Published in final edited form as:

Math Biosci. 2016 November ; 281: 62–73. doi:10.1016/j.mbs.2016.09.003.

A mathematical model of endothelial nitric oxide synthase activation with time delay exhibiting Hopf bifurcation and oscillations

L. R. Ritter^{a,1}, C. A. Chrestensen^b, and J. C. Salerno^c

L. R. Ritter: lritter@kennesaw.edu; C. A. Chrestensen: cchreste@kennesaw.edu

^aDepartment of Mathematics, Kennesaw State University, Marietta, GA 30060 USA

^bDepartment of Chemistry and Biochemistry, Kennesaw State University, Kennesaw, GA 30144 USA

^cDepartment of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA 30144 USA

Abstract

Nitric Oxide (NO) is a gaseous compound that serves as a signaling molecule in cellular interactions. In the vasculature, NO is synthesized from endogenous agents by endothelial nitric oxide synthase (eNOS) where it plays key roles in several functions related to homeostasis, adaptation, and development. Recent experimental studies have revealed cycles of increasing and decreasing NO production when eNOS is stimulated by factors such as glucose or insulin. We offer a mathematical model of a generic amino acid receptor site on eNOS wherein this species is subject to activation/deactivation by a pair of interactive kinase and phosphatase species. The enzyme kinetic model is presented as a system of ordinary differential equations including time delay to allow for various intermediate, unspecified complexes. We show that under conditions on the model parameters, varying the delay time may give rise to a Hopf bifurcation. Properties of the bifurcating solutions are explored via a center manifold reduction, and a numerical illustration is provided.

Keywords

Delay Differential Equations; Stability Analysis; Hopf Bifurcation; Nitric Oxide Synthase

Correspondence to: L. R. Ritter, lritter@kennesaw.edu.

¹Kennesaw State University, 1100 S. Marietta Pkwy, MD #9085, Marietta, GA 30060 USA

Dedication This paper is dedicated to the memory of John Salerno (May 1949–December 2015), researcher and teacher, mentor and friend.

2010 MSC: 34K60, 92C45, 92C40

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Nitric Oxide (NO) is a significant biological signaling molecule in intracellular and cell-cell interactions. In the vasculature, for example, NO is used by endothelial cells for regulatory and developmental processes including maintenance of vascular tone, vasodilation, insulin secretion, and angiogenesis [1, 2]. Within a variety of organisms, NO is synthesized from endogenous L-arginine, oxygen and NADPH by a class of enzymes called nitric oxide synthases. Among these, endothelial nitric oxide synthase (eNOS) is the primary integrator for the signaling network in the human vasculature. The eNOS isoform is known as a key element in vasoprotective NO production for smooth muscle cell relaxation, inhibition of platelet aggregation, and suppression of immune cell adhesion at the arterial wall. Understanding the regulatory network in NO production and endothelial function and dysfunction has direct application to the study of diabetes and cardiovascular health and disease [3, 4, 5]

Endothelial NOS is an important enzyme in a complex regulatory network involving kinases and phosphatases, signal inducing hormones and growth factors (e.g. insulin, estrogen, VEGF), as well as mechanical stress. The discovery of eNOS activation via phosphorylation by various kinases opened a new window on the understanding of NO production [6, 2]. And several studies have focused on identification of reaction sites to specific agonists and elucidation of signaling pathways (see for example the review articles [6, 7, 8] and the references therein). More recently, experimental and computational approaches have been implemented in an effort to study the time course of related biological signaling. Complex dynamics, in particular bistability and oscillatory behavior, have been observed in some kinase cascade models characterized by phosphorylation mediated activation [9, 10, 11]. In [10] for example, it was observed *in vitro* that competition between substrates and phosphatase gives rise to oscillation in activation of extracellular signal-regulated kinase (ERK). The mathematical analysis carried out by Liu *et al.* in [9] is based on substrate dependent control of phosphorylation observed in both *in vitro* and *in vivo* studies. Experimental results involving single dose insulin stimulation following serum starvation of bovine aortic endothelial cells by Wang *et al.* [11] showed a time course characterized by cycles of rapid increase and decline in NO production. The current authors have observed oscillations of phosphorylation at the amino acid site serine 1177 (S1177) in response to insulin and fetal bovine serum treatment (unpublished). These dynamic processes lend themselves to mathematical models of both the discrete and continuous variety. Most notably, enzyme kinetic models formulated as systems of differential equations provide insight into feedback mechanisms and the dependence of observed behavior on key parameters [12, 13, 14, 9, 10].

