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...
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:

\[
\begin{align*}
S_0 + K &\underset{k_2}{\overset{k_1}{\rightleftharpoons}} S_0 K \xrightarrow{k_3} S_1 K \xrightarrow{k_4} S_2 + K \\
S_2 + P &\overset{k_5}{\underset{k_6}{\rightleftharpoons}} S_2 P \xrightarrow{k_7} S_1 + P \overset{k_8}{\underset{k_9}{\rightleftharpoons}} S_1 P \xrightarrow{k_{10}} S_0 + P
\end{align*}
\]  

(1)

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 mechanism.

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 $K_{\text{tot}}$, $P_{\text{tot}}$, and $S_{\text{tot}}$, 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].

| $k_i$ | $K_{\text{tot}}$ | $P_{\text{tot}}$ | $S_{\text{tot}}$ |
|------|-----------------|-----------------|-----------------|
| 1    | 17.5            | 5               | 40              |
| 1    | 100             | 1               | 100             |
| 1    | 100             | 0.9             | 3               |
| 1    | 100             | 3               | 1               |

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

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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]).
steady state is locally stable

≈ 13.03 (oscillations)

≈ 29.23

steady state is unstable

steady state is locally stable

$K_{tot}$

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., $K_{tot} = 100$). 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 $(K_{tot}, P_{tot}, S_{tot}) \in \mathbb{R}_+^3$ 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) [10]. 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.
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:

\[
\begin{align*}
S_0 + K & \rightleftharpoons S_0K \rightarrow S_1 + K \rightleftharpoons S_1K \rightarrow S_2 + K \quad \text{(distributive)} \\
S_0 + K & \rightleftharpoons S_0K \rightleftharpoons S_1K \rightarrow S_2 + K \quad \text{(processive)}
\end{align*}
\]

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 [11] 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 [11].

For the mixed-mechanism network, we let $x_1, x_2, \ldots, x_9$ 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:

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

(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:

\[
\begin{align*}
K_{\text{tot}} &= x_2 + x_3 + x_4, \\
P_{\text{tot}} &= x_6 + x_7 + x_9, \\
S_{\text{tot}} &= x_1 + x_3 + x_4 + x_5 + x_7 + x_8 + x_9.
\end{align*}
\]

(5)

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

\[\mathcal{P} = \{x \in \mathbb{R}_{>0}^9 \mid \text{the conservation equations (5) hold}\}.\]

(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:

\[
\frac{dx}{dt} = \Gamma \cdot R(x) =: g(x),
\]

(7)

where $\Gamma$ and $R$ are as follows. Letting $s$ denote the number of species and $r$ the number of reactions, $\Gamma$ 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, $R : \mathbb{R}_{>0}^s \to \mathbb{R}_{>0}^r$ 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 nonnegative 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 \( \sigma \) 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 \( p(\lambda) = a_0 \lambda^n + a_1 \lambda^{n-1} + \cdots + a_n \) is the following \( i \times i \) matrix:

\[
H_i = \begin{pmatrix}
    a_1 & a_0 & 0 & 0 & \cdots & 0 \\
    a_3 & a_2 & a_1 & a_0 & \cdots & 0 \\
    \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\
    a_{2i-1} & a_{2i-2} & a_{2i-3} & a_{2i-4} & \cdots & a_i
\end{pmatrix},
\]

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 \( p(\lambda) = a_0 \lambda^n + a_1 \lambda^{n-1} + \cdots + a_n \) 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 \( i = 1, \ldots, n \)).

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

\[
\mu \mapsto x(\mu)
\]

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

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

and, for \( i = 1, \ldots, n \), 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 \( \mu \) if and only if the following hold:

(i) \( a_n(\mu_0) > 0 \),
(ii) $\det H_1(\mu_0) > 0$, $\det H_2(\mu_0) > 0$, …, $\det H_{n-2}(\mu_0) > 0$, 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 $\chi = \chi_{k_1,\ldots,k_{10}}$:

$$\chi : \mathbb{R}^3_+ \to \mathbb{R}^9_+$$

$$(x_1, x_2, x_6) \mapsto (x_1, x_2, \ldots, x_9),$$

(9)

