

Emergence of oscillations in a mixed-mechanism phosphorylation system

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6 September 2018

Abstract

This work investigates the emergence of oscillations in one of the simplest cellular signaling networks exhibiting oscillations, namely, the dual-site phosphorylation and dephosphorylation network (futile cycle), in which the mechanism for phosphorylation is processive while the one for dephosphorylation is distributive (or vice-versa). The fact that this network yields oscillations was shown recently by Suwanmajo and Krishnan. Our results, which significantly extend their analyses, are as follows. First, in the three-dimensional space of total amounts, the border between systems with a stable versus unstable steady state is a surface defined by the vanishing of a single Hurwitz determinant. Second, this surface consists generically of simple Hopf bifurcations. Next, simulations suggest that when the steady state is unstable, oscillations are the norm. Finally, the emergence of oscillations via a Hopf bifurcation is enabled by the catalytic and association constants of the distributive part of the mechanism: if these rate constants satisfy two inequalities, then the system generically admits a Hopf bifurcation. Our proofs are enabled by the Routh-Hurwitz criterion, a Hopf-bifurcation criterion due to Yang, and a monomial parametrization of steady states.

Keywords: multisite phosphorylation, monomial parametrization, oscillation, Hopf bifurcation, Routh-Hurwitz criterion

1 Introduction

Oscillations have been observed experimentally in signaling networks formed by phosphorylation and dephosphorylation [20, 21], which suggests that these networks are involved in timekeeping and synchronization. Indeed, multisite phosphorylation is the main mechanism for establishing the 24-hour period in eukaryotic circadian clocks [30, 42]. Our motivating question, therefore, is, How do oscillations arise in phosphorylation networks?

We tackle this question for the network that, according to Suwanmajo and Krishnan, “could be the simplest enzymatic modification scheme that can intrinsically exhibit oscillation” [39, §3.1]. This network, in (1), is the mixed-mechanism (partially processive, partially

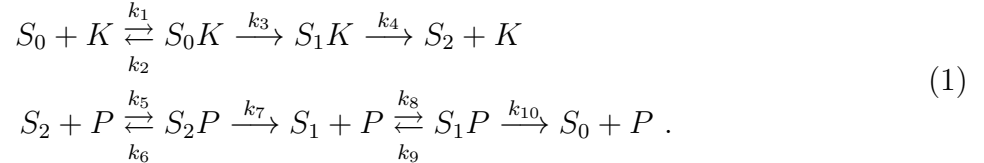
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distributive) dual-site phosphorylation network (or **mixed-mechanism network** for short). Examples of networks that include both processive and distributive elements include the “processive model” of Aoki *et al.* [1, Table S2] and a model of ERK regulation via enzymes MEK and MKP3 [37, Fig. 2].

In the mixed-mechanism network, S_i denotes a substrate with i phosphate groups attached, and K and P are, respectively, a *kinase* and a *phosphatase* enzyme:



When the kinase *phosphorylates* – that is, adds phosphate groups to – a substrate in the mixed-mechanism network (via the reactions labeled by k_1 to k_4), the kinase and substrate do *not* dissociate before both phosphate groups are added. Accordingly, the mechanism for phosphorylation is *processive*. In contrast, when the phosphatase *dephosphorylates* – i.e., removes phosphate groups from – a substrate (via reactions k_5 to k_{10}), this mechanism is *distributive*: the phosphatase and substrate dissociate each time a phosphate group is removed. Accordingly, network (1) is said to have a mixed mechanism¹.

The dynamical systems arising from the mixed-mechanism network live in a 9-dimensional space, but, due to three conservation laws, are essentially 6-dimensional. Specifically, the total amounts of kinase, phosphatase, and substrate – denoted by K_{tot} , P_{tot} , and S_{tot} , respectively – are conserved. For each choice of three such total amounts and each choice of positive rate constants k_i , there is a unique positive steady state [39]. One focus of our work is determining when such a steady state undergoes a Hopf bifurcation leading to oscillations (with any of the k_i ’s or total amounts as bifurcation parameter).

1.1 Summary of main results

How do oscillations of the mixed-mechanism network emerge, and how robust are they? These questions are the motivation for our work. Let us describe Suwanmajo and Krishnan’s progress in this direction. They first found rate constants k_i and total amounts, displayed in Table 1, that yield oscillations [39, Supplementary Information].

k_1	k_2	k_3	k_4	k_5	k_6	k_7	k_8	k_9	k_{10}	K_{tot}	P_{tot}	S_{tot}
1	1	1	1	100	1	0.9	3	1	100	17.5	5	40

Table 1: Rate constants (left) and total amounts (right), from [39, Supplementary Information], which lead to oscillations in the mixed-mechanism network (1).

Next, they examined whether oscillations persist as K_{tot} varies. What they found, summarized in Figure 1, is that oscillations persist when K_{tot} is in the (approximate) interval (13.03, 29.23), and oscillations arise as the unique steady state undergoes a Hopf bifurcation.

¹Network (1) is symmetric to the mixed-mechanism network in which phosphorylation is distributive (instead of processive) and dephosphorylation is processive (instead of distributive), so our results apply equally well to that network (cf. [39, networks 21–22]).

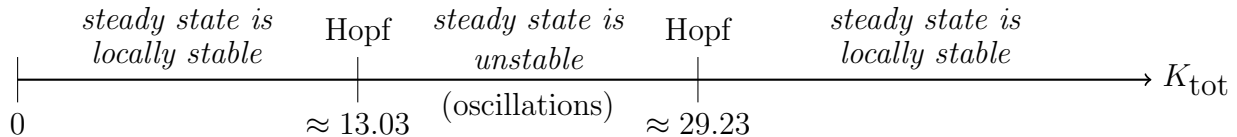


Figure 1: Stability of the unique steady state of the mixed-mechanism network (1) as a function of K_{tot} , as analyzed by Suwanmajo and Krishnan [39, Fig. 4]. (The other total amounts, P_{tot} and S_{tot} , and the rate constants k_i are those in Table 1.) Oscillations were found when K_{tot} is in the “unstable” interval [39].

Subsequently, Conradi and Shiu [7] found that when P_{tot} also is allowed to vary, oscillations exist for larger values of K_{tot} (e.g., $K_{\text{tot}} = 100$). So, how exactly do oscillations depend on the three total amounts (or, equivalently, the initial conditions)? Concretely, our goal is to expand Figure 1 to encompass all possible perturbations to the initial conditions (i.e., the total amounts):

Question 1.1. *Consider the mixed-mechanism network (1), with k_i 's from Table 1.*

1. *For which values of $(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}}) \in \mathbb{R}_{>0}^3$ is the unique steady state unstable?*
2. *Whenever (by perturbing parameters or total amounts) a steady state switches from being locally stable to unstable, does this always give rise to a Hopf bifurcation?*

The direct method for solving Question 1.1(1) is to solve the steady-state equations, and then apply the six-dimensional Routh-Hurwitz stability criterion. However, this approach is intractable: the resulting Hurwitz determinants are pages-long.

Accordingly, we take an algebraic shortcut. Namely, we find a parametrization of the set of steady states, and then use this for the input to Routh-Hurwitz. The result is somewhat surprising: each Hurwitz determinant except the last two (which are positive multiples of each other) is always positive. This yields our answer to Question 1.1(1): *For every ODE system arising from the mixed-mechanism network (1), a (two-dimensional) surface in the three-dimensional space of total amounts defines the border between steady states that are stable and those that are unstable.* (Our result even applies to many systems for which the k_i 's are not those in Table 1; see Proposition 4.1.)

We can now translate Question 1.1(2) as follows: does the surface mentioned above consist of Hopf bifurcations? We prove, using a Hopf-bifurcation criterion stated in terms of Hurwitz determinants, due to Yang [43], that the answer, at least generically, is “yes”: *When the unique steady state of the mixed-mechanism network (1) switches from being stable to unstable, then, generically, it undergoes a Hopf bifurcation.*

For general one-parameter ODE systems, there are two types of local bifurcations: saddle nodes (which require a zero eigenvalue of the Jacobian matrix) and Hopf bifurcations (which require a pair of pure imaginary eigenvalues of the Jacobian) [16]. We show that a saddle node bifurcation can not occur for any parameter values (see the proof of Proposition 4.1). Therefore, only Hopf bifurcations are possible for the mixed-mechanism system.

A second question we aim to answer is the following:

Question 1.2. Consider the mixed-mechanism network (1). What conditions on the k_i 's guarantee a Hopf-bifurcation for some (positive) values of the total concentrations?

