UNIVERSITY OF COPENHAGEN

# Oscillations and bistability in a model of ERK regulation

Obatake, Nida; Shiu, Anne; Tang, Xiaoxian; Torres, Angélica

# Oscillations and bistability in a model of ERK regulation

Nida Obatake[1], Anne Shiu[1], Xiaoxian Tang[1], and Angélica Torres[2]

[1]*Department of Mathematics, Texas A&M University, USA*
[2]*Department of Mathematical Sciences, University of Copenhagen, Denmark*

March 6, 2019

## 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 [14]. Such networks are of great biological importance [9]. 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 ($F$) [40]. This network, which we call the ERK network, has an important

role in regulating many cellular activities, with dysregulation implicated in many cancers [42]. 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 [21].
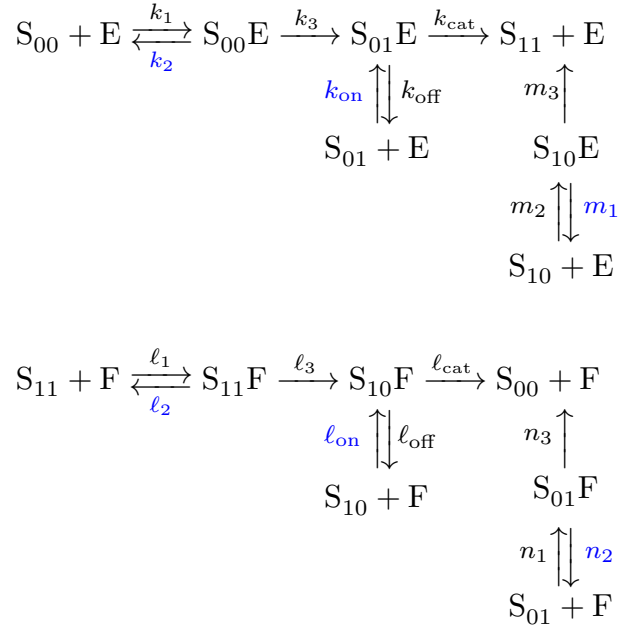
$$S_{00} + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} S_{00}E \xrightarrow{k_3} S_{01}E \xrightarrow{k_{cat}} S_{11} + E$$

$$k_{on} \updownarrow k_{off} \qquad m_3 \uparrow$$

$$S_{01} + E \qquad S_{10}E$$

$$m_2 \updownarrow m_1$$

$$S_{10} + E$$

$$S_{11} + F \underset{\ell_2}{\overset{\ell_1}{\rightleftharpoons}} S_{11}F \xrightarrow{\ell_3} S_{10}F \xrightarrow{\ell_{cat}} S_{00} + F$$

$$\ell_{on} \updownarrow \ell_{off} \qquad n_3 \uparrow$$

$$S_{10} + F \qquad S_{01}F$$

$$n_1 \updownarrow n_2$$

$$S_{01} + F$$

Figure 1: The (full) ERK network, from [40], with notation of [17]. 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, and Shvartsman [40] 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 $\ell_{cat}$ are much larger than $k_{off}$ and $\ell_{off}$, 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 [13, 18]. 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 [14]:

**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

2

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 [40] | Yes [40] | Conjecture: 3 | 7 |
| Irreversible | Yes* | If $k_{on} > 0$ or $\ell_{on} > 0$ | 1 | 5* |
| Reduced | Yes | No | 1 | 3 |

Table 1: Summary of results. 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 $k_{on} = 0$. 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 of Sadeghimanesh and Feliu [41]. Indeed, there has been significant interest in understanding which operations on networks preserve oscillations [3], bistability [4, 20, 32], and other properties [24].

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 [12] or bistability [10, 11]. 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 [3, 4, 27, 28, 34].

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 [22, 31, 45]. Indeed, here we build on results in [10, 12, 17].

Specifically, following [12], we investigate oscillations by employing a steady-state parametrization together with a criterion of Yang [47] that characterizes Hopf bifurcations in terms of determinants of Hurwitz matrices. In [12], this approach 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. [10, 15, 17, 19, 22, 33, 37] – 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).
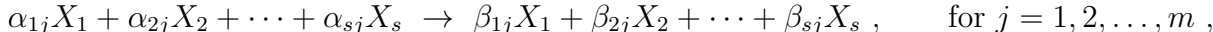
The outline of our work is as follows. Section 2 contains background on chemical reaction systems, steady-state parametrizations, and Hopf bifurcations. We present steady-state parametrizations for the ERK network and the reduced ERK network in Section 3. Section 4 contains our main results on oscillations 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

As in [17], our notation closely matches that of Conradi, Feliu, Mincheva, and Wiuf [10]. A <u>reaction network</u> $G$ (or *network* for short) comprises a set of $s$ species $\{X_1, X_2, \ldots, X_s\}$ and a set of $m$ reactions:

$$\alpha_{1j}X_1 + \alpha_{2j}X_2 + \cdots + \alpha_{sj}X_s \ \rightarrow \ \beta_{1j}X_1 + \beta_{2j}X_2 + \cdots + \beta_{sj}X_s \ , \qquad \text{for } j = 1, 2, \ldots, m \ ,$$

where each $\alpha_{ij}$ and $\beta_{ij}$ is a non-negative integer. The <u>stoichiometric matrix</u> 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 <u>stoichiometric subspace</u>, denoted by $S$, is the image of $N$. A <u>conservation-law matrix</u> 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 <u>conservative</u>.

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 <u>reaction rate constant</u>. 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 $f_{\kappa,i}$.

4

A trajectory $x(t)$ beginning at a nonnegative vector $x(0) = x^0 \in \mathbb{R}^s_{\geq 0}$ 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 nonnegative 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 $\mathrm{Im}\,(\mathrm{Jac}(f)(x^*)|_S)$ 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 $G$ is multistationary (respectively, bistable) if, for some choice of positive rate-constant vector $\kappa \in \mathbb{R}^m_{>0}$, there exists a stoichiometric compatibility class (2) with two or more positive steady states (respectively, exponentially stable positive steady states) of (1). 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 $c$.

## 2.2 Steady-state parametrizations

Here we introduce steady-state parametrizations (Definition 2.2) and recall from [17] 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 $\kappa^* \in \mathbb{R}^m_{>0}$,

---

[1]Some authors define *monostationary* to be non-multistationary; the two definitions are equivalent for the ERK networks in this work.

(ii) the <u>reparametrization map</u> below is surjective:

$$\begin{aligned}
\bar{a} \ : \ \mathbb{R}^m_{>0} \ &\to \ \mathbb{R}^{\bar{m}}_{>0} \\
\kappa \ &\mapsto \ (\bar{a}_1(\kappa), \bar{a}_2(\kappa), \ldots, \bar{a}_{\bar{m}}(\kappa)) \ ,
\end{aligned} \tag{4}$$

(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 $(f_{j_\ell})$ as follows:

$$(\bar{h}_{j_1}, \bar{h}_{j_2}, \ldots, \bar{h}_{j_{s-d}})^\top \ := \ M(\kappa) \ (f_{j_1}, f_{j_2}, \ldots, f_{j_{s-d}})^\top \ , \tag{5}$$

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 <u>effective steady-state function</u> of $G$.

The "steady-state parametrizations" that we will use in this work belong to a subclass of the ones introduced in [17]. Thus, for simplicity, Definition 2.2 below is more restrictive than [17, Definition 3.6]. Specifically, our parametrizations have the form $\phi(\hat{a}; x)$, while those in [17] 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 $h_{j_\ell}$'s as in (6). The positive steady states of $G$ <u>admit a positive parametrization with respect to $h_{c,a}$</u> 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 $\phi$ a <u>positive parametrization</u> or a <u>steady-state parametrization</u>.

**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 $\phi$, the <u>critical function</u> $C : \mathbb{R}_{>0}^{\hat{m}} \times \mathbb{R}_{>0}^{s} \to \mathbb{R}$ is given by:

$$C(\hat{a}; x) \quad = \quad (\det \text{Jac } h_{c,a})\,|_{(a;x)=\phi(\hat{a};x)} \ ,$$

where $\text{Jac}(h_{c,a})$ denotes the Jacobian matrix of $h_{c,a}$ with respect to $x$.

The following result is a specialization[2] of [17, Theorem 3.12]:

**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 $(\hat{a}^*; x^*) \in \mathbb{R}_{>0}^{\hat{m}} \times \mathbb{R}_{>0}^{s}$ such that*

$$\text{sign}(C(\hat{a}^*; x^*)) \quad = \quad (-1)^{\text{rank}(N)+1} \ .$$

*(B)* **Monostationarity.** *$G$ is monostationary if for all $(\hat{a}; x) \in \mathbb{R}_{>0}^{\hat{m}} \times \mathbb{R}_{>0}^{s}$,*

$$\text{sign}(C(\hat{a}; x)) \quad = \quad (-1)^{\text{rank}(N)} \ .$$

## 2.3 Hopf bifurcations

A <u>simple Hopf bifurcation</u> 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 <u>oscillations</u> or periodic orbits [35].

