



Emergence of Oscillations in a Mixed-Mechanism Phosphorylation System

Carsten Conradi¹ · Maya Mincheva² · Anne Shiu³

Received: 8 September 2018 / Accepted: 8 February 2019
© Society for Mathematical Biology 2019

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

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11538-019-00580-6>) contains supplementary material, which is available to authorized users.

✉ Maya Mincheva
mmincheva@niu.edu

¹ HTW Berlin, Berlin, Germany

² Northern Illinois University, DeKalb, USA

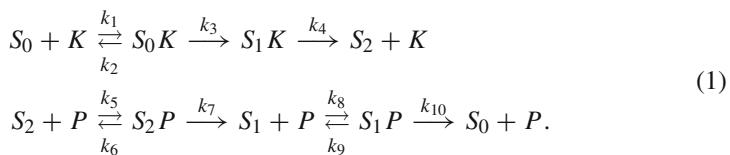
³ Texas A&M University, College Station, USA

1 Introduction

Oscillations have been observed experimentally in signaling networks formed by phosphorylation and dephosphorylation (Hilioti et al. 2008; Hu et al. 2013), which suggest that these networks are involved in timekeeping and synchronization. Indeed, multisite phosphorylation is the main mechanism for establishing the 24-h period in eukaryotic circadian clocks (Ode and Ueda 2017; Virshup and Forger 2009). 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 (2015, §3.1) “could be the simplest enzymatic modification scheme that can intrinsically exhibit oscillation.” 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. (2011, Table S2) and a model of ERK regulation via enzymes MEK and MKP3 (Rubinstein et al. 2016, 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:



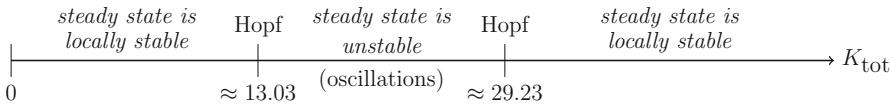
When the kinase *phosphorylates*—that is, adds phosphate groups to—a substrate in the mixed-mechanism network (via the reactions labeled by k_1 to k_4), the kinase and substrate do *not* dissociate before both phosphate groups are added. Accordingly, the mechanism for phosphorylation is *processive*. 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 mechanism.¹

The dynamical systems arising from the mixed-mechanism network live in a nine-dimensional space, but, due to three conservation laws, are essentially six-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 (Suwanmajo and Krishnan 2015). 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 Network (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. Suwanmajo and Krishnan 2015, networks 21, 22).

Table 1 Rate constants (left) and total amounts (right), from (Suwanmajo and Krishnan 2015, Supplementary Information), which lead to oscillations in the mixed-mechanism network (1)

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

**Fig. 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 (2015, 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 (Suwanmajo and Krishnan 2015)

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 (Suwanmajo and Krishnan 2015, Supplementary Information).

Next, they examined whether oscillations persist as K_{tot} varies. What they found, summarized in Fig. 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.

Subsequently, Conradi and Shiu (2018) 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 Fig. 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 (2002), 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) (Guckenheimer and Holmes 2013). We show that a saddle node bifurcation cannot 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 Sect. 1.2 below, the catalytic constants alone enable bistability (Conradi and Mincheva 2014). 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 (Johnston et al. 2018; Thomson and Gunawardena 2009) and methods for using them to determine whether a network is multistationary (Johnston 2014; Müller et al. 2016; Millán and Dickenstein 2018; Millán and Turjanski 2015). Going further, steady-state parametrizations can also be used to find a witness to multistationarity or even the precise parameter regions that yield multistationarity (Conradi et al. 2017; Conradi and Mincheva 2014). 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 Domijan and Kirkilionis 2009; Errami et al. 2015; Gatermann et al. 2005; Hadač et al. 2017.)

As mentioned earlier, there has been much interest in the dynamics of phosphorylation systems (Conradi and Shiu 2018). The mixed-mechanism network (1) fits into the related literature as follows. The mixed network is a dual-site network situated between

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

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

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 Conradi and Shiu (2018), fully processive networks are globally convergent to a unique steady state (Conradi and Shiu 2015; Eithun and Shiu 2017; Rao 2017), while mixed-mechanism networks admit oscillations but not bistability (Suwanmajo and Krishnan 2015), and fully distributive networks admit bistability (Hell and Rendall 2015) and the question of oscillations is open (Conradi and Shiu 2018).