As an answer to Question 1.2, we prove that the catalytic constants (k_7 and k_{10}) and association constants (k_5 and k_8) of the distributive part of the mechanism enable oscillations to emerge via a Hopf bifurcation. Specifically, under the simplifying assumption that all dissociation (backward-reaction) constants are equal ($k_2 = k_6 = k_9$), if the rate constants satisfy two inequalities – lower bounds on k_{10} and k_5/k_8 – then the system generically admits a Hopf bifurcation (Proposition 4.3 and Theorem 4.5). (As a comparison, for the fully distributive dual-site network described in Section 1.2 below, the catalytic constants alone enable bistability [5].) Finally, we encode the relevant inequalities in a procedure to generate many parameter values for which we expect oscillations (Procedure 5.1).

1.2 Connection to related work

Our work joins a growing number of works that harness steady-state parametrizations. Such results include criteria for when such parametrizations exist [26, 40] and methods for using them to determine whether a network is multistationary [25, 29, 32, 34]. Going further, steady-state parametrizations can also be used to find a witness to multistationarity or even the precise parameter regions that yield multistationarity [4, 5]. In this work, we use a steady-state parametrization in a novel way: to study oscillations via Hopf bifurcations. (Our approach is similar in spirit to using Clarke's convex parameters together with a Hopf-bifurcation criterion [9, 11, 14, 18]).

As mentioned earlier, there has been much interest in the dynamics of phosphorylation systems [7]. The mixed-mechanism network (1) fits into the related literature as follows. The mixed network is a dual-site network situated between two extremes: the *fully processive* dual-site network – in which the phosphorylation and dephosphorylation mechanisms are both processive – and the *fully distributive* dual-site network. One might therefore expect the dynamics of the mixed-mechanism network to straddle those of the two networks. This is indeed the case. As summarized in Table 2, and reviewed in [7], fully processive networks are globally convergent to a unique steady state [6, 10, 35], while mixed-mechanism networks admit oscillations but not bistability [39], and fully distributive networks admit bistability [19] (and the question of oscillations is open [7]).

Dual-site network	Oscillations?	Bistability?	Global convergence?
Fully processive	No	No	Yes
Mixed-mechanism	Yes	No	No
Fully distributive	(Open)	Yes	No

Table 2: Dual-site phosphorylation networks and their properties: whether they admit oscillations or bistability, and whether all trajectories converge to a unique steady state.

Finally, we revisit Suwanmajo and Krishnan's claim mentioned earlier that the mixed-mechanism network is among the simplest enzymatic mechanisms with oscillations. In support of this claim, Tung proved that the simpler system obtained from the mixed-mechanism network by taking its (two-dimensional) Michaelis-Menten approximation, is *not* oscillatory

[41]. Moreover, Rao showed that this approximation is globally convergent to a unique steady state [36]. The validity of the Michaelis-Menten approximation for phosphorylation systems has been called into question [38], and what we know about the mixed-mechanism system concurs: this system is oscillatory, but its Michaelis-Menten approximation is not.

The outline of our work is as follows. Section 2 provides background on multisite phosphorylation, steady states, and Hopf bifurcations. Section 3 gives a monomial parametrization of the steady states of mixed-mechanism network. In Section 4, we prove our main results (described above). We use these results in Section 5 to give a procedure for generating rate constants admitting Hopf bifurcations. In Section 6, we present simulations that suggest that oscillations are the norm in the unstable-steady-state regime. Finally, we end with a Discussion in Section 7.

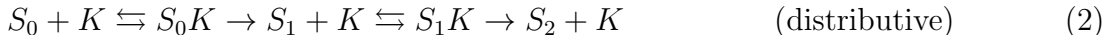
2 Background

In this section, we introduce the ODEs arising from the mixed-mechanism network, and recall two criteria: the Routh-Hurwitz criterion for steady-state stability and Yang’s criterion for Hopf bifurcations.

2.1 Multisite phosphorylation and the mixed-mechanism network

A biological process of great importance, *phosphorylation* is the enzyme-mediated addition of a phosphate group to a protein substrate, which often modifies the function of the substrate. This basic mechanism is: $S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E$, where S_i is the substrate with i phosphate groups attached and E is the enzyme.

Many substrates have more than one *site* at which phosphate groups can be attached. Such multisite phosphorylation may be *distributive* or *processive*, or somewhere in between [17, 31]. Compare distributive versus processive mechanisms for phosphorylation on two sites:



In distributive phosphorylation, such as (2), each binding of substrate and enzyme results in at most one addition of a phosphate group. In contrast, in processive phosphorylation, such as (3), when an enzyme catalyzes the addition of a phosphate group, then phosphate groups are added to all sites before the enzyme and substrate dissociate. In the mixed-mechanism network (1) introduced earlier, the phosphorylation mechanism is processive, while dephosphorylation is distributive.

x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9
S_0	K	S_0K	S_1K	S_2	P	S_2P	S_1	S_1P

Table 3: Assignment of variables to species for the mixed-mechanism network (1).

For the mixed-mechanism network, we let x_1, x_2, \dots, x_9 denote the species concentrations in the order given in Table 3. The dynamical system (arising from mass-action kinetics)

defined by the mixed-mechanism network (1) is given by the following ODEs:

$$\begin{aligned}
\dot{x}_1 &= -k_1x_1x_2 + k_2x_3 + k_{10}x_9 \\
\dot{x}_2 &= -k_1x_1x_2 + k_2x_3 + k_4x_4 \\
\dot{x}_3 &= k_1x_1x_2 - (k_2 + k_3)x_3 \\
\dot{x}_4 &= k_3x_3 - k_4x_4 \\
\dot{x}_5 &= k_4x_4 - k_5x_5x_6 + k_6x_7 \\
\dot{x}_6 &= -k_5x_5x_6 - k_8x_8x_6 + (k_6 + k_7)x_7 + (k_9 + k_{10})x_9 \\
\dot{x}_7 &= k_5x_5x_6 - (k_6 + k_7)x_7 \\
\dot{x}_8 &= k_7x_7 - k_8x_6x_8 + k_9x_9 \\
\dot{x}_9 &= k_8x_6x_8 - (k_9 + k_{10})x_9 .
\end{aligned} \tag{4}$$

The conservation laws arise from the fact that the total amounts of free and bound enzyme or substrate remain constant. That is, as the dynamical system (4) progresses, the following three conservation values, denoted by $K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}} \in \mathbb{R}_{>0}$, remain constant:

$$\begin{aligned}
K_{\text{tot}} &= x_2 + x_3 + x_4 , \\
P_{\text{tot}} &= x_6 + x_7 + x_9 , \\
S_{\text{tot}} &= x_1 + x_3 + x_4 + x_5 + x_7 + x_8 + x_9 .
\end{aligned} \tag{5}$$

Also, a trajectory $x(t)$ beginning in $\mathbb{R}_{\geq 0}^9$ remains in $\mathbb{R}_{\geq 0}^9$ for all positive time t , so it remains in a *stoichiometric compatibility class*, which we denote as follows:

$$\mathcal{P} = \{x \in \mathbb{R}_{\geq 0}^9 \mid \text{the conservation equations (5) hold}\} . \tag{6}$$

2.2 Stability of steady states and the Routh-Hurwitz criterion

The dynamical system (4) arising from the mixed-mechanism network is an example of a *reaction kinetics system*. That is, the system of ODEs takes the following form:

$$\frac{dx}{dt} = \Gamma \cdot R(x) =: g(x) , \tag{7}$$

where Γ and R are as follows. Letting s denote the number of species and r the number of reactions, Γ is an $s \times r$ matrix whose k -th column is the reaction vector of the k -th reaction, i.e., it encodes the net change in each species that results when that reaction takes place. Also, $R : \mathbb{R}_{\geq 0}^s \rightarrow \mathbb{R}_{\geq 0}^r$ encodes the reaction rates of the r reactions as functions of the s species concentrations.

A *steady state* (respectively, *positive steady state*) of a reaction kinetics system is a non-negative concentration vector $x^* \in \mathbb{R}_{\geq 0}^s$ (respectively, $x^* \in \mathbb{R}_{> 0}^s$) at which the ODEs (7) vanish: $g(x^*) = 0$. Letting $S := \text{im}(\Gamma)$ denote the *stoichiometric subspace*, a steady state x^* is *nondegenerate* if $\text{Im}(dg(x^*)|_S) = S$, where $dg(x^*)$ denotes the Jacobian matrix of g at x^* .

A nondegenerate steady state is locally asymptotically stable if each of the $\sigma := \dim(S)$ nonzero eigenvalues of $dg(x^*)$ has negative real part. Hence, a steady state is locally stable

if and only if the characteristic polynomial of the Jacobian evaluated at the steady state has σ roots with negative real part (the remaining roots will be 0).

