Dynamics of ERK regulation in the processive limit

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Abstract
We consider a model of extracellular signal-regulated kinase 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.

Keywords Chemical reaction network · Extracellular signal-regulated kinase · Bistability · Oscillation · Hopf bifurcation

Mathematics Subject Classification 92C40 · 92C45

1 Introduction
We focus on the following question, posed by Rubinstein et al. (2016), pertaining to a model of extracellular signal-regulated kinase (ERK) regulation (Fig. 1):

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

\textsuperscript{1} This level is the probability that the enzyme acts processively, that is, adds a second phosphate group after adding the first (Salazar and Höfer 2009). A somewhat similar idea, from Sun et al. (2014), is the "degree of processivity".

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure1.png}
\caption{Schematic representation of the ERK network.
\textbf{A} Catalysis and dephosphorylation via a kinase (K) and phosphatase (P).
\textbf{B} Processes involving cross-talk between Erk1 and Erk2.
\textbf{C} An example of a bistable switch.
\textbf{D} A network with multiple feedback loops.
\textbf{E} Oscillations in the network dynamics.
}\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure2.png}
\caption{Experimental data showing the response of the ERK network to various stimuli.
\textbf{A} Normal response to a stimulus.
\textbf{B} Abnormal response due to a genetic mutation.
\textbf{C} Comparison of the ERK network in different cell types.
}\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure3.png}
\caption{Simulation results for the ERK network under different conditions.
\textbf{A} Simulation of the network in the presence of a competitive inhibitor.
\textbf{B} Simulation of the network with a constant input.
\textbf{C} Simulation of the network under varying environmental conditions.
}\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure4.png}
\caption{Theoretical models of the ERK network.
\textbf{A} A simple model of bistability.
\textbf{B} A model with multiple stable states.
\textbf{C} A model incorporating feedback loops.
}\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure5.png}
\caption{Quantitative analysis of the ERK network dynamics.
\textbf{A} Graphical representation of the bistability threshold.
\textbf{B} Graph showing the oscillation amplitude.
\textbf{C} Graph depicting the relationship between processivity and stability.
}\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure6.png}
\caption{Comparison of the ERK network with other signaling pathways.
\textbf{A} Comparison of bistability in different signaling pathways.
\textbf{B} Comparison of oscillatory behavior in different signaling pathways.
}\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure7.png}
\caption{The significance of bistability and oscillations in cellular signaling.
\textbf{A} Bistability and its role in decision-making.
\textbf{B} Oscillations and their implications for cell cycle control.
}\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure8.png}
\caption{Applications of ERK network models.
\textbf{A} Model for predicting cellular responses to different stimuli.
\textbf{B} Model for understanding cancer cell proliferation.
\textbf{C} Model for studying the effect of drug combinations.
}\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure9.png}
\caption{The future directions for research on the ERK network.
\textbf{A} Potential areas for experimental validation.
\textbf{B} Potential areas for theoretical modeling.
}\end{figure}
Fig. 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 $k_2$, $m_1$, $l_2$, $\ell_{on}$, $n_2$ (in blue) yields the **minimally bistable ERK subnetwork** (the explanation for this name is given before Question 1.2).

The motivation behind this question was given earlier (Futran et al. 2013; Obatake et al. 2019; Rubinstein et al. 2016), which we summarize here. Briefly, as both $p_k$ and $p_\ell$ 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 (Conradi and Shiu 2015). As bistability and oscillations may allow networks to act as a biological switch or clock (Tyson et al. 2008), we want to know how far “along the way” to the limit, the network maintains the capacity for these important dynamical properties.

A partial result toward resolving Question 1.1 was given by Rubinstein et al. (2016), who exhibited, in simulations, oscillations for $p_k, p_\ell \approx 0.97$. 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 (described below).

Additional prior results aimed at answering Question 1.1 appeared in work of three of the present authors with Torres (Obatake et al. 2019). We showed that bistability is preserved when reactions in the ERK network are made irreversible, as long as at least one of the reactions labeled by $k_{on}$ and $\ell_{on}$ 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 $\ell_{on}$ and $\ell_{off}$, 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 $p_\ell$ close to 1, is the minimally bistable ERK subnetwork, bistable?

If yes, then by results lifting bistability from subnetworks to larger networks (Joshi and Shiu 2013), 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 (Obatake et al. 2019). For this network, called the “reduced ERK network” (Figure 2), we now ask a variant of Question 1.1 for oscillations (an affirmative answer to Question 1.3 likely “lifts” to an affirmative answer to Question 1.1; see Remark 5.3):

**Question 1.3** For $p_k$ and $p_\ell$ 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. We also investigate how varying processivity levels affects the range of parameter values that yield multistationarity and also how multistationarity (and thus bistability) is lost as the ERK network limits to a (fully processive) network without bistability. Our numerical observations suggest that as the processivity levels approach 1, the classical S-shaped curve often associated with multistationarity deforms to a steep Hill function (see Figure 5 in Sect. 4.3).

Similarly, for Question 1.3, again at (nearly) all processivity levels, the reduced ERK network admits a Hopf bifurcation (Theorem 5.1), a precursor to oscillations. We also numerically investigate such oscillations (see Figure 6).

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 of Obatake et al. (2019) 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 (Conradi et al. 2020; Giaroli et al. 2019; Sadeghimanesh and Feliu 2019), 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 Banaji 2018; Banaji and Pantea 2018; Cappelletti et al. 2020; Feliu and Wiuf 2013; Joshi and Shiu 2013). Also, our proofs harness two results from previous work: a Hopf-bifurcation criterion for the reduced ERK network (Obatake et al. 2019), and a criterion for multistationarity arising from degree theory (Conradi et al. 2017; Dickenstein et al. 2019).
This work proceeds as follows. Section 2 provides background on chemical reaction systems and other topics. In Sect. 3, we give some details about the networks we study. Next, we present our main results on multistationarity and bistability (Sect. 4), Hopf bifurcations and oscillations (Sect. 5), and coexistence of bistability and oscillations (Sect. 6). In Sect. 7, we prove results on the maximum number of steady states in the minimally bistable ERK network. We conclude with a Discussion in Sect. 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 (Dickenstein et al. 2019), our notation closely matches that of Conradi et al. (2017). 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:

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

where each $\alpha_{ij}$ and $\beta_{ij}$ 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 $d = s - \text{rank}(N)$. 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:

\[
\dot{x} = f(x) := N \cdot \begin{pmatrix}
\kappa_1 x_1^{\alpha_{11}} x_2^{\alpha_{21}} \cdots x_s^{\alpha_{s1}} \\
\kappa_2 x_1^{\alpha_{12}} x_2^{\alpha_{22}} \cdots x_s^{\alpha_{s2}} \\
\vdots \\
\kappa_m x_1^{\alpha_{1m}} x_2^{\alpha_{2m}} \cdots x_s^{\alpha_{sm}}
\end{pmatrix},
\]