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 (Tung 2018). Moreover, Rao showed that this approximation is globally convergent to a unique steady state (Rao 2018). The validity of the Michaelis–Menten approximation for phosphorylation systems has been called into question (Salazar and Höfer 2009), 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 Sect. 4, we prove our main results (described above). We use these results in Sect. 5 to give a procedure for generating rate constants admitting Hopf bifurcations. In Sect. 6, we present simulations that suggest that oscillations are the norm in the unstable steady-state regime. Finally, we end with a Discussion in Sect. 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.

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

x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9
S_0	K	$S_0 K$	$S_1 K$	S_2	P	$S_2 P$	S_1	$S_1 P$

2.1 Differential Equations of the Mixed-Mechanism Network

For the mixed-mechanism network (1), 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:

$$\begin{aligned}
 \dot{x}_1 &= -k_1 x_1 x_2 + k_2 x_3 + k_{10} x_9 \\
 \dot{x}_2 &= -k_1 x_1 x_2 + k_2 x_3 + k_4 x_4 \\
 \dot{x}_3 &= k_1 x_1 x_2 - (k_2 + k_3) x_3 \\
 \dot{x}_4 &= k_3 x_3 - k_4 x_4 \\
 \dot{x}_5 &= k_4 x_4 - k_5 x_5 x_6 + k_6 x_7 \\
 \dot{x}_6 &= -k_5 x_5 x_6 - k_8 x_8 x_6 + (k_6 + k_7) x_7 + (k_9 + k_{10}) x_9 \\
 \dot{x}_7 &= k_5 x_5 x_6 - (k_6 + k_7) x_7 \\
 \dot{x}_8 &= k_7 x_7 - k_8 x_6 x_8 + k_9 x_9 \\
 \dot{x}_9 &= k_8 x_6 x_8 - (k_9 + k_{10}) x_9.
 \end{aligned} \tag{2}$$

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 (2) progresses, the following three conservation values, denoted by $K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}} \in \mathbb{R}_{>0}$, remain constant:

$$\begin{aligned}
 K_{\text{tot}} &= x_2 + x_3 + x_4, \\
 P_{\text{tot}} &= x_6 + x_7 + x_9, \\
 S_{\text{tot}} &= x_1 + x_3 + x_4 + x_5 + x_7 + x_8 + x_9.
 \end{aligned} \tag{3}$$

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:

$$\mathcal{P} = \{x \in \mathbb{R}_{\geq 0}^9 \mid \text{the conservation equations (3) hold}\}. \tag{4}$$

2.2 Stability of Steady States and the Routh–Hurwitz Criterion

The dynamical system (2) arising from the mixed-mechanism network is an example of a *reaction kinetics system*. That is, the system of ODEs takes the following form:

$$\frac{dx}{dt} = \Gamma \cdot R(x) =: g(x), \tag{5}$$

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 nonnegative concentration vector $x^* \in \mathbb{R}_{\geq 0}^s$ (respectively, $x^* \in \mathbb{R}_{>0}^s$) at which the ODEs (5) 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 $dg(x^*)$ have 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 (Gantmacher 1959).

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:

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

in which the (k, l) th entry is a_{2k-l} as long as $0 \leq 2k-l \leq n$, 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 (Liu 1994).

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:

$$\mu \mapsto x(\mu) \quad (6)$$

(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:

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

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 Yang 2002) *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 (1994) 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 (Proposition 3.2). We then use this parametrization to analyze the space of compatibility classes (Proposition 3.6).

3.1 Uniqueness of Steady States

Susanmajo 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 (Susanmajo and Krishnan 2015, §A.2). Additionally, there are no boundary steady states in any compatibility class \mathcal{P} , as in (4), and \mathcal{P} is compact. Hence, via a standard application of the Brouwer fixed-point theorem (e.g., Millán et al. 2012, 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 (2) arising from the mixed-mechanism network has a unique steady state in \mathcal{P} , and it is a positive steady state.*

Proposition 3.1 precludes the existence of multiple positive steady states, and hence, the existence of a saddle node bifurcation. Thus, a Hopf bifurcation is the only other one-parameter bifurcation which may occur. Indeed, we will show that a Hopf bifurcation exists for some parameter values in Sect. 4.