To check whether a polynomial has only roots with negative real parts, we appeal to the Routh-Hurwitz criterion below [13].

Definition 2.1. The i -th Hurwitz matrix of a univariate polynomial $p(\lambda) = a_0\lambda^n + a_1\lambda^{n-1} + \dots + a_n$ is the following $i \times i$ matrix:

$$H_i = \begin{pmatrix} a_1 & a_0 & 0 & 0 & 0 & \dots & 0 \\ a_3 & a_2 & a_1 & a_0 & 0 & \dots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & & \vdots \\ a_{2i-1} & a_{2i-2} & a_{2i-3} & a_{2i-4} & a_{2i-5} & \dots & a_i \end{pmatrix},$$

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

Proposition 2.2 (Routh-Hurwitz criterion). *A polynomial $p(\lambda) = a_0\lambda^n + a_1\lambda^{n-1} + \dots + a_n$ with $a_0 > 0$ has all roots with negative real part if and only if all n of its Hurwitz matrices have positive determinant ($\det H_i > 0$ for all $i = 1, \dots, n$).*

2.3 Hopf bifurcations and a criterion due to Yang

A *simple Hopf bifurcation* is a bifurcation in which a single complex-conjugate pair of eigenvalues of the Jacobian matrix crosses the imaginary axis, while all other eigenvalues remain with negative real parts. Such a bifurcation, if it is supercritical, generates nearby *oscillations* or periodic orbits [27].

To detect simple Hopf bifurcations, we will use a criterion of Yang that characterizes Hopf bifurcations in terms of Hurwitz-matrix determinants (Proposition 2.3).

Setup for Yang's criterion. We consider an ODE system parametrized by $\mu \in \mathbb{R}$:

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

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

$$\mu \mapsto x(\mu) \tag{8}$$

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

$$p_\mu(\lambda) := \det(\lambda I - \text{Jac } g_\mu)|_{x=x(\mu)} = \lambda^n + a_1(\mu)\lambda^{n-1} + \dots + a_n(\mu),$$

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

Proposition 2.3 (Yang's criterion [43]). *Assume the above setup. Then, there is a simple Hopf bifurcation at x_0 with respect to μ if and only if the following hold:*

- (i) $a_n(\mu_0) > 0$,

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

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

Remark 2.4. Liu [27] gave an earlier version of Yang’s Hopf-bifurcation criterion (Proposition 2.3), using a variant of the Hurwitz matrices that differs from ours.

3 Steady states of the mixed-mechanism network

In this section, we recall that the mixed-mechanism network admits a unique steady state in each compatibility class (Proposition 3.1), and prove that the set of steady states admits a monomial parametrization (Theorem 3.2). We then use this parametrization to analyze the space of compatibility classes (Proposition 3.6).

3.1 Uniqueness of steady states

Suwanmajo and Krishnan proved that, for *every* choice of positive rate constants and positive total amounts, the mixed-mechanism network does *not* admit multiple positive steady states [39, §A.2]. Additionally, there are no boundary steady states in any compatibility class \mathcal{P} , as in (6), and \mathcal{P} is compact. Hence, via a standard application of the Brouwer fixed-point theorem (e.g., [33, Remark 3.9]), there is always a unique steady state:

Proposition 3.1 (Uniqueness of steady states). *For any choice of positive rate constants k_i and positive total amounts K_{tot}, P_{tot} , and S_{tot} , the dynamical system (4) arising from the mixed-mechanism network has a unique steady state in \mathcal{P} , and it is a positive steady state.*

Proposition 3.1 proves part of a conjecture that we posed [6]. The other half of the conjecture, however, posited that mixed-mechanism systems, like fully processive systems [6, 10], are globally convergent to the unique steady state. Suwanmajo and Krishnan demonstrated that this is false: the system can exhibit oscillatory behavior [39]!

This capacity for oscillations is the focus of this work, and our analysis will harness a monomial parametrization of the steady states. We turn to this topic now.

3.2 A monomial parametrization of the steady states

The steady states of the mixed-mechanism network can be parametrized by monomials (and thus is said to have “toric steady states” [33]):

Theorem 3.2 (Parametrization of the steady states). *For every choice of rate constants $k_i > 0$, the set of positive steady states of the mixed-mechanism system (4) is three-dimensional and is the image of the following map $\chi = \chi_{k_1, \dots, k_{10}}$:*

$$\begin{aligned} \chi : \mathbb{R}_+^3 &\rightarrow \mathbb{R}_+^9 \\ (x_1, x_2, x_6) &\mapsto (x_1, x_2, \dots, x_9), \end{aligned} \tag{9}$$

given by

$$\begin{aligned} x_3 &:= \frac{k_1}{k_2 + k_3} x_1 x_2, & x_4 &:= \frac{k_1 k_3}{(k_2 + k_3) k_4} x_1 x_2, & x_5 &:= \frac{k_1 k_3 (k_6 + k_7)}{(k_2 + k_3) k_5 k_7} \frac{x_1 x_2}{x_6}, \\ x_7 &:= \frac{k_1 k_3}{(k_2 + k_3) k_7} x_1 x_2, & x_8 &:= \frac{k_1 k_3 (k_9 + k_{10})}{(k_2 + k_3) k_8 k_{10}} \frac{x_1 x_2}{x_6}, & x_9 &:= \frac{k_1 k_3}{(k_2 + k_3) k_{10}} x_1 x_2. \end{aligned}$$

Proof. It is straightforward to check that the image of χ is contained in the set of steady states: after substituting $\chi(x_1, x_2, x_3)$, the right-hand side of the mixed-mechanism network ODEs (4) vanishes. Conversely, let $x^* = (x_1, x_2, \dots, x_9)$ be a positive steady state. The right-hand side of the ODEs (4) vanish at x^* , so, in the following order, we use $\dot{x}_3 = 0$ to solve for x_3 in terms of x_1 and x_2 , use $\dot{x}_4 = 0$ to solve for x_4 via x_3 which was already obtained, use $\dot{x}_1 = 0$ to obtain x_9 , use $\dot{x}_9 = 0$ to obtain x_8 , use $\dot{x}_8 = 0$ to obtain x_7 , and finally use $\dot{x}_7 = 0$ to obtain x_5 . This yields precisely the parametrization (9), so x^* is in the image of χ . \square

Remark 3.3. The parametrization (9) appeared earlier in [7].

Remark 3.4. That we could achieve a steady-state parametrization was expected, due to Thomson and Gunawardena's rational parametrization theorem for multisite systems [40].

Remark 3.5. In the parametrization χ in Theorem 3.2, we divide by x_6 , so χ is technically not a monomial map. However, χ can be made monomial: we introduce $y := \frac{x_1}{x_6}$, so that the parametrization accepts as input (y, x_2, x_6) , and then x_1 is replaced by $y x_6$.

3.3 A parametrization of the compatibility classes

Every compatibility class \mathcal{P} of the mixed-mechanism network, by definition (6), is uniquely determined by a choice of total amounts $(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}}) \in \mathbb{R}_{>0}^3$. Thus, we identify the set of compatibility classes with $\{(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}})\} = \mathbb{R}_{>0}^3$. We parametrize this set below (Proposition 3.6).