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).

**Definition 2.5.** The $i$-th <u>Hurwitz matrix</u> 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:

$$H_i \quad = \quad \begin{pmatrix} b_1 & b_0 & 0 & 0 & 0 & \cdots & 0 \\ b_3 & b_2 & b_1 & b_0 & 0 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & & \vdots \\ b_{2i-1} & b_{2i-2} & b_{2i-3} & b_{2i-4} & b_{2i-5} & \cdots & b_i \end{pmatrix} \ ,$$

in which the $(k, l)$-th entry is $b_{2k-l}$ as long as $n \geq 2k - l \geq 0$, and 0 otherwise.

---

[2]As noted earlier, here we consider parametrizations of the form $\phi(\hat{a}; x)$, while [17] allowed those of the form $\phi(\hat{a}; \hat{x})$. Also, "conservative" in Proposition 2.4 can be generalized to "dissipative" [17].

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 $\mu$ and $x$. Assume that $x^0 \in \mathbb{R}^n$ is a steady state of the system defined by $\mu_0$, 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, $g_\mu(x(\mu)) = 0$ for all $\mu$) and that $x(\mu_0) = x^0$. Denote the characteristic polynomial of the Jacobian matrix of $g_\mu$, evaluated at $x(\mu)$, as follows:

$$p_\mu(\lambda) \;:=\; \det(\lambda I - \mathrm{Jac}\; g_\mu)\,|_{x=x(\mu)} \;=\; \lambda^n + b_1(\mu)\lambda^{n-1} + \cdots + b_n(\mu) \;,$$

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 [47])**.** *Assume the above setup. Then, there is a simple Hopf bifurcation at $x_0$ with respect to $\mu$ 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 in [12]. 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 $\phi$ 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 $\kappa_i$, and so we write $\phi : \mathbb{R}_{>0}^{\hat{m}} \times \mathbb{R}_{>0}^{s} \to \mathbb{R}_{>0}^{m} \times \mathbb{R}_{>0}^{s}$ as $(\hat{\kappa}; x) \mapsto \phi(\hat{\kappa}; x)$. Assume moreover that each coordinate of $\phi_i$ is a rational function: $\phi_i(\hat{\kappa}; x) \in \mathbb{Q}(\hat{\kappa}; x)$ for $i = 1, 2, \ldots, \hat{m} + s$. Then the following is a univariate, degree-$(s - d)$ polynomial in $\lambda$, with coefficients in $\mathbb{Q}(\hat{\kappa}; x)$:*

$$q(\lambda) \;:=\; \frac{1}{\lambda^d}\; \det(\lambda I - \mathrm{Jac}\; f)\,|_{(\kappa;x)\;=\;\phi(\hat{\kappa};x)} \;. \tag{9}$$

*Proof.* This result is straightforward from the fact that the characteristic polynomial of $\mathrm{Jac}(f)$ is a polynomial of degree $s$ and has zero as a root with multiplicity $d$ (because of the $d$ conservation laws). $\qquad\square$

**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 $\kappa_j$ 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 $\kappa_j$ 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* $\frac{\partial \mathfrak{h}_{s-d-1}}{\partial \kappa_j}\big|_{(\hat{\kappa};x)=(\hat{\kappa}^*;x^*)} \neq 0$.

*Moreover, given $\hat{\kappa}^*$ and $x^*$ as in (2), a simple Hopf bifurcation with respect to $\kappa_j$ 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:

$$\frac{1}{\lambda^d} \det(\lambda I - \mathrm{Jac}\ f)\big|_{x=x^*,\ \kappa_i = \kappa_i^*\ for\ all\ i \neq j} \ .$$

Now our result follows directly from Proposition 2.6 and Definition 2.2. $\qquad\square$

**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 in [12] 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).

## 3.1 The (full) ERK network

| $x_1$ | $x_2$ | $x_3$ | $x_4$ | $x_5$ | $x_6$ | $x_7$ | $x_8$ | $x_9$ | $x_{10}$ | $x_{11}$ | $x_{12}$ |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|----------|----------|----------|
| $S_{00}$ | $E$ | $F$ | $S_{11}F$ | $S_{10}F$ | $S_{01}F$ | $S_{01}E$ | $S_{10}E$ | $S_{01}$ | $S_{10}$ | $S_{00}E$ | $S_{11}$ |

Table 2: Assignment of variables to species for the ERK network in Figure 1.

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:

$$\dot{x}_1 = -k_1 x_1 x_2 + k_2 x_{11} + \ell_{\text{cat}} x_5 + n_3 x_6$$
$$\dot{x}_2 = -k_1 x_1 x_2 - k_{\text{on}} x_2 x_9 - m_2 x_2 x_{10} + k_2 x_{11} + k_{\text{cat}} x_7 + k_{\text{off}} x_7 + m_1 x_8 + m_3 x_8$$
$$\dot{x}_3 = -\ell_1 x_3 x_{12} - \ell_{\text{on}} x_3 x_{10} - n_1 x_3 x_9 + \ell_2 x_4 + \ell_{\text{cat}} x_5 + \ell_{\text{off}} x_5 + n_2 x_6 + n_3 x_6$$
$$\dot{x}_4 = \ell_1 x_3 x_{12} - \ell_2 x_4 - \ell_3 x_4$$
$$\dot{x}_5 = \ell_{\text{on}} x_3 x_{10} + \ell_3 x_4 - \ell_{\text{cat}} x_5 - \ell_{\text{off}} x_5$$
$$\dot{x}_6 = n_1 x_3 x_9 - n_2 x_6 - n_3 x_6$$
$$\dot{x}_7 = k_{\text{on}} x_2 x_9 + k_3 x_{11} - k_{\text{cat}} x_7 - k_{\text{off}} x_7 \qquad (10)$$
$$\dot{x}_8 = m_2 x_2 x_{10} - m_1 x_8 - m_3 x_8$$
$$\dot{x}_9 = -k_{\text{on}} x_2 x_9 - n_1 x_3 x_9 + k_{\text{off}} x_7 + n_2 x_6$$
$$\dot{x}_{10} = -\ell_{\text{on}} x_3 x_{10} - m_2 x_2 x_{10} + \ell_{\text{off}} x_5 + m_1 x_8$$
$$\dot{x}_{11} = k_1 x_1 x_2 - k_2 x_{11} - k_3 x_{11}$$
$$\dot{x}_{12} = -\ell_1 x_3 x_{12} + k_{\text{cat}} x_7 + \ell_2 x_4 + m_3 x_8$$

There are 18 rate constants $k_i, \ell_i, m_i, n_i$. The 3 conservation laws correspond to the total amounts of substrate $S$, kinase $E$, and phosphatase $F$, respectively:

$$
\begin{aligned}
x_1 + x_4 + x_5 + x_6 + x_7 + x_8 + x_9 + x_{10} + x_{11} + x_{12} &= \text{S}_{\text{tot}} =: c_1 \\
x_2 + x_7 + x_8 + x_{11} &= \text{E}_{\text{tot}} =: c_2 \qquad (11) \\
x_3 + x_4 + x_5 + x_6 &= \text{F}_{\text{tot}} =: c_3.
\end{aligned}
$$

A steady-state parametrization for the full ERK network was given in [17, Examples 3.1 and 3.7]. That parametrization, however, can not specialize to accommodate irreversible versions of the network (in the effective parameters given in [17], two of the denominators are $k_{\text{on}}$ and $\ell_{\text{on}}$, 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 in [17], 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 $k_{\text{off}}$ or $\ell_{\text{off}}$, so that we can still pursue Question 1.1 (which involves $k_{\text{off}}$ and $\ell_{\text{off}}$) in a model with fewer reactions. We instead allow the removal of reactions $k_{\text{on}}$ and $\ell_{\text{on}}$.