Also, Proposition 3.1 proves part of a conjecture that we posed (Conradi and Shiu 2015). The other half of the conjecture, however, posited that mixed-mechanism systems, like fully processive systems (Conradi and Shiu 2015; Eithun and Shiu 2017),

are globally convergent to the unique steady state. Suwanmajo and Krishnan (2015) demonstrated that this is false: the system can exhibit oscillatory behavior!

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” (Millán et al. 2012):

Proposition 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 (2) is three-dimensional and is the image of the following map $\chi = \chi_{k_1, \dots, k_{10}}$:*

$$\begin{aligned} \chi : \mathbb{R}_+^3 &\rightarrow \mathbb{R}_+^9 \\ (x_1, x_2, x_6) &\mapsto (x_1, x_2, \dots, x_9), \end{aligned} \tag{7}$$

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 (2) vanishes. Conversely, let $x^* = (x_1, x_2, \dots, x_9)$ be a positive steady state. The right-hand side of the ODEs (2) 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 (7), so x^* is in the image of χ . \square

Remark 3.3 The parametrization (7) appeared earlier in Conradi and Shiu (2018).

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 (Thomson and Gunawardena 2009).

Remark 3.5 In the parametrization χ in Preposition 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 (4), 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 (3):

$$\phi(x) := (x_2 + x_3 + x_4, x_6 + x_7 + x_9, x_1 + x_3 + x_4 + x_5 + x_7 + x_8 + x_9). \quad (8)$$

Each compatibility class \mathcal{P} contains a unique positive steady state (Proposition 3.1), and the positive steady states are parametrized by χ from Preposition 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 (8) and χ is the steady-state parametrization (7). The map $\phi \circ \chi$ is given by

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

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

$$(x_1, x_2, x_6) \mapsto \left(x_1 x_2 + x_2, x_6 + \frac{1009}{1800} x_1 x_2, x_1 + \frac{2809}{1800} x_1 x_2 + \frac{161}{900} \frac{x_1 x_2}{x_6} \right). \quad (9)$$

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 2018) for the unique positive solution:

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

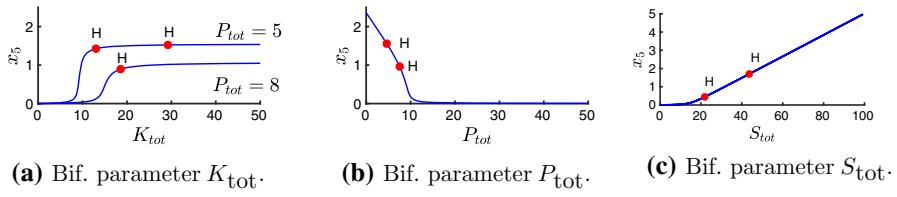


Fig. 2 Numerical continuation of the unique positive steady state, in (10), when $(K_{tot}, P_{tot}, S_{tot}) = (17.5, 5, 40)$: **a** for $P_{tot} = 5, 8$ and $S_{tot} = 40$, we observe (supercritical) Hopf bifurcations at $K_{tot} \approx 13.0296, 29.2251$ ($P_{tot} = 5$) and $K_{tot} \approx 18.5758$ ($P_{tot} = 8$). **b** For $K_{tot} = 5$ and $S_{tot} = 40$, we observe (supercritical) Hopf bifurcations at $P_{tot} \approx 4.6310$ and $P_{tot} \approx 7.5479$. **c** For $K_{tot} = 17.5$ and $P_{tot} = 5$, we observe (supercritical) Hopf bifurcations at $S_{tot} \approx 21.8213$ and $S_{tot} \approx 43.5944$. All figures in this work were made using Matcont (Dhooge et al. 2003) (colour figure online)