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 $x(0) = x^0 \in \mathbb{R}_{\geq 0}^s$ remains, for all positive time, in the following stoichiometric compatibility class with respect to the
**total-constant vector** \( c := Wx^0 \in \mathbb{R}^d \) (Sontag 2001, Lemma II.3):

\[
S_c := \{ x \in \mathbb{R}^s_{\geq 0} \mid Wx = c \}.
\] (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** \( x^* \in \mathbb{R}^s_{\geq 0} \setminus \mathbb{R}^s_{> 0} \). A steady state \( x^* \) is **nondegenerate** if \( \text{Im}(\text{Jac}(f)(x^*))|_{S} \) is the stoichiometric subspace \( S \). (Here, \( \text{Jac}(f)(x^*) \) 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 \( \text{Jac}(f)(x^*) \), 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 \( f_{c,\kappa} : \mathbb{R}^s_{\geq 0} \rightarrow \mathbb{R}^s \) defined by

\[
f_{c,\kappa,i} = f_{c,\kappa}(x)_i := \begin{cases} f_i(x) & \text{if } i \notin I, \\ (Wx - c)_k & \text{if } i = i_k \in I. \end{cases}
\] (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 (Dickenstein et al. 2019, 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 conservation-law matrix \( W \). Let \( f_{c,\kappa} \) arise from \( G \) and \( W \) as in (3). A **steady-state parametrization** is a map \( \phi : \mathbb{R}^m_{> 0} \times \mathbb{R}^s_{> 0} \rightarrow \mathbb{R}^m_{> 0} \times \mathbb{R}^s_{> 0} \), 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 \( \pi : \mathbb{R}^m_{> 0} \times \mathbb{R}^s_{> 0} \rightarrow \mathbb{R}^m_{> 0} \times \mathbb{R}^s_{> 0} \), the map \( \pi \circ \phi \) is the identity map.

(ii) The image of \( \phi \) equals the following set:

\[
\{(\kappa^*; x^*) \in \mathbb{R}^{m+s}_{> 0} \mid x^* \text{ is a steady state of the system defined by } G \text{ and } \kappa = \kappa^* \}.
\]
Table 1 Assignment of variables to species for the 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} | S_{10} E | S_{01} | S_{10} | S_{00} E | S_{11} |

For such a parametrization $\phi$, the **critical function** $C : \mathbb{R}^m_+ \times \mathbb{R}^s_+ \rightarrow \mathbb{R}$ is given by:

$$C(\hat{\kappa}; \hat{x}) = \left( \det \text{Jac}(f_{c,\kappa}) \right) \big|_{(\kappa; x) = \phi(\hat{\kappa}; \hat{x})},$$

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

The following result is implied by (Dickenstein et al. 2019, Theorem 3.12):

**Proposition 2.2** (Multistationarity and critical functions) *Let $\phi$ 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 $(\hat{\kappa}^*; \hat{x}^*) \in \mathbb{R}^m_+ \times \mathbb{R}^s_+$ such that

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

(B) **Witness to multistationarity.** Every $(\hat{\kappa}^*; \hat{x}^*) \in \mathbb{R}^m_+ \times \mathbb{R}^s_+$ with $\text{sign}(C(\hat{\kappa}^*; x^*)) = (-1)^{\text{rank}(N)+1}$ yields a witness to multistationarity $(\kappa^*; c^*)$ 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 $\kappa^*$, 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 Obatake et al. (2019) 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

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):

$$\dot{x}_1 = -k_1 x_1 x_2 + \ell_{\text{cat}} x_5 + n_3 x_6 =: f_1$$
\[ \begin{align*}
x_2 &= -k_{1\text{on}}x_2 - k_{\text{off}}x_9 - m_2x_{10}x_2 + k_{\text{cat}}x_7 + k_{\text{off}}x_7 + m_3x_8 &=: f_2 \\
x_3 &= -\ell_1x_3x_{12} - n_1x_3x_9 + \ell_{\text{cat}}x_5 + \ell_{\text{off}}x_5 + n_3x_6 &=: f_3 \\
x_4 &= \ell_1x_3x_{12} - \ell_3x_4 &=: f_4 \\
x_5 &= \ell_3x_4 - \ell_{\text{cat}}x_5 - \ell_{\text{off}}x_5 &=: f_5 \\
x_6 &= n_1x_3x_9 - n_3x_6 &=: f_6 \\
x_7 &= k_{\text{on}}x_2x_9 + k_{3\text{cat}}x_{11} - k_{\text{cat}}x_7 - k_{\text{off}}x_7 &=: f_7 \\
x_8 &= m_2x_2x_{10} - m_3x_8 &=: f_8 \\
x_9 &= -k_{\text{on}}x_2x_9 - n_1x_3x_9 + k_{\text{cat}}x_7 &=: f_9 \\
x_{10} &= -m_2x_2x_{10} + \ell_{\text{off}}x_5 &=: f_{10} \\
x_{11} &= k_{1\text{on}}x_2 - k_3x_{11} &=: f_{11} \\
x_{12} &= -\ell_1x_3x_{12} + k_{\text{cat}}x_7 + m_3x_8 &=: f_{12} \quad (4)
\end{align*} \]

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

\[
\begin{align*}
x_1 + x_4 + x_5 + x_6 + x_7 + x_8 + x_9 + x_{10} + x_{11} + x_{12} &= S_{\text{tot}} =: c_1 \\
x_2 + x_7 + x_8 + x_{11} &= E_{\text{tot}} =: c_2 \\
x_3 + x_4 + x_5 + x_6 &= F_{\text{tot}} =: c_3. \quad (5)
\end{align*}
\]

This network admits a steady-state parametrization (Proposition 3.1 below). Another parametrization for this network was given in (Obatake et al. 2019, Sect. 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:

\[
\phi : \mathbb{R}^{13}_{>0} \times \mathbb{R}^3_{>0} \to \mathbb{R}^{13}_{>0} \times \mathbb{R}^{12}_{>0} \\
(\kappa; x_1, x_2, x_3) \mapsto (\kappa; x_1, x_2, \ldots, x_{12}),
\]