In this paper, we propose a model of a generic amino acid residue in eNOS (representing for example any of several serine amino acids) wherein this species is subject to activation or deactivation by a pair (in general a pair of families) of interactive kinase and phosphatase species. This is a dramatic simplification of eNOS regulation, since it has multiple regulatory phosphorylation sites both activating and inhibitory [8, 15]. We note that this simplification is further enhanced because of the long list of candidate kinases to phosphorylate those targets. For phosphorylated S1177 (the most studied site), AKT, PKA,

AMPK, PKG and CaMKII are all candidates². Less is known about phosphatase action on eNOS, but PP2A, PP2B and PTEN³ have all been shown to dephosphorylate eNOS [8, 16]. The genesis of this mathematical analysis came from work initiated with the neuronal isoform of nitric oxide synthase (nNOS) [17] that looked specifically at pulse generation of NO caused by feedback inhibition of nNOS by NO. Direct feedback inhibition of eNOS by NO is less potent but eNOS has been shown to produce NO in a transient fashion peaking at 1 and 5 minutes post stimulation with insulin [11]. In our simplification we attempt to understand a mechanism by which this could be plausible for a single phosphorylation modification as well as create a general model for eNOS and other signaling pathways.

In the following section, we present our simple model in the form of a system of differential equations with time delays. The time delays in particular allow for complex behavior induced by intermediaries that are allowed to remain unspecified. Taking a further simplification of a single delay parameter, the third section is devoted to the establishment of stability criteria of an equilibrium state. Stability of the steady state is then analyzed further in sections 3 and 4 where we consider the delay as a bifurcation parameter. The existence and resulting properties of periodic solutions—local oscillations about the constant equilibrium—are provided in section 4 via a center manifold reduction. A numerical example, using a set of test parameter values, is provided. We close with a brief discussion of our system as a model of the eNOS activation process and potential implications of the mathematical findings.

2. Mathematical Model

The activation and deactivation of an eNOS amino acid site occurs downstream of a sequence of interactions between various kinase and phosphatase species typically initiated by some chemical or mechanical stimulus (e.g. insulin, glucose, bradykinin, etc.). Figure 1 shows a schematic of the eNOS regulatory network depicting the signal cascade resulting in NO production. Kinases and phosphatases may activate, deactivate, and inhibit activation of one another via phosphorylation and dephosphorylation. And at the final step considered herein, an eNOS site is phosphorylated by a kinase (such as AKT) and dephosphorylated by a phosphatase (such as PP2A) to influence NO production.

We introduce a three species model including a single kinase **A**, a single phosphatase **B**, and one eNOS receptor site **C** whose interactions are illustrated in figure 2. These species are taken as a simplified model of the final stage of a complex signal cascade (e.g. the PP2A–AKT–eNOS interactions seen in figure 1). Consideration of the multi-step interactions could be accounted for by allowing each of **A** and **B** to be matrix valued functions of time whose rows represent individual enzyme types and having two columns to differentiate between inactive and active forms of each specific enzyme. Preliminary numerical investigations (unpublished) show that such a multi-step system involving a large number of ordinary differential equations is capable of producing the oscillatory behavior expected. Here

²AKT–protein kinase B, PKA–protein kinase A, AMPK–activated protein kinase, PKG–protein kinase G, CaMKII–calmodulin-dependent protein kinase II

³PP2A–protein phosphatase 2, PP2B–protein phosphatase 2b, PTEN–phosphatase and tensin homolog

however, we will assume that each species is vector valued of the form $\mathbf{A} = (a_1, a_2)$, $\mathbf{B} = (b_1, b_2)$, and $\mathbf{C} = (c_1, c_2)$ where the subscript 1 denotes the inactive form and 2 the active form of the respective enzyme. To account for the unspecified upstream reactions, the obligatory formation and decay of various protein complexes, diffusion, mobilization to other compartments, and the interactions of intermediaries, we allow for time delays. In addition, these biochemical processes are conservative so that $a_1 + a_2 = \text{const}$, $b_1 + b_2 = \text{const}$, and $c_1 + c_2 = \text{const}$. Hence our model presents as a limited system of delay differential equations.

Each of the interactions is assumed to be of Michaelis-Menton type with cross species interactions appearing as factors in the related reaction rates. The kinase $\mathbf{A}$ can convert from inactive to active $a_1 \rightarrow a_2$ and vice versa $a_2 \rightarrow a_1$, and these conversions can be influenced by the active phosphatase b_2 through both direct dephosphorylation as well as through inhibition of phosphorylation of $\mathbf{A}$ by upstream kinases (figure 2, cell (i)). Combining the regulatory and feedback terms, the equations for the kinase are

$$\frac{da_1}{dt} = -K_{a1}f_p(b_2(t-\tau_b))\frac{a_1}{K_{an}+a_1} - K_{a2}g_p(b_2(t-\tau_b))\frac{a_1}{K_{am}+a_1} + K_{a3}b_2(t-\tau_{b'})\frac{a_2}{K_{ao}+a_2} + K_{a4}\frac{a_2}{K_{ap}+a_2},$$

(1)

$$\frac{da_2}{dt} = -\frac{da_1}{dt}.$$

(2)

Each parameter K_{ai} , $i = 1, \dots, 4$ is a positive rate constant. The effective Michaelis constants $K_{an}, \dots, K_{ap}$ are positive and allow for up to four distinct substrates. The inhibition of activation of kinase by the active phosphatase b_2 appears in the first two terms on the right hand side of the a_1 equation which is further fractionated to account for multiple substrates [18] by the steady state partition functions

$$f_p(x) = \frac{r_1}{x+r_1+r_2}, \quad \text{and} \quad g_p(x) = \frac{x+r_2}{x+r_1+r_2} = 1 - f_p(x).$$