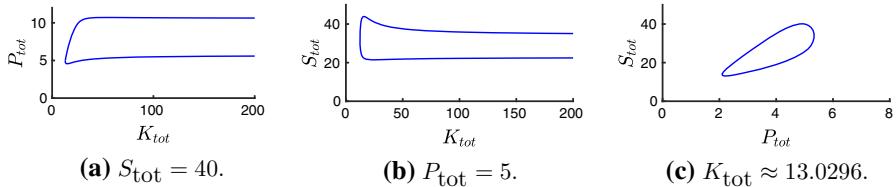


Fig. 3 Slices of the Hopf bifurcation surface \mathcal{H} , from Theorem 4.5. Specifically, displayed are the intersections of \mathcal{H} with the hyperplanes defined by **a** $S_{tot} = 40$, **b** $P_{tot} = 5$, and **c** $K_{tot} \approx 13.0296$. Each such curve was obtained numerically, using Matcont (Dhooge et al. 2003), by a two-parameter continuation of the Hopf bifurcation arising from $K_{tot} \approx 13.0296$, $P_{tot} = 5$, and $S_{tot} = 40$. 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 (colour figure online)

We obtain the remaining coordinates of x^* using the parametrization χ in (7):

$$\begin{aligned} x^* &= \chi(x_1^*, x_2^*, x_6^*) \\ &\approx (1.0134, 8.6916, 4.4041, 4.4041, 1.4893, 0.0624, 4.8935, 23.7512, 0.0440). \end{aligned} \quad (10)$$

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 Fig. 2, where the total amounts are the bifurcation parameters (c.f. Fig. 1 which is with respect to K_{tot}). Figure 2 suggests that in the three-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 Fig. 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 (Atkins et al. 2018). We choose small values for the dissociation rate constants in Sect. 5, similar to what was done in Ferrell and Ha (2014).

Proposition 4.1 *Consider the dynamical system (2) 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 (2), 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 (7). 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

$$\begin{aligned} \text{numerator}(b_6) &= k_1^2 k_3^2 k_4 (k_{10} + k_7) (k_{10} k_5 k_7 + k_5 k_7 k_b + k_{10} k_8 (k_7 + k_b)) x_1 x_2^2 \\ &\quad + k_1 k_{10} k_3 k_4 k_7 (k_3 + k_b) (k_{10} k_5 k_7 + k_5 k_7 k_b + k_{10} k_8 (k_7 + k_b)) x_2 x_6 \\ &\quad + k_{10}^2 k_4 k_5 k_7^2 k_8 (k_3 + k_b)^2 x_6^2 + k_1 k_{10}^2 (k_3 + k_4) k_5 k_7^2 k_8 (k_3 + k_b) x_1 x_6^2 \\ &\quad + k_1 k_{10} k_5 k_7 (k_{10} k_4 k_7 + k_3 k_4 k_7 + k_{10} k_3 (k_4 + k_7)) k_8 (k_3 + k_b) x_2 x_6^2 \\ \text{numerator}(b_5) &= k_1^2 k_3^2 k_4 (k_{10} + k_7) (k_{10} + k_b) (k_7 + k_b) x_1 x_2^2 \\ &\quad + k_1 k_{10} k_3 k_4 k_7 (k_{10} + k_b) (k_3 + k_b) (k_7 + k_b) x_2 x_6 + \dots \\ \text{numerator}(b_4) &= k_1 k_3 k_4 (k_{10} + k_7) (k_{10} + k_b) (k_3 + k_b) (k_7 + k_b) x_1 x_2 + \dots \end{aligned}$$

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

$$\begin{aligned}
\text{numerator}(b_3) &= \dots + k_1^2 k_3 \left(k_{10}^2 (k_7 + k_b) + k_7 k_b (k_3 + k_4 + k_7 + k_b) \right. \\
&\quad \left. + k_{10} ((k_7 + k_b)^2 + k_3 (2k_7 + k_b) + k_4 (2k_7 + k_b)) \right) x_1^2 x_2 + \dots \\
\text{numerator}(b_2) &= \dots + k_1^2 k_3 (k_7 k_b + k_{10} (2k_7 + k_b)) x_1^2 x_2 + \dots \\
\text{numerator}(b_1) &= k_1 k_3 (k_7 k_b + k_{10} (2k_7 + k_b)) x_1 x_2 \\
&\quad + k_{10} k_7 (k_3 + k_b) (k_{10} + k_3 + k_4 + k_7 + 3k_b) x_6 \\
&\quad + k_1 k_{10} k_7 (k_3 + k_b) x_1 x_6 + k_1 k_{10} k_7 (k_3 + k_b) x_2 x_6 \\
&\quad + k_{10} k_7 (k_5 + k_8) (k_3 + k_b) x_6^2
\end{aligned} \tag{11}$$