**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 $k_2, k_{on}, m_1, \ell_2, \ell_{on}, n_2$ (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 $\mathbb{1}_{k_{on}}$, $\mathbb{1}_{m_1}$, $\mathbb{1}_{\ell_2}$, $\mathbb{1}_{\ell_{on}}$, and $\mathbb{1}_{n_2}$. Then $\mathcal{N}$ admits an effective steady-state function $h_{c,a} : \mathbb{R}^{12}_{>0} \to \mathbb{R}^{12}$ given by:*

$$
\begin{aligned}
h_{c,a,1} &= x_1 + x_4 + x_5 + x_6 + x_7 + x_8 + x_9 + x_{10} + x_{11} + x_{12} - c_1 \ , \\
h_{c,a,2} &= x_2 + x_7 + x_8 + x_{11} - c_2 \ , \\
h_{c,a,3} &= x_3 + x_4 + x_5 + x_6 - c_3 \ , \\
h_{c,a,4} &= a_{12}x_3x_{12} - x_4 \ , \\
h_{c,a,5} &= a_3x_4 - x_5 - a_2x_8 \ , \\
h_{c,a,6} &= a_{13}x_3x_9 - x_6 \ , \\
h_{c,a,7} &= a_5x_{11} - a_4x_6 - x_7 \ , \\
h_{c,a,8} &= a_{11}x_2x_{10} - x_8 \ , \\
h_{c,a,9} &= a_9x_7 - \mathbb{1}_{k_{on}}a_8x_2x_9 - x_6 \ , \\
h_{c,a,10} &= a_7x_5 - \mathbb{1}_{\ell_{on}}a_6x_3x_{10} - x_8 \ , \\
h_{c,a,11} &= a_{10}x_1x_2 - x_{11} \ , \\
h_{c,a,12} &= x_7 - a_1x_5 \ .
\end{aligned}
\tag{12}
$$

*Moreover, with respect to this effective steady-state function, the positive steady states of $\mathcal{N}$ admit the following positive parametrization:*

$$
\begin{aligned}
\phi : \mathbb{R}^{2+\mathbb{1}_{k_{on}}+\mathbb{1}_{\ell_{on}}+12}_{>0} &\to \mathbb{R}^{13+12}_{>0} \\
(\hat{a}; \ x_1, x_2, \ldots, x_{12}) &\mapsto (a_1, a_2, \ldots, a_{13}, \ x_1, x_2, \ldots, x_{12}) \ ,
\end{aligned}
$$

*given by*

$$
\begin{aligned}
a_1 &:= \frac{x_7}{x_5} & a_3 &:= \frac{a_2x_8 + x_5}{x_4} & a_5 &:= \frac{a_4x_6 + x_7}{x_{11}} \\
a_7 &:= \frac{\mathbb{1}_{\ell_{on}}a_6x_3x_{10} + x_8}{x_5} & a_9 &:= \frac{\mathbb{1}_{k_{on}}a_8x_2x_9 + x_6}{x_7} & a_{10} &:= \frac{x_{11}}{x_1x_2} \\
a_{11} &:= \frac{x_8}{x_2x_{10}} & a_{12} &:= \frac{x_4}{x_3x_{12}} & a_{13} &:= \frac{x_6}{x_3x_9} \ .
\end{aligned}
\tag{13}
$$

*Here, $\hat{a} = (a_2, a_4, a_6, a_8)$ if $\mathcal{N}$ contains the reactions labeled by $k_{on}$ and $\ell_{on}$, and $\hat{a} = (a_2, a_4, a_6)$ if $\mathcal{N}$ contains the reaction $\ell_{on}$ but not $k_{on}$, and so on.*

*Proof.* We will show that the map $\bar{a} : \mathbb{R}^{12+\mathbb{1}_{k_2}+\mathbb{1}_{k_{on}}+\mathbb{1}_{m_1}+\mathbb{1}_{\ell_2}+\mathbb{1}_{\ell_{on}}+\mathbb{1}_{n_2}}_{>0} \to \mathbb{R}^{11+\mathbb{1}_{k_{on}}+\mathbb{1}_{\ell_{on}}}_{>0}$, defined as follows, is a reparametrization map as in (4):

$$
\begin{aligned}
\bar{a}_1 &= \frac{\ell_{cat}}{k_{cat}}, & \bar{a}_2 &= \frac{m_3}{\ell_{cat}}, & \bar{a}_3 &= \frac{\ell_3}{\ell_{cat}}, & \bar{a}_4 &= \frac{n_3}{k_{cat}}, & \bar{a}_5 &= \frac{k_3}{k_{cat}}, \\
\bar{a}_6 &= \frac{\mathbb{1}_{\ell_{on}}\ell_{on}}{m_3}, & \bar{a}_7 &= \frac{\ell_{off}}{m_3}, & \bar{a}_8 &= \frac{\mathbb{1}_{k_{on}}k_{on}}{n_3}, & \bar{a}_9 &= \frac{k_{off}}{n_3}, & \bar{a}_{10} &= \frac{k_1}{\mathbb{1}_{k_2}k_2+k_3}, \\
\bar{a}_{11} &= \frac{m_2}{\mathbb{1}_{m_1}m_1+m_3}, & \bar{a}_{12} &= \frac{\ell_1}{\mathbb{1}_{\ell_2}\ell_2+\ell_3}, & \bar{a}_{13} &= \frac{n_1}{\mathbb{1}_{n_2}n_2+n_3}.
\end{aligned}
\tag{14}
$$

In particular, we remove the effective parameter $\bar{a}_6$ (respectively, $\bar{a}_8$) if $\mathbb{1}_{\ell_{on}} = 0$ (respectively, $\mathbb{1}_{k_{on}} = 0$). Notice that each $\bar{a}_i$ (if it is not removed) is defined and positive for all $\kappa = (k_1, \ldots, n_3) \in \mathbb{R}_{>0}^{12 + \mathbb{1}_{k_2} + \mathbb{1}_{k_{on}} + \mathbb{1}_{m_1} + \mathbb{1}_{\ell_2} + \mathbb{1}_{\ell_{on}} + \mathbb{1}_{n_2}}$.

We must show that the map $\bar{a}$ is surjective. Indeed, given $a \in \mathbb{R}_{>0}^{11 + \mathbb{1}_{k_{on}} + \mathbb{1}_{\ell_{on}}}$, 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:

$$(k_1, k_2, k_3, k_{cat}, k_{on}, k_{off}, \ell_1, \ell_2, \ell_3, \ell_{cat}, \ell_{on}, \ell_{off}, m_1, m_2, m_3, n_1, n_2, n_3) =$$
$$((\mathbb{1}_{k_2} + a_5)a_{10}, \mathbb{1}_{k_2}, a_5, 1, \mathbb{1}_{k_{on}}a_4a_8, a_4a_9, (\mathbb{1}_{\ell_2} + a_1a_3)a_{12}, \mathbb{1}_{\ell_2}, a_1a_3, a_1, \mathbb{1}_{\ell_{on}}a_1a_2a_6, a_1a_2a_7, \mathbb{1}_{m_1},$$
$$(\mathbb{1}_{m_1} + a_1a_2)a_{11}, a_1a_2, (\mathbb{1}_{n_2} + a_4)a_{13}, \mathbb{1}_{n_2}, a_4).$$

Next, consider the following $9 \times 9$ matrix:

$$M(\kappa) = \begin{pmatrix} \frac{1}{\mathbb{1}_{\ell_2}\ell_2 + \ell_3} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \frac{1}{\ell_{cat}} & 0 & 0 & \frac{1}{\ell_{cat}} & 0 & \frac{1}{\ell_{cat}} & 0 & 0 \\ 0 & 0 & \frac{1}{\mathbb{1}_{n_2}n_2 + n_3} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \frac{1}{k_{cat}} & \frac{1}{k_{cat}} & 0 & \frac{1}{k_{cat}} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{\mathbb{1}_{m_1}m_1 + m_3} & 0 & 0 & 0 & 0 \\ 0 & 0 & \frac{1}{n_3} & 0 & 0 & \frac{1}{n_3} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{m_3} & 0 & \frac{1}{m_3} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{1}{\mathbb{1}_{k_2}k_2 + k_3} & 0 \\ \frac{1}{k_{cat}} & \frac{1}{k_{cat}} & 0 & 0 & \frac{1}{k_{cat}} & 0 & \frac{1}{k_{cat}} & 0 & \frac{1}{k_{cat}} \end{pmatrix}. \quad (15)$$

It is straightforward to check that $\det M(\kappa)$ is the product of all diagonal terms, and hence is positive for all $\kappa \in \mathbb{R}_{>0}^{12 + \mathbb{1}_{k_2} + \mathbb{1}_{k_{on}} + \mathbb{1}_{m_1} + \mathbb{1}_{\ell_2} + \mathbb{1}_{\ell_{on}} + \mathbb{1}_{n_2}}$.