Parameters r_1 and r_2 are effective reaction rate ratios for kinase activation and deactivation processes. This inhibition depends on a previous state of the active phosphatase, $b_2(t - \tau_b)$, with fixed delay time τ_b . (Here, and throughout this paper, the time dependence of nondelayed terms is suppressed. So for example, $a_1 = a_1(t)$ and $a_2 = a_2(t)$ in (1)–(2).) Direct dephosphorylation of active kinase ($a_2 \rightarrow a_1$) by active phosphatase at its previous state $b_2(t - \tau_{b'})$ appears in the third term of (1). In this general model, we allow for two distinct delay times for the different phosphatase actions. The final term on the right side of equation (1) accounts for the intra-species regulation of the kinase.

We note here that there are roughly 500 kinase proteins in the human genome as contrasted with approximately 200 phosphatases accounting for less interactive specificity of any one protein phosphatase [19, 20, 21, 22]. Hence we allow for the active phosphatase to both inhibit activation and to directly deactivate the kinase. This also accounts for both of these actions while maintaining the level of simplification imposed (e.g. one phosphatase species) while admitting inhibition of phosphorylation of the kinase subject to multiple substrates through the steady state partition. Moreover, the inhibition mechanism can be mathematically "turned off" in our model by simply setting $r_1 = 0$.

The phosphatase **B** is similarly transformed between its inactive and active state ($b_1 \rightarrow b_2$) and visa versa ($b_2 \rightarrow b_1$) through both intra-species reaction and via phosphorylation by active kinase (figure 2, cell (ii)). The equations governing these process are

$$\frac{db_1}{dt} = -K_{b1} \frac{b_1}{K_{bn} + b_1} - K_{b2} a_2 (t - \tau_a) \frac{b_1}{K_{bm} + b_1} + K_{b3} \frac{b_2}{K_{bo} + b_2}, \quad (3)$$

$$\frac{db_2}{dt} = -\frac{db_1}{dt}. \quad (4)$$

Here, K_{b1} , K_{b2} , and K_{b3} are positive rate constants, and K_{bn} , K_{bm} and K_{bo} are the effective Michaelis constants for the respective reactions. The direct phosphorylation by active kinase converting b_1 to active b_2 appears as the factor $a_2(t - \tau_a)$ on the right hand side of equation (3). As with the feedback in the kinase equations, this effect is determined by the previous state of a_2 with the constant delay τ_a . Due to the large number of kinases relative to phosphatases and the increase in specificity [19], the influence of the kinase arm on the phosphatase is through this single phosphorylation term.

The third species, the eNOS, is passive in that it does not contribute feedback to the kinase-phosphatase interactions. The transformation between the inactive and active states ($c_1 \rightarrow c_2$) is in part directed by the kinase through phosphorylation by the active a_2 . The reverse reaction ($c_2 \rightarrow c_1$) is in part directed by the phosphatase by dephosphorylation by the active b_2 (figure 2, cell (iii)). The equations for **C** are given by

$$\frac{dc_1}{dt} = -K_{c1} \frac{c_1}{K_{cn} + c_1} - K_{c2} a_2 \frac{c_1}{K_{cm} + c_1} + K_{c3} b_2 \frac{c_2}{K_{co} + c_2} + K_{c4} \frac{c_2}{K_{cp} + c_2}, \quad (5)$$

$$\frac{dc_2}{dt} = -\frac{dc_1}{dt}. \quad (6)$$

As before, the rate constants $K_{c1}, \dots, K_{c4}$ are positive. And $K_{cm}, K_{cm}, K_{co},$ and K_{cp} denote effective Michaelis constants. Activation, phosphorylation by the active kinase, of c_1 to form c_2 appears as the factor a_2 in the second term on the right side of (5); dephosphorylation by the active phosphatase converting c_2 to c_1 is accounted for in the third term by the factor b_2 . The authors are unaware of any results indicating that eNOS in particular contributes direct feedback to the upstream signal cascade. However, the NO it produces has been shown to inhibit ERK [23]. This type of feedback is outside the scope of our simplified model; so we consider eNOS as the effector in the signaling process. As such we do not include any action of C in equations (1)–(4), and we assume that the actions of a_2 and b_2 on C are determined by the present state of the respective species.

Numerical simulations of (1)–(6) for a wide array of parameter values demonstrate that in the absence of delay all species decay to a steady state. Oscillations appear with the introduction of nonzero delay. With the mass conservation, nothing is lost if we only consider equations (1), (3), and (5). Furthermore, since $c_{1,2}$ is completely determined by solutions to equations (1) and (3) we focus our analysis on these two equations. In the following sections, we assume a single delay time $\tau := \tau_a = \tau_b = \tau_{b'}$ (leaving distinct multiple delays for future work). We first produce stability criteria for a constant equilibrium state and then consider possible oscillatory behavior arising due to the delay time. In particular, we establish conditions under which a Hopf bifurcation occurs taking the delay time as a bifurcation parameter.