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 three conservation laws (3), 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 three Hurwitz determinants are also positive:

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

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.nnb`.) 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 versus 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):

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

where the coefficients α_{ij} are sums of (many) positive monomials and are given in the file `mixed_analyis_H5N_x1_LT.nnb`. Therefore (for fixed x_2 and x_6), when x_1 is sufficiently large, the expression (12) 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 (2) arising from the mixed-mechanism network. Assume the positive rate constants satisfy $k_2 = k_6 = k_9$ and the following inequality:

$$k_{10}k_3k_4 - (k_3 + k_4)(k_3 + k_7)(k_4 + k_7) > 0. \quad (13)$$

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 (13). By the proof of Proposition 4.1, a steady state $\chi(x_1, x_2, x_6)$ of the mixed-mechanism system (2) 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`):

$$\begin{aligned} \text{numerator}(\det H_5) = & k_1^9 \left(\alpha_0 x_6^3 + \alpha_1 x_6^2 + \alpha_2 x_6 + \alpha_3 \right) \\ & \left(x_1^5 + \frac{k_{10}k_7(k_3 + k_b)}{k_3(k_{10}(2k_7 + k_b) + k_7k_b)} x_1^4 x_6 \right) x_2^9 \\ & + \text{lower degree terms}, \end{aligned} \quad (14)$$

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`):

$$\alpha_0 = k_8^3 \left(\beta_0 \left(\frac{k_5}{k_8} \right)^3 + \beta_1 \left(\frac{k_5}{k_8} \right)^2 + \beta_2 \left(\frac{k_5}{k_8} \right) + \beta_3 \right), \quad (15)$$

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:

$$\beta_0 = -k_1^9 k_3^5 k_7^3 (k_{10}k_3k_4 - (k_3 + k_4)(k_3 + k_7)(k_4 + k_7))(k_{10} + k_b)^3 (k_7k_b + k_{10}(2k_7 + k_b))^2.$$

It follows that $\beta_0 < 0$, when inequality (13) holds.

Thus, when (13) holds, then, by Eq. (15), the inequality $\alpha_0 < 0$ holds for k_5/k_8 sufficiently large. In this case, the cubic polynomial in x_6 appearing in (14), 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 (2) arising from the mixed-mechanism network and any positive rate constants with $k_2 = k_6 = k_9$ and $k_{10}k_3k_4 - (k_3 + k_4)(k_3 + k_7)(k_4 + k_7) > 0$. 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:*

$$\frac{d(\text{numerator}(\det H_5)|_{x_1=x_1^*, x_6=x_6^*})}{dx_2}|_{x_2=x_2^*} \neq 0. \quad (16)$$

Proof Fix positive rate constants for which $k_2 = k_6 = k_9$ and $k_{10}k_3k_4 - (k_3 + k_4)(k_3 + k_7)(k_4 + k_7) > 0$. 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 (11), 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 to x_2 if and only if (16) 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 (13) (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 (16) is maintained under small perturbations of the x_i 's), the fact that simple roots of a polynomial depend continuously—in fact, infinitely differentiably—on the coefficients (Lozada-Cruz 2012), and the fact that the inequality (13) 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 (16) 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 $f = c_n x^n + c_{n-1} x^{n-1} + \cdots + c_0$ 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 (Gelfand et al. 1994). 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 (2) 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 (13). 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

$$\mathcal{H}' := V_{>0}(\det H_5) := \left\{ (x_1, x_2, x_6) \in \mathbb{R}_{>0}^3 \mid \det H_5(x_1, x_2, x_6) = 0 \right\}, \text{ and}$$

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

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 $\dim(\mathcal{S}) \leq 1$.

To this end, note that if $(x_1^*, x_2^*, x_6^*) \in \mathcal{S}$, then x_2^* 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 $1889568000000000000000000x_6^5$, 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:

$$\begin{aligned} \mathcal{S} &\rightarrow \{(x_1, x_6) \in \mathbb{R}^2 \mid D(x_1, x_6) = 0\} =: \mathcal{D} \\ (x_1, x_2, x_6) &\mapsto (x_1, x_6). \end{aligned}$$

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 four 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 nine (distinct) complex roots:

$$\begin{aligned} & -131.425, -102.999, -78.022, -66.423, -39.194, \\ & -3.946 \pm 0.734i, -3.677, 268.606. \end{aligned}$$

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

In Fig. 3, we show some slices of the Hopf bifurcation surface \mathcal{H} (where the rate constants are from Table 1). Accordingly, this figure extends the one-dimensional Fig. 1.