Let $\phi : \mathbb{R}_{>0}^9 \rightarrow \mathbb{R}_{>0}^3$ denote the map sending a vector of concentrations to the corresponding total amounts $(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}})$, as in (5):

$$\phi(x) := (x_2 + x_3 + x_4, x_6 + x_7 + x_9, x_1 + x_3 + x_4 + x_5 + x_7 + x_8 + x_9). \quad (10)$$

Each compatibility class \mathcal{P} contains a unique positive steady state (Proposition 3.1), and the positive steady states are parametrized by χ from Theorem 3.2, so the space of compatibility classes is parametrized as follows:

Proposition 3.6 (Parametrization of the compatibility classes). *Identify every compatibility class \mathcal{P} of the mixed-mechanism network (1), with the corresponding total amounts $(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}}) \in \mathbb{R}_{>0}^3$. Then, for every choice of positive rate constants k_i , the following is a bijection that sends a vector $(x_1, x_2, x_6) \in \mathbb{R}_{>0}^3$ to the compatibility class in which the unique steady state is $\chi(x_1, x_2, x_6)$:*

$$\phi \circ \chi : \mathbb{R}_{>0}^3 \rightarrow \mathbb{R}_{>0}^3 = \{(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}})\},$$

where ϕ is as in (10) and χ is the steady-state parametrization (9). The map $\phi \circ \chi$ is given by

$$(x_1, x_2, x_6) \mapsto \left(x_2 + \frac{k_1}{k_2 + k_3} \left(1 + \frac{k_3}{k_4} \right) x_1 x_2, \quad x_6 + \frac{k_1 k_3}{k_2 + k_3} \left(\frac{1}{k_7} + \frac{1}{k_{10}} \right) x_1 x_2, \right. \\ \left. x_1 + \frac{k_1 k_3}{k_2 + k_3} \left[\left(\frac{1}{k_3} + \frac{1}{k_4} + \frac{1}{k_7} + \frac{1}{k_{10}} \right) + \frac{1}{x_6} \left(\frac{k_6 + k_7}{k_5 k_7} + \frac{k_{10} + k_9}{k_{10} k_8} \right) \right] x_1 x_2 \right),$$

which becomes, when the rate constants are those in Table 1, the following:

$$(x_1, x_2, x_6) \mapsto \left(x_1 x_2 + x_2, \quad x_6 + \frac{1009}{1800} x_1 x_2, \quad x_1 + \frac{2809}{1800} x_1 x_2 + \frac{161}{900} \frac{x_1 x_2}{x_6} \right). \quad (11)$$

Example 3.7. Consider the mixed-mechanism system with rate constants from Table 1. To compute the unique steady state x^* in the compatibility class given by $(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}}) = (17.5, 5, 40)$, we use Proposition 3.6. Namely, we know that $\phi \circ \chi(x_1^*, x_2^*, x_6^*) = (17.5, 5, 40)$, so we solve (using, e.g., `Mathematica` [22]) for the unique positive solution:

$$(x_1^*, x_2^*, x_6^*) \approx (1.0134, 8.6916, 0.0624).$$

We obtain the remaining coordinates of x^* using the parametrization χ in (9):

$$x^* = \chi(x_1^*, x_2^*, x_6^*) \quad (12) \\ \approx (1.0134, 8.6916, 4.4041, 4.4041, 1.4893, 0.0624, 4.8935, 23.7512, 0.0440).$$

3.4 Steady states and Hopf bifurcations

Our analysis of oscillations in the mixed-mechanism system is based on Hopf bifurcations. Hopf-bifurcation diagrams are displayed in Figure 2, where the total amounts are the bifurcation parameters (c.f. Figure 1 which is with respect to K_{tot}). Figure 2 suggests that, in the 3-dimensional space of total amounts, there is a surface of Hopf bifurcations. Indeed, we will see in the next section that this is the case (see Theorem 4.5 and Figure 3).

4 Hopf bifurcations in the mixed-mechanism system

We saw in the previous section that the mixed-mechanism network yields a unique positive steady state in each compatibility class. Now we show that the compatibility classes with a *stable* steady state are separated from those with an *unstable* steady state by a single surface \mathcal{H} (Proposition 4.1 and Theorem 4.2), and, under stronger hypotheses, crossing the surface \mathcal{H} generically corresponds to undergoing a Hopf bifurcation (Theorem 4.5). (Recall that *generically* means that the exceptional set has zero measure. So, we will show that the subset of the surface corresponding to non-Hopf points has dimension at most 1.)

To simplify computations, we assume that dissociation (backward-reaction) constants are equal: $k_2 = k_6 = k_9$. In chemistry, the forward reaction is usually more thermodynamically favorable than the backward reaction. Therefore, the rate constant of a forward reaction is much larger than the rate constant of the backward reaction [2]. We choose small values for the dissociation rate constants in Section 5, similar to what was done in [12].

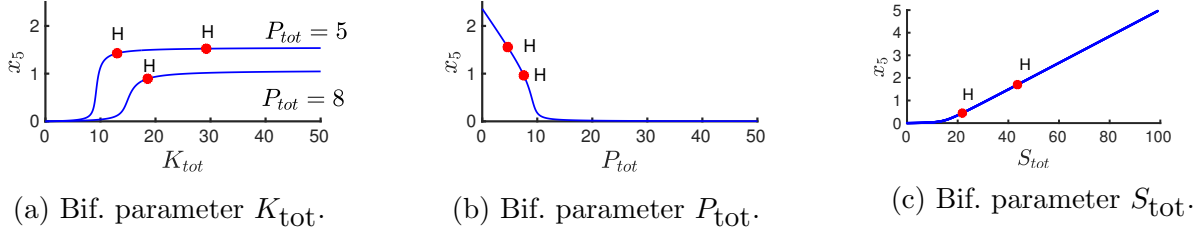


Figure 2: Numerical continuation of the unique positive steady state, in (12), when $(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}}) = (17.5, 5, 40)$: (a) For $P_{\text{tot}} = 5, 8$ and $S_{\text{tot}} = 40$, we observe (supercritical) Hopf bifurcations at $K_{\text{tot}} \approx 13.0296, 29.2251$ ($P_{\text{tot}} = 5$) and $K_{\text{tot}} \approx 18.5758$ ($P_{\text{tot}} = 8$). (b) For $K_{\text{tot}} = 5$ and $S_{\text{tot}} = 40$, we observe (supercritical) Hopf bifurcations at $P_{\text{tot}} \approx 4.6310$ and $P_{\text{tot}} \approx 7.5479$. (c) For $K_{\text{tot}} = 17.5$ and $P_{\text{tot}} = 5$, we observe (supercritical) Hopf bifurcations at $S_{\text{tot}} \approx 21.8213$ and $S_{\text{tot}} \approx 43.5944$. All figures in this work were made using `Matcont` [8].

Proposition 4.1. *Consider the dynamical system (4) arising from the mixed-mechanism network and any positive rate constants for which $k_2 = k_6 = k_9$. Then:*

1. Every compatibility class \mathcal{P} contains a unique (positive) steady state x^* .
2. Exactly one of the following holds:
 - (a) The unique steady state x^* in each compatibility class \mathcal{P} is locally asymptotically stable.
 - (b) In the space of total amounts $\{(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}})\} = \mathbb{R}_{>0}^3$, which we identify with the space of compatibility classes \mathcal{P} , a surface \mathcal{H} defines the border between those \mathcal{P} whose unique steady state x^* is locally asymptotically stable and those \mathcal{P} for which x^* is unstable.

Proof. Item 1 follows from Proposition 3.1.

For item 2, let J denote the Jacobian matrix of the mixed-mechanism system (4), with equal dissociation constants: $k_2 = k_6 = k_9 =: k_b$, evaluated at the parametrized steady state $\chi(x_1, x_2, x_6)$, from (9). The characteristic polynomial of J is:

$$p(\lambda) := \det(\lambda I - J) = \lambda^3(\lambda^6 + b_1\lambda^5 + b_2\lambda^4 + \dots + b_6),$$

where the coefficients b_i (displayed below) are rational functions in x_1, x_2, x_6 and the k_i 's. To streamline reading we only give the complete numerator of b_6 and b_1 . The full coefficients can be found in the `Mathematica` file `mixed_coeffs_charpoly_kb.nb`².

$$\begin{aligned} \text{numerator}(b_6) = & k_1^2 k_3^2 k_4 (k_{10} + k_7) (k_{10} k_5 k_7 + k_5 k_7 k_b + k_{10} k_8 (k_7 + k_b)) x_1 x_2^2 \\ & + k_1 k_{10} k_3 k_4 k_7 (k_3 + k_b) (k_{10} k_5 k_7 + k_5 k_7 k_b + k_{10} k_8 (k_7 + k_b)) x_2 x_6 \\ & + k_{10}^2 k_4 k_5 k_7^2 k_8 (k_3 + k_b)^2 x_6^2 + k_1 k_{10}^2 (k_3 + k_4) k_5 k_7^2 k_8 (k_3 + k_b) x_1 x_6^2 \\ & + k_1 k_{10} k_5 k_7 (k_{10} k_4 k_7 + k_3 k_4 k_7 + k_{10} k_3 (k_4 + k_7)) k_8 (k_3 + k_b) x_2 x_6^2 \end{aligned} \quad (13)$$

²This file and others mentioned below are in the Supporting Information; see Appendix A.