8
given by

\[
\begin{align*}
x_3 &:= \frac{k_1}{k_2 + k_3}x_1x_2, &
x_4 &:= \frac{k_1k_3}{(k_2 + k_3)k_4}x_1x_2, &
x_5 &:= \frac{k_1k_3(k_0 + k_7)}{(k_2 + k_3)k_5k_7}x_1x_2, \\
x_7 &:= \frac{k_1k_3}{(k_2 + k_3)k_7}x_1x_2, &
x_8 &:= \frac{k_1k_3(k_0 + k_10)}{(k_2 + k_3)k_8k_10}x_1x_2, &
x_9 &:= \frac{k_1k_3}{(k_2 + k_3)k_10}x_1x_2.
\end{align*}
\]

**Proof.** It is straightforward to check that the image of \( \chi \) 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 \( \text{(4)} \) vanishes. Conversely, let \( x^* = (x_1, x_2, \ldots, x_9) \) be a positive steady state. The right-hand side of the ODEs \( \text{(4)} \) 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 \( \text{(9)} \), so \( x^* \) is in the image of \( \chi \).

**Remark 3.3.** The parametrization \( \text{(9)} \) 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 \( \chi \) in Theorem 3.2, we divide by \( x_6 \), so \( \chi \) is technically not a monomial map. However, \( \chi \) can be made monomial: we introduce \( y := \frac{x_2}{x_6} \), so that the parametrization accepts as input \((y, x_2, x_6)\), and then \( x_1 \) is replaced by \( yx_6 \).

### 3.3 A parametrization of the compatibility classes

Every compatibility class \( \mathcal{P} \) of the mixed-mechanism network, by definition \( \text{(6)} \), is uniquely determined by a choice of total amounts \((K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}}) \in \mathbb{R}^3_{>0}\). Thus, we identify the set of compatibility classes with \( \{(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}})\} = \mathbb{R}^3_{>0} \). We parametrize this set below (Proposition 3.4).

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

\[
\phi(x) := (x_2 + x_3 + x_4, x_6 + x_7 + x_9, x_1 + x_3 + x_4 + x_5 + x_7 + x_8 + x_9).
\]  

Each compatibility class \( \mathcal{P} \) contains a unique positive steady state (Proposition 3.1), and the positive steady states are parametrized by \( \chi \) 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 \( \text{(1)} \), with the corresponding total amounts \((K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}}) \in \mathbb{R}^3_{>0} \). Then, for every choice of positive rate constants \( k_i \), the following is a bijection that sends a vector \((x_1, x_2, x_6) \in \mathbb{R}^3_{>0} \) to the compatibility class in which the unique steady state is \( \chi(x_1, x_2, x_6) \):

\[
\phi \circ \chi : \mathbb{R}^3_{>0} \to \mathbb{R}^3_{>0} = \{(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}})\},
\]
where $\phi$ is as in (10) and $\chi$ 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.

$$

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

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 $(K_{tot}, P_{tot}, S_{tot}) = (17.5, 5, 40)$, 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 $\chi$ in (9):

$$
x^* = \chi(x^*_1, x^*_2, x^*_6) \approx (1.0134, 8.6916, 4.4041, 4.4041, 1.4893, 0.0624, 4.8935, 23.7512, 0.0440).
$$

### 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].
Consider the dynamical system 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 \( \{(K_{tot}, P_{tot}, S_{tot})\} = \mathbb{R}^3_{>0} \), which we identify with the space of compatibility classes \( \mathcal{P} \), a surface \( H \) 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. Item 2, let \( J \) denote the Jacobian matrix of the mixed-mechanism system arising from the mixed-mechanism network with equal dissociation constants: \( k_2 = k_6 = k_9 = k_0 \), evaluated at the parametrized steady state \( \chi(x_1, x_2, x_6) \), from (11). The characteristic polynomial of \( J \) is:

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

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

\[
\text{numerator}(b_6) = k_2^2k_3^2k_4(k_{10} + k_7)(k_{10}k_5k_7 + k_5k_7k_6 + k_{10}k_8(k_7 + k_6))x_1x_2^2
\]

\[
+ k_1k_{10}k_3k_4k_7(k_3 + k_6)(k_{10}k_3k_7 + k_3k_7k_6 + k_{10}k_8(k_7 + k_6))x_2x_6
\]

\[
+ k_1^2k_3k_4^2k_7^2(k_3 + k_6)^2x_6^2 + k_1k_2^2(k_3 + k_1)_4k_3^2k_7^2k_8(k_3 + k_6)x_1x_2^2
\]

\[
+ k_1k_{10}k_3k_7(k_{10}k_4k_7 + k_3k_4k_7 + k_{10}k_3(k_4 + k_7))k_8(k_3 + k_6)x_2x_6^2
\]

