Dynamics of ERK regulation in the processive limit

Carsten Conradi[1], Nida Obatake[2], Anne Shiu[2], and Xiaoxian Tang[2,3]

[1] *HTW Berlin, Germany*
[2] *Department of Mathematics, Texas A&M University, USA*
[3] *School of Mathematics and Systems Science, Beihang University, China*

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Abstract

We consider a model of extracellular signal-regulated kinase (ERK) regulation by dual-site phosphorylation and dephosphorylation, which exhibits bistability and oscillations, but loses these properties in the limit in which the mechanisms underlying phosphorylation and dephosphorylation become processive. Our results suggest that anywhere along the way to becoming processive, the model remains bistable and oscillatory. More precisely, in simplified versions of the model, precursors to bistability and oscillations (specifically, multistationarity and Hopf bifurcations, respectively) exist at all "processivity levels". Finally, we investigate whether bistability and oscillations can exist together.

1 Introduction

We focus on the following question, posed by Rubinstein *et al.* [17], pertaining to a model of extracellular signal-regulated kinase (ERK) regulation (Figure 1):

Question 1.1. *For all* ***processivity levels***[1] $p_k := k_{\text{cat}}/(k_{\text{cat}} + k_{\text{off}})$ *and* $p_\ell := \ell_{\text{cat}}/(\ell_{\text{cat}} + \ell_{\text{off}})$ *close to 1, is the ERK network in Figure 1, bistable and oscillatory?*

The motivation behind this question was given earlier [11, 15, 17], which we summarize here. Briefly, as both p_k and p_ℓ approach 1, the ERK network "limits" to a (fully processive) network that is globally convergent to a unique steady state, and thus lacks bistability and oscillations. As bistability and oscillations may allow networks to act as a biological switch or clock [23], we want to know how far "along the way" to the limit, the network maintains the capacity for these important dynamical properties.

[1] This level is the probability that the enzyme acts processively, that is, adds a second phosphate group after adding the first [19]. A somewhat similar idea, from [20], is the "degree of processivity".

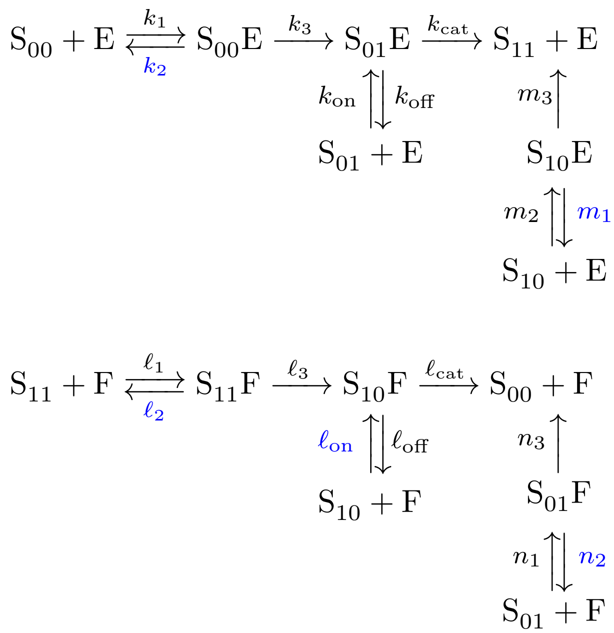

Figure 1: The **ERK network** consists of ERK regulation through dual-site phosphorylation by the kinase MEK (denoted by E) and dephosphorylation by the phosphatase MKP3 (F). Each S_{ij} denotes an ERK phosphoform, with subscripts indicating at which of two sites phosphate groups are attached. Deleting from this network the reactions labeled $k_2, m_1, l_2, \ell_{\text{on}}, n_2$ (in blue) yields the **minimally bistable ERK subnetwork** (the explanation for this name is given before Question 1.2).

A partial result toward resolving Question 1.1 was given by Rubinstein *et al.*, who exhibited, in simulations, oscillations for $p_k, p_\ell \approx 0.97$ [17]. This left open the question of oscillations for $0.97 < p_k, p_\ell < 1$. Our result in this direction is given in Theorem 5.1.

Additional prior results aimed at answering Question 1.1 appeared in work of three of the present authors with Torres [15]. We showed that bistability is preserved when reactions in the ERK network are made irreversible, as long at least one of the reactions labeled by k_{on} and ℓ_{on} is preserved. We therefore give the name "minimally bistable ERK subnetwork" to the network obtained by making all reaction irreversible except the reversible-reaction pair k_{on} and k_{off} (Figure 1). (By symmetry, the network preserving ℓ_{on} and ℓ_{off}, rather than k_{on} and k_{off} is equivalent.) We therefore state the following version of Question 1.1 for bistability:

Question 1.2. *For p_k and p_ℓ close to 1, is the minimally bistable ERK subnetwork, bistable?*

If yes, then by results lifting bistability from subnetworks to larger networks [13], this also answers in the affirmative the part of Question 1.1 pertaining to bistability.

Similarly, for oscillations, we showed that when reactions are made irreversible and also two "intermediates" (namely, $S_{10}E$ and $S_{01}F$) are removed, oscillations are preserved [15]. For this network, called the "reduced ERK network" (Figure 2), we now ask a variant of Question 1.1 for oscillations (see Remark 5.3 for a discussion of the relation between Questions 1.1 and 1.3):

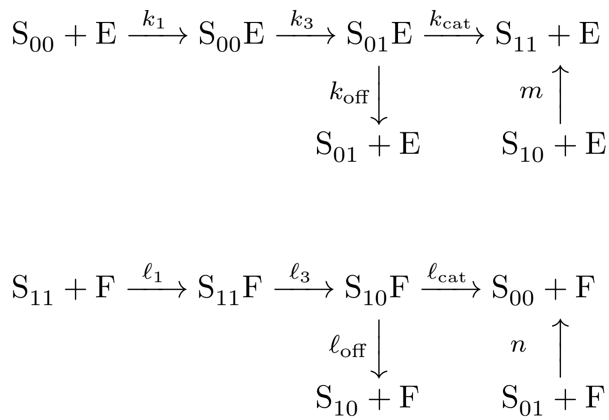

Figure 2: **Reduced ERK network** [15]

Question 1.3. *For p_k and p_ℓ close to 1, is the reduced ERK network, oscillatory?*

Our answers to Questions 1.2 and 1.3 are as follows. For the first question, at *all* processivity levels – not just near 1 – the minimally bistable ERK subnetwork admits multiple steady states, a necessary condition for bistability (Theorem 4.1). Furthermore, computational evidence suggests that indeed we have bistability. Similarly, for the second question, again at (nearly) all processivity levels, the reduced ERK network admits a Hopf bifurcation (Theorem 5.1), a precursor to oscillations.

Finally, we pursue several more questions pertaining to ERK networks. We investigate in the ERK network whether – for some choice of rate constants – bistability and Hopf bifurcations can coexist (see Theorem 6.1). We also pursue a conjecture from [15] on the maximum number of steady states in the minimally bistable ERK network.

Our results fit into related literature as follows. First, as other authors have done for their models of interest [5, 12, 18], we analyze simplified versions of the ERK network obtained by removing intermediate species and/or reactions (in some cases, bistability and oscillations can be "lifted" from smaller networks to larger ones [1, 2, 4, 10, 13]). Also, our proofs harness two results from previous work: a Hopf-bifurcation criterion for the reduced ERK network [15], and a criterion for multistationarity arising from degree theory [6, 9].

This work proceeds as follows. Section 2 provides background on chemical reaction systems and other topics. In Section 3, we give some details about the networks we study. Next, we present our main results on multistationarity and bistability (Section 4), Hopf bifurcations and oscillations (Section 5), and coexistence of bistability and oscillations (Section 6). In Section 7, we prove results on the maximum number of steady states in the minimally bistable ERK network. We conclude with a Discussion in Section 8.

2 Background

This section contains background on chemical reaction systems and their steady states. We also recall how "steady-state parametrizations" can be used to assess whether a network is multistationary (Proposition 3.3).

2.1 Chemical reaction systems

As in [9], our notation closely matches that of Conradi, Feliu, Mincheva, and Wiuf [6]. A **reaction network** G (or, for brevity, *network*) consists of a set of s species $\{X_1, X_2, \ldots, X_s\}$ and a set of m reactions:

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

where each α_{ij} and β_{ij} is a non-negative integer. The **stoichiometric matrix** of G, denoted by N, is the $s \times m$ matrix with $N_{ij} = \beta_{ij} - \alpha_{ij}$. Let $d = s - \operatorname{rank}(N)$. The image of N is the **stoichiometric subspace**, denoted by S. A **conservation-law matrix** of G, denoted by W, is a row-reduced $d \times s$-matrix such that the rows form a basis of the orthogonal complement of S. If there exists a choice of W such that each entry is nonnegative and each column contains at least one nonzero entry (equivalently, each species occurs in at least one nonnegative conservation law), then G is **conservative**.

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 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 a **reaction rate constant**. By considering the rate constants as a vector of parameters $\kappa = (\kappa_1, \kappa_2, \ldots, \kappa_m)$, we have polynomials $f_{\kappa,i} \in \mathbb{Q}[\kappa, x]$, for $i = 1, 2, \ldots, s$. For ease of notation, we often write f_i rather than $f_{\kappa,i}$.