3. Single delay time: stability analysis

Nondimensionalization of the system may be carried out so that the new time variable $t_{new} = (K_{c1}/[C])t$ where $[C]$ is the total concentration of species C , and the nondimensional versions of A and B are normalized by their respective total concentrations. As such, the form of equations (1) and (3) remain the same except that we may write the scaled active forms of the kinase and phosphatase as $a_2 = 1 - a_1$, and $b_2 = 1 - b_1$. To simplify the notation, let

$$\phi_{\alpha,\beta}(x) = \frac{x}{K_{\alpha\beta} + x}, \quad \text{e. g.} \quad \phi_{a,n}(a_1) = \frac{a_1}{K_{an} + a_1}$$

represent the rational Michaelis-Menten reaction terms. Letting $a := a_1$ and $b := b_1$, the equations considered can be restated as

$$\begin{aligned} \frac{da}{dt} = & -K_{a1}f_p(1-b(t-\tau))\phi_{a,n}(a) - K_{a2}(1-f_p(1-b(t-\tau)))\phi_{a,m}(a) + K_{a3}(1-b(t-\tau))\phi_{a,o}(1-a) + K_{a4}\phi_{a,p}(1-a) \\ & =: F(a(t), b(t-\tau)) \end{aligned}$$

(7)

$$\begin{aligned}\frac{db}{dt} &= -K_{b1}\phi_{b,n}(b) - K_{b2}(1-a(t-\tau))\phi_{b,m}(b) + K_{b3}\phi_{b,o}(1-b) \\ &=: G(a(t-\tau), b(t))\end{aligned}\quad (8)$$

3.1. Stability of a constant equilibrium state

For a wide range of parameter values, it is readily, numerically demonstrated that there exists a constant equilibrium state (a_e, b_e) . Let us define the perturbation variables u and v via

$$a = a_e + u(t), \quad \text{and} \quad b = b_e + v(t), \quad |u|, |v| \ll 1.$$

Upon substitution, and keeping only linear terms in u and v we obtain the system

$$\frac{d\mathbf{u}}{dt} = \Psi \mathbf{u}(t-\tau) - \Phi \mathbf{u} \quad (9)$$

where $\mathbf{u} = (u, v)^T$. The coefficient matrices are

$$\Psi = \begin{pmatrix} 0 & B \\ C & 0 \end{pmatrix}, \quad \text{and} \quad \Phi = \begin{pmatrix} |A| & 0 \\ 0 & |D| \end{pmatrix}$$

whose entries are determined to be

$$\begin{aligned}A &= -K_{a1}f_p(1-b_e)\phi'_{a,n}(a_e) - K_{a2}[1-f_p(1-b_e)]\phi'_{a,m}(a_e) - K_{a3}(1-b_e)\phi'_{a,0}(1-a_e) - K_{a4}\phi'_{a,p}(1-a_e), \\ B &= K_{a1}f'_p(1-b_e)\phi_{a,n}(a_e) - K_{a2}f'_p(1-b_e)\phi_{a,m}(a_e) - K_{a3}\phi_{a,0}(1-a_e), \\ C &= K_{b2}\phi_{b,m}(b_e), \quad \text{and} \\ D &= -K_{b1}\phi'_{b,n}(b_e) - K_{b2}(1-a_e)\phi'_{b,m}(b_e) - K_{b3}\phi'_{b,o}(1-b_e).\end{aligned}$$

(Here, the primes denote derivatives with respect to the appropriate variable.) Given that the rate coefficients $K_{ai} > 0$, these entries will satisfy $A, D < 0$, and $C > 0$, with B having undetermined sign.

In the following, we are interested in the space $\mathcal{C}([-\tau, 0], \mathbb{R}^2)$ of continuous functions (representing, for example, all possible initial histories) along with the norm

$$\|\phi\| = \sup_{t \in [-\tau, 0]} \|\phi(t)\|_2.$$

We can state the following result:

Theorem 3.1—If $\sqrt{|BC|} < \frac{\sqrt{3}}{2} \min(|A|, |D|)$, then every solution $\mathbf{u}$ of (9) decays to zero as $t \rightarrow \infty$.

Proof: Let $\mathbf{u} = e^{\omega t} \mathbf{u}_0$. Upon substitution, (9) yields the transcendental equation

$$\omega \mathbf{u}_0 = e^{-\omega \tau} \Psi \mathbf{u}_0 - \Phi \mathbf{u}_0$$

from which we obtain the quasi-polynomial characteristic equation

$$\det(e^{-\omega \tau} \Psi - \Phi - \omega I) = 0. \quad (10)$$

Next, let $\omega = \mu + i\sigma$. Expanding (10) and separating the real and imaginary parts we obtain the system

$$\text{Re: } 0 = (\mu + |A|)(\mu + |D|) - \sigma^2 - BCe^{-2\mu\tau} \cos(2\sigma\tau), \quad (11)$$

$$\text{Im: } 0 = \sigma(2\mu + |A| + |D|) + BCe^{-2\mu\tau} \sin(2\sigma\tau). \quad (12)$$