²This file and others mentioned below are in the Supplementary Information; see Appendix A.
numerator(b_6) = k_1^2 k_2^3 k_4(k_{10} + k_7)(k_{10} + k_8)(k_7 + k_9)x_1 x_2^2 \\
\phantom{\text{numerator(b_6)}} + k_1 k_{10} k_3 k_4 k_7(k_{10} + k_8)(k_7 + k_9)(k_7 + k_9)x_2 x_6 + \ldots
\numerator(b_4) = k_1 k_3 k_4(k_{10} + k_7)(k_{10} + k_8)(k_3 + k_9)(k_7 + k_8)(k_7 + k_9)x_1 x_2 + \ldots
\numerator(b_3) = \ldots + k_1^2 k_3 \left( k_1^2(k_7 + k_9) + k_7 k_9(k_3 + k_4 + k_7 + k_9) \right) x_1^2 x_2 + \ldots
\numerator(b_2) = \ldots + k_1^2 k_3 \left( k_7 k_9 + k_{10}(2k_7 + k_9) \right) x_1^2 x_2 + \ldots
\numerator(b_1) = k_1 k_3(k_7 k_9 + k_{10}(2k_7 + k_9)) x_1 x_2 + k_{10} k_7 k_9 x_6 + k_1 k_{10} k_7 (k_3 + k_9) x_2 x_6 + k_{10} k_7 (k_5 + k_8)(k_3 + k_9) x_6^2
\ 
And for the denominators:
\ 
\denominator(b_6) = k_{10}(k_6 + k_3) k_7
\denominator(b_i) = k_{10}(k_6 + k_3) k_7 x_6 , \text{ for } i = 2, 3, 4, 5 .
\ 
As x_1, x_2, x_6 and the k_i are positive, thus b_1, b_2, \ldots, b_6 > 0 \text{ (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:

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

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

\[
\numerator(\det H_2) = k_1^2 k_2^2(k_7 k_9 + k_{10}(2k_7 + k_9))^2 x_1^3 x_2^2 \\
\phantom{\text{numerator(\det H_2)}} + k_1^2 k_{10} k_3 k_7 \left( k_7 k_9 + k_{10}(2k_7 + k_9) \right) x_1^3 x_2 x_6 + \ldots
\numerator(\det H_3) = k_1^2 k_2^3 \left( k_{10} k_3(k_7 + k_9 + k_{10} k_7 + k_9) k_9(k_7 + k_9)(k_7 + k_9) \right) (k_7 k_9 + k_{10}(2k_7 + k_9))^2 x_1^3 x_2^3 x_6 + \ldots
\numerator(\det H_4) = k_1^2 k_2^3 \left( k_{10} k_5 k_7 + k_5 k_7 + k_5 k_7 + k_5 k_7 + k_5 k_7 + k_5 k_7 + k_5 k_7 + k_5 k_7 \right) \left( k_7 k_9 + k_{10}(2k_7 + k_9) \right)^2 \\
\phantom{\text{numerator(\det H_4)}} \left( k_7 k_7 (k_3 + k_4 + k_7) \right) k_9 + k_5 k_7 (k_7 + k_9) + k_{10}(k_3 + k_4 + k_7)(k_3 k_7 + k_3(k_7 + k_9)) x_1^3 x_2^3 x_6 + \ldots
\]

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

\[
\denominator(\det H_2) = k_{10}^2 k_2^2(k_7 + k_9)^2 x_6^2
\denominator(\det H_3) = k_{10}^2 k_2^3(k_7 + k_9)^3 x_6^3
\denominator(\det H_4) = k_{10}^2 k_2^4(k_7 + k_9)^4 x_6^4
\]