where

\[
\begin{align*}
x_4 &= \frac{k_1k_{\text{cat}}(\ell_{\text{cat}}+\ell_{\text{off}})(k_{\text{on}}x_2+n_1x_3)x_1x_2}{\ell_3n_1k_{\text{on}}x_2+n_1k_{\text{off}}x_2} =: k_1k_{\text{cat}}(k_{\text{on}}x_2+n_1x_3)x_1x_2 \\
x_6 &= \frac{n_3(k_{\text{cat}}x_9+n_1x_3+n_{\text{off}}x_1x_3)}{k_1k_{\text{cat}}n_{\text{off}}(k_{\text{on}}x_2+n_1x_3)x_1x_2} =: k_1k_{\text{cat}}(k_{\text{on}}x_2+n_1x_3)x_1x_2 \\
x_8 &= \frac{k_1k_{\text{cat}}(k_{\text{on}}x_2+n_1x_3)x_1x_2}{k_1k_{\text{cat}}n_{\text{off}}(k_{\text{on}}x_2+n_1x_3)x_1x_2} =: k_1k_{\text{cat}}(k_{\text{on}}x_2+n_1x_3)x_1x_2 \\
x_{10} &= \frac{k_1k_{\text{cat}}(k_{\text{on}}x_2+n_1x_3)x_1x_2}{k_1k_{\text{cat}}(k_{\text{on}}x_2+n_1x_3)x_1x_2} =: k_1k_{\text{cat}}(k_{\text{on}}x_2+n_1x_3)x_1x_2 \\
x_{12} &= \frac{k_1k_{\text{cat}}(k_{\text{on}}x_2+n_1x_3)x_1x_2}{k_1k_{\text{cat}}(k_{\text{on}}x_2+n_1x_3)x_1x_2} =: k_1k_{\text{cat}}(k_{\text{on}}x_2+n_1x_3)x_1x_2.
\end{align*}
\]
Table 2  Assignment of variables to species for the reduced ERK network in Fig. 2

| x_1 | x_2 | x_3 | x_4 | x_5 | x_6 | x_7 | x_8 | x_9 | x_10 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| S_00 | E | S_00E | S_01E | S_11 | S_01 | S_10 | F | S_11F | S_10F |

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 as 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 \( x_{12} \). \( \square \)

3.2 Reduced ERK network

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:

\[
\begin{align*}
\dot{x}_1 &= -k_1 x_1 x_2 + n x_6 x_8 + \ell_{\text{cat}} x_{10} =: f_1 \\
\dot{x}_2 &= -k_1 x_1 x_2 + k_{\text{cat}} x_4 + k_{\text{off}} x_4 =: f_2 \\
\dot{x}_3 &= k_1 x_1 x_2 - k_3 x_3 =: f_3 \\
\dot{x}_4 &= k_3 x_3 - k_{\text{cat}} x_4 - k_{\text{off}} x_4 =: f_4 \\
\dot{x}_5 &= m x_2 x_7 - \ell_1 x_5 x_8 + k_{\text{cat}} x_4 =: f_5 \\
\dot{x}_6 &= -n x_6 x_8 + k_{\text{off}} x_4 =: f_6 \\
\dot{x}_7 &= -m x_2 x_7 + \ell_{\text{off}} x_{10} =: f_7 \\
\dot{x}_8 &= -\ell_1 x_5 x_8 + \ell_{\text{off}} x_{10} + \ell_{\text{cat}} x_{10} =: f_8 \\
\dot{x}_9 &= \ell_1 x_5 x_8 - \ell_3 x_9 =: f_9 \\
\dot{x}_{10} &= -\ell_{\text{off}} x_{10} + \ell_3 x_9 - \ell_{\text{cat}} x_{10} =: f_{10}.
\end{align*}
\]

(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. Such a bifurcation generates oscillations or periodic orbits (see, e.g., the book of Kuznetsov (1995)).
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:

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

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

The following result is (Obatake et al. 2019, 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 $\hat{k} := (k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}})$ and $x := (x_1, x_2, \ldots, x_{10})$. Consider the map\footnote{The map $\phi$ is a steady-state parametrization (Obatake et al. 2019).} $\phi : \mathbb{R}^{3+10} \rightarrow \mathbb{R}^{10+10}$, 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 $x_{10} \neq 0$.

\[
\begin{align*}
 k_1 & := \frac{(k_{\text{cat}} + k_{\text{off}})x_4}{x_1x_2}, &
 k_3 & := \frac{(k_{\text{cat}} + k_{\text{off}})x_4}{x_3}, &
 m & := \frac{\ell_{\text{off}}x_{10}}{x_2x_7}, \\
 \ell_1 & := \frac{\ell_{\text{off}}x_{10} + k_{\text{cat}}x_4}{x_5x_8}, &
 \ell_3 & := \frac{\ell_{\text{off}}x_{10} + k_{\text{cat}}x_4}{x_9}, &
 \ell_{\text{cat}} & := \frac{k_{\text{cat}}x_4}{x_{10}}, \\
 n & := \frac{k_{\text{off}}x_4}{x_6x_8}.
\end{align*}
\]

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

\[
q(\lambda) := \frac{1}{\lambda^3} \det (\lambda I - \text{Jac}(f))|_{(x; x) = \phi(\hat{k}; x)}.
\]

(8)

Now let $h_i$, 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:

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

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

\[
\begin{align*}
 h_4(\hat{k}^*; x^*) & > 0, &
 h_5(\hat{k}^*; x^*) & > 0, &
 h_6(\hat{k}^*; x^*) & = 0, &
 \frac{\partial}{\partial k_{\text{cat}}} h_6(\hat{k}; x)|_{(\hat{k}; x) = (\hat{k}^*; x^*)} & \neq 0.
\end{align*}
\]

(9)

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

In this section, we show that, for every choice of processivity levels, the minimally bistable ERK network is multistationary (Theorem 4.1). We also give evidence suggesting that in fact, when we have multistationarity, we always have bistability (Sect. 4.2). Finally, we investigate multistationarity numerically for processivity levels close to 1 (Sect. 4.3).

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 \((k_1^*, k_2^*, k_{cat}^*, k_{on}^*, k_{off}^*, \ell_1^*, \ell_3^*, \ell_{cat}^*, \ell_{off}^*, m_2^*, m_3^*, n_1^*, n_3^*) \in \mathbb{R}_{>0}^{13}\) such that

1. \( p_k = k_{cat}^*/(k_{cat}^* + k_{off}^*) \) and \( p_\ell = \ell_{cat}^*/(\ell_{cat}^* + \ell_{off}^*) \), 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 \( k_{off}^* = \ell_{off}^* = 1 \) and allowing \( k_{cat}^* \) and \( \ell_{cat}^* \) to be arbitrary positive values, we obtain all processivity levels \( p_k = k_{cat}^*/(k_{cat}^* + k_{off}^*) \) and \( p_\ell = \ell_{cat}^*/(\ell_{cat}^* + \ell_{off}^*) \) 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 \( k_{cat}^* > 0 \) and \( \ell_{cat}^* > 0 \), the following specialization of the critical function is positive when we further specialize at some choice of \((k_1, k_3, k_{on}, \ell_1, \ell_3, m_2, m_3, n_1, n_3) \in \mathbb{R}_{>0}^9\), and \( \hat{x} \in \mathbb{R}_{>0}^3 \):

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

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

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

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

\[
\tilde{C}|_{k_1=t^{-1}, k_3=t^{-1}, k_{on}=1, \ell_1=t, \ell_3=t^{-1}, m_2=1, m_3=1, n_1=1, n_3=1, x_1=t, x_2=t, x_3=1} = (2k_{cat}^2\ell_{cat}^2 + 2k_{cat}^2\ell_{cat})t^5
\]

\[
+ (-4k_{cat}^2\ell_{cat}^2 - 3k_{cat}^2\ell_{cat} + 3k_{cat}^2\ell_{cat}^2 - k_{cat}^3 + 9k_{cat}^2\ell_{cat})t^4 + \text{lower-order terms in } t .
\]
Therefore, for all $k_{\text{cat}} > 0$ and $\ell_{\text{cat}} > 0$, the leading coefficient with respect to $t$ in (12) is positive and so the specialization of $\tilde{C}$ is positive for sufficiently large $t$, which yields the desired values for the rate constants shown in (11).