A solution $x(t)$ with nonnegative initial values $x(0) = x^0 \in \mathbb{R}^s_{\geq 0}$ 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 in (1) vanish: $f(x^*) = 0$. We distinguish between **positive steady states** $x^* \in \mathbb{R}^s_{>0}$ and **boundary steady states** $x^* \in \mathbb{R}^s_{\geq 0} \backslash \mathbb{R}^s_{>0}$. A steady state x^* is **nondegenerate** if $\operatorname{Im}\left(\operatorname{Jac}(f)(x^*)|_S\right)$ is the stoichiometric subspace S. (Here, $\operatorname{Jac}(f)(x^*)$ is the Jacobian matrix of f, with respect to x, at x^*.) A nondegenerate steady state is **exponentially stable** if for each of the $\sigma := \dim(S)$ nonzero eigenvalues of $\mathrm{Jac}(f)(x^*)$, the real part is negative.

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) that contains two or more positive steady states (respectively, exponentially stable positive steady states) of (1).

We analyze steady states within a stoichiometric compatibility class, by using conservation laws in place of linearly dependent steady-state equations, as follows. Let $I = \{i_1 < i_2 < \cdots < i_d\}$ denote the set of indices of the first nonzero coordinate of the rows of the 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 polynomial system $f_{c,\kappa} = 0$ coincide with the positive steady states of (1) in the stoichiometric compatibility class (2) defined by the total-constant vector c.

2.2 Steady-state parametrizations

The parametrizations defined below form a subclass of the ones in [9, Definition 3.6] (specifically, we do not use "effective parameters" here).

Definition 2.1. Let G be a network with m reactions, s species, and (row-reduced) conservation-law matrix W. Let $f_{c,\kappa}$ arise from G and W as in (3). A **steady-state parametrization** is a map $\phi : \mathbb{R}^{\hat{m}}_{>0} \times \mathbb{R}^{\hat{s}}_{>0} \to \mathbb{R}^m_{>0} \times \mathbb{R}^s_{>0}$, for some $\hat{m} \leq m$ and $\hat{s} \leq s$, which we denote by $(\hat{\kappa}; \hat{x}) \mapsto \phi(\hat{\kappa}; \hat{x})$, such that:

(i) $\phi(\hat{\kappa}; \hat{x})$ extends the vector $(\hat{\kappa}; \hat{x})$. More precisely, for the natural projection $\pi : \mathbb{R}^m_{>0} \times \mathbb{R}^s_{>0} \to \mathbb{R}^{\hat{m}}_{>0} \times \mathbb{R}^{\hat{s}}_{>0}$, the map $\pi \circ \phi$ is the identity map.

(ii) The image of ϕ equals the following set:

$$\{(\kappa^*; x^*) \in \mathbb{R}^{m+s}_{>0} \mid x^* \text{ is a steady state of the system defined by } G \text{ and } \kappa = \kappa^*\} \ .$$

For such a parametrization ϕ, the **critical function** $C : \mathbb{R}^{\hat{m}}_{>0} \times \mathbb{R}^{\hat{s}}_{>0} \to \mathbb{R}$ is given by:

$$C(\hat{\kappa}; \hat{x}) \quad = \quad (\det \mathrm{Jac}\ f_{c,\kappa})\,|_{(\kappa;x)=\phi(\hat{\kappa};\hat{x})} \ ,$$

where $\mathrm{Jac}(f_{c,\kappa})$ denotes the Jacobian matrix of $f_{c,\kappa}$ with respect to x.

The following result is implied by [9, Theorem 3.12]:

Proposition 2.2 (Multistationarity and critical functions)**.** *Let ϕ be a steady-state parametrization (as in Definition 2.1) for a network G that is conservative and has no boundary steady states in any compatibility class. Let N be the stoichiometric matrix of G.*

(A) **Multistationarity.** *G is multistationary if there exists $(\hat{\kappa}^*; \hat{x}^*) \in \mathbb{R}^{\hat{m}}_{>0} \times \mathbb{R}^{\hat{s}}_{>0}$ such that*

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

(B) **Witness to multistationarity.** *Every $(\hat{\kappa}^*; \hat{x}^*) \in \mathbb{R}^{\hat{m}}_{>0} \times \mathbb{R}^{\hat{s}}_{>0}$ with $\mathrm{sign}(C(\hat{\kappa}^*, x^*)) = (-1)^{\mathrm{rank}(N)+1}$ yields a witness to multistationarity (κ^*, c^*) as follows. Let $(\kappa^*, x^*) = \phi(\hat{\kappa}^*, \hat{x}^*)$. Let $c^* = Wx^*$ (so, c^* is the total-constant vector defined by x^*, where W is the conservation-law matrix). Then, for the mass-action system (1) arising from G and κ^*, there are two or more positive steady states in the stoichiometric compatibility class (2) defined by c^*.*

3 ERK networks

As mentioned in the Introduction, this work primarily concerns two networks, the minimally bistable ERK subnetwork and the reduced ERK network. Here we recall from [15] the ODEs arising from these networks and a Hopf-bifurcation criterion for the reduced ERK network (Proposition 3.3). We also present a steady-state parametrization for the minimally bistable ERK subnetwork (Proposition 3.1).

3.1 Minimally bistable ERK subnetwork

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 1: Assignment of variables to species for the minimally bistable ERK subnetwork.

For the minimally bistable ERK subnetwork, let $x_1, x_2, \ldots, x_{12}$ denote the concentrations of the species in the order given in Table 1. We obtain the following ODE system (1):

$$\begin{aligned}
\dot{x_1} &= -k_1x_1x_2 + \ell_{\mathrm{cat}}x_5 + n_3x_6 &&=: f_1\\
\dot{x_2} &= -k_1x_1x_2 - k_{\mathrm{on}}x_2x_9 - m_2x_{10}x_2 + k_{\mathrm{cat}}x_7 + k_{\mathrm{off}}x_7 + m_3x_8 &&=: f_2\\
\dot{x_3} &= -\ell_1x_3x_{12} - n_1x_3x_9 + \ell_{\mathrm{cat}}x_5 + \ell_{\mathrm{off}}x_5 + n_3x_6 &&=: f_3\\
\dot{x_4} &= \ell_1x_3x_{12} - \ell_3x_4 &&=: f_4\\
\dot{x_5} &= \ell_3x_4 - \ell_{\mathrm{cat}}x_5 - \ell_{\mathrm{off}}x_5 &&=: f_5\\
\dot{x_6} &= n_1x_3x_9 - n_3x_6 &&=: f_6\\
\dot{x_7} &= k_{\mathrm{on}}x_2x_9 + k_3x_{11} - k_{\mathrm{cat}}x_7 - k_{\mathrm{off}}x_7 &&=: f_7\\
\dot{x_8} &= m_2x_2x_{10} - m_3x_8 &&=: f_8\\
\dot{x_9} &= -k_{\mathrm{on}}x_2x_9 - n_1x_3x_9 + k_{\mathrm{off}}x_7 &&=: f_9\\
\dot{x_{10}} &= -m_2x_2x_{10} + \ell_{\mathrm{off}}x_5 &&=: f_{10}\\
\dot{x_{11}} &= k_1x_1x_2 - k_3x_{11} &&=: f_{11}\\
\dot{x_{12}} &= -\ell_1x_3x_{12} + k_{\mathrm{cat}}x_7 + m_3x_8 &&=: f_{12}
\end{aligned} \tag{4}$$

The 3 conservation equations correspond to the total amounts of substrate, kinase E, and phosphatase F, respectively:

$$
\begin{aligned}
x_1 + x_4 + x_5 + x_6 + x_7 + x_8 + x_9 + x_{10} + x_{11} + x_{12} &= S_{\text{tot}} =: c_1 \\
x_2 + x_7 + x_8 + x_{11} &= E_{\text{tot}} =: c_2 \\
x_3 + x_4 + x_5 + x_6 &= F_{\text{tot}} =: c_3.
\end{aligned} \tag{5}
$$

This network admits a steady-state parametrization (Proposition 3.1 below). Another parametrization for this network was given in [15, Section 3.2], involving "effective parameters" (replacing, for instance, $\ell_{\text{cat}}/k_{\text{cat}}$ by a new parameter a_1). That parametrization, however, does not give (direct) access to the rate constants $k_{\text{cat}}, \ell_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}}$ involved in processivity levels. We therefore need a new parametrization, as follows.

Proposition 3.1 (Steady-state parametrization for minimally bistable ERK subnetwork). *For the minimally bistable ERK subnetwork, with rate-constant vector denoted by* $\kappa := (k_1, k_3, k_{\text{cat}}, k_{\text{on}}, k_{\text{off}}, \ell_1, \ell_3, \ell_{\text{cat}}, \ell_{\text{off}}, m_2, m_3, n_1, n_3)$*, a steady-state parametrization is given by:*

$$
\begin{aligned}
\phi : \mathbb{R}^{13}_{>0} \times \mathbb{R}^{3}_{>0} &\to \mathbb{R}^{13}_{>0} \times \mathbb{R}^{12}_{>0} \\
(\kappa;\ x_1, x_2, x_3) &\mapsto (\kappa;\ x_1, x_2, \ldots, x_{12}) ,
\end{aligned}
$$