(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 \[2\]), 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 $\mathbb{R}^3_{>0}$, then case (b) of the proposition holds. Otherwise, if $\mathcal{H} \cap \mathbb{R}^3_{>0} = \emptyset$, 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 $(x_1, x_2, x_6) \in \mathbb{R}^3_{>0}$. The denominator of $\det H_5(x_1, x_2, x_6)$ is strictly positive:

$$\text{denominator}(\det H_5) = k_1^5 k_7^5 (k_3 + k_6)^5 x_6^5.$$ 

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

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):

$$\text{numerator}(\det H_5) = x_1^2 x_2 \left( \frac{k_1 k_7 x_6 (k_3 + k_6)}{k_3 (k_10 (2k_7 + k_b) + k_7 k_6)} + x_2 \right)$$

$$k_8 x_6 \left( \alpha_{01} + \alpha_{10} \frac{k_5}{k_8} \right) + k_8^2 x_6^2 \left( \alpha_{02} + \alpha_{11} \frac{k_5}{k_8} + \alpha_{20} \left( \frac{k_5}{k_8} \right)^2 \right)$$

$$+ k_8^3 x_6^3 \left( \alpha_{03} + \alpha_{12} \frac{k_5}{k_8} + \alpha_{21} \left( \frac{k_5}{k_8} \right)^2 + \alpha_{30} \left( \frac{k_5}{k_8} \right)^3 \right) + \text{lower order terms},$$

where the coefficients $\alpha_{ij}$ are sums of (many) positive monomials and are given in the file mixed_analysis_HSN_x1LT.nb. Therefore (for fixed $x_2$ and $x_6$) when $x_1$ is sufficiently large, the expression (14) is positive, as desired.

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 $\mathbb{R}^3_{>0}$, and indeed we show that this is the case when certain relationships hold among the rate constants.

**Theorem 4.2.** Consider the dynamical system (11) arising from the mixed-mechanism network. Assume the positive rate constants satisfy $k_2 = k_6 = k_9$ and the following inequality:

$$k_1 k_3 k_4 - (k_3 + k_4)(k_3 + k_7)(k_4 + k_7) > 0.$$  

(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 (11) 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 $(x_1, x_2, x_6) \in \mathbb{R}^3_{>0}$. So, by Proposition 2.2 it suffices to show that if $k_5/k_8$ is sufficiently large, then there exists $(x_1^*, x_2^*, x_6^*) \in \mathbb{R}^3_{>0}$ 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):

\[
\text{numerator}(\det H_5) = k_i^9 \left( \alpha_0 x_6^3 + \alpha_1 x_6^2 + \alpha_2 x_6 + \alpha_3 \right) \left( x_1^5 + \frac{k_10k_7(k_3 + k_b)}{k_3(k_10(2k_7 + k_b) + k_7k_b)} x_1^4 x_6 \right) x_2^9 \\
+ \text{lower order terms},
\]

where \( \alpha_0, \ldots, \alpha_3 \) are rational functions in \( k_b, k_3, k_4, k_5, k_7, k_8, k_{10} \). These functions \( \alpha_i \) are given in mixed_analysis_H5N_x2_LT.nb.

We now analyze \( \alpha_0 \), which has the following form (see mixed_analysis_H5N_x2_LT.nb):

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

where each coefficient \( \beta_i \) 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, \( \beta_0 \) is the following polynomial:

\[
\beta_0 = -k_1^3 k_3^3 k_7^3 \left( (k_10 k_3 k_4 - (k_3 + k_4)(k_3 + k_7)(k_4 + k_7)) \right) \left( k_{10} + k_b \right)^3 \left( 2k_7 + k_b \right)^2.
\]

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 \( x_2^3 \) 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.

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 \( 1 \) 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 \( (x_1^*, x_2^*, x_6^*) \in \mathbb{R}^3_0 \) 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:

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

**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 \( \det H_5 : \mathbb{R}^3_0 \to \mathbb{R} \) 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 \( (x_1^*, x_2^*, x_6^*) \in \mathbb{R}^3_0 \) (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 \( x(\mu) = \ldots \).
\( \chi(x_1^*, \mu, x_6^*) \) 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 factor of \( H_5 \). So, by Proposition 2.3, \( \chi(x_1^*, x_2^*, x_6^*) \) is a Hopf bifurcation with respect to \( x_2 \) if and only if (18) holds.