We will argue by contradiction. To this end, suppose that there exists a solution ω with $\mu = 0$. Then rearranging (11) and (12) we get

$$\sigma = -\frac{BCe^{-2\mu\tau} \sin(2\sigma\tau)}{(2\mu + |A| + |D|)},$$

and

$$\begin{aligned} (\mu + |A|)(\mu + |D|) &= BCe^{-2\mu\tau} \cos(2\sigma\tau) + \sigma^2 \\ &= BCe^{-2\mu\tau} \cos(2\sigma\tau) + \frac{(BC)^2 e^{-4\mu\tau} \sin^2(2\sigma\tau)}{(2\mu + |A| + |D|)^2} \\ &\leq BCe^{-2\mu\tau} \cos(2\sigma\tau) + \frac{(BC)^2 e^{-4\mu\tau} \sin^2(2\sigma\tau)}{4(\mu + \tilde{m})^2} \\ &\leq BCe^{-2\mu\tau} \cos(2\sigma\tau) + \frac{(BC)^2 e^{-4\mu\tau} \sin^2(2\sigma\tau)}{4\tilde{m}^2} \\ &\leq |BC| + \frac{(BC)^2}{4\tilde{m}^2} \end{aligned}$$

where $\tilde{m} = \min(|A|, |D|)$. Next, the hypothesis regarding the product BC implies that

$$\frac{\sqrt{|BC|}}{\tilde{m}} < 1, \quad \text{i.e.} \quad \frac{(BC)^2}{4\tilde{m}^2} < \frac{\tilde{m}^2}{4}.$$

Hence the above inequality can be extended to obtain

$$\tilde{m}^2 \leq (\mu + \tilde{m})^2 \leq (\mu + |A|)(\mu + |D|) \leq |BC| + \frac{(BC)^2}{4\tilde{m}^2} < |BC| + \frac{\tilde{m}^2}{4}.$$

Here we reach the contradiction to the aforementioned hypothesis, namely

$$\frac{3\tilde{m}^2}{4} < |BC|.$$

It follows that any solution ω of equation (10) has negative real part, and each solution $\mathbf{u}$ of (9) has a bound of the form [24]

$$\|\mathbf{u}(t)\| \leq M e^{-\gamma t}, \quad \text{for some } M, \gamma > 0$$

completing the argument.

A few remarks are in order.

Remark 3.1—*First, in the absence of delay (setting $\tau = 0$), the equilibrium is asymptotically stable provided $BC < AD$, and this condition is necessarily met if $B < 0$.*

Remark 3.2—*Although there are still several (sixteen distinct) parameters in this system, the values of a_e and b_e can be determined numerically and the condition*

$\sqrt{|BC|} < \frac{\sqrt{3}}{2} \min\{|A|, |D|\}$ can be numerically checked once these parameter values are specified.

Remark 3.3—*Equations (11) and (12) can permit a pure real solution $\sigma = 0$. If $B > 0$, then (11) admits one positive solution when $BC > AD$.*

3.2. Dependence of stability on the delay parameter

In light of remark 3.1, we next investigate dependence of stability of an equilibrium on the value of the delay parameter. If a stable equilibrium solution (a_e, b_e) becomes unstable due to a change in the delay length, then at least one eigenvalue must traverse the imaginary axis from the left into the right half plane. Again, considering the linearized system (9), we can look for solutions of the form $\mathbf{u}(t) = e^{i\sigma t} \mathbf{u}_0(t)$ for $\sigma \in \mathbb{R}_+$ to obtain a boundary on the parameter regime corresponding to stability.

When $\mu = 0$, the system (11)–(12) yields the conditions

$$\frac{|AD| - \sigma^2}{BC} = \cos(2\sigma\tau), \quad \text{and} \quad (13)$$

$$\frac{(|A| + |D|)\sigma}{BC} = -\sin(2\sigma\tau). \quad (14)$$

Upon elimination of the parameter τ these produce the polynomial equation

$$\sigma^4 + (|A|^2 + |D|^2)\sigma^2 + (AD)^2 - (BC)^2 = 0. \quad (15)$$

A solution $\sigma \in \mathbb{R}_+$ exists if and only if $|BC| > |AD|$ —(note that this doesn't necessarily conflict with the existence condition for a stable equilibrium in the non-delay case since $B < 0$ satisfies that condition regardless of the magnitude of B). The above construction argues the following statement:

Lemma 3.2—*Suppose that the non-delay ($\tau = 0$) system (1)–(3) possess a stable equilibrium state (a_e, b_e) . If $|BC| > |AD|$, then there exists at least one critical delay value τ^* such that the characteristic equation for (9) corresponding to this delay admits a pure imaginary eigenvalue $\omega = i\sigma_1$.*