where

$$
\begin{aligned}
x_4 &= \frac{k_1 k_{\text{cat}} (\ell_{\text{cat}} + \ell_{\text{off}})(k_{\text{on}} x_2 + n_1 x_3) x_1 x_2}{\ell_3 \ell_{\text{cat}} (k_{\text{cat}} k_{\text{on}} x_2 + k_{\text{cat}} n_1 x_3 + k_{\text{off}} n_1 x_3)}, &\quad x_5 &= \frac{k_1 k_{\text{cat}} (k_{\text{on}} x_2 + n_1 x_3) x_1 x_2}{\ell_{\text{cat}} (k_{\text{cat}} k_{\text{on}} x_2 + k_{\text{cat}} n_1 x_3 + k_{\text{off}} n_1 x_3)} \\
x_6 &= \frac{n_1 k_1 k_{\text{off}} x_1 x_2 x_3}{n_3 (k_{\text{cat}} k_{\text{on}} x_2 + k_{\text{cat}} n_1 x_3 + k_{\text{off}} n_1 x_3)}, &\quad x_7 &= \frac{k_1 (k_{\text{on}} x_2 + n_1 x_3) x_1 x_2}{k_{\text{cat}} k_{\text{on}} x_2 + k_{\text{cat}} n_1 x_3 + k_{\text{off}} n_1 x_3}, \\
x_8 &= \frac{k_1 k_{\text{cat}} \ell_{\text{off}} (k_{\text{on}} x_2 + n_1 x_3) x_1 x_2}{\ell_{\text{cat}} m_3 (k_{\text{cat}} k_{\text{on}} x_2 + k_{\text{cat}} n_1 x_3 + k_{\text{off}} n_1 x_3)}, &\quad x_9 &= \frac{k_1 k_{\text{off}} x_1 x_2}{k_{\text{cat}} k_{\text{on}} x_2 + k_{\text{cat}} n_1 x_3 + k_{\text{off}} n_1 x_3}, \\
x_{10} &= \frac{k_1 k_{\text{cat}} \ell_{\text{off}} (k_{\text{on}} x_2 + n_1 x_3) x_1}{\ell_{\text{cat}} m_2 (k_{\text{cat}} k_{\text{on}} x_2 + k_{\text{cat}} n_1 x_3 + k_{\text{off}} n_1 x_3)}, &\quad x_{11} &= \frac{k_1 x_1 x_2}{k_3}, \\
x_{12} &= \frac{k_1 k_{\text{cat}} (\ell_{\text{cat}} + \ell_{\text{off}})(k_{\text{on}} x_2 + n_1 x_3) x_1 x_2}{\ell_{\text{cat}} \ell_1 (k_{\text{cat}} k_{\text{on}} x_2 + k_{\text{cat}} n_1 x_3 + k_{\text{off}} n_1 x_3) x_3}.
\end{aligned} \tag{6}
$$

Proof. Due to the conservation laws (5), it suffices to show that by solving the equations $f_i = 0$ from (4), for all $i \neq 2, 3, 12$, we obtain the expressions in (6). We accomplish this follows. By solving for x_{11} in the equation $f_{11} = 0$, we obtain the desired expression for x_{11}. Next, we solve for x_7 and x_9 in $f_7 = f_9 = 0$, and use the expression for x_{11}, plus the fact that each x_i and each rate constant is positive, to obtain the expressions for x_7 and x_9. Our remaining steps proceed similarly: we use $f_6 = 0$ to obtain x_6, then $f_1 = 0$ for x_5, then $f_{10} = 0$ for x_{10}, then $f_8 = 0$ for x_8, then $f_5 = 0$ for x_4, and finally $f_4 = 0$ for x_{12}. □

3.2 Reduced ERK network

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

The reduced ERK network has 10 rate constants: $k_1, k_3, k_{\text{cat}}, k_{\text{off}}, m, \ell_1, \ell_3, \ell_{\text{cat}}, \ell_{\text{off}}, n$. Letting $x_1, x_2, \ldots, x_{10}$ denote the species concentrations in the order given in Table 2, the resulting mass-action kinetics ODEs are as follows:

$$\begin{aligned}
\dot{x_1} &= -k_1x_1x_2 + nx_6x_8 + \ell_{\text{cat}}x_{10} &&=: f_1 \\
\dot{x_2} &= -k_1x_1x_2 + k_{\text{cat}}x_4 + k_{\text{off}}x_4 &&=: f_2 \\
\dot{x_3} &= k_1x_1x_2 - k_3x_3 &&=: f_3 \\
\dot{x_4} &= k_3x_3 - k_{\text{cat}}x_4 - k_{\text{off}}x_4 &&=: f_4 \\
\dot{x_5} &= mx_2x_7 - \ell_1x_5x_8 + k_{\text{cat}}x_4 &&=: f_5 \\
\dot{x_6} &= -nx_6x_8 + k_{\text{off}}x_4 &&=: f_6 \\
\dot{x_7} &= -mx_2x_7 + \ell_{\text{off}}x_{10} &&=: f_7 \\
\dot{x_8} &= -\ell_1x_5x_8 + \ell_{\text{off}}x_{10} + \ell_{\text{cat}}x_{10} &&=: f_8 \\
\dot{x_9} &= \ell_1x_5x_8 - \ell_3x_9 &&=: f_9 \\
\dot{x_{10}} &= -\ell_{\text{off}}x_{10} + \ell_3x_9 - \ell_{\text{cat}}x_{10} &&=: f_{10}.
\end{aligned} \tag{7}$$

3.3 Hopf-bifurcation criterion for the reduced ERK network

At a **simple Hopf bifurcation**, a single complex-conjugate pair of eigenvalues of the Jacobian matrix crosses the imaginary axis at nonzero speed, while all other eigenvalues remain with negative real parts. If such a bifurcation is supercritical, **oscillations** or periodic orbits are generated [14].

Definition 3.2. The i-th **Hurwitz matrix** of a univariate polynomial $p(\lambda) = b_0\lambda^n + b_1\lambda^{n-1} + \cdots + b_n$ is the following $i \times i$ matrix:

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

where the (k,l)-th entry is b_{2k-l} as long as $0 \le 2k - l \le 2k - l$, and 0 otherwise.

The following result is [15, Proposition 4.1].

Proposition 3.3 (Hopf criterion for reduced ERK)**.** *Consider the reduced ERK network, and let $f_1, f_2, \ldots, f_{10}$ denote the right-hand sides of the resulting ODEs, as in (7). Let $\hat{\kappa} := (k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}})$ and $x := (x_1, x_2, \ldots, x_{10})$. Consider the map[2] $\phi : \mathbb{R}^{3+10}_{>0} \to \mathbb{R}^{10+10}_{>0}$, denoted by $(k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}},\ x_1, x_2, \ldots, x_{10}) \mapsto (k_1, k_3, k_{\text{cat}}, k_{\text{off}}, m, \ell_1, \ell_3, \ell_{\text{cat}}, \ell_{\text{off}}, n,\ x_1, x_2, \ldots, x_{10})$, where*

$$k_1 := \frac{(k_{\text{cat}} + k_{\text{off}})x_4}{x_1x_2} \qquad k_3 := \frac{(k_{\text{cat}} + k_{\text{off}})x_4}{x_3} \qquad m := \frac{\ell_{\text{off}}x_{10}}{x_2x_7} \qquad \ell_1 := \frac{\ell_{\text{off}}x_{10} + k_{\text{cat}}x_4}{x_5x_8}$$

$$\ell_3 := \frac{\ell_{\text{off}}x_{10} + k_{\text{cat}}x_4}{x_9} \qquad \ell_{\text{cat}} := \frac{k_{\text{cat}}x_4}{x_{10}} \qquad n := \frac{k_{\text{off}}x_4}{x_6x_8}.$$

[2] The map ϕ is a steady-state parametrization [15].

Then the following is a univariate, degree-7 polynomial in λ, with coefficients in $\mathbb{Q}(x)[\hat{\kappa}]$:

$$q(\lambda) \ := \ \frac{1}{\lambda^3} \ \det\left(\lambda I - \text{Jac}(f)\right)|_{(\kappa;x)=\phi(\hat{\kappa};x)} \ . \tag{8}$$

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 (8). Then the following are equivalent:

(1) there exists a rate-constant vector $\kappa^* \in \mathbb{R}^{10}_{>0}$ such that the resulting system (7) exhibits a simple Hopf bifurcation, with respect to k_{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

$$\mathfrak{h}_4(\hat{\kappa}^*; x^*) > 0 \ , \ \mathfrak{h}_5(\hat{\kappa}^*; x^*) > 0 \ , \ \mathfrak{h}_6(\hat{\kappa}^*; x^*) = 0 \ , \ \frac{\partial}{\partial k_{\text{cat}}}\mathfrak{h}_6(\hat{\kappa}; x)|_{(\hat{\kappa};x)=(\hat{\kappa}^*;x^*)} \neq 0 \ . \tag{9}$$

Moreover, given $\hat{\kappa}^*$ and x^* as in (2), a simple Hopf bifurcation with respect to k_{cat} occurs at x^* when the rate-constant vector is $\kappa^* := \widetilde{\pi}(\phi(\hat{\kappa}^*; x^*))$. Here, $\widetilde{\pi} : \mathbb{R}^{10}_{>0} \times \mathbb{R}^{10}_{>0} \to \mathbb{R}^{10}_{>0}$ is the natural projection to the first 10 coordinates.

4 Bistability

In this section, we show that, for every choice of processivity levels, the irreversible ERK network is multistationary (Theorem 4.1). We also give evidence suggesting that in fact, when we have multistationarity, we always have bistability (Section 4.2).

4.1 Multistationarity at all processivity levels

Theorem 4.1 (Multistationarity at all processivity levels)**.** *Consider the minimally bistable ERK subnetwork. For every choice of processivity levels $p_k \in (0, 1)$ and $p_\ell \in (0, 1)$, there is a rate-constant vector $(k_1^*, k_3^*, k_{\text{cat}}^*, k_{\text{on}}^*, k_{\text{off}}^*, \ell_1^*, \ell_3^*, \ell_{\text{cat}}^*, \ell_{\text{off}}^*, m_2^*, m_3^*, n_1^*, n_3^*) \in \mathbb{R}^{13}_{>0}$ such that*

1. *$p_k = k_{\text{cat}}^*/(k_{\text{cat}}^* + k_{\text{off}}^*)$ and $p_\ell = \ell_{\text{cat}}^*/(\ell_{\text{cat}}^* + \ell_{\text{off}}^*)$, and*
2. *the resulting system admits multiple positive steady states (in some compatibility class).*

Proof. Let $C(\kappa; \hat{x})$ (where $\hat{x} = (x_1, x_2, x_3)$) denote the critical function of the steady-state parametrization (6) in Proposition 3.1.