\[ \phi \circ \chi(H') \subset H' \setminus \mathcal{S} \]

\[ \mathcal{S} := \left\{ (x_1^*, x_2^*, x_6^*) \in \mathcal{H} \mid \frac{d\det H_5}{dx_2}|_{x_2=x_2^*} = 0 \right\} \subset H' \]

We have that \( \mathcal{H} = \phi \circ \chi(H') \), 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: \( \phi \circ \chi(H' \setminus \mathcal{S}) \). So, it suffices to show that \( \dim(\mathcal{S}) < \dim(\mathcal{H}') \). Note that \( \dim(\mathcal{H}') \geq 2 \), so we will show that \( \dim(\mathcal{S}) \leq 1 \).
To this end, note that if \((x_1^*, x_2^*, x_6^*) \in \mathcal{S}\), then \(x_5^*\) is a multiple root of the univariate polynomial numerator\((\det H_5)|_{x_1=x_1^*, x_6=x_6^*}\) (this also uses the fact the denominator of \(\det H_5\), which is 188956800000000000000\(x_6^*\), does not depend on \(x_2\)). Thus, any \((x_1^*, x_2^*, x_6^*) \in \mathcal{S}\) satisfies \(D(x_1^*, x_6^*) = 0\), where \(D\) is the discriminant of \(\det H_5\) and \(H_5\) is viewed as a univariate polynomial in the variable \(x_2\). So, we have the map:

\[
\mathcal{S} \rightarrow \{(x_1, x_6) \in \mathbb{R}^2 \mid D(x_1, x_6) = 0\} =: \mathcal{D} \\
(x_1, x_2, x_6) \mapsto (x_1, x_6).
\]

The preimage of any point of this map has size at most 4 (because numerator\((\det H_5)|_{x_1=x_1^*, x_6=x_6^*}\) has degree 9, so it has at most 4 multiple roots).

Thus, to achieve our desired inequality (namely, \(\dim(\mathcal{S}) \leq 1\)), we need only prove the following claim: \(\dim(\mathcal{D}) \leq 1\) or, equivalently, the bivariate polynomial \(D\) is not the zero polynomial. It suffices to show that \(D(1,1)\) is nonzero, which in turn would follow if we can show that the univariate, degree-9 polynomial numerator\((\det H_5)|_{x_1=x_1^*, x_6=x_6^*} = H_5(1, x_2, 1)\) does not have a multiple root over \(\mathbb{C}\). Indeed, using Mathematica, we see that the numerator of \(\det H_5(1, x_2, 1)\) has 9 (distinct) complex roots:

\[
-131.425, \ -102.999, \ -78.022, \ -66.423, \ -39.194, \ -3.946 \pm 0.734i, \ -3.677, \ 268.606 .
\]

Thus, \(D\) is a nonzero polynomial, and this completes the proof.

Figure 3: Slices of the Hopf-bifurcation surface \(\mathcal{H}\), from Theorem \ref{thm:main}. Specifically, displayed are the intersections of \(\mathcal{H}\) with the hyperplanes defined by (a) \(S_{\text{tot}} = 40\), (b) \(P_{\text{tot}} = 5\), and (c) \(K_{\text{tot}} \approx 13.0296\). Each such curve was obtained numerically, using Matcont \cite{Matcont}, by a two-parameter continuation of the Hopf bifurcation arising from \(K_{\text{tot}} \approx 13.0296\), \(P_{\text{tot}} = 5\), and \(S_{\text{tot}} = 40\). Each point of the curves in (a) – (c) corresponds to a Hopf bifurcation with respect to either of the two varying total concentrations. Points “inside” \(\mathcal{H}\) correspond to unstable steady states and thus the potential for oscillations.

In Figure \ref{fig:hopf} we show some slices of the Hopf-bifurcation surface \(\mathcal{H}\) (where the rate constants are from Table \ref{tab:rates}). Accordingly, this figure extends the one-dimensional Figure \ref{fig:bifurcation}.

The bifurcations analyzed in Proposition \ref{prop:main} and Theorem \ref{thm:main} are with respect to the bifurcation parameter \(x_2\), the steady-state value of the kinase \(K\). It is natural to ask whether we also obtain a bifurcation with respect to a more biologically meaningful parameter, such as a rate constant or a total amount. We now explain how to perform such an analysis.