The bifurcations analyzed in Proposition 4.3 and Theorem 4.5 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_{tot}) as a bifurcation parameter (perturbing this parameter corresponds to perturbing the compatibility class), consider the following maps:

$$\{(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}})\} = \mathbb{R}_{>0}^3 \xleftarrow{\phi \circ \chi} \mathbb{R}_{>0}^3 \xrightarrow{h_5 := \det H_5} \mathbb{R}_{>0}$$

Recall that $(\phi \circ \chi) : \mathbb{R}_{>0}^3 \rightarrow \mathbb{R}_{>0}^3$ is a bijection. Let $g := h_5 \circ (\phi \circ \chi)^{-1} : \mathbb{R}_{>0}^3 \rightarrow \mathbb{R}$. 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 (9) (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:

$$\begin{aligned} \frac{\partial g}{\partial P_{\text{tot}}} &= \frac{1}{\partial p / \partial x_1} \frac{\partial h_5}{\partial x_1} + \frac{1}{\partial p / \partial x_2} \frac{\partial h_5}{\partial x_2} + \frac{1}{\partial p / \partial x_6} \frac{\partial h_5}{\partial x_6} \\ &= \frac{1800}{1009x_2} \frac{\partial h_5}{\partial x_1} + \frac{1800}{1009x_1} \frac{\partial h_5}{\partial x_2} + \frac{\partial h_5}{\partial x_6}. \end{aligned} \quad (17)$$

For specific values of x_1, x_2, x_6 , it is straightforward to check whether the sum (17) 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 (13) holds, and $\alpha_0 < 0$ [as in (15)], 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 (15),
- (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 (14), 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 $k_{10} > \frac{(k_3+k_4)(k_3+k_7)(k_4+k_7)}{k_3 k_4}$.
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.
6. Return the k_i 's and $(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}}) := \phi \circ \chi(x_1, x_2, x_6)$, 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` (Dhooge et al. 2003). See Fig. 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:

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

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

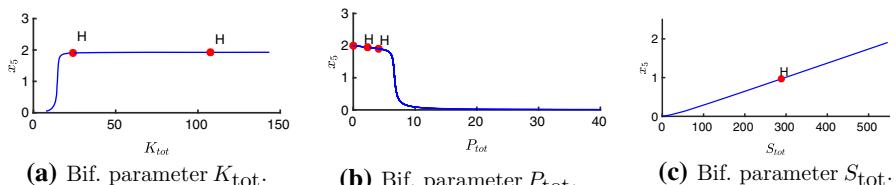


Fig. 4 Numerical continuation of the steady state (18), when total amounts are as in (19): **a** A (supercritical) Hopf bifurcations are at $K_{\text{tot}} \approx 24.0623$ and 107.5635 . **b** (Supercritical) Hopf bifurcations are at $P_{\text{tot}} \approx 4.1022$ and $P_{\text{tot}} \approx 2.3275$. 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 $S_{\text{tot}} \approx 288.4384$ (Color figure online)

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 (7):

$$\begin{aligned}
 (x_1, x_2, \dots, x_9) &= \chi(x_1, x_2, x_6) \\
 &= (5.82148, 10, 8.30052, 6.39056, 1.90691, 0.1, \\
 &\quad 3.49146, 520.229, 0.358855). \tag{18}
 \end{aligned}$$

Using this steady state, we obtain the total amounts, using (8):

$$(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}}) = \phi(x_1, x_2, \dots, x_9) = (24.6911, 3.95031, 546.499). \tag{19}$$

The resulting bifurcation analysis is shown in Fig. 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 stable periodic orbit.