By setting $k_{\text{off}} = \ell_{\text{off}} = 1$ and allowing k_{cat} and ℓ_{cat} to be arbitrary positive values, we obtain all processivity levels $p_k = k_{\text{cat}}/(k_{\text{cat}} + k_{\text{off}})$ and $p_\ell = \ell_{\text{cat}}/(\ell_{\text{cat}} + \ell_{\text{off}})$ in $(0, 1)$. Also, the rank of stoichiometric matrix N for this network is 9; hence, $(-1)^{\text{rank}(N)+1} = 1$. So, by Proposition 2.2, it suffices to show that for all $k_{\text{cat}}^* > 0$ and $\ell_{\text{cat}}^* > 0$, the following specialization of the critical function is positive when we further specialize at some choice of $(k_1, k_3, k_{\text{on}}, \ell_1, \ell_3, m_2, m_3, n_1, n_3) \in \mathbb{R}^9_{>0}$, and $\hat{x} \in \mathbb{R}^3_{>0}$:

$$C(\kappa; \hat{x})|_{k_{\text{off}}=\ell_{\text{off}}=1, \ k_{\text{cat}}=k_{\text{cat}}^*, \ \ell_{\text{cat}}=\ell_{\text{cat}}^*} \tag{10}$$

To see that the function (10) can be positive, first note that the denominator of $C(\kappa;\hat{x})|_{k_{\rm off}=\ell_{\rm off}=1}$, shown here, is always positive (all rate constants and x_i's are positive):

$$(k_{\rm cat}k_{\rm on}x_2 + k_{\rm cat}n_1x_3 + n_1x_3)^2\ell_{\rm cat}x_3 \ .$$

(See the supplementary file `minERK-mss-bistab.mw`.) Thus, it suffices to analyze the numerator of $C(\kappa;\hat{x})|_{k_{\rm off}=\ell_{\rm off}=1}$. We denote this numerator by $\widetilde{C}$, and specialize as follows to obtain (see the supplementary file):

$$\widetilde{C}|_{k_1=t^{-1},k_3=t^{-1},k_{\rm on}=1,\ell_1=t,\ell_3=t^{-1},m_2=1,m_3=1,n_1=1,n_3=1,x_1=t,x_2=t,x_3=1} \tag{11}$$

$$= (2k_{\rm cat}^2\ell_{\rm cat}^2 + 2k_{\rm cat}^2\ell_{\rm cat})t^5 \tag{12}$$

$$+ (-4k_{\rm cat}^3\ell_{\rm cat}^2 - 3k_{\rm cat}^3\ell_{\rm cat} + 3k_{\rm cat}^2\ell_{\rm cat}^2 - k_{\rm cat}^3 + 9k_{\rm cat}^2\ell_{\rm cat} + 3k_{\rm cat}\ell_{\rm cat}^2 + 2k_{\rm cat}^2 + 3k_{\rm cat}\ell_{\rm cat})t^4$$

$$+ \text{lower-order terms in } t.$$

Therefore, for all $k_{\rm cat} > 0$ and $\ell_{\rm cat} > 0$, the leading coefficient with respect to t in (12) is positive and so the specialization of $\widetilde{C}$ is positive for sufficiently large t, which yields the desired values for the rate constants shown in (11). □

Remark 4.2. In the proof of Theorem 4.1, the specialization (11) was obtained by viewing $\widetilde{C}$ as a polynomial in which each coefficient is a polynomial in $k_{\rm cat}$ and $\ell_{\rm cat}$, and then analyzing the resulting Newton polytope in a standard way (cf. [15, Lemma B.3]), as follows. We first found a vertex of the polytope whose corresponding coefficient is a positive polynomial (namely, the leading coefficient in (12)). Next, we chose a vector v in the interior of the corresponding cone in the polytope's outer normal fan. Hence, by substituting $t^{v_1}, t^{v_2}, \ldots$ for the variables, we obtained a polynomial that is positive for sufficiently large t.

4.2 Evidence for bistability

Theorem 4.1 states that the minimally bistable ERK network is multistationary at all processivity levels. Multistationarity is a necessary condition for bistability, which is the focus of the original Question 1.2 from the Introduction. Accordingly, we show bistability at many processivity levels with $p_k = p_\ell$ (Proposition 4.4). Furthermore, we give additional evidence for bistability at *all* processivity levels (Remark 4.5), which we state as Conjecture 4.6.

Remark 4.3 (Assessing bistability is difficult)**.** Although there are many criteria for checking whether a network is multistationary, there are relatively few for checking bistability [22]. Moreover, here we consider a more difficult question: does our network exhibit bistability for an infinite family of parameters (rather than a single parameter vector), encompassing all processivity levels? Thus, it is perhaps unsurprising that we obtain only partial results in this direction. Another "infinite" analysis of bistability was performed recently by Tang and Wang, who proved that an infinite family of sequestration networks all are bistable [21].

Proposition 4.4 (Bistability at many processivity levels)**.** *Consider the minimally bistable ERK subnetwork. For each of the following processivity levels:*

$$p_k \ = \ p_\ell \ \in \ \{0.1, 0.2, \ldots, 0.9, \ 0.91, 0.92, \ldots, 0.99\} \ , \tag{13}$$

there is a rate-constant vector $(k_1^*, k_3^*, k_{\rm cat}^*, k_{\rm on}^*, k_{\rm off}^*, \ell_1^*, \ell_3^*, \ell_{\rm cat}^*, \ell_{\rm off}^*, m_2^*, m_3^*, n_1^*, n_3^*) \in \mathbb{R}^{13}_{>0}$ *such that* $p_k = k_{\rm cat}^*/(k_{\rm cat}^* + k_{\rm off}^*)$ *and* $p_\ell = \ell_{\rm cat}^*/(\ell_{\rm cat}^* + \ell_{\rm off}^*)$*, and the resulting system admits multiple exponentially stable positive steady states (in some compatibility class).*

Proof. As in the proof of Theorem 4.1, we achieve each value of $p_k^* = p_\ell^*$, as in (13), by setting $k_{\rm off}^* = \ell_{\rm off}^* = 1$ and $k_{\rm cat}^* = \ell_{\rm cat}^* = p_k^*/(1-p_k^*)$.

Next, we follow the proof of Theorem 4.1 to find a witness to multistationarity. Recall that the specialized numerator of the critical function given in (11), which is a polynomial in $k_{\rm cat}$, $\ell_{\rm cat}$, and t, is positive (indicating multistationarity) for sufficiently large t. That is, there exists a $T \in \mathbb{R}_{>0}$, which depends on the value of $p_k^* = p_\ell^*$, at which the specialized critical function is positive for all $t \geq T$. For each value of $p_k^* = p_\ell^*$, we pick such a positive number T, as follows:

$p_k^* = p_\ell^*$	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
T	3	3	3	3	4	5	7	10	20

$p_k^* = p_\ell^*$	0.91	0.92	0.93	0.94	0.95	0.96	0.97	0.98	0.99
T	22	25	28	33	40	50	66	100	200

It follows, from (11) and Proposition 2.2(B), that with the following rate-constant vector:

$$\begin{aligned}\kappa^* &:= (k_1^*, k_3^*, k_{\rm cat}^*, k_{\rm on}^*, k_{\rm off}^*, \ell_1^*, \ell_3^*, \ell_{\rm cat}^*, \ell_{\rm off}^*, m_2^*, m_3^*, n_1^*, n_3^*) \\ &= (T^{-1}, T^{-1}, p_k^*/(1-p_k^*), 1, 1, T, T^{-1}, p_k^*/(1-p_k^*), 1, 1, 1, 1, 1)\ ,\end{aligned}$$

there are multiple steady states in the compatibility class containing $x^* := \pi(\phi(\kappa^*; 1, T, 1))$, where $\phi : \mathbb{R}^{13}_{>0} \times \mathbb{R}^3_{>0} \to \mathbb{R}^{13}_{>0} \times \mathbb{R}^{12}_{>0}$ is the steady-state parametrization in Proposition 3.1 and $\pi : \mathbb{R}^{13}_{>0} \times \mathbb{R}^{12}_{>0} \to \mathbb{R}^{12}_{>0}$ denotes the canonical projection to the last 12 coordinates.

Finally, for each such x^* (one for each choice of $p_k^* = p_\ell^*$), the stoichiometric compatibility class of x^* contains exactly three positive steady states (arising from the rate-constant vector κ^*); see `minERK-mss-bistab.mw`. Moreover, two of the steady states each have three zero eigenvalues and the remaining eigenvalues having strictly negative real parts (indicating that these two steady states are exponentially stable), and one steady state has a (single) non-zero eigenvalue with positive real part (indicating it is unstable); see the supplementary file. Therefore, we have bistability for each of the processivity levels in (13). □

Proposition 4.4 showed bistability for certain processivity levels with $p_k = p_\ell$. Even when $p_k \neq p_\ell$ (see Remark 4.5), we found – in every instance we examined – bistability.

Remark 4.5 (Bistability at random processivity levels)**.** For the minimally bistable ERK subnetwork, we generated random pairs of processivity levels p_k and p_ℓ between 0 and 1 (Table 3). For all such pairs, following the procedure described in the proof of Proposition 4.4, we found bistability. For details, see the supplementary file `minERK-MSS-bistab.mw`.