We note that if σ_1 solves (15) and τ^* solves (13)–(14) at $\sigma = \sigma_1$, then $\tau_j = \tau^* + \frac{j\pi}{\sigma_1}$ solves the latter system for each $j \in \mathbb{Z}$. Of course we are only interested in $\tau > 0$ hence we will label τ^* the smallest positive solution. The question remains whether an eigenvalue actually undergoes a sign change in its real part. To investigate this, suppose that a pair (σ_1, τ^*) exists, and assume $\omega = \omega(\tau) = \mu(\tau) + i\sigma(\tau)$ is sufficiently smooth with $\omega(\tau^*) = 0 + i\sigma_1$. Then

$$\frac{d\omega}{d\tau} = \frac{d\mu}{d\tau} + i \frac{d\sigma}{d\tau}.$$

Differentiating (10) with respect to τ produces

$$2\omega \frac{d\omega}{d\tau} + (|A| + |D|) \frac{d\omega}{d\tau} + 2BCe^{-2\omega\tau} \left(\omega + \tau \frac{d\omega}{d\tau} \right) = 0. \quad (16)$$

⁴Nothing is lost by assuming that $\sigma > 0$ as such eigenvalues will occur in conjugate pairs.

Evaluating the above at $\omega = i\sigma_1$ and $\tau = \tau^*$, making use of (13)–(14), and collecting the real and imaginary parts we arrive at

$$\begin{aligned} (|A|+|D|+2\tau^*(|AD|-\sigma_1^2))\frac{d\mu}{d\tau} &= 2(|A|+|D|)\sigma_1^2+2\sigma_1(1+(|A|+|D|)\tau^*)\frac{d\sigma}{d\tau} \\ 2\sigma_1(1+(|A|+|D|)\tau^*)\frac{d\mu}{d\tau} &= -2\sigma_1(|AD|-\sigma_1^2)-(|A|+|D|+2\tau^*(|AD|-\sigma_1^2))\frac{d\sigma}{d\tau}. \end{aligned}$$

A change of stability bifurcation requires $\frac{d\mu}{d\tau} \neq 0$ at $\omega = i\sigma_1$, $\tau = \tau^*$. If $\frac{d\mu}{d\tau} = 0$, the above system can be rearranged to obtain

$$\frac{d\sigma}{d\tau} = -\frac{(|A|+|D|)\sigma_1}{1+(|A|+|D|)\tau^*} = -\frac{2(|AD|-\sigma_1^2)\sigma_1}{|A|+|D|+2(|AD|-\sigma_1^2)\tau^*},$$

implying that

$$A^2+D^2+2\sigma_1^2=0.$$

Since σ_1 is real, this equation possesses no solutions. Thus we have established the following result.

Theorem 3.3—*Suppose the system (1)–(3) has a stable equilibrium (a_e, b_e) solution in the absence of delay ($\tau = 0$), and let (9) be the linearization of the system about this equilibrium. Then there exists a critical delay τ^* for which the equilibrium state changes stability provided $|BC| > |AD|$.*

Theorem 3.3 indicates that the model may give rise to a Hopf bifurcation. This will be investigated further in the following section where we consider the direction of the bifurcation and properties of the resulting solution. Before doing so, we provide an illustrative example⁵. Taking the parameter values shown in table 1, the equilibrium point, coefficients of the linearized system, critical eigenvalue and critical delay for the system (7)–(8) are found to be

$$\begin{aligned} a_e &= 0.9360, & b_e &= 0.6451, & \sigma_1 &= 0.4344, & \tau^* &= 1.8054, \\ A &= -2.9576, & B &= -1.5153, & C &= 0.8658, & D &= -0.0627. \end{aligned}$$

Figure 3 shows the longtime solution of the linearized system (9) as a function of the delay parameter.

Using MATLAB[®] dde23, (9) was solved over a (machine) time interval $[0, t_F]$ taking the constant history $(u(t), v(t)) = (0.01, -0.1)$ for $-\tau \leq t < 0$. The final time $(u(t_F), v(t_F))$ is plotted against the delay parameter τ where the steady state $(0, 0)$ undergoes a stability

⁵The values used are illustrative in that numerical values for reaction rates and Michaelis constants are not available.

change as τ surpasses the critical value determined numerically. Figure 4 shows examples of plots of $u(t)$ and $v(t)$ (left) and a *phase plane* type diagram (right) for values of τ below the critical value (top) and above the critical value (bottom). Below the critical delay value, the solution spirals into the origin. It appears that the bifurcation results in a stable limit cycle with small amplitude oscillations about the equilibrium for the nonlinear system (see figure 5 in section 4.2).