Some simulations are shown in Fig. 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

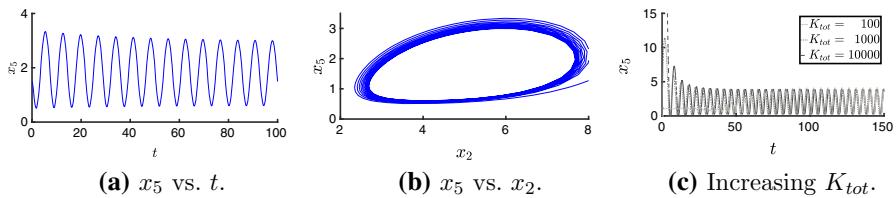


Fig. 5 Numerical verification of oscillations in the mixed-mechanism system with rate constants as in Table 1. For **a**, **b**, we used $(K_{\text{tot}}, P_{\text{tot}}, S_{\text{tot}}) = (14, 5, 40)$ and initial values as in (10). Here, the solution converges to a periodic orbit. For **c**, we used $(P_{\text{tot}}, S_{\text{tot}}) = (8, 40)$ and three values for K_{tot} (namely, 100, 1000, and 10,000), and again initial values as in (10), 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 (colour figure online)

that Suwanmajo and Krishnan found to support oscillations. In contrast, in Fig. 5(C), we see oscillations, when $(P_{\text{tot}}, S_{\text{tot}}) = (8, 40)$, for three large values for K_{tot} : 100, 1000, and 10,000. 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, we conjecture that oscillations indeed persist for arbitrarily large K_{tot} ; and, that the periodic orbit in x_5 indeed does not depend on K_{tot} .

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 to analyze the robustness of the period and the amplitude with respect to K_{tot} using the theory developed in Bure and Rozenvasser (1974), Ingalls (2004) and Ingalls et al. (2017).

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 (Rao 2018).

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 admit 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 Rubinstein et al. (2016) 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 (Conradi and Shiu 2018), 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 as supplementary material:

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 (3)
- `mixed_xt.txt` ...contains xt , the parameterization (7)
- `mixed_Jx.txt` ...contains Jx , the Jacobian evaluated at the parameterization (7)

Mathematica Notebooks:

- `mixed_analysis_H5N_x1_LT.nb`:

Functionality: This file can be used to obtain numerator($\det H_5$) as in (12), 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 (14), 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 (2). 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.

References

Aoki K, Yamada M, Kunida K, Yasuda S, Matsuda M (2011) Processive phosphorylation of ERK MAP kinase in mammalian cells. *Proc Natl Acad Sci USA* 108(31):12675–12680

Atkins P, De Paula J, Keeler J (2018) Atkins' physical chemistry. Oxford University Press, Oxford

Bure EG, Rozenvasser YN (1974) On investigations of autooscillating system sensitivity, no 7. *Avtomat. i Telemekh*, pp 9–17

Conradi C, Mincheva M (2014) Catalytic constants enable the emergence of bistability in dual phosphorylation. *J R Soc Interface* 11(95):20140158

Conradi C, Shiu A (2015) A global convergence result for processive multisite phosphorylation systems. *Bull Math Biol* 77(1):126–155

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

Conradi C, Feliu E, Mincheva M, Wiuf C (2017) Identifying parameter regions for multistationarity. *PLoS Comput Biol* 13(10):e1005751

Dhooge A, Govaerts W, Kuznetsov YA (2003) MATCONT: a MATLAB package for numerical bifurcation analysis of ODEs. *ACM Trans Math Softw* 29(2):141–164

Domijan M, Kirkilionis M (2009) Bistability and oscillations in chemical reaction networks. *J Math Biol* 59(4):467–501

Eiethun M, Shiu A (2017) An all-encompassing global convergence result for processive multisite phosphorylation systems. *Math Biosci* 291:1–9

Errami H, Eiswirth M, Grigoriev D, Seiler WM, Sturm T, Weber A (2015) Detection of Hopf bifurcations in chemical reaction networks using convex coordinates. *J Comput Phys* 291:279–302

Ferrell JE, Ha SH (2014) Ultrasensitivity part II: multisite phosphorylation, stoichiometric inhibitors, and positive feedback. *Trends Biochem Sci* 39(11):556–569

Gantmacher FR (1959) Matrix theory, vol 21. Chelsea, New York

Gatermann K, Eiswirth M, Sensse A (2005) Toric ideals and graph theory to analyze Hopf bifurcations in mass action systems. *J Symb Comput* 40(6):1361–1382

Gelfand IM, Kapranov MM, Zelevinsky AV (1994) Discriminants, resultants and multidimensional determinants. Birkhäuser, Basel