In light of Proposition 4.4 and Remark 4.5, we conjecture that, in Theorem 4.1, multistationarity can be strengthened to bistability. In other words, we conjecture that the answer to Question 1.2 is "yes":

p_k	0.01570	0.02229	0.06748	0.2203	0.2268	0.2576	0.2897	0.4613	0.5378
p_ℓ	0.05004	0.3476	0.6011	0.6076	0.9461	0.2263	0.9883	0.4217	0.3770
p_k	0.5893	0.6613	0.6968	0.9076	0.9307	0.9598	0.9771	0.9845	
p_ℓ	0.5289	0.04355	0.1351	0.2668	0.9010	0.6118	0.07128	0.9809	

Table 3: Randomly generated pairs of processivity levels, rounded to four significant digits. At every such pair, the minimally bistable ERK network exhibits bistability (in some compatibility class). Computations are in the supplementary file `minERK-MSS-bistab.mw`.

Conjecture 4.6 (Bistability at all processivity levels)**.** *Consider the minimally bistable ERK subnetwork. For every choice of processivity levels $p_k \in (0,1)$ and $p_\ell \in (0,1)$, there is a rate-constant vector $(k_1^*, k_3^*, k_{\text{cat}}^*, k_{\text{on}}^*, k_{\text{off}}^*, \ell_1^*, \ell_3^*, \ell_{\text{cat}}^*, \ell_{\text{off}}^*, m_2^*, m_3^*, n_1^*, n_3^*) \in \mathbb{R}_{>0}^{13}$ such that $p_k = k_{\text{cat}}^*/(k_{\text{cat}}^* + k_{\text{off}}^*)$ and $p_\ell = \ell_{\text{cat}}^*/(\ell_{\text{cat}}^* + \ell_{\text{off}}^*)$, and the resulting system admits multiple exponentially stable positive steady states (in some compatibility class).*

If Conjecture 4.6 holds, then [13, Theorem 3.1] implies that bistability "lifts" to the original ERK network. In other words, this would answer in the affirmative the original Question 1.1, for bistability.

5 Hopf bifurcations

In this section, we answer Question 1.3 in the affirmative: Theorem 5.1 asserts that a Hopf bifurcation exists for the reduced ERK network at all processivity levels p_k and p_ℓ arbitrarily close to 1 – and in fact for all levels greater than 0.003.

Theorem 5.1 (Hopf bifurcations at all processivity levels)**.** *Consider the reduced ERK network. For all $0.002295 < \epsilon < 1$, there exists a rate-constant vector $\kappa^* = (k_1^*, k_3^*, k_{\text{cat}}^*, k_{\text{off}}^*, m^*, \ell_1^*, \ell_3^*, \ell_{\text{cat}}^*, \ell_{\text{off}}^*, n^*)$ such that*

1. *$p_k = k_{\text{cat}}^*/(k_{\text{cat}}^* + k_{\text{off}}^*) > \epsilon$ and $p_\ell = \ell_{\text{cat}}^*/(\ell_{\text{cat}}^* + \ell_{\text{off}}^*) > \epsilon$, and*
2. *the resulting system (7) admits a simple Hopf bifurcation (with respect to k_{cat}).*

Proof. Fix $0.002295 < \epsilon < 1$. Observe that, for every choice of rate constants for which (a) $k_{\text{cat}}^* > \epsilon/(1-\epsilon) > 0.002295/(1-0.002295) \approx 0.0023$, (b) $\ell_{\text{cat}}^* := t^2 k_{\text{cat}}^*$ (for any choice of $t > 1$), and (c) $k_{\text{off}}^* = \ell_{\text{off}}^* := 1$, we obtain the desired inequalities for p_k and p_ℓ:

$$\epsilon \ < \ \frac{k_{\text{cat}}^*}{k_{\text{cat}}^*+1} \ = \ p_k \ < \ \frac{t^2 k_{\text{cat}}^*}{t^2 k_{\text{cat}}^*+1} \ = \ p_\ell \ . \tag{14}$$

Next, we show that a Hopf bifurcation exists, by verifying the conditions on $\mathfrak{h}_4$, $\mathfrak{h}_5$, and $\mathfrak{h}_6$ (as in Proposition 3.3). First, we show in the supplementary file `redERK-Hopf.mw` that $\mathfrak{h}_4(\hat{\kappa};\ x)$ is a sum of positive terms, and thus $\mathfrak{h}_4(\hat{\kappa};\ x) > 0$ for all $\hat{\kappa} = (k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}}) \in \mathbb{R}_{>0}^3$ and $x \in \mathbb{R}_{>0}^{10}$.

Next, let $(\widehat{\kappa};\ x) := (k^*_{\text{cat}}, 1, 1;\ 1, 1, 1, t^2, 1, t^2, 1/t, 1, t^2, 1)$. We verify (using `Mathematica`) that if $k^*_{\text{cat}} > 0.0023$, then $\mathfrak{h}_5(\hat{\kappa}^*; x) > 0$ for all $t > 0$; see the supplementary file `h5pos.nb`. Fix $k^*_{\text{cat}} > 0.0023$. Substituting $t^* = 1$ into $\mathfrak{h}_6(\widehat{\kappa}^*; x^*)$ yields a positive polynomial (in k^*_{cat}):

$$\begin{aligned}\mathfrak{h}_6(\widehat{\kappa}^*; x^*)|_{t^*=1} = (k^*_{\text{cat}} + 1)^2 \Big(& 31824000{k^*_{\text{cat}}}^{18} + 713988320{k^*_{\text{cat}}}^{17} + 7660517072{k^*_{\text{cat}}}^{16} + 52115784592{k^*_{\text{cat}}}^{15} + 251452795392{k^*_{\text{cat}}}^{14} \\ & + 912214161728{k^*_{\text{cat}}}^{13} + 2574990720896{k^*_{\text{cat}}}^{12} + 5775757031984{k^*_{\text{cat}}}^{11} + 10424374721840{k^*_{\text{cat}}}^{10} \\ & + 15237491111424{k^*_{\text{cat}}}^{9} + 18065664178000{k^*_{\text{cat}}}^{8} + 17318286301088{k^*_{\text{cat}}}^{7} + 13314668410544{k^*_{\text{cat}}}^{6} \\ & + 8093460125184{k^*_{\text{cat}}}^{5} + 3802097816832{k^*_{\text{cat}}}^{4} + 1331324403072{k^*_{\text{cat}}}^{3} + 327072356352{k^*_{\text{cat}}}^{2} \\ & + 50292006912k^*_{\text{cat}} + 3641573376 \Big) .\end{aligned}$$

Also, as $t \to \infty$, the limit of $\mathfrak{h}_6(\hat{\kappa}^*; x^*)$ is $-\infty$. Hence, there exists $t^* > 1$ such that $\mathfrak{h}_6(\widehat{\kappa}^*; x^*) = 0$ (where $x^* = \big(1, 1, 1, t^{*2}, 1, t^{*2}, 1/t^*, 1, t^{*2}, 1\big)$); see the supplementary file `redERK-Hopf.mw`. Finally, we check that $\frac{\partial \mathfrak{h}_6}{\partial k_{\text{cat}}}(\widehat{\kappa}^*; x^*) \neq 0$ whenever $\mathfrak{h}_6(\widehat{\kappa}^*; x^*) = 0$ – we verified this using the `Julia` package `HomotopyContinuation.jl` [3] (see the supplementary file `nondegen-close-to-1.txt`).

Thus, the reduced ERK system admits a Hopf bifurcation at

$$x^* \ := \ (x^*_1, x^*_2, \ldots, x^*_{10}) \ = \ \big(1, 1, 1, t^{*2}, 1, t^{*2}, 1/t^*, 1, t^{*2}, 1\big) \ , \tag{15}$$

when the rate-constant vector is

$$\begin{aligned}\kappa^* \ :=& \ (k^*_1, k^*_3, k^*_{\text{cat}}, k^*_{\text{off}}, m^*, \ell^*_1, \ell^*_3, \ell^*_{\text{cat}}, \ell^*_{\text{off}}, n^*) \\ =& \ \big((k^*_{\text{cat}} + 1)t^{*2}, (k^*_{\text{cat}} + 1)t^{*2}, k^*_{\text{cat}}, 1, t^*, k^*_{\text{cat}} t^{*2} + 1, (k^*_{\text{cat}} t^{*2} + 1)/t^{*2}, k^*_{\text{cat}} t^{*2}, 1, 1\big) \ .\end{aligned} \tag{16}$$

By construction, these rate constants satisfy the conditions (a), (b) (with $t = t^* > 1$), and (c) listed at the beginning of the proof. So, the inequalities (14) hold. □

Remark 5.2. Following the proof of Theorem 5.1, we provide witnesses for the Hopf bifurcation for several values of p_k and p_ℓ in the supplementary file `redERK-Hopf.mw` (under the "First Vertex Analysis" section) for the interested reader. For instance, when $\epsilon = 0.89$, then then the choices $k^*_{\text{cat}} = 9$ and $t^* \approx 124.02$ satisfy the conditions imposed in the proof, and so we obtain, as in (14), the processivity levels $p_k = 0.9$ and $p_\ell \approx 0.999993$. Thus, from (15), there is a Hopf bifurcation at $x^* \approx (1, 1, 1, 15380.68, 1, 15380.68, 0.008, 1, 15380.68, 1)$ when the rate-constant vector is as in (16):

$$\kappa^* \ \approx (153806.78, 153806.78, 9, 1, 124.02, 138427.1, 9.00, 138426.11, 1, 1) \ .$$

Remark 5.3 (Relation to Question 1.1)**.** As noted earlier, Theorem 5.1 addresses Question 1.3, the reduced-ERK version of the original Question 1.1. We focused on the reduced ERK network rather than the original ERK network, because analyzing the original one is computationally challenging.