\textbf{Remark 4.2} In the proof of Theorem 4.1, the specialization (11) was obtained by viewing $\tilde{C}$ as a polynomial in which each coefficient is a polynomial in $k_{\text{on}}$, $k_{\text{cat}}$ and $\ell_{\text{cat}}$, and then analyzing the resulting Newton polytope in a standard way (cf. Obatake et al. 2019, LemmaB.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 $v$ (specifically, $v = [1, 1, 0, -1, -1, 1, -1, 0, 0, 0, 0]$) in the interior of the corresponding cone in the polytope’s outer normal fan. So, by substituting $k_{\text{on}} = 1$ and $t^{\nu_1}, t^{\nu_2}, \ldots$ for the variables $x_1, x_2, x_3, k_1, k_3, \ell_1, \ell_3, m_2, m_3, n_1, n_3$, the resulting polynomial is positive for large $t$.

\subsection*{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 \textit{all} processivity levels (Remark 4.5), which we state as Conjecture 4.6.

\textbf{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 (Torres and Feliu 2019). 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 (2019), who proved that an infinite family of sequestration networks all are bistable.

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

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

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).

\textbf{Proof} As in the proof of Theorem 4.1, we achieve each value of $p^*_k = p^*_\ell$, as in (13), by setting $k^*_{\text{off}} = \ell^*_{\text{off}} = 1$ and $k^*_{\text{cat}} = \ell^*_{\text{cat}} = p^*_k/(1 - p^*_k)$. 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 $k_{\text{cat}}$, $\ell_{\text{cat}}$, 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:

$$\kappa^* := \left( k_1^*, k_3^*, k_{\text{cat}}^*, k_{\text{on}}^*, k_{\text{off}}^*, \ell_1^*, \ell_3^*, \ell_{\text{cat}}^*, \ell_{\text{off}}^*, m_2^*, m_3^*, n_1^*, n_3^* \right) = \left( T^{-1}, T^{-1}, p_k^*/(1 - p_k^*), 1, 1, T, T^{-1}, p_\ell^*/(1 - p_\ell^*), 1, 1, 1, 1 \right),$$

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

**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 $p_\ell \in (0, 1)$,
Table 3  Randomly generated pairs of processivity levels, rounded to four significant digits

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

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.

**Fig. 3**  Numerical investigation of multistationarity as $p_\ell \to 1$ (for $p_k = 0.1$; see Sect. 4.3.1 and Appendix B for figure setup and generation). An increase of $p_\ell$ leads to a (small) decrease of $\tilde{c}_1$ at $\tilde{c}_2 \approx 1.25$ (display a) and to a larger multistationarity interval (from approximately $0.99 \leq \tilde{c}_2 \leq 1.01$ to $0.92 \leq \tilde{c}_2 \leq 1.02$) (display b).

If Conjecture 4.6 holds, then (Joshi and Shiu 2013, 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.

### 4.3 Numerical investigation for processivity levels near 1

In this subsection, we numerically investigate multistationarity of the minimally bistable ERK network, for processivity levels close to 1. Specifically, we examine how processivity levels near 1 affect the S-shaped steady-state curves (as in DiStefano III 2013, Figure 9.6) usually associated with multistationarity. We focus in particular on the concentration of the fully phosphorylated substrate ($x_{12}$), as this species is arguably the most interesting in our signaling network. Indeed, this substrate is generally further processed by other signaling modules.
Fig. 4 Numerical investigation of multistationarity as $p_k \to 1$ (for $p_\ell = 0.9$). An increase in $p_k$ leads to a substantial decrease in $\tilde{x}_{12}/\tilde{c}_1$ at $\tilde{c}_2 \approx 1.25$ (display a) and a smaller multistationarity interval (from $0.992 \leq \tilde{c}_2 \leq 1.01$ to $1 \leq \tilde{c}_2 \leq 1.007$) (displays b–c)

4.3.1 Setup for Figs. 3–5

Figure 3–5 were generated by numerical continuation using Matlab and Matcont. Further details on how we obtained these figures are in Appendix B. In particular, parameter values, total concentrations, and initial conditions were obtained by equation (14) and also (in the appendix) (25)–(26) and the values in Tables 6–7. In all figures, the x-axis is the relative total amount of kinase ($c_2/\tilde{c}_2$ obtained in step (iii) of the procedure described in the appendix), and the y-axis is the relative amount of fully phosphorylated substrate ($\tilde{x}_{12}/\tilde{c}_1$), also obtained in step (iii)). The reason for examining relative (rather than actual) amounts is that, as $p_k$ and/or $p_\ell$ approach 1, certain total amounts differ by orders of magnitude, and so it is more meaningful to compare values relative to a reference point.

4.3.2 Results

Figure 3 shows that, for $p_k = 0.1$ and various values of $p_\ell$, we obtain classical S-shaped curves often associated with multistationarity. We also see that increasing $p_\ell$ alone has only a modest effect on the curve: at the relative total concentration $\tilde{c}_2 \approx 1.25$, the fraction of fully phosphorylated substrate $\tilde{x}_{12}/\tilde{c}_1$ (at steady state) decreases but only by a small amount (see Figure 3a).

Next, we investigate the interval of values of $c_2/\tilde{c}_2$ at which multistationarity occurs, which we call the multistationarity interval. We see in Figure 3b (which is a “zoomed in” version of Figure 3a) that as $p_\ell$ increases (with $p_k = 0.1$), the multistationarity interval enlarges (see the caption of Figure 3b). We can view the size of this interval as a measure of the robustness of multistationarity with respect to fluctuations of the total amount of kinase. Hence, Figure 3 motivates us to conjecture that increasing only one processivity level leads to increased robustness of multistationarity, as follows: When one processivity level is fixed and close to 0, increasing the other processivity level leads to a larger multistationarity interval.

Next, we fix $p_\ell$ at a high value (namely, $p_\ell = 0.9$) and increase $p_k$ (see Figure 4). Again, increasing $p_k$ reduces the fraction of fully phosphorylated substrate $\tilde{x}_{12}/\tilde{c}_1$ at
Fig. 5 Numerical investigation of multistationarity for $p_k = p_\ell$ close to 1. An increase in $p_k$ and $p_\ell$ leads to a decrease in $\frac{c_1}{c_2}$ at $\frac{c_2}{c_1} \approx 1.25$ (display a). Also, when there is multistationarity, the values of $\frac{c_1}{c_2}$ at all three steady states decrease (possibly approaching 0) as $p_k$ and $p_\ell$ approach 1. (display b). Finally, as $p_k$ and $p_\ell$ approach 1, the multistationarity interval becomes so small that the curve approaches a step function (displays a–c).