$$\begin{aligned}
\text{numerator}(b_5) &= k_1^2 k_3^2 k_4 (k_{10} + k_7)(k_{10} + k_b)(k_7 + k_b)x_1 x_2^2 \\
&\quad + k_1 k_{10} k_3 k_4 k_7 (k_{10} + k_b)(k_3 + k_b)(k_7 + k_b)x_2 x_6 + \dots \\
\text{numerator}(b_4) &= k_1 k_3 k_4 (k_{10} + k_7)(k_{10} + k_b)(k_3 + k_b)(k_7 + k_b)x_1 x_2 + \dots \\
\text{numerator}(b_3) &= \dots + k_1^2 k_3 \left(k_{10}^2 (k_7 + k_b) + k_7 k_b (k_3 + k_4 + k_7 + k_b) \right. \\
&\quad \left. + k_{10} \left((k_7 + k_b)^2 + k_3 (2k_7 + k_b) + k_4 (2k_7 + k_b) \right) \right) x_1^2 x_2 + \dots \\
\text{numerator}(b_2) &= \dots + k_1^2 k_3 (k_7 k_b + k_{10} (2k_7 + k_b)) x_1^2 x_2 + \dots \\
\text{numerator}(b_1) &= k_1 k_3 (k_7 k_b + k_{10} (2k_7 + k_b)) x_1 x_2 + k_{10} k_7 (k_3 + k_b) (k_{10} + k_3 + k_4 + k_7 + 3k_b) x_6 \\
&\quad + k_1 k_{10} k_7 (k_3 + k_b) x_1 x_6 + k_1 k_{10} k_7 (k_3 + k_b) x_2 x_6 + k_{10} k_7 (k_5 + k_8) (k_3 + k_b) x_6^2
\end{aligned}$$

And for the denominators:

$$\begin{aligned}
\text{denominator}(b_6) &= k_{10} (k_b + k_3) k_7 \\
\text{denominator}(b_i) &= k_{10} (k_b + k_3) k_7 x_6, \quad \text{for } i = 2, 3, 4, 5.
\end{aligned}$$

As x_1, x_2, x_6 and the k_i are positive, thus $b_1, b_2, \dots, b_6 > 0$ (in the aforementioned `Mathematica` file, we checked the above numerators are sums of only positive monomials).

Recall that, due to the 3 conservation laws (5), the Jacobian matrix has rank 6, not 9. Accordingly, the relevant Hurwitz matrix, namely, for $p(\lambda)/\lambda^3$, is as follows:

$$\begin{pmatrix}
b_1 & 1 & 0 & 0 & 0 & 0 \\
b_3 & b_2 & b_1 & 1 & 0 & 0 \\
b_5 & b_4 & b_3 & b_2 & b_1 & 1 \\
0 & b_6 & b_5 & b_4 & b_3 & b_2 \\
0 & 0 & 0 & b_6 & b_5 & b_4 \\
0 & 0 & 0 & 0 & 0 & b_6
\end{pmatrix}$$

Consider the Hurwitz determinants. First $\det H_1 = b_1 > 0$. The next 3 Hurwitz determinants are also positive:

$$\begin{aligned}
\text{numerator}(\det H_2) &= k_1^3 k_3^2 (k_7 k_b + k_{10} (2k_7 + k_b))^2 x_1^3 x_2^2 \\
&\quad + k_1^3 k_{10} k_3 k_7 (k_3 + k_b) (k_7 k_b + k_{10} (2k_7 + k_b)) x_1^3 x_2 x_6 + \dots \\
\text{numerator}(\det H_3) &= k_1^5 k_3^3 (k_{10} k_5 k_7 + k_5 k_7 k_b + k_{10} k_8 (k_7 + k_b)) (k_7 k_b + k_{10} (2k_7 + k_b))^2 x_1^5 x_2^3 x_6 + \dots \\
\text{numerator}(\det H_4) &= k_1^7 k_3^4 (k_{10} k_5 k_7 + k_5 k_7 k_b + k_{10} k_8 (k_7 + k_b)) (k_7 k_b + k_{10} (2k_7 + k_b))^2 \\
&\quad \left(k_5 k_7 (k_3 + k_4 + k_7) k_b + k_{10}^2 k_8 (k_7 + k_b) + \right. \\
&\quad \left. k_{10} (k_3 + k_4 + k_7) (k_5 k_7 + k_8 (k_7 + k_b)) \right) x_1^7 x_2^4 x_6^2 + \dots
\end{aligned}$$

where the denominators, which are positive, are, respectively:

$$\begin{aligned}
\text{denominator}(\det H_2) &= k_{10}^2 k_7^2 (k_b + k_3)^2 x_6^2 \\
\text{denominator}(\det H_3) &= k_{10}^3 k_7^3 (k_b + k_3)^3 x_6^3 \\
\text{denominator}(\det H_4) &= k_{10}^4 k_7^4 (k_b + k_3)^4 x_6^4
\end{aligned}$$

(We display only the leading terms of the polynomials; the complete polynomials together with an algorithmic verification of positivity are in `mixed_Hi.nb`.) The final Hurwitz determinant is $\det H_6 = (b_6)(\det H_5)$, and we saw that $b_6 > 0$. So, by the Routh-Hurwitz criterion (Proposition 2.2), the steady state $\chi(x_1, x_2, x_6)$ is locally stable if and only if $\det H_5 > 0$.

Hence, the surface \mathcal{H} that delineates the boundary between compatibility classes with stable steady states vs. those with unstable steady states is defined by $\det H_5 \circ (\phi \circ \chi)^{-1} = 0$, where $\phi \circ \chi$ is the parametrization of compatibility classes from Proposition 3.6. If \mathcal{H} intersects the positive orthant $\mathbb{R}_{>0}^3$, then case (b) of the proposition holds. Otherwise, if $\mathcal{H} \cap \mathbb{R}_{>0}^3 = \emptyset$, then we claim that we are in case (a). To show this, we need to verify that $\det H_5(x_1, x_2, x_6) > 0$ for some $(x_1, x_2, x_6) \in \mathbb{R}_{>0}^3$. The denominator of $\det H_5(x_1, x_2, x_6)$ is strictly positive:

$$\text{denominator}(\det H_5) = k_{10}^5 k_7^5 (k_3 + k_b)^5 x_6^5.$$

So we need only show that the numerator of $\det H_5(x_1, x_2, x_6)$ is strictly positive for some $(x_1, x_2, x_6) \in \mathbb{R}_{>0}^3$.

To this end, we view this numerator as a polynomial in x_1 (so the coefficients are rational functions of x_2, x_6 , and the k_i 's):

$$\begin{aligned} \text{numerator}(\det H_5) = & x_1^9 x_2^4 \left(\frac{k_{10} k_7 x_6 (k_3 + k_b)}{k_3 (k_{10} (2k_7 + k_b) + k_7 k_b)} + x_2 \right) \\ & \left[k_8 x_6 \left(\alpha_{01} + \alpha_{10} \frac{k_5}{k_8} \right) + k_8^2 x_6^2 \left(\alpha_{02} + \alpha_{11} \frac{k_5}{k_8} + \alpha_{20} \left(\frac{k_5}{k_8} \right)^2 \right) \right. \\ & \left. + k_8^3 x_6^3 \left(\alpha_{03} + \alpha_{12} \frac{k_5}{k_8} + \alpha_{21} \left(\frac{k_5}{k_8} \right)^2 + \alpha_{30} \left(\frac{k_5}{k_8} \right)^3 \right) \right] + \text{lower order terms} , \end{aligned} \quad (14)$$

where the coefficients α_{ij} are sums of (many) positive monomials and are given in the file `mixed_analysis_H5N_x1_LT.nb`. Therefore (for fixed x_2 and x_6) when x_1 is sufficiently large, the expression (14) is positive, as desired. \square

The proof of Proposition 4.1 focused on the surface \mathcal{H} defined by the equation $\det H_5 \circ (\phi \circ \chi)^{-1} = 0$. This surface sometimes meets the positive orthant $\mathbb{R}_{>0}^3$, and indeed we show that this is the case when certain relationships hold among the rate constants.

Theorem 4.2. *Consider the dynamical system (4) arising from the mixed-mechanism network. Assume the positive rate constants satisfy $k_2 = k_6 = k_9$ and the following inequality:*

$$k_{10} k_3 k_4 - (k_3 + k_4)(k_3 + k_7)(k_4 + k_7) > 0 . \quad (15)$$

If k_5/k_8 is sufficiently large, then there is a compatibility class \mathcal{P} whose unique steady state x^ is unstable.*

Proof. Assume that the rate constants satisfy $k_2 = k_6 = k_9 =: k_b$ and (15). By the proof of Proposition 4.1, a steady state $\chi(x_1, x_2, x_6)$ of the mixed-mechanism system (4) is locally stable if and only if $\det H_5(x_1, x_2, x_6) > 0$. We also saw in that proof that the denominator of $\det H_5(x_1, x_2, x_6)$ is strictly positive for all $(x_1, x_2, x_6) \in \mathbb{R}_{>0}^3$. So, by Proposition 2.2, it suffices to show that if k_5/k_8 is sufficiently large, then there exists $(x_1^*, x_2^*, x_6^*) \in \mathbb{R}_{>0}^3$ such that the numerator of $\det H_5(x_1^*, x_2^*, x_6^*)$ is strictly negative: this would show that the steady state $x^* := \chi(x_1^*, x_2^*, x_6^*)$ is unstable.