The mass-action ODEs of $\mathcal{N}$ are obtained from those (10) of the full ERK network by replacing the rate constants $k_2, k_{on}, m_1, \ell_2, \ell_{on}, n_2$, respectively, by $\mathbb{1}_{k_2}k_2, \mathbb{1}_{k_{on}}k_{on}, \mathbb{1}_{m_1}m_1, \mathbb{1}_{\ell_2}\ell_2, \mathbb{1}_{\ell_{on}}\ell_{on}$, 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 $\phi$ is a positive parametrization with respect to (12) (as in Definition 2.2) follows directly from comparing equations (12) and (13). $\qquad\square$

**Remark 3.2** (Multistationarity depends on only $k_{on}$ and $\ell_{on}$). Proposition 3.1 considers any network obtained by deleting any (or none) of the six reactions labeled by $k_2, k_{on}, m_1, \ell_2, \ell_{on}, n_2$. Nonetheless, the resulting steady-state parametrization (13) depends on $k_{on}$ and $\ell_{on}$ but not any of the other rate constants. Thus, multistationarity for these irreversible networks depends only on whether the network contains $k_{on}$ and $\ell_{on}$ (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.
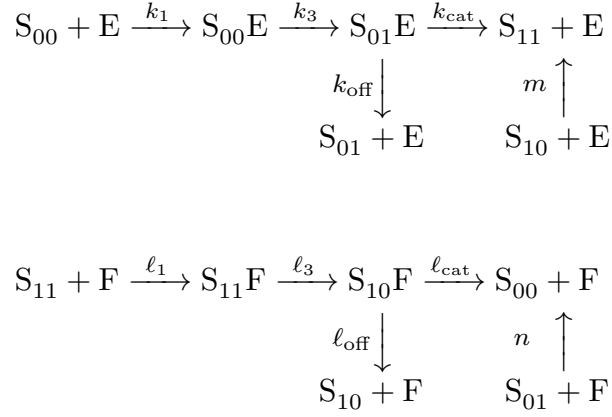
$$S_{00} + E \xrightarrow{k_1} S_{00}E \xrightarrow{k_3} S_{01}E \xrightarrow{k_{\text{cat}}} S_{11} + E$$
$$k_{\text{off}} \downarrow \qquad\qquad m \uparrow$$
$$S_{01} + E \qquad S_{10} + E$$

$$S_{11} + F \xrightarrow{\ell_1} S_{11}F \xrightarrow{\ell_3} S_{10}F \xrightarrow{\ell_{\text{cat}}} S_{00} + F$$
$$\ell_{\text{off}} \downarrow \qquad\qquad n \uparrow$$
$$S_{10} + F \qquad S_{01} + F$$

Figure 2: Reduced ERK network.

Our notion of removing intermediates matches that of Feliu and Wiuf [20], 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 [7, 36, 41].

| $x_1$ | $x_2$ | $x_3$ | $x_4$ | $x_5$ | $x_6$ | $x_7$ | $x_8$ | $x_9$ | $x_{10}$ |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|----------|
| $S_{00}$ | $E$ | $S_{00}E$ | $S_{01}E$ | $S_{11}$ | $S_{01}$ | $S_{10}$ | $F$ | $S_{11}F$ | $S_{10}F$ |

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:

$$
\begin{aligned}
\dot{x}_1 &= -k_1 x_1 x_2 + n x_6 x_8 + \ell_{\text{cat}} x_{10} & =: f_1 \\
\dot{x}_2 &= -k_1 x_1 x_2 + k_{\text{cat}} x_4 + k_{\text{off}} x_4 & =: f_2 \\
\dot{x}_3 &= k_1 x_1 x_2 - k_3 x_3 & =: f_3 \\
\dot{x}_4 &= k_3 x_3 - k_{\text{cat}} x_4 - k_{\text{off}} x_4 & =: f_4
\end{aligned}
$$

13

$$\dot{x}_5 = mx_2x_7 - \ell_1 x_5 x_8 + k_{\text{cat}}x_4 \qquad =: f_5 \qquad (16)$$
$$\dot{x}_6 = -nx_6x_8 + k_{\text{off}}x_4 \qquad =: f_6$$
$$\dot{x}_7 = -mx_2x_7 + \ell_{\text{off}}x_{10} \qquad =: f_7$$
$$\dot{x}_8 = -\ell_1 x_5 x_8 + \ell_{\text{off}}x_{10} + \ell_{\text{cat}}x_{10} \qquad =: f_8$$
$$\dot{x}_9 = \ell_1 x_5 x_8 - \ell_3 x_9 \qquad =: f_9$$
$$\dot{x}_{10} = -\ell_{\text{off}}x_{10} + \ell_3 x_9 - \ell_{\text{cat}}x_{10} \qquad =: f_{10}.$$

The 3 conservation equations are:

$$x_1 + x_3 + x_4 + x_5 + x_6 + x_7 + x_9 + x_{10} = S_{\text{tot}} =: c_1$$
$$x_2 + x_3 + x_4 = E_{\text{tot}} =: c_2 \qquad (17)$$
$$x_8 + x_9 + x_{10} = F_{\text{tot}} =: c_3.$$

**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:*

$$h_{c,a,1} = x_1 + x_3 + x_4 + x_5 + x_6 + x_7 + x_9 + x_{10} - c_1, \quad h_{c,a,2} = x_2 + x_3 + x_4 - c_2,$$
$$h_{c,a,3} = -(k_{\text{cat}} + k_{\text{off}})\ell_{\text{cat}}x_{10} + k_1 k_{\text{cat}}x_1 x_2, \quad h_{c,a,4} = k_3 x_3 - (k_{\text{cat}} + k_{\text{off}})x_4,$$
$$h_{c,a,5} = \ell_{\text{off}}x_{10} - mx_2 x_7, \quad h_{c,a,6} = \ell_1 x_5 x_8 - (\ell_{\text{cat}} + \ell_{\text{off}})x_{10},$$
$$h_{c,a,7} = \ell_3 x_9 - (\ell_{\text{cat}} + \ell_{\text{off}})x_{10}, \quad h_{c,a,8} = x_8 + x_9 + x_{10} - c_3,$$
$$h_{c,a,9} = k_{\text{cat}}x_4 - \ell_{\text{cat}}x_{10}, \quad h_{c,a,10} = k_{\text{off}}\ell_{\text{cat}}x_{10} - k_{\text{cat}}nx_6 x_8.$$
$$(18)$$

*Moreover, with respect to this effective steady-state function, the positive steady states admit the following positive parametrization:*

$$\phi : \mathbb{R}^{3+10}_{>0} \to \mathbb{R}^{10+10}_{>0} \qquad (19)$$
$$(k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}}, \ x_1, x_2, \ldots, x_{10}) \mapsto (\kappa_1, \kappa_3, k_{\text{cat}}, k_{\text{off}}, m, \ell_1, \ell_3, \lambda_{\text{cat}}, \ell_{\text{off}}, n, \ x_1, x_2, \ldots, x_{10}) ,$$

*given by*

$$k_1 := \frac{(k_{\text{cat}} + k_{\text{off}})x_4}{x_1 x_2} \qquad k_3 := \frac{(k_{\text{cat}} + k_{\text{off}})x_4}{x_3} \qquad m := \frac{\ell_{\text{off}}x_{10}}{x_2 x_7} \qquad \ell_1 := \frac{\ell_{\text{off}}x_{10} + k_{\text{cat}}x_4}{x_5 x_8}$$

$$\ell_3 := \frac{\ell_{\text{off}}x_{10} + k_{\text{cat}}x_4}{x_9} \qquad \ell_{\text{cat}} := \frac{k_{\text{cat}}x_4}{x_{10}} \qquad n := \frac{k_{\text{off}}x_4}{x_6 x_8}. \qquad (20)$$

*In particular, the image of $\phi$ 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 $\phi$ 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 $(k^*; x^*) \in \mathbb{R}_{>0}^{10+10}$, 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 "$\Rightarrow$" 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, $\lambda_{\text{cat}}$ – when evaluated at $(k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}}; x) = (k_{\text{cat}}^*, k_{\text{off}}^*, \ell_{\text{off}}^*; x^*)$ – equals $\ell_{\text{cat}}^*$. Next, the equality $h_3(k^*; x^*) = 0$ implies that

$$
k_1^* = \frac{(k_{\text{cat}}^* + k_{\text{off}}^*)\ell_{\text{cat}}^* x_{10}^*}{k_{\text{cat}}^* x_1^* x_2^*} = \frac{(k_{\text{cat}}^* + k_{\text{off}}^*)x_4^*}{x_1^* x_2^*} , \tag{22}
$$

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 $\kappa_3$ (respectively, $m$, $\ell_1$, $\ell_3$, or $n$) – when

---

[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 in [17] hold in that generality.

evaluated at $(k_{\mathrm{cat}}, k_{\mathrm{off}}, \ell_{\mathrm{off}}; x) = (k^*_{\mathrm{cat}}, k^*_{\mathrm{off}}, \ell^*_{\mathrm{off}}; x^*)$ – equals $k^*_3$ (respectively, $m^*$, $\ell^*_1$, $\ell^*_3$, or $n^*$). Thus, $\phi(k^*_{\mathrm{cat}}, k^*_{\mathrm{off}}, \ell^*_{\mathrm{off}}; x^*) = (k^*; x^*)$.

