use the following well-known lemma and its proof.

Lemma B.3. *For a real, multivariate polynomial $f = b_1 x^{\sigma_1} + b_2 x^{\sigma_2} + \cdots + b_\ell x^{\sigma_\ell} \in \mathbb{R}[x_1, x_2, \ldots, x_s]$, if σ_i is a positive vertex (respectively, negative vertex) of* $\mathrm{Newt}(f)$*, then there exists $x^* \in \mathbb{R}^s_{>0}$ such that $f(x^*) > 0$ (respectively, $f(x^*) < 0$).*

Proof. Let σ_i be a vertex of $\mathrm{Newt}(f)$. Pick $w = (w_1, w_2, \ldots, w_s)$ in the relative interior of the outer normal cone $N_f(\sigma_i)$, which exists because σ_i is a vertex. Then, by construction, the linear functional $\langle w, - \rangle$ is maximized over the exponent-vectors $\sigma_1, \sigma_2, \ldots, \sigma_\ell$ at σ_i. Thus, we have the following univariate "polynomial with real exponents" in t:

$$f(t^{w_1}, t^{w_2}, \ldots, t^{w_s}) \;=\; b_1 t^{\langle w, \sigma_1 \rangle} + b_2 t^{\langle w, \sigma_1 \rangle} + \cdots + b_\ell t^{\langle w, \sigma_\ell \rangle} \;=\; b_i t^{\langle w, \sigma_i \rangle} + \text{lower-order terms} \,.$$

So, for t large, $\mathrm{sign}(f(t^{w_1}, t^{w_2}, \ldots, t^{w_s})) = \mathrm{sign}(b_i)$. Note that $(t^{w_1}, t^{w_2}, \ldots, t^{w_s}) \in \mathbb{R}^s_{>0}$. ☐

Our proof of Proposition B.2 is constructive, through the following algorithm, where we use the notation $f_w(t) := f(t^{w_1}, t^{w_2}, \ldots, t^{w_s})$, for $t \in \mathbb{R}$ and $w = (w_1, w_2, \ldots, w_s) \in \mathbb{R}^s$.

Algorithm 1: Newton-polytope method

```
   input : polynomials $f, g$, and vertices $\alpha, \beta_+, \beta_-$, as in Proposition B.2
   output: a point $x^* \in \mathbb{R}^s_{>0}$ s.t. $f(x^*) > 0$ and $g(x^*) = 0$
1  define $C_0 := \mathrm{int}(N_f(\alpha)) \cap \mathrm{int}(N_g(\beta_+))$ and $C_1 := \mathrm{int}(N_f(\alpha)) \cap \mathrm{int}(N_g(\beta_-))$;
2  pick $\ell = (\ell_1, \ell_2, \ldots, \ell_s) \in C_0$ and $m = (m_1, m_2, \ldots, m_s) \in C_1$;
3  define $f_\ell(t) := f(t^{\ell_1}, t^{\ell_2}, \ldots, t^{\ell_s})$; define $f_m(t)$; define $g_\ell(t)$; define $g_m(t)$;
4  define $\tau_\ell := \inf\{t^* \in \mathbb{R}_{>0} \mid f_\ell(t) > 0$ and $g_\ell(t) > 0$ for all $t > t^*\}$;
5  define $\tau_m := \inf\{t^* \in \mathbb{R}_{>0} \mid f_m(t) > 0$ and $g_m(t) < 0$ for all $t > t^*\}$;
6  define $T := \max\{\tau_\ell, \tau_m\} + 1$;
7  define $h(r) := f_{r\ell+(1-r)m}(T)$;
8  while $\min\{h(r) \mid r \in [0,1]\} \leq 0$ do
9      $T := 2T$;
10     $h(r) := f_{r\ell+(1-r)m}(T)$;
11 define $r^* := \mathrm{argmin}\{\left(g_{r\cdot\ell+(1-r)m}(T)\right)^2 \mid r \in [0,1]\}$ (pick one $r^*$ if there are multiple);
   return : $T^{r^*\ell+(1-r^*)m} := \left(T^{r^*\ell_1+(1-r^*)m_1}, T^{r^*\ell_2+(1-r^*)m_2}, \ldots, T^{r^*\ell_s+(1-r^*)m_s}\right)$
```