Finally, in Figure 5, we investigate values of $p_k = p_\ell$ close to 1. Now the multistationarity interval becomes vanishingly small (see, in particular, Figure 5c), leading to a steady-state function that approaches a steep Hill function. We conjecture that this phenomenon is the norm: As both processivity levels approach 1, the length of the multistationarity interval approaches 0.

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 $\kappa^* = (k_1^*, k_3^*, k_{\text{cat}}^*, k_{\text{off}}^*, m^*, \ell_{\text{cat}}^*, \ell_{\text{off}}^*, n^*)$ 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 $k_{\text{cat}}$).
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) $\ell_{\text{cat}}^* := t^2k_{\text{cat}}^*$ (for any choice of $t > 1$), and (c) $k_{\text{off}}^* = \ell_{\text{off}}^* := 1$, we obtain the desired inequalities for $p_k$ and $p_\ell$:

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

(15)

Next, we show that a Hopf bifurcation exists, by verifying the conditions on $h_4$, $h_5$, and $h_6$ (as in Proposition 3.3). First, we show in the supplementary file redERK-Hopf.mw that $h_4(\kappa; x)$ is a sum of positive terms, and thus $h_4(\kappa; x) > 0$ for all $\kappa = (k_{\text{cat}}, k_{\text{off}}, \ell_{\text{off}}) \in \mathbb{R}^3_0$ and $x \in \mathbb{R}^{10}_0$.

Next, let $(\kappa; x) := (k_{\text{cat}}^*, 1, 1, 1, 1, t^2, 1, t^2, 1, t^2, 1)$. We verify (using Mathematica) that if $k_{\text{cat}}^* > 0.0023$, then $h_5(\kappa^*; x) > 0$ for all $t > 0$; see the supplementary file $h5pos.nb$. Fix $k_{\text{cat}}^* > 0.0023$. Substituting $t^* = 1$ into $h_6(\kappa^*; x^*)$ yields a positive polynomial (in $k^*$):

$$
h_6(\kappa^*; x^*)|_{t^*=1} = (k_{\text{cat}}^* + 1)^2 \left( 31824000k_{\text{cat}}^{18} + 713988320k_{\text{cat}}^{17} + 7660517072k_{\text{cat}}^{16} + 52115784592k_{\text{cat}}^{15} + 251452795392k_{\text{cat}}^{14} + 912214161728k_{\text{cat}}^{13} + 2574990720896k_{\text{cat}}^{12} + 15237491111424k_{\text{cat}}^{9} + 18065666417800k_{\text{cat}}^{8} + 1731828630188k_{\text{cat}}^{7} + 1314668410544k_{\text{cat}}^{6} + 8093460125184k_{\text{cat}}^{5} + 3802097816832k_{\text{cat}}^{4} + 131324403072k_{\text{cat}}^{3} + 327072356352k_{\text{cat}}^{2} + 50292006912k_{\text{cat}} + 3641573736 \right).
$$

Also, as $t \to \infty$, the limit of $h_6(\kappa^*; x^*)$ is $-\infty$. Hence, there exists $t^* > 1$ such that $h_6(\kappa^*; x^*) = 0$ (where $x^* = (1, 1, 1, t^{*2}, 1, t^{*2}, 1/t^*, 1, t^{*2}, 1)$); see the supplementary file redERK-Hopf.mw. Finally, we check that $\frac{\partial h_6}{\partial k_{\text{cat}}}(\kappa^*; x^*) \neq 0$ whenever $h_6(\kappa^*; x^*) = 0$ — we verified this using the Julia package HomotopyContinuation.jl (Breiding and Timme 2018) (see the supplementary file nondegen-close-to-1.txt).

Thus, the reduced ERK system admits a Hopf bifurcation at

$$
x^* := (x_1^*, x_2^*, \ldots, x_{10}^*) = (1, 1, 1, t^{*2}, 1, t^{*2}, 1/t^*, 1, t^{*2}, 1),
$$

(16)

when the rate-constant vector is

$$
\kappa^* := (k_1^*, k_2^*, k_{\text{cat}}^*, k_{\text{off}}^*, m^*, \ell_1^*, \ell_2^*, \ell_{\text{cat}}^*, \ell_{\text{off}}^*, n^*)
$$

$$
= \left( (k_{\text{cat}}^* + 1)t^{*2}, (k_{\text{cat}}^* + 1)t^{*2}, k_{\text{cat}}^*, 1, t^*, k_{\text{cat}}^*t^{*2} + 1, (k_{\text{cat}}^*t^{*2} + 1)/t^{*2}, k_{\text{cat}}^*t^{*2}, 1, 1 \right).
$$

(17)

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 (15) 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 $p_\ell$ in the supplementary file `redERK-Hopf.mw` (under the “First Vertex Analysis” section) for the interested reader. For instance, when $\epsilon = 0.89$, 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 (15), the processivity levels $p_k = 0.9$ and $p_\ell \approx 0.999993$. Thus, from (16), there is a Hopf bifurcation at $x^* \approx (1, 1, 15380.68, 1, 15380.68, 0.008, 1, 15380.68, 1)$ when the rate-constant vector is as in (17):

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

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 (Banaji 2018)) and add some intermediate complexes (which is conjectured to preserve oscillations (Banaji 2018)). 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. An approach to achieving such a result is to use the results of Feliu et al. (2019) to write the reduced system as a limiting case of the original system, where some parameter goes to zero, and then give an argument like that in (Hell and Rendall 2016, §3).

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, $(\tilde{\kappa}; x) := (k^*_\text{cat}, 1, 1, 1, 1, t^*, 1, t^2, 1, t, 1, 1)$. Another specialization (that admits a Hopf bifurcation) would give rise to other bounds on $p_k$ and $p_\ell$. Nevertheless, as our interest is in $p_k$ and $p_\ell$ 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 cannot also simultaneously control $p_\ell$.

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 $\kappa^* = (k^*_1, k^*_2, k^*_\text{cat}, k^*_\text{off}, m^*, \ell^*_1, \ell^*_2, \ell^*_3, \ell^*_\text{cat}, \ell^*_\text{off}, n^*)$ 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 $k_{\text{cat}}$ and $\ell_{\text{cat}}$ in the reduced ERK network, we have the analogous result for all choices of $p_\ell$.

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
For the reduced ERK network, oscillations in $x_5$ arising from three pairs of processivity levels $(p_k, p_\ell)$. The rate-constant vectors $\kappa^*$ were obtained from (17), using the values in Table 4. The initial conditions were chosen to be close to—and in the same compatibility class as—the corresponding Hopf bifurcation $x^*$ from (16) (using the values in Table 4); specifically, we perturbed $x^*$ by adding 0.05 to $x^*_5$ and subtracting 0.05 from $x^*_6$.