The "$\Leftarrow$" direction is similar. Assume $\phi(k^*_{\mathrm{cat}}, k^*_{\mathrm{off}}, \ell^*_{\mathrm{off}}; x^*) = (k^*; x^*)$. That is, the expressions for $k_1$, $k_3$, $m$, $\ell_1$, $\ell_3$, $\ell_{\mathrm{cat}}$, and $n$ evaluate to, respectively, $k^*_1$, $k^*_3$, $m^*$, $\ell^*_1$, $\ell^*_3$, $\ell^*_{\mathrm{cat}}$, and $n^*$, when $(k_{\mathrm{cat}}, k_{\mathrm{off}}, \ell_{\mathrm{off}}; x) = (k^*_{\mathrm{cat}}, k^*_{\mathrm{off}}, \ell^*_{\mathrm{off}}; x^*)$. 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)). $\qquad\square$

**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 [17, §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" [39]. 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` [23]). As for the irreversible versions of the ERK network, when the reactions with rate constants $k_{\mathrm{on}}$ and $\ell_{\mathrm{on}}$ are deleted, we see from (12) that the steady-state ideal becomes binomial. Hence, irreversible ERK networks that are missing both $k_{\mathrm{on}}$ and $\ell_{\mathrm{on}}$ are "linearly binomial" as in [17, §4].

**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 [1] or [43]).

# 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 [40]. 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:

$$
\begin{aligned}
(k_1, k_3, k_{\text{cat}}, k_{\text{off}}, \ell_1, \ell_3, \ell_{\text{cat}}, \ell_{\text{off}}, m_2, m_3, n_1, n_3) \ = \ & (5241, 5314.5, 1291, 76.203, 64.271, \quad (23) \\
& 44.965, 924970, 27238, 2.76250 \times 10^6, \\
& 2.0451, 2.1496 \times 10^6, 1.3334) \ .
\end{aligned}
$$

These rate constants (23) come from the ones that Rubinstein *et al.* showed generate oscillations for the full ERK network [40, 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`):

$$
\begin{aligned}
(x_1, x_2, \ldots, x_{12}) \ \approx \ & (1.215 \times 10^{-5}, \ 4.722 \times 10^{-5}, \ 8.777 \times 10^{-4}, \ 1.396 \times 10^{-3}, \\
& 6.590 \times 10^{-8}, \ 2.698 \times 10^{-3}, \ 2.873 \times 10^{-4}, \ 1.150 \times 10^{-3}, \quad (24) \\
& 3.072 \times 10^{-3}, \ 2.262 \times 10^{-6}, \ 0.042, \ 0.849) \ .
\end{aligned}
$$

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 a result of Banaji that "lifts" oscillations when one or more reactions are made reversible [3, Proposition 4.1].

### 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.

**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 $\hat{\kappa} :=$*
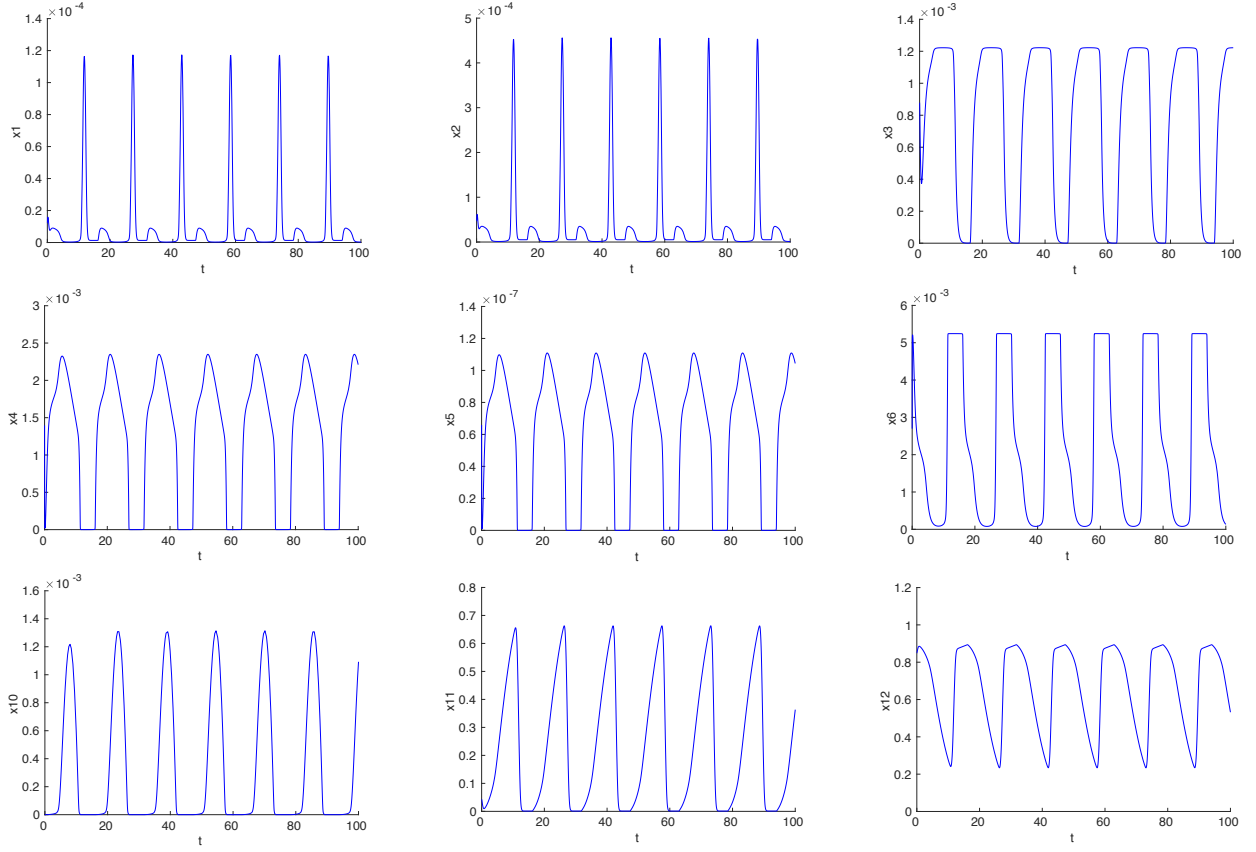
Figure 3: The fully irreversible ERK network undergoes oscillations when the rate constants are as in (23) and the initial species concentrations are as in (24). Displayed in this figure are all species concentrations, except $x_7, x_8$, and $x_9$. This figure was generated using `MATCONT`, a numerical bifurcation package [16]. For details, see the supplementary file `ERK-Matcont.txt`.

$(k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}})$ and $x := (x_1, x_2, \ldots, x_{10})$, and let $\phi$ be the steady-state parametrization (19). Then the following is a univariate, degree-7 polynomial in $\lambda$, with coefficients in $\mathbb{Q}(x)[\hat{\kappa}]$:

$$q(\lambda) \ := \ \frac{1}{\lambda^3} \ \det\left(\lambda I - \text{Jac}(f)\right)\big|_{(\kappa;x)=\phi(\hat{\kappa};x)} \ . \tag{25}$$

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 $k_{\text{cat}}$, at some $x^* \in \mathbb{R}^{10}_{>0}$, and

(2) there exist $x^* \in \mathbb{R}^{10}_{>0}$ and $\hat{\kappa}^* \in \mathbb{R}^3_{>0}$ such that

$$\mathfrak{h}_4(\hat{\kappa}^*; x^*) > 0 \ , \ \ \mathfrak{h}_5(\hat{\kappa}^*; x^*) > 0 \ , \ \ \mathfrak{h}_6(\hat{\kappa}^*; x^*) = 0 \ , \ \text{and} \tag{26}$$

$$\frac{\partial}{\partial k_{\text{cat}}} \mathfrak{h}_6(\hat{\kappa}; x)\big|_{(\hat{\kappa};x)=(\hat{\kappa}^*;x^*)} \neq 0 \ .$$
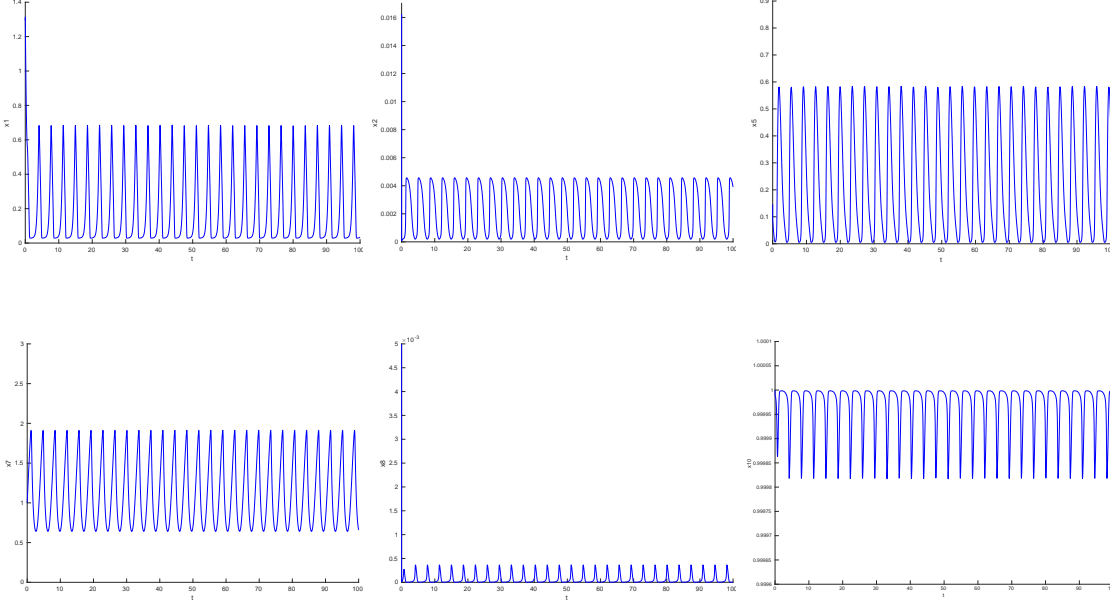
18

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 $x_9$.