4. Functional formulation and center manifold reduction

Here we wish to investigate further the nature of the bifurcation where we are particularly interested in determining its direction. The system (7)–(8) can be restated in the form

$$\frac{da}{dt} = F(a(t), b(t-\tau)), \quad (17)$$

$$\frac{db}{dt} = G(a(t-\tau), b(t)). \quad (18)$$

Assuming the existence of an equilibrium solution (a_e, b_e) , let τ^* denote the critical delay time determined under the required conditions in section 3.2. We introduce the change of variables

$$\tau = \tau^* + \gamma, \quad t = s\tau, \quad u(s) = a(s\tau) - a_e, \quad \text{and} \quad v(s) = b(s\tau) - b_e.$$

Upon application of the variable change, and relabeling the time variable t as opposed to s , we arrive at

$$\frac{du}{dt} = (\tau^* + \gamma) [-A|u(t) + Bv(t-1)] + \hat{F}(u(t), v(t-1)) \quad (19)$$

$$\frac{dv}{dt} = (\tau^* + \gamma) [Cu(t-1) - D|v(t)] + \hat{G}(u(t-1), v(t)) \quad (20)$$

where the coefficients $A, \dots, D$ are those defined in section 3.2 and the hat functions represent the nonlinear parts of (17)–(18), scaled by the factor $(\tau^* + \gamma)$, in a neighborhood of the now shifted equilibrium. Note both of these hat functions are *near zero* in then sense that they are zero with all first partials zero at $(0, 0)$. In what follows, we will only require expansions of the nonlinear parts up to at most order three [25, 26]. If we omit the dependence, stipulating that all partials are evaluated at the equilibrium point (a_e, b_e) , we can

express the nonlinear part as the cubic polynomial⁶ with remainder (subscripts denote partials with respect to the first or second variable)

$$\begin{pmatrix} \hat{F}(u, v) \\ \hat{G}(u, v) \end{pmatrix} = (\tau^* + \gamma) \times \begin{pmatrix} \frac{1}{2}F_{11}u^2 + F_{12}uv + \frac{1}{2}F_{22}v^2 + \frac{1}{6}F_{111}u^3 + \frac{1}{2}F_{112}u^2v + \frac{1}{2}F_{122}uv^2 + \frac{1}{6}F_{222}v^3 \\ \frac{1}{2}G_{11}u^2 + G_{12}uv + \frac{1}{2}G_{22}v^2 + \frac{1}{6}G_{111}u^3 + \frac{1}{2}G_{112}u^2v + \frac{1}{2}G_{122}uv^2 + \frac{1}{6}G_{222}v^3 \end{pmatrix} + O(\|\mathbf{u}\|^4) \quad (21)$$

Let $\mathbf{u} = (u, v)^T$ and in the tradition of [25] denote the time shifted function by

$$\mathbf{u}_t(\theta) := \mathbf{u}(t + \theta), \quad -1 \leq \theta \leq 0.$$

This allows us to write (19)–(20) in the functional formulation highlighting the linear and nonlinear parts as

$$\dot{\mathbf{u}}_t = \mathbf{L}\mathbf{u}_t + \mathcal{F}(\mathbf{u}_t). \quad (22)$$

For each $\phi \in \mathcal{C}^1([-1, 0], \mathbb{R}^2)$ the linear part of the above equation corresponds to the operator

$$\mathbf{L}\phi(\theta) = \begin{cases} \frac{d\phi}{d\theta}, & -1 \leq \theta < 0 \\ \hat{\Psi}\phi(-1) - \hat{\Phi}\phi(0), & \theta = 0. \end{cases}$$

In keeping with our hat convention, the coefficient matrices in this definition are the matrices defined in section 3.2 scaled by the coefficient $(\tau^* + \gamma)$. Also extending from the previous section, when $\gamma = 0$, this operator is assumed to possess a single simple, pure imaginary eigenvalue $i\sigma$ (equal to $i\tau^* \sigma_1$ found in section 3.2). Finally, the nonlinear part of (22) based on (19)–(20) is

$$\mathcal{F}(\phi)(\theta) = \begin{cases} \mathbf{0}, & -1 \leq \theta < 0 \\ \begin{pmatrix} \hat{F}(\phi_1(0), \phi_2(-1)) \\ \hat{G}(\phi_1(-1), \phi_2(0)) \end{pmatrix}, & \theta = 0. \end{cases}$$

To assess the possible stability of periodic solutions we wish to reduce our consideration to that of a finite dimensional space; the center manifold of the operator in equation (22) [25].

⁶ G is linear in a , so $G_{11} = G_{111} = G_{112} = 0$

To facilitate this, we introduce the adjoint space $\mathcal{C}^1([0, 1], \mathbb{R}^2)$ and the bilinear form for $\phi \in \mathcal{C}^1([-1, 0], \mathbb{R}^2)$ and $\psi \in \mathcal{C}^1([0, 1], \mathbb{R}^2)$ given by (see for example [25, 26, 27])

$$\langle \psi, \phi \rangle = \psi^*(0)\phi(0) + \int_{-1}^0 \psi^*(x+1)\hat{\Psi}\phi(x) dx.$$

With respect to this bilinear form, the adjoint of the operator $\mathbf{L}$ is

$$\mathbf{L}^*\psi(r) = \begin{cases} -\frac{d\psi}{dr}, & 0 < r \leq 1 \\ \hat{\Psi}^*\psi(1) - \hat{\Phi}^*\psi(0), & r=0, \end{cases} \quad \text{for } \psi \in \mathcal{C}^1([0, 1], \mathbb{R}^2).$$