Indeed, we verify in the supplementary file redERK-Hopf-all-pk-values.mw that $h_4(\hat{\kappa}; x) > 0$ and $h_5(\hat{\kappa}; x) > 0$ 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 $h_6(\hat{\kappa}^*; x^*) = 0$ for some $t^* > 0$. Finally, in the supplementary file nondegen-all-process.txt, we show that $\frac{\partial h_6}{\partial k_{\text{cat}}}(\hat{\kappa}^*; x^*) \neq 0$ whenever $h_6(\hat{\kappa}^*; x^*) \neq 0$.

We end this section with a numerical investigation into the effect of processivity levels on oscillations arising from the Hopf bifurcations analyzed above. Again we focus on the concentration of the fully phosphorylated substrate, in this case $x_5$. We see in Figure 6 that indeed processivity levels have a large effect on the dynamics: as $p_k$ and $p_\ell$ approach 1, the amplitude decreases while the period increases—at least for the rate-constant vectors $\kappa^*$ and initial conditions we investigated (see the caption of Figure 6). It is an interesting question whether or not this phenomenon arises at other regions of parameter space. We conjecture that indeed oscillations always dampen as $p_k$ and $p_\ell$ approach 1.
Table 4  Values of $k^\ast_{\text{cat}}$ and $\tau^\ast$ used for Fig. 6, and resulting processivity levels, as in (15)

| $k^\ast_{\text{cat}}$ | $\tau^\ast$ | $p_k$  | $p_L$  |
|---------------------|-------------|--------|--------|
| 1                   | 11.3685     | 0.5    | 0.992322 |
| 3                   | 28.7451     | 0.75   | 0.999597 |
| 9                   | 130.22      | 0.9    | 0.999993 |

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 (Sect. 6.1), and then we consider coexistence in distinct compatibility classes (Sect. 6.2).

6.1 Precluding coexistence in a compatibility class

The next result, which applies to general 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 $(G, \kappa)$. Let $S_c$ be a compatibility class such that (1) the system is dissipative\(^3\) with respect to $S_c$, and (2) $S_c$ contains at most 3 steady states and no boundary steady states. Then $S_c$ 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 $x^\ast$ in $S_c$, the coefficient of $\lambda^d$ in det($\lambda I - \text{Jac } f)|_{x=x^\ast}$. If $x^\ast$ is a Hopf bifurcation, then (by a criterion of Yang (2002), restated in (Conradi et al. 2019, Proposition 2.3)) the coefficient is positive. Similarly, if $x^\ast$ is a stable steady state, then (by the Routh-Hurwitz criterion) the coefficient is positive. Finally, by a straightforward generalization of (Wiuf and Feliu 2013, Proposition 5.3), the coefficient equals $(-1)^{s-d} \text{det } \text{Jac } f_{c, \kappa}|_{x=x^\ast}$.

Assume for contradiction that $S_c$ 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 (Conradi et al. 2017) and by above) the Brouwer degree of $f_{c, \kappa}$ with respect to $S_c$ is as follows:

$$\text{sign } \text{det } \text{Jac } f_{c, \kappa}|_{x=x^{(1)}} + \text{sign } \text{det } \text{Jac } f_{c, \kappa}|_{x=x^{(2)}} + \text{sign } \text{det } \text{Jac } f_{c, \kappa}|_{x=x^{(3)}} = (-1)^{s-d} + (-1)^{s-d} + (-1)^{s-d}.$$  

\(^3\)Dissipative means that there is a compact subset of $S_c$ that every trajectory eventually enters; being dissipative is automatic when the network is conservative (Conradi et al. 2017).
which yields a contradiction, as the degree must be $\pm 1$ (see (Conradi et al. 2017)).

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 (Obatake et al. 2019), and a version of this conjecture was stated earlier (see (Obatake et al. 2019, Propositions 5.8–5.9 and Conjecture 5.10)). We pursue the conjecture in Sect. 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 steady states in one compatibility class and a simple Hopf bifurcation in another?

As an initial investigation 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 (Obatake et al. 2019, Equation (23)) (these non-$k_{\text{on}}$ 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 (Obatake et al. 2019, 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 (2008) took to analyze the fully distributive, dual-site phosphorylation system. Namely, we substitute a steady-state parametrization from (Obatake et al. 2019) 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).

\(^4\) The reduced ERK network is not in this list, as it does not admit bistability (Obatake et al. 2019).
Proposition 7.1 Consider the family of polynomial systems in $x_1, x_2, x_3$ given by:

$$c_1 - c_2 - c_3 = x_1 - x_2 - x_3 + \frac{a_{5\alpha\alpha}a_{10}x_1 x_2}{a_8 x_2 + a_{13} x_3 + a_{4\alpha\alpha}a_{13} x_3} + \frac{a_{5\alpha\alpha}a_{10}x_1 x_2(a_{8} x_2 + a_{2\alpha\alpha}a_{8} x_2 + a_{13} x_3 + a_{2\alpha\alpha}a_{13} x_3)}{a_{1\alpha\alpha}(a_{8} x_2 + a_{13} x_3 + a_{4\alpha\alpha}a_{13} x_3)}.$$  \hspace{2cm} (18)

$$c_2 = x_2 + \frac{a_{5\alpha\alpha}a_{10}x_1 x_2(a_{8} x_2 + a_{13} x_3 + a_{4\alpha\alpha}a_{13} x_3)}{a_{8} x_2 + a_{13} x_3 + a_{4\alpha\alpha}a_{13} x_3} + \frac{a_{5\alpha\alpha}a_{10}x_1 x_2(a_{8} x_2 + a_{13} x_3 + a_{4\alpha\alpha}a_{13} x_3)}{a_{1}(a_{8} x_2 + a_{13} x_3 + a_{4\alpha\alpha}a_{13} x_3)} + a_{10} x_1 x_2.$$ \hspace{2cm} (19)

$$c_3 = x_3 + \frac{a_{5\alpha\alpha}a_{10}x_1 x_2(a_{8} x_2 + a_{13} x_3 + a_{4\alpha\alpha}a_{13} x_3)}{a_{1}(a_{8} x_2 + a_{13} x_3 + a_{4\alpha\alpha}a_{13} x_3)} + \frac{a_{5\alpha\alpha}a_{10}x_1 x_2 x_3}{a_{8} x_2 + a_{13} x_3 + a_{4\alpha\alpha}a_{13} x_3}.$$ \hspace{2cm} (20)

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

Proof The Eqs. (18)–(20) are obtained as follows. Using the “effective steady-state function” $h_{c,a}$ from (Obatake et al. 2019, 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” (Dickenstein et al. 2019; Obatake et al. 2019).