*Moreover, given $\hat{\kappa}^*$ and $x^*$ as in (2), a simple Hopf bifurcation with respect to $k_{\mathrm{cat}}$ occurs at $x^*$ when the rate constants are taken to be $\kappa^* := \widetilde{\pi}(\phi(\hat{\kappa}^*; x^*))$. Here, $\widetilde{\pi} : \mathbb{R}_{>0}^{10} \times \mathbb{R}_{>0}^{10} \to \mathbb{R}_{>0}^{10}$ 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 $(\hat{\kappa}; x) \in \mathbb{R}_{>0}^3 \times \mathbb{R}_{>0}^{10}$. 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.) $\qquad\square$

**Remark 4.2.** Note that $k_{\mathrm{cat}}$ 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 $k_{\mathrm{cat}}$ 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:*

$$(k_1^*, k_3^*, k_{\mathrm{cat}}^*, k_{\mathrm{off}}^*, m^*, \ell_1^*, \ell_3^*, \ell_{\mathrm{cat}}^*, \ell_{\mathrm{off}}^*, n^*) \approx (5.562806640 \times 10^6,\ 730,\ 729,\ 1,\ 453.5941390,$$
$$3.879519315 \times 10^8,\ 730,\ 729,\ 1,\ 80675.77183)$$

*Here, $\phi$ 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`. $\qquad\square$

**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 [40], 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 $k_{\mathrm{on}}$ and $\ell_{\mathrm{on}}$ (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 $\mathcal{N}$:

$$C(\hat{a}; x) \quad = \quad (\det \mathrm{Jac}\ h_{c,a})\,|_{(a;x) = \phi(\hat{a};x)}\,, \tag{27}$$

where $\hat{a} = (k_{\mathrm{cat}}, k_{\mathrm{off}}, \ell_{\mathrm{off}})$, 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 $(\hat{a}; x) \in \mathbb{R}^3_{>0} \times \mathbb{R}^{10}_{>0}$:

$$-k_{\mathrm{cat}}^3 (k_{\mathrm{cat}} + k_{\mathrm{off}})^2 x_4^3 (k_{\mathrm{cat}} x_4 + \ell_{\mathrm{off}} x_{10})^2 \ell_{\mathrm{off}} k_{\mathrm{off}} (x_1 x_2 x_8 + x_1 x_3 x_8 + x_1 x_4 x_8 + x_{10} x_2 x_5$$
$$+ x_{10} x_2 x_6 + x_{10} x_2 x_8 + x_2 x_3 x_8 + x_2 x_4 x_8 + x_2 x_5 x_8 + x_2 x_5 x_9 + x_2 x_6 x_8 + x_2 x_6 x_9$$
$$+ x_2 x_7 x_8 + x_2 x_8 x_9 + x_3 x_7 x_8 + x_4 x_7 x_8)\,.$$

Thus, the following holds for all $(\hat{a}; x) \in \mathbb{R}^3_{>0} \times \mathbb{R}^{10}_{>0}$:

$$\mathrm{sign}(C(\hat{a}; x)) = -1 = (-1)^{\mathrm{rank}(N)}\,,$$

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 $k_{\mathrm{on}}$ and $\ell_{\mathrm{on}}$ 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) while preserving bistability.

**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 $k_2, k_{\mathrm{on}}, m_1, \ell_2, \ell_{\mathrm{on}}, n_2$ (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 $k_{\mathrm{on}}$ and $\ell_{\mathrm{on}}$.*

*Proof.* By definition, every bistable network is multistationary, so $(2) \Rightarrow (1)$. We therefore need only show $(1) \Rightarrow (3) \Rightarrow (2)$. (All computations below are found in our supplementary file `irreversibleERK.mw`).

For $(1) \Rightarrow (3)$, we will prove $\neg(3) \Rightarrow \neg(1)$: Assume that $\mathcal{N}$ contains neither the reaction labeled by $k_{\mathrm{on}}$ nor the reaction $\ell_{\mathrm{on}}$. 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 $\mathbb{1}_{k_{\mathrm{on}}} = \mathbb{1}_{\ell_{\mathrm{on}}} = 0$) and $\phi(\hat{a}; x)$ is as in (13) (with $\hat{a} = (a_2, a_4)$).

Here, $\det \mathrm{Jac}(h_{c,a})$ is a rational function with denominator equal to $k_{\mathrm{off}} x_2 (n_2 + n_3) \ell_{\mathrm{cat}} l_3 k_3 m_3$, 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 $\det \mathrm{Jac}(h_{c,a})$. Hence, for all $(\hat{a}; x) \in \mathbb{R}^2_{>0} \times \mathbb{R}^{12}_{>0}$, the equality $\mathrm{sign}(C(\hat{a}; x)) = -1 = (-1)^{\mathrm{rank}(N)}$ 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 $k_{\mathrm{on}}$ and $\ell_{\mathrm{on}}$ 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 $k_{\mathrm{on}}$.

Consider the network $\mathcal{N}'$ obtained from the full ERK network by deleting all reactions marked in blue in Figure 1, except for $k_{\mathrm{on}}$ (equivalently, we set $k_2 = m_1 = \ell_2 = \ell_{\mathrm{on}} = n_2 = 0$). We will show that the following total constants and rate constants yield bistability:

$$
\begin{aligned}
(c_1, \ c_2, \ c_3) \ &= \ (46, 13, 13), \quad \text{and} \\
(k_1, k_3, k_{\mathrm{cat}}, k_{\mathrm{on}}, k_{\mathrm{off}}, \ell_1, \ell_3, \ell_{\mathrm{cat}}, \ell_{\mathrm{off}}, m_2, m_3, n_1, n_3) \ &= \\
(2, 1.1, 1, 5, 15, 2, 1.1, 1, 10, 20, 10, 20, 10) \ .
\end{aligned}
\tag{28}
$$

Among the resulting three steady states (see the supplementary file), one of them is approximately:

(20.72107755, 0.2956877203, 3.248789181, 7.821850626, 0.7821850626, 1.147175131, 0.7821850626, 0.7821850626, 0.1765542587, 1.322653950, 11.13994215, 1.324191138) .

At the above steady state, the Jacobian matrix (of the system obtained from (10) by making the substitutions (28) and $k_2 = m_1 = \ell_2 = \ell_{on} = n_2 = 0$) has three zero eigenvalues (due to the three conservation laws). For the remaining eigenvalues, the real parts are approximately:

$$-76.0913958200572, \quad -70.7106617930401 , \quad -16.3022723748274,$$
$$-10.9324829878475, \quad -10.9324829878475, \quad -8.81318904794782 ,$$
$$-4.88866989801728, \quad -4.88866989801728 , \quad -0.0545784672515179 ,$$

Thus, the nonzero eigenvalues have strictly negative real part, so the steady state is exponentially stable.

Another steady state is approximately

(0.1782157709, 8.088440520, 0.2275355904, 11.45336411, 1.145336411, 0.1737638914, 1.145336411, 1.145336411, 0.3818389270, 0.07080081803, 2.620886659, 27.68512059) .

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:

$$-163.308657649675, \quad -68.5596972162577 , \quad -57.0205793889569 ,$$
$$-16.4435472947534, \quad -12.1029003142539, \quad -9.27515541335710,$$
$$-9.27515541335710, \quad -3.08709626767693 , \quad -0.209550347944487.$$

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 [32, Theorem 3.1], bistability "lifts" from $\mathcal{N}'$ to $\mathcal{N}$. Thus, $\mathcal{N}$ is bistable. □

We obtain the following immediate consequence of Theorem 4.6.

**Corollary 4.7.** *The fully irreversible ERK network is monostationary.*

# 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 $\ell_{on}$) 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 from [34] a network's maximum number of positive steady states, 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).

In [34], $k = \infty$ was allowed when there are infinitely many steady states in a stoichiometric compatibility class. Here 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 Ziegler's book [48]. 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 $P_1, P_2, ..., P_s$.

The mixed volume counts the number of solutions in $(\mathbb{C}^*)^s$ of a generic polynomial system.