Nevertheless, we conjecture that Theorem 5.1 "lifts" to the original ERK network. Indeed, to go from the reduced ERK network to the original ERK network, we make some reactions reversible (which is known to preserve oscillations [1]) and add some intermediate complexes (which is conjectured to preserve oscillations [1]). More precisely, we hope for a future result that states that adding intermediates preserves oscillations and Hopf bifurcations, while the "old" rate constants are only slightly perturbed. Such a result would help us to elevate Theorem 5.1 to an answer to Question 1.1 for the original ERK network.

Remark 5.4. The bounds $p_k, p_\ell > 0.002295$ in Theorem 5.1 arose from our choice of specialization in the proof, namely, $(\widehat{\kappa};\ x) := (k^*_{\text{cat}}, 1, 1;\ 1, 1, 1, t^2, 1, t^2, 1/t, 1, t^2, 1)$. Another specialization (that admits a Hopf bifurcation) would give rise to other bounds on p_k and p_ℓ. Nevertheless, as our interest is in p_k and p_ℓ close to 1, our bounds are not restrictive.

Next, we relax the hypothesis $p_k > 0.002295$ in Theorem 5.1 to allow for all values of $p_k > 0$. However, we can not also control p_ℓ at the same time.

Proposition 5.5 (Hopf bifurcations at all p_k). *Consider the reduced ERK network. For every choice of processivity level $p_k \in (0, 1)$, there exists a rate-constant vector $\kappa^* = (k^*_1, k^*_3, k^*_{\text{cat}}, k^*_{\text{off}}, m^*, \ell^*_1, \ell^*_3, \ell^*_{\text{cat}}, \ell^*_{\text{off}}, n^*)$ such that*

1. *$p_k = k^*_{\text{cat}}/(k^*_{\text{cat}} + k^*_{\text{off}})$, and*
2. *the resulting system admits a Hopf bifurcation.*

Moreover, by symmetry of k_{cat} and ℓ_{cat} in the reduced ERK network, we have the analogous result for all choices of p_ℓ.

Proof. As in the proof of Theorem 5.1, we achieve any desired value of $p_k \in (0, 1)$ by setting $k^*_{\text{off}} = 1$ and $k^*_{\text{cat}} = p_k/(1 - p_k)$. Accordingly, consider any $k^*_{\text{cat}} \in \mathbb{R}_{>0}$. We will show, using Proposition 3.3, that there exists $t^* > 0$ such that the reduced ERK network admits a Hopf bifurcation at

$$x^* \ := \ (x^*_1, x^*_2, \ldots, x^*_{10}) \ = \ \left(1, 1, 1, 1/t^{*2}, 1, 1, t^*, 1, 1/t^{*2}, 1\right) \ ,$$

when the rate-constant vector is

$$\begin{aligned}&(k^*_1, k^*_3, k^*_{\text{cat}}, k^*_{\text{off}}, m^*, \ell^*_1, \ell^*_3, \ell^*_{\text{cat}}, \ell^*_{\text{off}}, n^*)\\ &\ = \left((k^*_{\text{cat}}+1)/t^{*2}, (k^*_{\text{cat}}+1)/t^{*2}, k^*_{\text{cat}}, 1, 1/t^*, (t^{*2}+k^*_{\text{cat}})/t^{*2}, (t^{*2}+k^*_{\text{cat}})/t^{*4}, k^*_{\text{cat}}/t^{*2}, 1, 1/t^{*2}\right).\end{aligned}$$

Indeed, we verify in the supplementary file `redERK-Hopf-all-pk-values.mw` that $\mathfrak{h}_4(\widehat{\kappa};\ x) > 0$ and $\mathfrak{h}_5(\widehat{\kappa};\ x) > 0$ for all $\hat{\kappa} = (k_{\text{cat}}, 1, 1) \in \mathbb{R}^3_{>0}$ and $x = (1, 1, 1, x_4, 1, 1, x_7, 1, x_9, 1) \in \mathbb{R}^{10}_{>0}$, and that $\mathfrak{h}_6(\widehat{\kappa}^*;\ x^*) = 0$ for some $t^* > 0$. Finally, in the supplementary file `nondegen-all-process.txt`, we show that $\frac{\partial \mathfrak{h}_6}{\partial k_{\text{cat}}}(\widehat{\kappa}^*; x^*) \neq 0$ whenever $\mathfrak{h}_6(\widehat{\kappa}^*; x^*) = 0$. □

6 Coexistence of bistability and oscillations

Having shown that multistationarity and Hopf bifurcations exist in certain ERK systems for (nearly) all possible processivity levels, we now investigate whether these two dynamical phenomena can occur together. The first question is whether bistability and oscillations can coexist in the same compatibility class (Section 6.1), and then we consider coexistence in distinct compatibility classes (Section 6.2).

6.1 Precluding coexistence in a compatibility class

The next result, which is is not specific to ERK networks, forbids bistability and Hopf bifurcations from occurring in the same compatibility class, when there are up to 3 steady states and certain other conditions are satisfied. These conditions allow us to apply (in the proof) results from degree theory.

Theorem 6.1. *Consider a reaction system (G, κ). Let $\mathcal{S}_c$ be a compatibility class such that (1) the system is dissipative[3] with respect to $\mathcal{S}_c$, and (2) $\mathcal{S}_c$ contains at most 3 steady states and no boundary steady states. Then $\mathcal{S}_c$ does not contain both a simple Hopf bifurcation and two stable steady states.*

Proof. Let W be a $d \times s$ (row-reduced) conservation-law matrix, where d is the number of conservation laws and s is the number of species. Let $f_{c,\kappa}$ be the resulting augmented system.

We examine, for certain x^* in $\mathcal{S}_c$, the coefficient of λ^d in $\det(\lambda I - \mathrm{Jac} f)|_{x=x^*}$. If x^* is a Hopf bifurcation, then (by Yang's criterion [26], restated in [7, Proposition 2.3]) the coefficient is positive. Similarly, if x^* is a stable steady state, then (by the Routh-Hurwitz criterion) the coefficient is positive. Finally, by a straightforward generalization of [25, Proposition 5.3], the coefficient equals $(-1)^{s-d} \det \mathrm{Jac} f_{c,\kappa}|_{x=x^*}$.

Assume for contradiction that $\mathcal{S}_c$ contains a simple Hopf bifurcation $x^{(1)}$ and two stable steady states $x^{(2)}$ and $x^{(3)}$ (and hence no more steady states by hypothesis). Then (by definition [6] and by above) the Brouwer degree of $f_{c,\kappa}$ with respect to $\mathcal{S}_c$ is as follows:

$$\begin{aligned} &\mathrm{sign}\det \mathrm{Jac} f_{c,\kappa}|_{x=x^{(1)}} + \mathrm{sign}\det \mathrm{Jac} f_{c,\kappa}|_{x=x^{(2)}} + \mathrm{sign}\det \mathrm{Jac} f_{c,\kappa}|_{x=x^{(3)}} \\ &\qquad = (-1)^{s-d} + (-1)^{s-d} + (-1)^{s-d} , \end{aligned}$$

which yields a contradiction, as the degree must be ± 1 (see [6]). □

For the minimally bistable ERK subnetwork, Theorem 6.1 implies that, *if the following conjecture holds, Hopf bifurcations and bistability do not coexist in compatibility classes*:

Conjecture 6.2. *For the minimally bistable ERK subnetwork, the maximum number of positive steady states (in any compatibility class, for any choice of rate constants) is 3.*

The maximum number of positive steady states is at most 5 [15], and a version of this conjecture was stated earlier (see [15, Propositions 5.8–5.9 and Conjecture 5.10]). We pursue the conjecture in Section 7.

6.2 Coexistence in distinct compatibility classes

Theorem 6.1 precludes, for certain reaction systems, the coexistence of bistability and a simple Hopf bifurcation in a single compatibility class. Next, for ERK systems, we ask about coexistence in *distinct* compatibility classes.

Question 6.3. *Is it possible in one of the ERK networks (the original one or the minimally bistable ERK subnetwork[4]) to have – for some choice of positive rate constants – 2 stable*

[3] *Dissipative* means that there is a compact subset of $\mathcal{S}_c$ that every trajectory eventually enters; being dissipative is automatic when the network is conservative [6].

[4] The reduced ERK network is not in this list, as it does not admit bistability [15].

steady states in one compatibility class and a simple Hopf bifurcation in another?

As an initial investigation, which hints at a negative answer to Question 6.3, we examine the minimally bistable ERK network (see the supplementary file `min-bistab-ERK-Hopf-and-Bistability.mw`). This network yields a Hopf bifurcation when $k_{\text{on}} = 4.0205$ and the other rate constants are as in [15, Equation (23)] (these non-k_{on} rate constants yield oscillations in the fully irreversible ERK network). However, for this choice of rate constants, there is no bistability (in any compatibility class), which we determined by computing the critical function, much like in the proof of [15, Proposition 4.5].

7 Maximum number of steady states

In this section, we pursue Conjecture 6.2, which states that the maximum number of positive steady states of the minimally bistable ERK subnetwork is 3. The idea is first to reduce to a system of 3 equations in 3 variables (Proposition 7.1) and then, using resultants, to further reduce to a single univariate polynomial (Proposition 7.3).

Our methods are similar to the approach that Wang and Sontag took to analyze the fully distributive, dual-siste phosphorylation system [24]. Namely, we substitute a steady-state parametrization from [15] for the minimally bistable ERK subnetwork into the conservation laws, which yields a polynomial system in only 3 variables. We then show that the maximum number of positive roots of this family of polynomial systems is equal to the maximum number of steady states (as in Conjecture 6.2).