Guckenheimer J, Holmes P (2013) Nonlinear oscillations, dynamical systems, and bifurcations of vector fields, vol 42. Springer, Berlin

Hadač O, Muzika F, Nevoral V, Přibyl M, Schreiber I (2017) Minimal oscillating subnetwork in the Huang–Ferrell model of the MAPK cascade. *PLoS ONE* 12(6):1–25

Hell J, Rendall AD (2015) A proof of bistability for the dual futile cycle. *Nonlinear Anal Real* 24:175–189

Hilioti Z, Sabbagh W, Paliwal S, Bergmann A, Goncalves MD, Bardwell L, Levchenko A (2008) Oscillatory phosphorylation of yeast Fus3 MAP kinase controls periodic gene expression and morphogenesis. *Curr Biol* 18(21):1700–1706

Hu H, Goltsov A, Bown JL, Sims AH, Langdon SP, Harrison DJ, Faratian D (2013) Feedforward and feedback regulation of the MAPK and PI3K oscillatory circuit in breast cancer. *Cell Signal* 25(1):26–32

Ingalls BP (2004) Autonomously oscillating biochemical systems: parametric sensitivity of extrema and period. *Syst Biol* 1(1):62–70

Ingalls B, Mincheva M, Roussel MR (2017) Parametric sensitivity analysis of oscillatory delay systems with an application to gene regulation. *Bull Math Biol* 79(7):1539–1563

Johnston MD (2014) Translated chemical reaction networks. *Bull Math Biol* 76(6):1081–1116

Johnston MD, Müller S, Pantea C (2018) A deficiency-based approach to parametrizing positive equilibria of biochemical reaction systems. Preprint. [arXiv:1805.09295](https://arxiv.org/abs/1805.09295)

Liu WM (1994) Criterion of Hopf bifurcations without using eigenvalues. *J Math Anal Appl* 182(1):250–256

Lozada-Cruz G (2012) The simple application of the implicit function theorem, vol XIX, no 1. Boletín de la Asociación Matemática Venezolana

Millán MP, Dickenstein A (2018) The structure of MESSI biological systems. *SIAM J Appl Dyn Syst* 17(2):1650–1682

Millán MP, Turjanski AG (2015) MAPK’s networks and their capacity for multistationarity due to toric steady states. *Math Biosci* 262:125–137

Millán MP, Dickenstein A, Shiu A, Conradi C (2012) Chemical reaction systems with toric steady states. *Bull Math Biol* 74(5):1027–1065

Müller S, Feliu E, Regensburger G, Conradi C, Shiu A, Dickenstein A (2016) Sign conditions for injectivity of generalized polynomial maps with applications to chemical reaction networks and real algebraic geometry. *Found Comput Math* 16(1):69–97

Ode KL, Ueda HR (2017) Design principles of phosphorylation-dependent timekeeping in eukaryotic circadian clocks. *Cold Spring Harb Perspect Biol* 10:a028357

Rao S (2017) Global stability of a class of futile cycles. *J Math Biol* 74:709–726

Rao S (2018) Stability analysis of the Michaelis–Menten approximation of a mixed mechanism of a phosphorylation system. *Math Biosci* 301:159–166

Rubinstein BY, Mattingly HH, Berezhkovskii AM, Shvartsman SY (2016) Long-term dynamics of multisite phosphorylation. *Mol Biol Cell* 27(14):2331–2340

Salazar C, Höfer T (2009) Multisite protein phosphorylation—from molecular mechanisms to kinetic models. *FEBS J* 276(12):3177–3198

Suwanmajo T, Krishnan J (2015) Mixed mechanisms of multi-site phosphorylation. *J R Soc Interface* 12(107):20141405

Thomson M, Gunawardena J (2009) The rational parameterisation theorem for multisite post-translational modification systems. *J Theor Biol* 261(4):626–636

Tung H-R (2018) Precluding oscillations in Michaelis–Menten approximations of dual-site phosphorylation systems. *Math Biosci* 306:56–59

Virshup DM, Forger DB (2009) Keeping the beat in the rising heat. *Cell* 137(4):602–604

Wolfram Research Inc. (2018) Mathematica, Version 11.3, Champaign, IL

Yang X (2002) Generalized form of Hurwitz–Routh criterion and Hopf bifurcation of higher order. *Appl Math Lett* 15(5):615–621