**Proposition 5.5** (Bernstein's theorem [5]). *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 and Hill [25].

**Remark 5.7.** The mixed volume (Definition 5.6) is well defined. Indeed, it is straightforward to check that the exponents appearing in $f_{c^*,\kappa^*}$ are the same as long as $c^* \in \mathbb{R}^d_{\neq 0}$ and $\kappa^*$ is chosen generically (so that no coefficients of $f_{c^*,\kappa^*}$ vanish, or equivalently certain linear combinations of the $\kappa_j$'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):*

max # of positive steady states $\leq$ max # of steady states over $\mathbb{C}^*$ $\leq$ 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 $k_{\mathrm{on}}$ 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 | $\geq 3$ | 7 | 7 |
| Full with $k_{\mathrm{on}} = 0$ | $\geq 3$ | 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` [26] package in `Macaulay2` [23]. 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) [17, 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 $(k_1, k_2, k_3, k_{cat}, k_{on}, k_{off}, \ell_1, \ell_2, \ell_3, \ell_{cat}, \ell_{on}, \ell_{off}, m_1, m_2, m_3, n_1, n_2, n_3) = (3, 25, 1, 5, 0, 6, 5, 23, 11, 13, 43, 41, 12, 7, 8, 12, 31, 21)$, 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 $(k_1, k_3, k_{cat}, k_{off}, \ell_1, \ell_3, \ell_{cat}, \ell_{off}, m_2, m_3, n_1, n_3) = (3, 1, 5, 6, 5, 11, 13, 41, 7, 8, 12, 21)$, 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 $(k_1, k_3, k_{cat}, k_{off}, m, n, \ell_1, \ell_3, \ell_{cat}, \ell_{off}) = (3, 4, 1, 5, 6, 8, 7, 11, 12, 5)$. 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 reversible 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 [17, 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 $k_{\mathrm{on}} = 0$. 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 $k_{\mathrm{on}} = 0$, 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) [8]. 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 [2, 6]. Oscillations also have been observed [29, 30], hinting at a role in timekeeping. Indeed, multisite phosphorylation is the main mechanism for establishing the 24-hour period in eukaryotic circadian clocks [38, 46].

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" [44, §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* [4, 20, 32]. 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 [17], 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 [12], 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

26

particular, is there a mixed-volume analogue of our bistability criterion, which is in terms of the reactions $k_{\mathrm{on}}$ and $\ell_{\mathrm{on}}$? 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.

## Acknowledgements

# References

[1] David Angeli, Patrick De Leenheer, and Eduardo Sontag. *A Petri net approach to persistence analysis in chemical reaction networks*, pages 181–216. Springer-Verlag, Berlin, 2007.

[2] Christoph P Bagowski and James E Ferrell. Bistability in the JNK cascade. *Curr. Biol.*, 11(15):1176–1182, 2001.

[3] Murad Banaji. Inheritance of oscillation in chemical reaction networks. *Appl. Math. Comput.*, 325:191–209, 2018.

[4] Murad Banaji and Casian Pantea. The inheritance of nondegenerate multistationarity in chemical reaction networks. *SIAM J. Appl. Math.*, 78(2):1105–1130, 2018.

[5] David N. Bernshtein. The number of roots of a system of equations. *Functional Analysis and its Applications (translated from Russian)*, 9(2):183, 1975.

[6] Upinder S Bhalla, Prahlad T Ram, and Ravi Iyengar. MAP kinase phosphatase as a locus of flexibility in a mitogen-activated protein kinase signaling network. *Science*, 297(5583):1018–1023, 2002.

[7] Daniele Cappelletti and Carsten Wiuf. Uniform approximation of solutions by elimination of intermediate species in deterministic reaction networks. *SIAM J. Appl. Dyn. Syst.*, 16(4):2259–2286, 2017.

[8] Lufen Chang and Michael Karin. Mammalian MAP kinase signalling cascades. *Nature*, 410(6824):37–40, 2001.

[9] Philip Cohen. The regulation of protein function by multisite phosphorylation–a 25 year update. *Trends Bioch. Sci.*, 25(12):596–601, 2000.

[10] Carsten Conradi, Elisenda Feliu, Maya Mincheva, and Carsten Wiuf. Identifying parameter regions for multistationarity. *PLoS Comput. Biol.*, 13(10):e1005751, 2017.

[11] Carsten Conradi, Alexandru Iosif, and Thomas Kahle. Multistationarity in the space of total concentrations for systems that admit a monomial parametrization. *Preprint,* `arXiv:1810.08152`, 2018.

[12] Carsten Conradi, Maya Mincheva, and Anne Shiu. Emergence of oscillations in a mixed-mechanism phosphorylation system. *B. Math. Biol., to appear*, 2019.

[13] Carsten Conradi and Anne Shiu. A global convergence result for processive multisite phosphorylation systems. *B. Math. Biol.*, 77(1):126–155, 2015.

[14] Carsten Conradi and Anne Shiu. Dynamics of post-translational modification systems: recent progress and future challenges. *Biophys. J.*, 114(3):507–515, 2018.

[15] Gheorghe Craciun and Martin Feinberg. Multiple equilibria in complex chemical reaction networks: extensions to entrapped species models. *IEE P. Syst. Biol.*, 153:179–186, 2006.

[16] Annick Dhooge, Willy Govaerts, and Yuri A. Kuznetsov. MATCONT: A Matlab package for numerical bifurcation analysis of ODEs. *SIGSAM Bull.*, 38(1):21–22, March 2004.

[17] Alicia Dickenstein, Mercedes Perez Millan, Anne Shiu, and Xiaoxian Tang. Multistationarity in structured reaction networks. *B. Math. Biol., to appear*, 2019.

[18] Mitchell Eithun and Anne Shiu. An all-encompassing global convergence result for processive multisite phosphorylation systems. *Math. Biosci.*, 291:1–9, 2017.

[19] Martin Feinberg. *Dynamics and Modelling of Reactive Systems*, chapter Chemical oscillations, multiple equilibria, and reaction network structure, pages 59–130. Academic Press, 1980.

[20] Elisenda Feliu and Carsten Wiuf. Simplifying biochemical models with intermediate species. *J. R. Soc. Interface*, 10(87), 2013.

[21] Alan S. Futran, A. James Link, Rony Seger, and Stanislav Y. Shvartsman. ERK as a model for systems biology of enzyme kinetics in cells. *Curr. Biol.*, 23(21):R972–R979, 2013.

[22] Magalí Giaroli, Frédéric Bihan, and Alicia Dickenstein. Regions of multistationarity in cascades of Goldbeter–Koshland loops. *J. Math. Biol.*, Nov 2018.

[23] Daniel R. Grayson and Michael E. Stillman. Macaulay2, a software system for research in algebraic geometry. Available at `http://www.math.uiuc.edu/Macaulay2/`.

[24] Elizabeth Gross, Heather A Harrington, Nicolette Meshkat, and Anne Shiu. Joining and decomposing reaction networks. *Preprint,* `arXiv:1810.05575`, 2018.

[25] Elizabeth Gross and Cvetelina Hill. Volumes of polytopes associated with chemical reaction networks. *In preparation*, 2019.

[26] Elizabeth Gross, Sonja Petrovic, and Jan Verschelde. PHCpack in Macaulay2. *The Journal of Software for Algebra and Geometry: Macaulay2*, 5:20–25, 2013.

[27] Otto Hadač, František Muzika, Vladislav Nevoral, Michal Přibyl, and Igor Schreiber. Minimal oscillating subnetwork in the Huang-Ferrell model of the MAPK cascade. *PLOS ONE*, 12(6):1–25, 06 2017.

[28] Rosa D Hernansaiz-Ballesteros, Luca Cardelli, and Attila Csikász-Nagy. Single molecules can operate as primitive biological sensors, switches and oscillators. *BMC Syst. Biol.*, 12(1):70, 2018.

[29] Zoe Hilioti, Walid Sabbagh, Saurabh Paliwal, Adriel Bergmann, Marcus D Goncalves, Lee Bardwell, and Andre Levchenko. Oscillatory phosphorylation of yeast Fus3 MAP kinase controls periodic gene expression and morphogenesis. *Curr. Biol.*, 18(21):1700–1706, 2008.

[30] Huizhong Hu, Alexey Goltsov, James L Bown, Andrew H Sims, Simon P Langdon, David J Harrison, and Dana Faratian. Feedforward and feedback regulation of the MAPK and PI3K oscillatory circuit in breast cancer. *Cell. Signal.*, 25(1):26–32, 2013.

[31] Matthew D Johnston, Stefan Müller, and Casian Pantea. A deficiency-based approach to parametrizing positive equilibria of biochemical reaction systems. *B. Math. Biol.*, 81:1143–1172, 2019.

[32] Badal Joshi and Anne Shiu. Atoms of multistationarity in chemical reaction networks. *J. Math. Chem.*, 51(1):153–178, Jan 2013.