Proposition 7.1. *Consider the family of polynomial systems in x_1, x_2, x_3 given by:*

$$\begin{aligned} c_1 - c_2 - c_3 &= x_1 - x_2 - x_3 + \frac{a_5 a_9 a_{10} x_1 x_2}{a_8 x_2 + a_{13} x_3 + a_4 a_9 a_{13} x_3} + \frac{a_5 a_7 a_{10} x_1 (a_8 x_2 + a_{13} x_3)}{a_1 a_{11} (a_8 x_2 + a_{13} x_3 + a_4 a_9 a_{13} x_3)} \\ &\quad + \frac{a_5 a_{10} x_1 x_2 (a_8 x_2 + a_2 a_7 a_8 x_2 + a_{13} x_3 + a_2 a_7 a_{13} x_3)}{a_1 a_3 a_{12} x_3 (a_8 x_2 + a_{13} x_3 + a_4 a_9 a_{13} x_3)}, \qquad (17) \\ c_2 &= x_2 + \frac{a_5 a_{10} x_1 x_2 (a_8 x_2 + a_{13} x_3)}{a_8 x_2 + a_{13} x_3 + a_4 a_9 a_{13} x_3} + \frac{a_5 a_7 a_{10} x_1 x_2 (a_8 x_2 + a_{13} x_3)}{a_1 (a_8 x_2 + a_{13} x_3 + a_4 a_9 a_{13} x_3)} + a_{10} x_1 x_2 \qquad (18) \\ c_3 &= x_3 + \frac{a_5 a_{10} x_1 x_2 (a_8 x_2 + a_2 a_7 a_8 x_2 + a_{13} x_3 + a_2 a_7 a_{13} x_3)}{a_1 a_3 (a_8 x_2 + a_{13} x_3 + a_4 a_9 a_{13} x_3)} \\ &\quad + \frac{a_5 a_{10} x_1 x_2 (a_8 x_2 + a_{13} x_3)}{a_1 (a_8 x_2 + a_{13} x_3 + a_4 a_9 a_{13} x_3)} + \frac{a_5 a_9 a_{10} a_{13} x_1 x_2 x_3}{a_8 x_2 + a_{13} x_3 + a_4 a_9 a_{13} x_3}, \qquad (19) \end{aligned}$$

where the coefficients a_i and c_i are arbitrary positive real numbers. Then the maximum number of positive roots $x^* \in \mathbb{R}^3_{>0}$, among all such systems, equals the maximum number of positive steady states of the minimally bistable ERK network.

Proof. The equations (17)–(19) are obtained as follows. Using the "effective steady-state function" $h_{c,a}$ from [15, Proposition 3.1], we solve for $x_4, x_5, \ldots, x_{12}$ in terms of x_1, x_2, x_3 (and the a_i's), and then substitute the resulting expressions into the conservation equations (5), except we replace the first conservation equation by the first one minus the sum of the second and third. Now the result follows from the definition of "effective steady-state function" [9, 15]. □

Next, we go from the 3 equations (in x_1, x_2, x_3) in (17)–(19) to 2 equations (in x_2 and x_3), as follows. All 3 equations in (17) are linear in x_1, so we solve each for x_1, obtaining equations of the form $x_1 = \gamma_1(x_2, x_3)$, $x_1 = \gamma_2(x_2, x_3)$, and $x_1 = \gamma_3(x_2, x_3)$, respectively. Now, let $g_1 := \gamma_3 - \gamma_2$ and $g_2 := \gamma_1 - \gamma_2$. These g_i's are polynomials in x_2 and x_3 (with coefficients which are polynomials in the a_i's and c_i's). By construction, and by Proposition 7.1, we immediately obtain the following result:

Proposition 7.2. *Let g_1, g_2, and γ_1 be as above. Then for the system $g_1 = g_2 = 0$ (where the coefficients a_i and c_i are arbitrary positive real numbers), the maximum number of positive roots $(x_2^*, x_3^*) \in \mathbb{R}^2_{>0}$ with $\gamma_1(x_2^*, x_3^*) > 0$, is equal to the maximum number of (positive) steady states of the minimally bistable ERK network.*

Let R be the resultant [8] of g_1 and g_2, with respect to x_2 (this resultant is shown in the supplementary files `maxNUMss.mw` and `resultant.txt`). We apply a standard argument using resultants to obtain the following result:

Proposition 7.3. *Let $(a^*; c^*) = (a_1^*, \ldots, a_{13}^*, c_1^*, c_2^*, c_3^*) \in \mathbb{R}^{16}_{>0}$. Let R be as above. If the univariate polynomial $R|_{(a^*;c^*)}$ has at most 3 roots in the interval $(0, \min\{c_1, c_3\})$, and if for every $x_3^* \in \mathbb{R}_{>0}$, the equation $g_1(x_2, x_3^*)|_{(a^*;c^*)} = 0$ has at most one positive solution for x_2, then system (17), when specialized at $(a^*; c^*)$, has at most 3 positive roots $x^* \in \mathbb{R}^3_{>0}$.*

Proof. By [8, Page 163, Chapter 3, Sec. 6, Proposition 1(i)],

$$R \in \langle g_1, g_2 \rangle \cap \mathbb{Q}[a_1, a_2, \ldots, a_{13}, c_1, c_2, c_3, x_3] \ . \tag{20}$$

By [8, Page 125, Chapter 3, Sec. 2, Theorem 3(i)],

$$\overline{\pi\left(\mathcal{V}\left(g_1, g_2\right)\right)} = \mathcal{V}\left(\langle g_1, g_2 \rangle \cap \mathbb{Q}[a_1, a_2, \ldots, a_{13}, c_1, c_2, c_3, x_3] \right) \ , \tag{21}$$

where $\pi : \mathbb{C}^{18} \to \mathbb{C}^{17}$ denotes the standard projection given by $(a; c; x_3, x_2) \mapsto (a; c; x_3)$, $\mathcal{V}(\cdot)$ denotes zero set over $\mathbb{C}$ of a set of polynomials, and $\overline{S}$ denotes the Zariski closure in $\mathbb{C}^n$ [8, Chapter 4] of a subset $S \subseteq \mathbb{C}^n$. So, by (20) and (21),

$$\overline{\pi\left(\mathcal{V}\left(g_1, g_2\right)\right)} \subseteq \mathcal{V}\left(R\right) \ .$$

Thus, for a given $(a^*; c^*) \in \mathbb{R}^{16}_{>0}$, because $R|_{(a^*;c^*)}$ has at most 3 positive roots x_3 in the interval $(0, \min\{c_1, c_3\})$, it follows that the solutions of the system $g_1|_{(a^*;c^*)} = g_2|_{(a^*;c^*)} = 0$ have up to 3 possibilities for x_3-coordinates in the interval $(0, \min\{c_1, c_3\})$. Next, we use the hypothesis that (for every $x_3^* \in \mathbb{R}_{>0}$) the equation $g_1(x_2, x_3^*)|_{(a^*;c^*)} = 0$ has at most 1 positive solution for x_2, to conclude that $g_1|_{(a^*;c^*)} = g_2|_{(a^*;c^*)} = 0$ has at most 3 positive solutions $(x_2, x_3) \in \mathbb{R}^2_{>0}$ with $x_3 < \min\{c_1, c_3\}$. Thus, by construction of g_1 and g_2 (see the paragraph before Proposition 7.2), the original system (17), when specialized at $(a^*; c^*)$, has at most 3 positive roots $x^* \in \mathbb{R}^3_{>0}$. □

As an example of how we can use Proposition 7.3 to tackle Conjecture 6.2, we next give two corollaries. We hope to pursue this direction more in future work.

Corollary 7.4. *For every choice of $c_1^*, c_2^*, c_3^*, a_9^* \in \mathbb{R}_{>0}$, if all other a_i^*'s are equal to* 1*, then the (specialized at $(a^*; c^*)$) original system* (17) *has at most 3 positive roots $x^* \in \mathbb{R}^3_{>0}$.*

Proof. To apply Proposition 7.3, we first show that the univariate polynomial $R|_{(a^*;c^*)}$ has at most 3 positive roots x_3. When all a_i^*'s except a_9^* are equal to 1, then this specialized resultant (see the supplementary file `maxNUMss.mw`) is as follows:

$$R|_{(a^*;c^*)} \;=\; a_9^* x_3^2 (a_9^* x_3 + 3c_2^* + 3x_3)(C_4 x_3^4 + C_3 x_3^3 + C_2 x_3^2 + C_1 x_3 + C_0) \;, \qquad (22)$$

where

$$C_4 \;=\; 2{a_9^*}^2 + 12 a_9^* \;, \qquad C_0 \;=\; -\,2c_3^*(c_2^* - c_3^*)^2,$$

and $C_1, C_2, C_3 \in \mathbb{Q}[a_9^*; c^*]$. By inspection, $C_4 > 0$ and $C_0 \le 0$, for all $c_1^*, c_2^*, c_3^*, a_9^* \in \mathbb{R}_{>0}$. We consider two cases. If $C_0 = 0$, then $x_3 = 0$ is solution of $R|_{(a^*;c^*)} = 0$, and so (because the "relevant" factor of $R|_{(a^*;c^*)} = 0$ in (22) has degree four) $R|_{(a^*;c^*)} = 0$ has at most 3 positive roots x_3. If $C_0 < 0$, then the sequence C_4, C_3, C_2, C_1, C_0 has at most 3 sign changes, and so, by Descartes' rule of signs, $R|_{(a^*;c^*)} = 0$ has at most 3 positive roots x_3.