To use a total amount (here we use \(P_{\text{tot}}\)) as a bifurcation parameter (perturbing this parameter corresponds to perturbing the compatibility class), consider the following maps:

\[
\{(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}})\} = \mathbb{R}_>^3 \xleftarrow{\phi^X} \mathbb{R}_>^3 \xrightarrow{h_5 := \det H_5} \mathbb{R}_>^3
\]
Recall that \((\phi \circ \chi) : \mathbb{R}^3_{>0} \to \mathbb{R}^3_{>0}\) is a bijection. Let \(g := h_5 \circ (\phi \circ \chi)^{-1} : \mathbb{R}^3_{>0} \to \mathbb{R}\). Also, let \(p := (\phi \circ \chi)_2 = x_6 + \frac{1009}{1800} x_1 x_2\) 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 \(\frac{\partial g}{\partial P_{\text{tot}}}\) is (generically) nonzero whenever \(g = 0\). Accordingly, we use the chain rule:

\[
\frac{\partial g}{\partial P_{\text{tot}}} = \frac{1}{\partial p/\partial x_1} \frac{\partial h_5}{\partial x_1} + \frac{1}{\partial p/\partial x_2} \frac{\partial h_5}{\partial x_2} + \frac{1}{\partial p/\partial x_6} \frac{\partial h_5}{\partial x_6}
\]

\[
= \frac{1800}{1009 x_2} \frac{\partial h_5}{\partial x_1} + \frac{1800}{1009 x_1} \frac{\partial h_5}{\partial x_2} + \frac{\partial h_5}{\partial x_6}.
\]

(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 \(P_{\text{tot}}\).

\section{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.

\begin{procedure}
\textbf{Procedure 5.1} (Generating rate constants likely to admit oscillations).
\begin{enumerate}
\item Choose positive values for \(k_b := k_2 = k_6 = k_9, x_1, k_1, k_3, k_4, k_7,\) and \(k_8\).
\item Choose a positive value for \(k_{10}\) for which \(k_{10} > \frac{(k_3+ k_4)(k_3+ k_7)(k_4+ k_7)}{k_3 k_4}\).
\item Choose the remaining rate constant \(k_5\) such that \(\alpha_0 < 0\).
\item Choose \(x_6\) so that \(q < 0\).
\item Choose \(x_2\) so that the numerator of \(\det H_5\) is negative but close to 0.
\end{enumerate}
\end{procedure}

\footnote{The 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 $k_6 = 0.143738$, $k_1 = 0.575284$, $k_3 = 3.89096$, $k_4 = 5.05386$, $k_7 = 9.25029$, $k_8 = 0.621813$, 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 $\alpha_0$ 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:

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

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):

$$(x_1, x_2, \ldots, x_9) = \chi(x_1, x_2, x_6) = (5.82148, 10, 8.30052, 6.39056, 1.90691, 0.1, 3.49146, 520.229, 0.358855).$$

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

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

The resulting bifurcation analysis is shown in Figure 4.
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 $K_{\text{tot}}$ (namely, 100, 1000, and 10000). Oscillations persist across these values, which yields a much larger range for $K_{\text{tot}}$ than Suwanmajo and Krishnan’s results would suggest.

Moreover, the value of $K_{\text{tot}}$ 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 $K_{\text{tot}}$? And, does the periodic orbit in $x_5$ indeed not depend on $K_{\text{tot}}$? 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 $P_{\text{tot}}$ and $S_{\text{tot}}$ such that for $K_{\text{tot}}$ arbitrarily large, the unique steady state in $\mathcal{P}$ is unstable.

2. For such values of $P_{\text{tot}}$ and $S_{\text{tot}}$ and for sufficiently large $K_{\text{tot}}$, 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 $K_{\text{tot}}$.

One way to tackle Conjecture 6.2 is analyze the robustness of the period and the amplitude with respect to $K_{\text{tot}}$ 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 $x_t$, the parameterization (9)
- mixed_Jx.txt ...contains $J_x$, 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 $\alpha_{01}, \alpha_{10}, ...$
  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 $\alpha_0, ..., \alpha_3$ and $\beta_0, ..., \beta_3$.
  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 $H_2, ..., H_5$. 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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