To this end, view the numerator of $\det H_5$ as a polynomial in x_2 with coefficients in x_1, x_6 , and the k_i 's. It is a degree-9 polynomial in x_2 of the following form (see the file

`mixed_analysis_H5N_x2_LT.nb`):

$$\begin{aligned} \text{numerator}(\det H_5) &= k_1^9 (\alpha_0 x_6^3 + \alpha_1 x_6^2 + \alpha_2 x_6 + \alpha_3) \left(x_1^5 + \frac{k_{10} k_7 (k_3 + k_b)}{k_3 (k_{10} (2k_7 + k_b) + k_7 k_b)} x_1^4 x_6 \right) x_2^9 \\ &\quad + \text{lower order terms} , \end{aligned} \tag{16}$$

where $\alpha_0, \dots, \alpha_3$ are rational functions in $k_b, k_3, k_4, k_5, k_7, k_8, k_{10}$. These functions α_i are given in `mixed_analysis_H5N_x2_LT.nb`.

We now analyze α_0 , which has the following form (see `mixed_analysis_H5N_x2_LT.nb`):

$$\alpha_0 = k_8^3 \left(\beta_0 \left(\frac{k_5}{k_8} \right)^3 + \beta_1 \left(\frac{k_5}{k_8} \right)^2 + \beta_2 \left(\frac{k_5}{k_8} \right) + \beta_3 \right) , \tag{17}$$

where each coefficient β_i is a rational function in $k_b, k_3, k_4, k_7, k_{10}$ (and hence does not depend on k_1, k_5 , or k_8). In particular, β_0 is the following polynomial:

$$\beta_0 = -k_1^9 k_3^5 k_7^3 (k_{10} k_3 k_4 - (k_3 + k_4)(k_3 + k_7)(k_4 + k_7)) (k_{10} + k_b)^3 (k_7 k_b + k_{10}(2k_7 + k_b))^2 .$$

It follows that $\beta_0 < 0$ when inequality (15) holds.

Thus, when (15) holds, then, by equation (17), the inequality $\alpha_0 < 0$ holds for k_5/k_8 sufficiently large. In this case, the cubic polynomial in x_6 appearing in (16), and hence also the coefficient of x_2^9 in the numerator of $\det H_5$, will be negative for x_6 sufficiently large. Hence, if we choose $x_1 := 1$ (or any positive value) and x_6 and x_2 sufficiently large, then the numerator of $\det H_5$ will be negative. \square

In the remainder of this section, we focus on the question of whether the surface \mathcal{H} consists of (at least generically) Hopf bifurcations. If so, this would imply that whenever a steady state of the mixed-mechanism network switches from stable to unstable, we expect it to undergo a Hopf bifurcation leading to oscillations. We begin our analyses of Hopf bifurcations by giving a criterion for such bifurcations.

Proposition 4.3. *Consider the dynamical system (4) arising from the mixed-mechanism network and any positive rate constants with $k_2 = k_6 = k_9$ and $k_{10} k_3 k_4 - (k_3 + k_4)(k_3 + k_7)(k_4 + k_7) > 0$. Then there exists $(x_1^*, x_2^*, x_6^*) \in \mathbb{R}_{>0}^3$ such that $\det H_5(x_1^*, x_2^*, x_6^*) = 0$ (in other words, $\phi \circ \chi(x_1^*, x_2^*, x_6^*)$ is on \mathcal{H}). Moreover, for such a vector (x_1^*, x_2^*, x_6^*) , the system undergoes a Hopf bifurcation with respect to x_2 at the steady state $\chi(x_1^*, x_2^*, x_6^*)$ if and only if the following inequality holds:*

$$\frac{d(\text{numerator}(\det H_5)|_{x_1=x_1^*, x_6=x_6^*})}{dx_2} \Big|_{x_2=x_2^*} \neq 0 . \tag{18}$$

Proof. Fix positive rate constants for which $k_2 = k_6 = k_9$ and $k_{10} k_3 k_4 - (k_3 + k_4)(k_3 + k_7)(k_4 + k_7) > 0$. By the proofs of Proposition 4.1 and Theorem 4.2, the function $\det H_5 : \mathbb{R}_{>0}^3 \rightarrow \mathbb{R}$ takes both positive and negative values. So, as $\det H_5$ is continuous, $\det H_5(x_1^*, x_2^*, x_6^*) = 0$ for some $(x_1^*, x_2^*, x_6^*) \in \mathbb{R}_{>0}^3$ (by the intermediate-value theorem).

Assume $\det H_5(x_1^*, x_2^*, x_6^*) = 0$. To see whether the steady state $\chi(x_1^*, x_2^*, x_6^*)$ is a Hopf bifurcation with respect to the parameter $\mu = x_2$, where the curve of steady states is $x(\mu) =$

$\chi(x_1^*, \mu, x_6^*)$ and $\mu_0 = x_2^*$, we use Proposition 2.3 (Yang's criterion). Parts (i) and (ii) of that criterion hold for *any* steady state $\chi(x_1^*, x_2^*, x_6^*)$, because $b_6 = b_6(x_1^*, x_2^*, x_6^*) > 0$, by (13), and also $\det H_i = \det H_i(x_1^*, x_2^*, x_6^*) > 0$ for $i = 1, 2, 3, 4$ (from the proof of Proposition 4.1). Recall from the proof of Proposition 4.1 that the denominator of $\det H_5$ is strictly positive and does not depend on x_2 ; thus, we can focus on the numerator of H_5 . So, by Proposition 2.3, $\chi(x_1^*, x_2^*, x_6^*)$ is a Hopf bifurcation with respect x_2 if and only if (18) holds. \square

Remark 4.4. Given rate constants k_i as in Proposition 4.3 for which there is a Hopf bifurcation, we can perturb slightly the rate constants involved in (15) (while maintaining the equality $k_2 = k_6 = k_9$) and preserve the existence of a Hopf bifurcation. Indeed, this assertion follows from Proposition 4.3 (inequality (18) is maintained under small perturbations of the x_i 's), the fact that simple roots of a polynomial depend continuously – in fact, infinitely differentially – on the coefficients [28], and the fact that the inequality (15) defines a (relatively) open set in the parameter space of the k_i 's.

Under the hypotheses of Proposition 4.3, we expect that inequality (18) holds generically on \mathcal{H} . We will confirm this when the rate constants are those in Table 1 (Theorem 4.5).

The proof of Theorem 4.5 makes use of discriminants, which we now review. Consider a degree- n , univariate polynomial $f = c_n x^n + c_{n-1} x^{n-1} + \dots + c_0$ with coefficients $c_i \in \mathbb{C}$. A *multiple root* of f is some $x^* \in \mathbb{C}$ for which $(x - x^*)^2$ divides f or equivalently $f(x^*) = f'(x^*) = 0$. It is well-known that f has a multiple root in \mathbb{C} if and only if a certain multivariate polynomial in the c_i 's, the *discriminant*, vanishes [15]. For instance, the discriminant of the quadratic polynomial $ax^2 + bx + c$ is the familiar expression $b^2 - 4ac$.

Theorem 4.5 (Hopf bifurcations of the mixed-mechanism network). *Consider the dynamical system (4) arising from the mixed-mechanism network and rate constants in Table 1. Let \mathcal{H} denote the surface, from Proposition 4.1, that defines the border between those \mathcal{P} whose unique steady state x^* is locally stable and those \mathcal{P} for which x^* is unstable. Then \mathcal{H} consists generically of compatibility classes \mathcal{P} whose unique steady state x^* undergoes a simple Hopf bifurcation (with x_2 as bifurcation parameter).*

Proof. It is straightforward to check that the rate constants in Table 1 satisfy the inequality (15). Therefore, the surface \mathcal{H} as in Proposition 4.1.2(b) exists, and is defined by $\det H_5 = 0$, where H_5 is the Hurwitz matrix (specialized to the rate constants in Table 1) as in the proof of Proposition 4.1.