Next, we go from the 3 equations (in $x_1, x_2, x_3$) in (18)–(20) to 2 equations (in $x_2$ and $x_3$), as follows. All 3 equations in (18)–(20) 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 $\gamma_1$ 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 $(x_2^*, x_3^*) \in \mathbb{R}_>^2$, 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 (Cox et al. 2007) 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 $(a^*; c^*) = (a^*_1, \ldots, a^*_4, c^*_1, c^*_2, c^*_3) \in \mathbb{R}_>^{16}$. 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 $x_2^* \in \mathbb{R}_>^3$, the equation $g_1(x_2, x_3^*)|_{(a^*, c^*)} = 0$ has at most one positive solution for $x_2$,

then system (18)–(20), when specialized at $(a^*, c^*)$, has at most 3 positive roots $x^* \in \mathbb{R}_>^3$. 

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For every choice of $c$ give two corollaries. We hope to pursue this direction more in future work.

By (Cox et al. 2007, Page 163, Chapter 3, Sec. 6, Proposition 1(i)),

$$R \in \langle g_1, g_2 \rangle \cap \mathbb{Q}[a_1, a_2, \ldots, a_{13}, c_1, c_2, c_3, x_3]. \quad (21)$$

By (Cox et al. 2007, Page 125, Chapter 3, Sec. 2, Theorem 3(i)),

$$\overline{\pi (\mathcal{V}(g_1, g_2))} = \mathcal{V}(\langle g_1, g_2 \rangle \cap \mathbb{Q}[a_1, a_2, \ldots, a_{13}, c_1, c_2, c_3, x_3]), \quad (22)$$

where $\pi : \mathbb{C}^{18} \rightarrow \mathbb{C}^{17}$ 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 $\mathbb{C}^n$ (Cox et al. 2007, Chapter 4) of a subset $S \subseteq \mathbb{C}^n$. So, by (21) and (22),

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

Thus, for a given $(a^*; c^*) \in \mathbb{R}_{>0}^{16}$, 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 $(x_2, x_3) \in \mathbb{R}_2^2$ 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 (18)–(20), when specialized at $(a^*; c^*)$, has at most 3 positive roots $x^*_3 \in \mathbb{R}_{>0}$.

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 $c^*_1, c^*_2, c^*_3, a^*_5 \in \mathbb{R}_{>0}$, if all other $a^*_i$'s are equal to 1, then the (specialized at $(a^*; c^*)$) original system (18) has at most 3 positive roots $x^*_3 \in \mathbb{R}_{>0}$.

**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 $a^*_5$ are equal to 1, then this specialized resultant (see the supplementary file maxNUMs.mw) is as follows:

$$R|_{(a^*; c^*)} = a^*_9 x^*_3 (a^*_9 x^*_3 + 3 c^*_2 + 3 x_3) (C_4 x^*_3 + C_3 x^*_3 + C_2 x^*_3 + C_1 x_3 + C_0), \quad (23)$$

where

$$C_4 = 2 a^*_9 + 12 a^*_5, \quad C_0 = -2 c^*_3 (c^*_2 - c^*_3)^2,$$

and $C_1, C_2, C_3 \in \mathbb{Q}[a^*_5; c^*]$. By inspection, $C_4 > 0$ and $C_0 \leq 0$, for all $c^*_1, c^*_2, c^*_3, a^*_5 \in \mathbb{R}_{>0}$. 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 (23) 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

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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 \( a_0^* \) are equal to 1, we have (see the supplementary file \texttt{maxNUMSS}.mw):

\[
g_1(x_2, x_3^*)_{(a^*;c^*)} = 3x_2^2 + (a_0^* x_3^* - 3c_2^* + 3c_3^*) x_2 - x_3^*(x_3^* + c_2^* - c_3^*) (a_0^* + 3) .
\]

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 \(-x_3(x_3 + c_3^* - c_3^*)(a_0^* + 3) > 0 \), then \( c_3^* > c_2^* \), and so the coefficient of \( x_2 \) is \( a_0^* x_3^* - 3c_2^* + 3c_3^* = a_0^* x_3^* + 3(c_3^* - c_2^*) > 0 \).

By the above two steps and Proposition 7.3, we conclude that the system (18) – when specialized at \((a^*;c^*)\) – has at most 3 positive roots \( x^* \in \mathbb{R}_{>0}^3 \). \( \square \)

**Corollary 7.5** For every choice of \( c_1^*, c_2^* \in \mathbb{R}_{>0} \), if

(i) \( a_0^* \) and \( c_2^* \) are sufficiently large,
(ii) all other \( a_i^* \)'s are equal to the same value \( b \) and are sufficiently large, and also
(iii) \( b > c_2^*/c_3^* > 1 \) and \( c_2^* > c_3^* + 1 \),

then the (specialized at \((a^*;c^*)\)) original system (18)–(20) has at most 3 positive roots \( x^* \in \mathbb{R}_{>0}^3 \).

**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 \( a_0^* \) are equal to \( b \), then (see \texttt{maxNUMSS}.mw) we have:

\[
R_{(a^*;c^*)} = - \Sigma \cdot (C_5 x_3^5 + C_4 x_3^4 + C_3 x_3^3 + C_2 x_3^2 + C_1 x_3 + C_0) ,
\]

where \( \Sigma = b^{17} a_0^* x_3^2 (2b c_2^* + c_3^* + a_0^* b x_3 + 2 b x_3 + x_3) \) (which is positive), and

\[
C_5 = 2a_0^* b^5 (b - 1)(b + 1)(a_0^* b + 2b + 1) ,
\]

\[
C_1 = b^3 (a_0^* c_2^* - c_2^* - 3a_0^* c_2^* e_3) + 2a_0^* c_1^* c_2^* - 2a_0^* c_1^* e_3 + 4a_0^* c_3^* - c_3^* c_2^* - 2c_1^* c_3^* + 3c_3^* c_2^* - c_3^* + c_3^* e_3^* + 2c_1^* c_3^* - 2c_3^* c_2^* - c_3^* - c_3^* ) + \text{lower-order terms in } b ,
\]

\[
C_0 = - c_3^* (b^2 + 1)(c_2^* - c_3^*) (a_0^* b^4 c_3^* - a_0^* b^3 c_3^* - a_0^* b^2 c_3^* - a_0^* b c_3^* - a_0^* c_3^* - b c_3^* - 2b^3 c_3^* - b^2 c_3^* - b c_3^* + b c_2^* + c_3^* :)
\]

and \( C_2, C_3, C_4 \in \mathbb{Q}[a_0^*;c^*] \). Assume that \( a_0^* \), \( b \), 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)

\[
g_1(x_2, x_3^*)|_{(a^*; c^*)} = (b^4 + b^3 + b^2)x_2^2 + (a_9^*b^4x_3^* - b^4c_2^* + 2b^4c_3^* - b^4x_3^* - b^3c_2^* + b^3c_3^* - b^2c_2^* + b^2x_3^*x_2 - b^2x_3^*(a_9^*b^2c_2^* - a_9^*b^2c_3^* + a_9^*b^2x_3^*) + b^2c_2^* - 2b^2c_3^* + 2b^2x_3^* + bc_2^* - bc_3^* + bx_3^* + c_2^*)
\]

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

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

Thus, if \( a_9^*, b, \) and \( c_3^* \) 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 (18) has at most 3 positive roots \( x^* \in \mathbb{R}_{>0}^3 \). \( \square \)

**Remark 7.6** In the two above proofs, we saw the (specialized) resultants (23) and (24) 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) (Plotnikov et al. 2011). 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 (Tyson et al. 2008). For MAPK cascades, to quote from Sun et al. (2014), “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”. 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 5 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       | Sect. 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   | Sect. 6.2         |
| maxNUMss.mw              | Maple       | Sect. 7           |
| resultant.txt            | Text        | Sect. 7           |
Thus, substitute into (26) the values of stationarity in the minimally bistable ERK network at various processivity levels \( p_k \) (cf. eq. (6)).