Second, we show that for every $x_3^* \in \mathbb{R}_{>0}$, the equation $g_1(x_2, x_3^*)|_{(a^*;c^*)} = 0$ has at most one positive solution for x_2. When all a_i^*'s except a_9^* are equal to 1, we have (see the supplementary file `maxNUMss.mw`):

$$g_1(x_2, x_3^*)|_{(a^*;c^*)} = 3x_2^2 + (a_9^* x_3^* - 3c_2^* + 3c_3^*)x_2 - x_3^*(x_3^* + c_2^* - c_3^*)(a_9^* + 3) \;.$$

Viewing $g_1(x_2, x_3^*)|_{(a^*;c^*)}$ as a polynomial in x_2, the leading coefficient is 3, which is positive. So, by Descartes' rule of signs, it suffices to show that either the constant term is non-positive or the coefficient of x_2 is positive. In other words, we must show that if the constant term is positive, then the coefficient of x_2 is positive. Indeed, if $-x_3(x_3 + c_2^* - c_3^*)(a_9^* + 3) > 0$, then $c_3^* > c_2^*$, and so the coefficient of x_2 is $a_9^* x_3^* - 3c_2^* + 3c_3^* = a_9^* x_3^* + 3(c_3^* - c_2^*) > 0$.

By the above two steps and Proposition 7.3, we conclude that the (specialized at $(a^*; c^*)$) system (17) has at most 3 positive roots $x^* \in \mathbb{R}^3_{>0}$. □

Corollary 7.5. *For every choice of $c_1^*, c_3^* \in \mathbb{R}_{>0}$, if a_9^* and c_2^* are sufficiently large, all other a_i^*'s are equal to the same value b and sufficiently large, and also $b > c_2^*/c_3^* > 1$ and $c_2^* > c_3^* + 1$; then the (specialized at $(a^*; c^*)$) original system* (17) *has at most 3 positive roots $x^* \in \mathbb{R}^3_{>0}$.*

Proof. First, we show that the univariate polynomial $R|_{(a^*;c^*)}$ has at most 3 positive roots x_3. When all a_i^*'s except a_9^* are equal to b, then (see `maxNUMss.mw`) we have:

$$R|_{(a^*;c^*)} \;=\; -\,\Sigma \cdot (C_5 x_3^5 + C_4 x_3^4 + C_3 x_3^3 + C_2 x_3^2 + C_1 x_3 + C_0) \;, \qquad (23)$$

where $\Sigma = b^{17} a_9^* x_3^2 (2bc_2^* + c_2^* + a_9^* b x_3 + 2bx_3 + x_3)$ (which is positive), and

$$C_5 \;=\; 2a_9^* b^5 (b-1)(b+1)(a_9^* b + 2b + 1) \;,$$

$$\begin{aligned} C_1 \;&=\; c_3^*(-a_9^* {c_2^*}^2 - {c_2^*}^2 - 3a_9^* c_2^* c_3^* + 2a_9^* c_1^* c_2^* - 2a_9^* c_1^* c_3^* + 4a_9^* {c_3^*}^2 + c_1^* c_2^* - 2c_1^* c_3^* + c_2^* c_3^* + 2{c_3^*}^2) b^7 \\ &\quad + \text{ lower-order terms in } b \;, \\ &=\; c_3^*(-a_9^* {c_2^*}^2 + \text{ [lower-order terms in } a_9^* \text{ and } c_2^*]) b^7 \;+\; \text{lower-order terms in } b \;, \end{aligned}$$

$$
\begin{aligned}
C_0 &= -c_3^*(b^2+1)(c_2^*-c_3^*)(a_9^*b^4c_3^*-a_9^*b^3c_2^*+a_9^*b^3c_3^*-a_9^*b^2c_3^*-b^3c_2^*+2b^3c_3^*-b^2c_3^*-bc_3^*+c_2^*) \\
&= -b^9c_3^*(b^2+1)(c_2^*-c_3^*)\left(a_9^*b^3(bc_3^*-c_2^*)+ \text{ [lower-order terms in } a_9^*,\, b,\, c_2^*]\right) \ ,
\end{aligned}
$$

and $C_2, C_3, C_4 \in \mathbb{Q}[a_9^*; c^*]$. Assume that a_9^*, b, and c_2^* are sufficiently large positive numbers. Assume also that $b > c_2^*/c_3^* > 1$. Then, by inspection, $C_5 > 0$, $C_1 < 0$, and $C_0 < 0$. So the sequence $C_5, C_4, C_3, C_2, C_1, C_0$ has at most 3 sign changes. Hence, Descartes' rule of signs implies that $R|_{(a^*;c^*)} = 0$ has at most 3 positive roots x_3.

Second, we show that for every $x_3^* \in \mathbb{R}_{>0}$, $g_1(x_2, x_3^*)|_{(a^*;c^*)} = 0$ has at most 1 positive solution for x_2. When all a_i^*'s except a_9^* are equal to b, then (see `maxNUMss.mw`)

$$
\begin{aligned}
g_1(x_2, x_3^*)|_{(a^*;c^*)} &= (b^4+b^3+b^2)x_2^2 \\
&\quad + (a_9^*b^4x_3^*-b^4c_2^*+2b^4c_3^*-b^4x_3^*-b^3c_2^*+b^3c_3^*-b^2c_2^*+b^2x_3^*)x_2 \\
&\quad - b^2x_3^*(a_9^*b^2c_2^*-a_9^*b^2c_3^*+a_9^*b^2x_3^*+b^2c_2^*-2b^2c_3^*+2b^2x_3^*+bc_2^*-bc_3^*+bx_3^*+c_2^*)
\end{aligned}
$$

In particular, the constant term can be rewritten and bounded above as follows, where we use the assumption that $c_2^* > c_3^* + 1$:

$$
\begin{aligned}
& -b^2x_3^*\left([a_9^*b^2][c_2^*-c_3^*+x_3^*+c_2^*/a_9^*]-2b^2c_3^*+2b^2x_3^*+bc_2^*-bc_3^*+bx_3^*+c_2^*\right) \\
< \quad & -b^2x_3^*\left([a_9^*b^2]+ \text{ [lower-order terms in } a_9^*,\, b,\, c_2^*]\right) \ .
\end{aligned}
$$

Thus, if a_9^*, b, and c_2^* are sufficiently large (and $c_2^* > c_3^* + 1$), then the constant term of $g_1(x_2, x_3^*)|_{(a^*;c^*)}$ is negative. Also, the leading coefficient, $b^4 + b^3 + b^2$, is positive. So, there is exactly 1 sign change in the sequence of coefficients, and hence, by Descartes' rule of signs, $g_1(x_2, x_3^*)|_{(a^*;c^*)}$ has at most 1 positive solution.

The above two steps and Proposition 7.3 together imply that the (specialized at $(a^*; c^*)$) system (17) has at most 3 positive roots $x^* \in \mathbb{R}^3_{>0}$. □

Remark 7.6. In the two above proofs, we saw the (specialized) resultants (22) and (23) have some "irrelevant" factors (those that are always positive) and one "relevant" factor, such that the sign of the resultant equals the sign of the relevant factor. This is true for the resultant, even before specialization; see the supplementary file `maxNUMss.mw`.

8 Discussion

The motivating question for this work is Question 1.1, which pertains to the important problem of how bistability and oscillations emerge in ERK networks. We essentially answered this question. What "essentially" means here is that we answered the question for some closely related ERK networks, and only two conjectures (Conjecture 4.6 and see also Remark 5.3) – which we believe to be true – stand in the way of complete answers.

We also pursued two related topics, the coexistence of oscillations and bistability, and the maximum number of positive steady states. We showed that if another conjecture we believe to be true (Conjecture 6.2) holds, then Hopf bifurcations and bistability do not coexist in compatibility classes in the minimally bistable ERK subnetwork. We then pursued

Conjecture 6.2 using resultants, achieving partial results and laying the groundwork for future progress on this conjecture. This question of the maximum number of positive steady states is important – it is one way to measure a network's capacity for processing information – and we would like in the future some easy criterion for computing this number for phosphorylation and other signaling networks.

Finally, our interest in phosphorylation networks is due to their role in mitogen-activated protein kinase (MAPK) cascades, which enable cells to make decisions (to differentiate, proliferate, die, and so on) [16]. We therefore want to understand which types of dynamics MAPK cascades and phosphorylation networks are capable of, as bistability and oscillations may be used by cells to make decisions and process information [23]. For MAPK cascades, to quote from Sun *et al.*, "By adjusting the degree of processivity in our model, we find that the MAPK cascade is able to switch among the ultrasensitivity, bistability, and oscillatory dynamical states" [20]. Our results here are complementary – even while keeping the processivity levels constant (at any amount), the ERK network can switch between a range of dynamical behaviors, from bistability to oscillations via a Hopf bifurcation.

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A Files in the Supporting Information

Table 4 lists the files in the Supporting Information, and the result or section each file supports. All files can be found at the online repository: https://github.com/neeedz/COST

Name	File type	Result or Section
`minERK-MSS-bistab.mw`	`Maple`	Theorem 4.1
`minERK-MSS-bistab.mw`	`Maple`	Section 4.2
`redERK-Hopf.mw`	`Maple`	Theorem 5.1
`h5pos.nb`	`Mathematica`	Theorem 5.1
`nondegen-close-to-1.txt`	`Text*`	Theorem 5.1
`redERK-Hopf-all-pk-values.mw`	`Maple`	Proposition 5.5
`nondegen-all-process.txt`	`Text*`	Proposition 5.5
`min-bistab-ERK-Hopf-and-Bistability.mw`	`Maple`	Section 6.2
`maxNUMss.mw`	`Maple`	Section 7
`resultant.txt`	`Text`	Section 7

Table 4: Supporting Information files and the results they support. Here, `Text*` indicates an output file from using the `Julia` package `HomotopyContinuation.jl` [3].