[33] Badal Joshi and Anne Shiu. A survey of methods for deciding whether a reaction network is multistationary. *Math. Model. Nat. Phenom., special issue on "Chemical dynamics"*, 10(5):47–67, 2015.

[34] Badal Joshi and Anne Shiu. Which small reaction networks are multistationary? *SIAM J. Appl. Dyn. Syst.*, 16(2):802–833, 2017.

[35] Wei Min Liu. Criterion of Hopf bifurcations without using eigenvalues. *J. Math. Anal. Appl.*, 182(1):250–256, 1994.

[36] Michael Marcondes de Freitas, Elisenda Feliu, and Carsten Wiuf. Intermediates, catalysts, persistence, and boundary steady states. *J. Math. Biol.*, 74(4):887–932, 2017.

[37] Stefan Müller, Elisenda Feliu, Georg Regensburger, Carsten Conradi, Anne Shiu, and Alicia Dickenstein. Sign conditions for injectivity of generalized polynomial maps with applications to chemical reaction networks and real algebraic geometry. *Found. Comput. Math.*, 16(1):69–97, 2016.

[38] Koji L. Ode and Hiroki R. Ueda. Design principles of phosphorylation-dependent time-keeping in eukaryotic circadian clocks. *Cold Spring Harbor Perspectives in Biology*, 2017.

[39] Mercedes Pérez Millán, Alicia Dickenstein, Anne Shiu, and Carsten Conradi. Chemical reaction systems with toric steady states. *B. Math. Biol.*, 74(5):1027–1065, 2012.

[40] Boris Y. Rubinstein, Henry H. Mattingly, Alexander M. Berezhkovskii, and Stanislav Y. Shvartsman. Long-term dynamics of multisite phosphorylation. *Mol. Biol. Cell*, 27(14):2331–2340, 2016.

[41] AmirHosein Sadeghimanesh and Elisenda Feliu. The multistationarity structure of networks with intermediates and a binomial core network. *Preprint,* `arXiv:1808.07548`, 2018.

[42] Yoav D. Shaul and Rony Seger. The MEK/ERK cascade: From signaling specificity to diverse functions. *BBA-Mol. Cell. Res., special issue on "Mitogen-Activated Protein Kinases: New Insights on Regulation, Function and Role in Human Disease"*, 1773(8):1213–1226, 2007.

[43] Anne Shiu and Bernd Sturmfels. Siphons in chemical reaction networks. *B. Math. Biol.*, 72(6):1448–1463, 2010.

[44] Thapanar Suwanmajo and Jeevanithya Krishnan. Mixed mechanisms of multi-site phosphorylation. *J. R. Soc. Interface*, 12(107), 2015.

[45] Matthew Thomson and Jeremy Gunawardena. The rational parameterisation theorem for multisite post-translational modification systems. *J. Theoret. Biol.*, 261(4):626–636, 2009.

[46] David M. Virshup and Daniel B. Forger. Keeping the beat in the rising heat. *Cell*, 137(4):602–604, 2009.

[47] Xiaojing Yang. Generalized form of Hurwitz-Routh criterion and Hopf bifurcation of higher order. *Appl. Math. Lett.*, 15(5):615–621, 2002.

[48] Günter M. Ziegler. *Lectures on polytopes*, volume 152 of *Graduate Texts in Mathematics*. Springer-Verlag, New York, 1995.

# 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 $\sigma_i \in \mathbb{Z}_{\geq 0}^s$ are distinct and $b_i \neq 0$ for all $i$. A vertex $\sigma_i$ of 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 $\sigma$ of Newt$(f)$, i.e., the cone generated by the outer normal vectors to all supporting hyperplanes of Newt$(f)$ containing the vertex $\sigma$. Finally, for a cone $C$, let int$(C)$ denote the relative interior of the cone.

For an extensive discussion on polytopes and normal cones, see [48].

**Proposition B.2.** *Let $f, g \in \mathbb{R}[x_1, x_2, \ldots x_s]$. Assume that $\alpha$ is a positive vertex of Newt$(f)$, $\beta_+$ is a positive vertex of Newt$(g)$, and $\beta_-$ is a negative vertex of Newt$(g)$. Then, if int$(N_f(\alpha)) \cap$ int$(N_g(\beta_+))$ and int$(N_f(\alpha)) \cap$ int$(N_g(\beta_-))$ are both nonempty, then there exists $x^* \in \mathbb{R}_{>0}^s$ 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 $\sigma_i$ is a positive vertex (respectively, negative vertex) of Newt$(f)$, then there exists $x^* \in \mathbb{R}_{>0}^s$ such that $f(x^*) > 0$ (respectively, $f(x^*) < 0$).*

*Proof.* Let $\sigma_i$ be a vertex of 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 $\sigma_i$ 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 $\sigma_i$. 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, sign$(f(t^{w_1}, t^{w_2}, \ldots, t^{w_s})) = $ sign$(b_i)$. Note that $(t^{w_1}, t^{w_2}, \ldots, t^{w_s}) \in \mathbb{R}_{>0}^s$. $\qquad \square$

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 $C_0 := \text{int}(N_f(\alpha)) \cap \text{int}(N_g(\beta_+))$ and $C_1 := \text{int}(N_f(\alpha)) \cap \text{int}(N_g(\beta_-))$;
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 $\tau_\ell := \inf\{t^* \in \mathbb{R}_{>0} \mid f_\ell(t) > 0 \text{ and } g_\ell(t) > 0 \text{ for all } t > t^*\}$;
5   define $\tau_m := \inf\{t^* \in \mathbb{R}_{>0} \mid f_m(t) > 0 \text{ and } g_m(t) < 0 \text{ for all } t > t^*\}$;
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 $r^* := \text{argmin}\{\left(g_{r\cdot\ell+(1-r)m}(T)\right)^2 \mid r \in [0,1]\}$ (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 $a_+ x^\alpha$ be the term of $f$ corresponding to the vertex $\alpha$ of Newt($f$), and similarly let $b_+ x^{\beta_+}$ (respectively, $b_- x^{\beta_-}$) be the term of $g$ corresponding to the vertex $\beta_+$ (respectively, $\beta_-$) of 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 $f = a_+ x^\alpha + (a_1 x^{\sigma_1} + a_2 x^{\sigma_2} + \cdots + a_d x^{\sigma_d})$, for some exponent vectors $\sigma_i \in \mathbb{Z}^s_{\geq 0}$.

Algorithm 1 terminates: First, $\ell$ and $m$ in line 2 exist by hypothesis. Also, $\tau_\ell$ and $\tau_m$ 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 $\ell$ 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 $t > 0$,

$$
\begin{aligned}
f_{r\ell+(1-r)m}(t) &= a_+ t^{\langle r\ell+(1-r)m, \alpha \rangle} + \left(a_1 t^{\langle r\ell+(1-r)m, \sigma_1 \rangle} + \cdots + a_d t^{\langle r\ell+(1-r)m, \sigma_d \rangle}\right) \\
&> a_+ t^\beta - (|a_1| + |a_2| + \cdots + |a_d|) t^{\beta-\delta} =: \widetilde{f}(t) .
\end{aligned}
\tag{29}
$$

In $\widetilde{f}(t)$, the term $a_+ t^\beta$ 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 $\psi$ 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 $\chi$ 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 $g(x^*) = 0$. $\qquad\square$

# 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 $\hat\kappa^* = (k^*_{\mathrm{cat}}, k^*_{\mathrm{off}}, \ell^*_{\mathrm{off}}) \in \mathbb{R}^3_{>0}$ satisfying the following conditions from Proposition 4.1:

$$\mathfrak{h}_4(\hat\kappa^*; x^*) > 0 \ , \ \ \mathfrak{h}_5(\hat\kappa^*; x^*) > 0 \ , \ \ \mathfrak{h}_6(\hat\kappa^*; x^*) = 0 \ , \ \text{and} \ \ \frac{\partial}{\partial k_{\mathrm{cat}}} \ \ \mathfrak{h}_6(\hat\kappa; x)|_{(\hat\kappa;x)=(\hat\kappa^*;x^*)} \neq 0 \ . \quad (30)$$

**Step One.** Specialize some of the parameters: set $k_{\mathrm{off}} = \ell_{\mathrm{off}} = 1$ 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 $\mathbb{Q}[k_{\mathrm{cat}}, y_1, y_2, y_5, y_8]$ after performing Steps One and Two. Accordingly, our updated goal is to find $(k^*_{\mathrm{cat}}, y^*_1, y^*_2, y^*_5, y^*_8) \in \mathbb{R}^5_{>0}$ 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 $(k^*_{\mathrm{cat}}, y^*_1, y^*_2, y^*_5, y^*_8)$ that accomplishes our updated goal, is guaranteed. To find such a point, we follow Algorithm 1 to obtain $k^*_{\mathrm{cat}} = 729, y^*_1 \approx 16.79978292, y^*_2 \approx 453.5941389, y^*_5 \approx 6.587368051$, 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.