Proof of Proposition B.2. Let a_+x^α be the term of f corresponding to the vertex α of $\mathrm{Newt}(f)$, and similarly let $b_+x^{\beta_+}$ (respectively, $b_-x^{\beta_-}$) be the term of g corresponding to the vertex β_+ (respectively, β_-) of $\mathrm{Newt}(g)$. Thus, $a_+ > 0$, $b_+ > 0$, and $b_- < 0$. Let $\{a_1, a_2, \ldots, a_d\} \subseteq \mathbb{R}$ denote the remaining set of coefficients of f, so that $f = a_+x^\alpha + (a_1x^{\sigma_1} + a_2x^{\sigma_2} + \cdots + a_dx^{\sigma_d})$, for some exponent vectors $\sigma_i \in \mathbb{Z}^s_{\geq 0}$.

Algorithm 1 terminates: First, ℓ and m in line 2 exist by hypothesis. Also, τ_ℓ and τ_m in lines 4–5 exist by the proof of Lemma B.3 and by construction. Next, $\min h(r)$ in line 8 exists because h is a continuous univariate function defined on a compact interval.

By construction and because cones are convex, the vector $r\ell + (1-r)m$, which is a convex combination of ℓ and m, is in the relative interior of $N_f(\alpha)$ for all $r \in [0,1]$. Thus, $\langle r\ell + (1-r)m,\ \alpha - \sigma_i\rangle > 0$ for all $i = 1, 2, \ldots, d$ and for all $r \in [0,1]$. This (together with a straightforward argument using continuity and compactness) implies the following:

$$\delta \ := \ \inf_{r\in[0,1]} \min_{i=1,2,\ldots,d} \langle r\ell + (1-r)m,\ \alpha - \sigma_i\rangle \ > \ 0.$$

Next, let $\beta := \inf_{r\in[0,1]}\langle r\ell + (1-r)m,\ \alpha\rangle$. Then, for all $r \in [0,1]$ and $t > 0$,

$$\begin{aligned} f_{r\ell+(1-r)m}(t) \ &= \ a_+t^{\langle r\ell+(1-r)m),\alpha\rangle} + \left(a_1t^{\langle r\ell+(1-r)m),\sigma_1\rangle} + \cdots + a_dt^{\langle r\ell+(1-r)m),\sigma_d\rangle}\right) \\ &> \ a_+t^\beta - (|a_1| + |a_2| + \cdots + |a_d|)t^{\beta-\delta} \ =: \ \widetilde{f}(t) \ . \end{aligned} \tag{29}$$

In $\widetilde{f}(t)$, the term a_+t^β dominates the other term, for t large, so there exists $T^* > 0$ such that $\widetilde{f}(t) \geq 0$ when $t \geq T^*$. So, by (29), the while loop in line 8 ends when $T \geq T^*$ (or earlier).

Algorithm 1 is correct: For T fixed, the minimum of $\psi(r) := \left(g(T^{r\ell+(1-r)m})\right)^2$ over the compact set $[0,1]$ is attained, because ψ is continuous. Next we show that this minimum value is 0, or equivalently that for $\chi(r) := g(T^{r\ell+(1-r)m})$ there exists some $r^* \in (0,1)$ such that $\chi(r^*) = 0$. Indeed, this follows from the Intermediate Value Theorem, because χ is continuous, $\chi(0) = g(T^m) < 0$ (because $T > \tau_m$), and $\chi(1) = g(T^\ell) > 0$ (because $T > \tau_\ell$).