To prove that \mathcal{H} consists generically of Hopf bifurcations, we use Proposition 4.3. That result states that $\chi(x_1^*, x_2^*, x_6^*)$ is a Hopf bifurcation with respect to x_2 if and only if $(x_1^*, x_2^*, x_6^*) \in \mathcal{H}' \setminus \mathcal{S}$, where

$$\begin{aligned} \mathcal{H}' &:= V_{>0}(\det H_5) := \{(x_1, x_2, x_6) \in \mathbb{R}_{>0}^3 \mid \det H_5(x_1, x_2, x_6) = 0\} \text{ , and} \\ \mathcal{S} &:= \left\{ (x_1^*, x_2^*, x_6^*) \in \mathcal{H}' \mid \frac{d(\det H_5|_{x_1=x_1^*, x_6=x_6^*})}{dx_2} \Big|_{x_2=x_2^*} = 0 \right\} \subseteq \mathcal{H}' \text{ .} \end{aligned}$$

We have that $\mathcal{H} = \phi \circ \chi(\mathcal{H}')$, and that the following subset of \mathcal{H} consists of compatibility classes whose unique steady state undergoes a simple Hopf bifurcation with x_2 as bifurcation parameter: $\phi \circ \chi(\mathcal{H}' \setminus \mathcal{S})$. So, it suffices to show that $\dim(\mathcal{S}) < \dim(\mathcal{H}')$. Note that $\dim(\mathcal{H}') \geq 2$, so we will show that $\dim(\mathcal{S}) \leq 1$.

Recall that $(\phi \circ \chi) : \mathbb{R}_{>0}^3 \rightarrow \mathbb{R}_{>0}^3$ is a bijection. Let $\mathbf{g} := h_5 \circ (\phi \circ \chi)^{-1} : \mathbb{R}_{>0}^3 \rightarrow \mathbb{R}$. Also, let $p := (\phi \circ \chi)_2 = x_6 + \frac{1009}{1800}x_1x_2$ denote the second coordinate function of $\phi \circ \chi$ from (11) (here we assume the rate constants from Table 1). We are interested in checking whether $\frac{\partial \mathbf{g}}{\partial P_{\text{tot}}}$ is (generically) nonzero whenever $\mathbf{g} = 0$. Accordingly, we use the chain rule:

$$\begin{aligned} \frac{\partial \mathbf{g}}{\partial P_{\text{tot}}} &= \frac{1}{\partial p / \partial x_1} \frac{\partial h_5}{\partial x_1} + \frac{1}{\partial p / \partial x_2} \frac{\partial h_5}{\partial x_2} + \frac{1}{\partial p / \partial x_6} \frac{\partial h_5}{\partial x_6} \\ &= \frac{1800}{1009x_2} \frac{\partial h_5}{\partial x_1} + \frac{1800}{1009x_1} \frac{\partial h_5}{\partial x_2} + \frac{\partial h_5}{\partial x_6}. \end{aligned} \quad (19)$$

For specific values of x_1, x_2, x_6 , it is straightforward to check whether the sum (19) is nonzero. More generally, we expect this sum to be generically nonzero; that is, we expect that the surface \mathcal{H} consists generically of Hopf bifurcations with respect to the total-amount P_{tot} .

5 Generating rate constants admitting oscillations

The proof of Theorem 4.2 yields a recipe for generating rate constants for the mixed-mechanism network at which we expect oscillations arising from a Hopf bifurcation. Specifically, we choose rate constants k_i for which the equalities $k_2 = k_6 = k_9$ hold, the inequality (15) holds, and $\alpha_0 < 0$ (as in (17)), and then pick x_2 and x_6 large enough so that $\det H_5$ is negative but close to 0. We summarize these choices in the following procedure.

Procedure 5.1 (Generating rate constants likely to admit oscillations).

Input: *The following functions³:*

- (i) α_0 as in (17),
- (ii) the numerator of $\det H_5$,
- (iii) $q := \alpha_0 x_6^3 + \alpha_1 x_6^2 + \alpha_2 x_6 + \alpha_3$ as in (16), and
- (iv) $\phi \circ \chi$ given in Proposition 3.6.

Output: *Rate constants and total amounts for which $\det H_5$ is negative and close to 0.*

Steps:

1. Choose positive values for $k_b := k_2 = k_6 = k_9$, x_1 , k_1 , k_3 , k_4 , k_7 , and k_8 .
2. Choose a positive value for k_{10} for which $k_{10} > \frac{(k_3+k_4)(k_3+k_7)(k_4+k_7)}{k_3k_4}$.
3. Choose the remaining rate constant k_5 such that $\alpha_0 < 0$.
4. Choose x_6 so that $q < 0$.
5. Choose x_2 so that the numerator of $\det H_5$ is negative but close to 0.

³The functions are provided as a text file in the Supporting Information. See Appendix A.

6. Return the k_i 's and $(K_{tot}, P_{tot}, S_{tot}) := \phi \circ \chi(x_1, x_2, x_6)$, where $\phi \circ \chi$ is evaluated at the k_i 's (and x_1, x_2, x_6) chosen in the previous steps.

Remark 5.2. Using the output of Procedure 5.1, one can attempt to exhibit and analyze oscillations or Hopf bifurcations using software, e.g., `Matcont` [8]. See Figure 4.

Example 5.3. We follow Procedure 5.1 as follows (to verify our computations see the file `mixed_generate_rc.nb`):

Step 1. We pick $k_b = 0.143738$, $k_1 = 0.575284$, $k_3 = 3.89096$, $k_4 = 5.05386$, $k_7 = 9.25029$, $k_8 = 0.621813$, and $x_1 = 5.82148$.

Step 2. The inequality for this step evaluates to $k_{10} > 85.5048$, so we choose $k_{10} = 90$.

Step 3. Evaluating α_0 at the chosen k_i 's, we obtain the following inequality:

$$-8.896 \times 10^{17} k_5^3 + 1.49735 \times 10^{20} k_5^2 + 4.79701 \times 10^{20} k_5 + 2.42695 \times 10^{20} < 0 ,$$

which we find, using `Mathematica`, is feasible for $k_5 > 171.471$. So, we pick $k_5 = 172$.

Step 4. By evaluating q at the values chosen above, we obtain the following inequality:

$$-1.41683 \times 10^{22} x_6^3 - 3.5508 \times 10^{25} x_6^2 - 1.80374 \times 10^{25} x_6 + 2.15078 \times 10^{24} < 0 .$$

This inequality holds when $x_6 > 0.0996797$, so we choose $x_6 = 0.1$.

Step 5. By evaluating the numerator of $\det H_5$, we obtain the following inequality:

$$\begin{aligned} & -5.42893 \times 10^{25} x_2^9 - 4.20944 \times 10^{29} x_2^8 - 5.05393 \times 10^{31} x_2^7 - 6.67609 \times 10^{32} x_2^6 \\ & + 4.66164 \times 10^{33} x_2^5 + 3.97617 \times 10^{34} x_2^4 + 1.01289 \times 10^{35} x_2^3 + 1.19894 \times 10^{35} x_2^2 \\ & + 6.7831 \times 10^{34} x_2 + 1.4718 \times 10^{34} < 0 . \end{aligned}$$

This inequality is feasible, as computed in `Mathematica`, for $x_2 > 9.0382$; we pick $x_2 = 10$.

Step 6. We have determined the following rate constants:

k_1	k_2	k_3	k_4	k_5	k_6	k_7	k_8	k_9	k_{10}
0.575284	0.143738	3.89096	5.05386	172	0.143738	9.25029	0.621813	0.143738	90

We obtain the following steady state, using (9):

$$\begin{aligned} (x_1, x_2, \dots, x_9) &= \chi(x_1, x_2, x_6) \\ &= (5.82148, 10, 8.30052, 6.39056, 1.90691, 0.1, 3.49146, 520.229, 0.358855) . \end{aligned} \tag{20}$$

Using this steady state, we obtain the total amounts, using (10):

$$(K_{tot}, P_{tot}, S_{tot}) = \phi(x_1, x_2, \dots, x_9) = (24.6911, 3.95031, 546.499) . \tag{21}$$

The resulting bifurcation analysis is shown in Figure 4.