Table 6 Values of \( p_k, p_\ell, \) and \( T \) used in Figs. 3 and 4

| \( p_k \) | 0.1 | 0.1 | 0.1 | 0.1 | 0.5 | 0.75 | 0.8 | 0.85 | 0.9 |
| \( p_\ell \) | 0.1 | 0.5 | 0.9 | 0.999 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| \( T \) | 2.21958 | 3.72221 | 4.98625 | 5.28023 | 4.80723 | 8.59917 | 10.6576 | 14.1522 | 21.2341 |

B Procedure to study multistationarity numerically

Here we describe the procedure we used in Sect. 4.3 for numerically studying multistationarity in the minimally bistable ERK network at various processivity levels \( p_k \) and \( p_\ell. \)

We begin by mirroring the analysis of Sect. 4.2. Specifically, we use the parameters given in (14) to study the critical function \( C(\kappa, \hat{x}) \) for \( x_1 = x_2 = T \) and \( x_3 = 1. \) Due to this choice of \( \kappa \) and \( \hat{c}, \) the critical function is a (rational) function of \( p_k, p_\ell, \) and \( T \) only, i.e., \( C(\kappa, \hat{x}) \equiv C(p_k, p_\ell, T). \) The numerator is the following polynomial:

\[
q(p_k, p_\ell, T) = -p_k \left( (3 - 2p_\ell)p_\ell + p_k \left( 1 - 3p_\ell + 2p_\ell^2 \right) \right) \\
+ \left( -5p_\ell^2 + p_kp_\ell(-9 + 11p_\ell) + p_\ell^3 \left( -1 + 3p_\ell - 2p_\ell^2 \right) - p_k^2 \left( 3 - 3p_\ell + p_\ell^2 \right) \right) T \\
+ \left( -8p_\ell^2 + p_kp_\ell(-13 + 9p_\ell) + p_\ell^3 \left( -1 + p_\ell - p_\ell^2 \right) + p_k^2 \left( -6 + 2p_\ell + 7p_\ell^2 \right) \right) T^2 \\
+ \left( -3p_\ell^2 - p_kp_\ell(5 + 8p_\ell) + p_\ell^3 \left( -4 + 3p_\ell + p_\ell^2 \right) + p_k^2 \left( -3 - 10p_\ell + 13p_\ell^2 \right) \right) T^3 \\
+ p_k \left( 5 - 8p_\ell \right) + p_k \left( 1 - 5p_\ell + p_\ell^2 \right) + p_\ell^2 \left( -7 + 4p_\ell + 2p_\ell^2 \right) T^4 \\
+ p_k \left( 3p_\ell + p_k^2 \left( -3 - 3p_\ell + 2p_\ell^2 \right) - p_k \left( -2 + p_\ell + 4p_\ell^2 \right) \right) T^5 - 2\left( 1 + p_k \right) p_\ell^2 p_k T^6
\]

As \( 0 < p_k, p_\ell < 1, \) the leading coefficient of \( q(p_k, p_\ell, T) \) as a polynomial in \( T \) is positive. Next, the steady-state parametrization \( \phi \) from Proposition 3.1 is as follows (cf. eq. (6)):

\[
x_1 = T, \ x_2 = T, \ x_3 = 1, \ x_4 = \frac{p_k T^2 (1 + T)}{p_\ell + p_k p_\ell T}, \ x_5 = \frac{-p_k \left( -1 + p_\ell \right) T (1 + T)}{p_\ell + p_k p_\ell T}, \\
x_6 = \frac{T - p_k T}{1 + p_k T}, \ x_7 = \frac{-\left( -1 + p_k \right) T (1 + T)}{1 + p_k T}, \ x_8 = \frac{-p_k \left( -1 + p_\ell \right) T (1 + T)}{p_\ell + p_k p_\ell T}, \\
x_9 = \frac{T - p_k T}{1 + p_k T}, \ x_{10} = \frac{-p_k \left( -1 + p_\ell \right) (1 + T)}{p_\ell + p_k p_\ell T}, \ x_{11} = T^2, \ x_{12} = \frac{p_k \left( 1 + T \right)}{p_\ell + p_k p_\ell T}
\]

(26)

To numerically study multistationarity for \( p_k, p_\ell \to 1, \) we proceed as follows:

(i) Pick values of \( 0 < \tilde{p}_k, \tilde{p}_\ell < 1 \) and \( \tilde{T} > 0 \) such that \( q(\tilde{p}_k, \tilde{p}_\ell, \tilde{T}) > 0 \) (recall eq. (25)).

(ii) Substitute into (26) the values of \( \tilde{p}_k, \tilde{p}_\ell, \) and \( \tilde{T} \) from the previous step to obtain a steady state \( \hat{x}. \)
### Table 7

| $p_k$ | $p_\ell$ | $T$ |
|------|---------|-----|
| 0.9  | 0.9    | 21.2341 |
| 0.95 | 0.95   | 43.2027 |
| 0.98 | 0.98   | 109.186 |
| 0.99 | 0.99   | 219.18  |

(iii) Compute, using (5), the total amounts $\tilde{c}_1$, $\tilde{c}_2$, and $\tilde{c}_3$ at $\tilde{x}$.

(iv) Use Matcont with initial condition near $\tilde{x}$ and bifurcation parameter $c_2$, to obtain a bifurcation curve.

(v) To compare curves corresponding to distinct $\tilde{p}_k$ and $\tilde{p}_\ell$, compute relative concentrations $\tilde{c}_i / c_1$ and $\tilde{c}_2 / c_2$ that relate $x_i$ and $c_2$ to the $\tilde{x}$ and $\tilde{c}_2$ computed in steps (ii) and (iii).

Step (v) is crucial for interpreting the numerical results obtained by the above procedure, because certain total amounts differ by orders of magnitude as $p_k$, $p_\ell \to 1$, and so it is more meaningful to compare values relative to the reference point $\tilde{x}$ obtained in step (ii) Figs. 6 and 7.

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