Finally, the inequality $f(T^{r^*\ell+(1-r^*)m}) > 0$ holds by construction of T, so defining $x^* := T^{r^*\ell+(1-r^*)m} \in \mathbb{R}^s_{>0}$ yields the desired vector satisfying $f(x^*) > 0$ and $g(x^*) = 0$. $\square$

C Using the Newton-polytope method

Here we show how we used Algorithm 1 to find the Hopf bifurcation in Theorem 4.3. (For details, see the supplementary files `reducedERK-hopf.mw` and `reducedERK-cones.sws`). Recall from the proof of that theorem, that our goal was to find some $x^* \in \mathbb{R}^{10}_{>0}$ and $\hat{\kappa}^* = (k^*_{\text{cat}}, k^*_{\text{off}}, \ell^*_{\text{off}}) \in \mathbb{R}^3_{>0}$ satisfying the following conditions from Proposition 4.1:

$$\mathfrak{h}_4(\hat{\kappa}^*; x^*) >0 \ , \ \mathfrak{h}_5(\hat{\kappa}^*; x^*) > 0 \ , \ \mathfrak{h}_6(\hat{\kappa}^*; x^*) = 0 \ , \ \text{and} \ \frac{\partial}{\partial k_{\text{cat}}} \ \mathfrak{h}_6(\hat{\kappa}; x)|_{(\hat{\kappa};x)=(\hat{\kappa}^*;x^*)} \neq 0 \ . \quad (30)$$

Step One. Specialize some of the parameters: set $k_{\text{off}} = \ell_{\text{off}} = 1$ and $x_3 = x_4 = x_6 = x_7 = x_9 = x_{10} = 1$. (Otherwise, $\mathfrak{h}_5$ and $\mathfrak{h}_6$ are too large to be computed.)

Step Two. Do a change of variables: let $y_i = 1/x_i$ for $i = 1, 2, 5, 8$. These variables x_i were in the denominator, so switching to the variables y_i yield polynomials.

Let $\mathcal{H}_4$, $\mathcal{H}_5$, and $\mathcal{H}_6$ denote the resulting polynomials in $\mathbb{Q}[k_{\text{cat}}, y_1, y_2, y_5, y_8]$ after performing Steps One and Two. Accordingly, our updated goal is to find $(k^*_{\text{cat}}, y^*_1, y^*_2, y^*_5, y^*_8) \in \mathbb{R}^5_{>0}$ at which $\mathcal{H}_4$ and $\mathcal{H}_5$ are positive and $\mathcal{H}_6$ is zero. (In a later step, we must also check the partial-derivative condition in (30).)

Step Three. Apply (a straightforward generalization of) Algorithm 1 as follows.

(i) Find a positive vertex of $\mathcal{H}_4$ and a positive vertex of $\mathcal{H}_5$ whose outer normal cones intersect (denote the intersection by C), and a positive vertex and a negative vertex of $\mathcal{H}_6$ (denote their outer normal cones by D_+ and D_-, respectively) for which:

   (a) the intersection $D_+ \cap D_-$ is 4-dimensional, and

   (b) the intersections $C \cap D_+$ and $C \cap D_-$ are both 5-dimensional.

(ii) By Proposition B.2, a vector $(k^*_{\text{cat}}, y^*_1, y^*_2, y^*_5, y^*_8)$ that accomplishes our updated goal, is guaranteed. To find such a point, we follow Algorithm 1 to obtain $k^*_{\text{cat}} = 729, y^*_1 \approx 16.79978292, y^*_2 \approx 453.5941389, y^*_5 \approx 6.587368051$, and $y^*_8 \approx 80675.77181$.

Recall the specializations in Step One and change of variables in Step Two, to obtain $\hat{\kappa} = (729, 1, 1)$ and

$$x^* \approx (0.05952457867,\ 0.002204614024,\ 1,\ 1,\ 0.1518056972,\ 1,\ 1,\ 0.00001239529511,\ 1,\ 1)\,.$$

Step Four. Verify that the conditions in (30) hold.