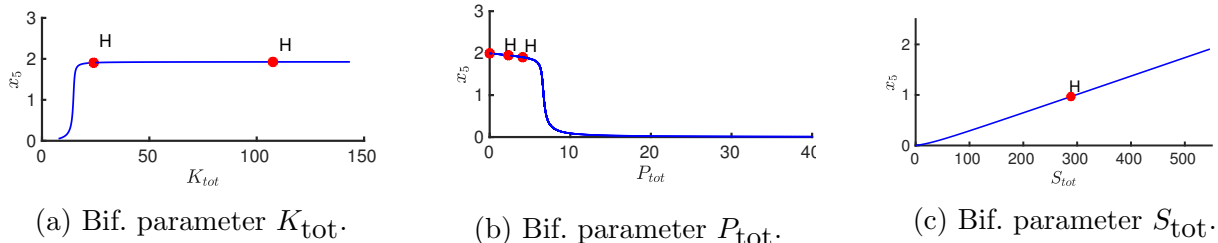


Figure 4: Numerical continuation of the steady state (20), when total amounts are as in (21): (a) A (supercritical) Hopf bifurcations are at $K_{\text{tot}} \approx 24.0623$ and 107.5635 . (b) (Supercritical) Hopf bifurcations are at $P_{\text{tot}} \approx 4.1022$ and $P_{\text{tot}} \approx 2.3275$. `Matcont` reported a branch point, the leftmost red circle, at $P_{\text{tot}} \approx -8.5427 \times 10^{-13}$, i.e., for $P_{\text{tot}} \approx 0$ and thus outside the domain of interest. (c) A (supercritical) Hopf bifurcation is at $S_{\text{tot}} \approx 288.4384$.

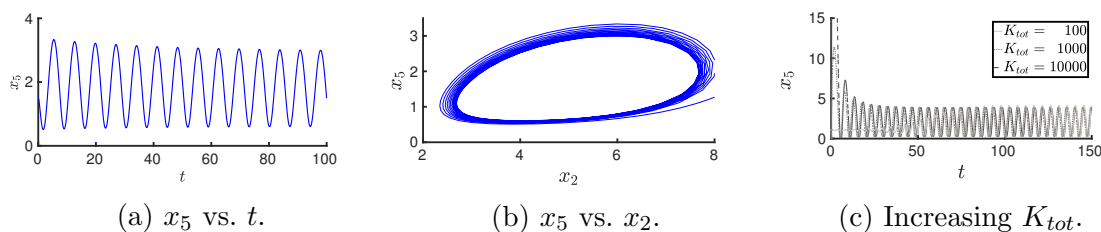


Figure 5: Numerical verification of oscillations in the mixed-mechanism system with rate constants as in Table 1. For (a) and (b), we used $(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}}) = (14, 5, 40)$ and initial values as in (12). Here the solution converges to a periodic orbit. For (c), we used $(P_{\text{tot}}, S_{\text{tot}}) = (8, 40)$ and three values for K_{tot} (namely, 100, 1000, and 10000), and again initial values as in (12), except that $x_5 = 1.1$. Again the solutions seem to converge to a periodic orbit, and moreover this periodic orbit appears not to depend on the value of K_{tot} . See Conjecture 6.2.

6 Dynamics: simulations and conjectures

Are oscillations the norm when the mixed-mechanism system has an unstable steady state? We conjecture that this is the case.

Conjecture 6.1. *Consider the mixed-mechanism network, and any choice of rate constants and total amounts. If the unique steady state in \mathcal{P} is unstable, then \mathcal{P} contains a periodic orbit that is locally asymptotically stable.*

Some simulations are shown in Figure 5. In (A) and (B) of that figure, we see solutions converging to a period orbit; this system arises from total-amounts similar to those that Suwanmajo and Krishnan found to support oscillations. In contrast, in Figure 5(C), we see oscillations, when $(P_{\text{tot}}, S_{\text{tot}}) = (8, 40)$, for three large values for K_{tot} : 100, 1000, and 10000. Oscillations persist across these values, which yields a much larger range for K_{tot} than Suwanmajo and Krishnan’s results would suggest.

Moreover, the value of K_{tot} appears *not* to affect the resulting periodic orbit (when projected to x_5 , the concentration of the doubly phosphorylated substrate S_2)! Could this be a biological design mechanism for robust timekeeping (for instance, in circadian clocks)?

Mathematically, do oscillations indeed persist for arbitrarily large K_{tot} ? And, does the periodic orbit in x_5 indeed not depend on K_{tot} ? We conjecture that the answers are “yes”.

Conjecture 6.2.

1. Consider the mixed-mechanism network with rate constants as in Table 1. Then there exist values of P_{tot} and S_{tot} such that for K_{tot} arbitrarily large, the unique steady state in \mathcal{P} is unstable.
2. For such values of P_{tot} and S_{tot} and for sufficiently large K_{tot} , the compatibility class \mathcal{P} contains a periodic orbit such that this orbit in x_5 (the concentration of S_2) does not depend on the value of K_{tot} .

One way to tackle Conjecture 6.2 is analyze the robustness of the period and the amplitude with respect to K_{tot} using the theory developed in [3, 24, 23].

Finally, we consider the dynamics in compatibility classes that contain a locally stable steady state. Our simulations suggest that such a steady state is in fact globally stable. Accordingly, we pose the question, *Consider the mixed-mechanism network, and any choice of rate constants and total amounts. If the unique steady state x^* in \mathcal{P} is locally stable, does it always follow that x^* is globally stable?* In the Michaelis-Menten limit, this is true [36].

7 Discussion

We return to the question, *How do oscillations emerge in phosphorylation networks?* Concretely, we would like (1) easy-to-check criteria for exactly which phosphorylation networks admits oscillations or Hopf bifurcations, and (2) for those networks that admit oscillations, a better understanding of the “geography of parameter space”, that is, a characterization of which rate constants and initial conditions yield oscillations. Both of these problems are still unresolved, and the second problem in particular is very difficult.

Nevertheless, here we made progress on characterizing some of the geography of parameter space for the mixed-mechanism phosphorylation network. Indeed, we found that a single surface defines the boundary between stable and unstable steady states, and this surface consists generically of Hopf bifurcations. Hence, when a steady state switches from stable to unstable, then we expect it to undergo a Hopf bifurcation leading to oscillations. Additionally, we gave a procedure for generating many parameter values leading to oscillations.

We now discuss the significance of our work. At a glance, it might seem that our results are specific to network (1) and rate constants related to those in Table 1. However, the approach is general: for other rate constants (e.g., estimated from data) or other networks (e.g., a version of the ERK network from [37] also has oscillations and a unique steady state), one could apply the same techniques. Therefore, the potential impact is broad.

Going forward, we hope that the novel techniques we used – specifically, using a steady-state parametrization together with a Hopf-bifurcation criterion – will contribute to solving other problems. For instance, we expect that such tools could help solve an important open problem in this area [7], namely, the question of whether oscillations or Hopf bifurcations arise from the fully distributive phosphorylation network.

Acknowledgements

AS was partially supported by the NSF (DMS-1312473/1513364 and DMS-1752672) and the Simons Foundation (#521874). AS thanks Jonathan Tyler for helpful discussions. CC was partially supported by the Deutsche Forschungsgemeinschaft DFG (DFG-284057449).

A Files in the Supporting Information

The following files can be found at <http://www.math.tamu.edu/~annejls/mixed.html>:

Text files:

- `mixed_H5N_kb.txt` ...contains H5N, the numerator of $\det H_5$ under the assumption $k_2 = k_6 = k_9 = k_b$
- `mixed_W.txt` ...contains a matrix W that defines (5)
- `mixed_xt.txt` ...contains xt, the parameterization (9)
- `mixed_Jx.txt` ...contains Jx, the Jacobian evaluated at the parameterization (9)

Mathematica Notebooks:

- `mixed_analysis_H5N_x1.LT.nb`:
Functionality: This file can be used to obtain numerator($\det H_5$) as in (14), in particular to examine the coefficients $\alpha_{01}, \alpha_{10}, \dots$
Input: the file `mixed_H5N_kb.txt`
- `mixed_analysis_H5N_x2.LT.nb`:
Functionality: This file can be used to obtain numerator($\det H_5$) as in (16), in particular to examine the coefficients $\alpha_0, \dots, \alpha_3$ and β_0, \dots, β_3 .
Input: the file `mixed_H5N_kb.txt`
- `mixed_coeffs_charpoly.nb`:
Functionality: This file can be used to obtain the characteristic polynomial of the Jacobian of the system (4). It contains the `Mathematica` commands to establish $b_i > 0$.
Input: the file `mixed_Jx.txt`
- `mixed_Hi.nb`:
Functionality: This file can be used to obtain the determinants of the Hurwitz matrices H_2, \dots, H_5 . It contains the `Mathematica` commands to establish $\det H_i > 0$, for $i = 2, 3, 4$ and that $\det H_5$ is of mixed sign.
Input: the file `mixed_Jx.txt`
- `mixed_generate_rc.nb`:
Functionality: This file contains a realization of Procedure 5.1.
Input: the files `mixed_H5N_kb.txt`, `mixed_W.txt`, `mixed_xt.txt`, `mixed_Jx.txt`.

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