Characterization of the center manifold requires finding eigenfunctions for $\mathbf{L}$ and $\mathbf{L}^*$ corresponding to the eigenvalues $\pm i\sigma$, respectively. From the operator definitions arise the pair of boundary value problems

$$\frac{d}{d\theta} \vec{\xi} = i\sigma \vec{\xi}, \quad -1 < \theta < 0, \quad \text{subject to } \hat{\Psi} \vec{\xi}(-1) - \hat{\Phi} \vec{\xi}(0) = i\sigma \vec{\xi}(0), \quad (23)$$

and

$$-\frac{d}{dr} \vec{\eta} = -i\sigma \vec{\eta}, \quad 0 < r < 1, \quad \text{subject to } \hat{\Psi}^* \vec{\eta}(1) - \hat{\Phi}^* \vec{\eta}(0) = -i\sigma \vec{\eta}(0). \quad (24)$$

Splitting the real and imaginary parts of $\vec{\xi}$ and $\vec{\eta}$, put

$$\vec{\xi} = \mathbf{s}_1 + i\mathbf{s}_2, \quad \text{and} \quad \vec{\eta} = \mathbf{n}_1 + i\mathbf{n}_2$$

with $\mathbf{s}_i, \mathbf{n}_i$ taking values in $\mathbb{R}^2$. The general solution to the ODEs is given by the eight parameter family

$$\begin{aligned} \mathbf{s}_1(\theta) &= \cos(\sigma\theta)\mathbf{c}_1 - \sin(\sigma\theta)\mathbf{c}_2 \\ \mathbf{s}_2(\theta) &= \sin(\sigma\theta)\mathbf{c}_1 + \cos(\sigma\theta)\mathbf{c}_2 \\ \mathbf{n}_1(r) &= \cos(\sigma r)\mathbf{d}_1 - \sin(\sigma r)\mathbf{d}_2 \\ \mathbf{n}_2(r) &= \sin(\sigma r)\mathbf{d}_1 + \cos(\sigma r)\mathbf{d}_2 \end{aligned} \quad \mathbf{c}_i, \mathbf{d}_i \in \mathbb{R}^2.$$

The assumption that the critical eigenvalue pair $\pm i\sigma_1$ is simple allows us to impose an orthogonality type condition $\langle \mathbf{n}_i, \mathbf{s}_j \rangle = \delta_{ij}$ on the eigenfunctions $\vec{\xi}$ and $\vec{\eta}$ [26]. Together with the boundary conditions in (23)–(24), this provides six equations. Hence the solution is not unique. In the spirit of [25, 27], we set

$$\mathbf{c}_1 = (1, c_{12})^T, \quad \text{and} \quad \mathbf{c}_2 = (0, c_{22})^T.$$

Then applying the boundary conditions in (23), we find that

$$c_{12} = \frac{1}{\hat{B}}(|\hat{A}|\cos\sigma - \sigma\sin\sigma), \quad \text{and} \quad c_{22} = \frac{1}{\hat{B}}(|\hat{A}|\sin\sigma + \sigma\cos\sigma).$$

(Of course, the validity of this result rests on the linear independence of $\mathbf{c}_1$ and $\mathbf{c}_2$. In particular, we require $\tan\sigma \neq -\sigma/|\hat{A}|$ which must be checked but does hold for the test parameters used in section 3.2.) The boundary conditions in (24) correspond to the system

$$\begin{bmatrix} -|\hat{A}| & \cos(\sigma)\hat{C} \\ \cos(\sigma)\hat{B} & -|\hat{D}| \end{bmatrix} \begin{pmatrix} d_{11} \\ d_{12} \end{pmatrix} - \begin{bmatrix} \sigma & \sin(\sigma)\hat{C} \\ \sin(\sigma)\hat{B} & \sigma \end{bmatrix} \begin{pmatrix} d_{21} \\ d_{22} \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \end{pmatrix}. \quad (25)$$

(Again, hat parameters are scaled, by $(\tau^* + \gamma)$, versions of those from section 3.2.) Finally, with the short hand

$$\tilde{x} = \frac{1}{2\sigma}(\sigma\cos(\sigma) + \sin(\sigma)), \quad \tilde{y} = \frac{1}{2\sigma}(\sigma\cos(\sigma) - \sin(\sigma)), \quad \text{and} \quad \tilde{z} = \frac{1}{2}\sin(\sigma),$$

the system obtained from the *orthogonality* equations $\langle n_i, s_j \rangle = \delta_{ij}$ is

$$\begin{bmatrix} \tilde{x}\hat{B}c_{12} + \tilde{z}\hat{B}c_{22} + 1 & \tilde{x}\hat{C} + c_{12} \\ \tilde{x}\hat{B}c_{22} - \tilde{z}\hat{B}c_{12} & c_{22} - \tilde{z}\hat{C} \end{bmatrix} \begin{pmatrix} d_{11} \\ d_{12} \end{pmatrix} + \begin{bmatrix} -\tilde{z}\hat{B}c_{12} + \tilde{y}\hat{B}c_{22} & -\tilde{z}\hat{C} \\ -\tilde{y}\hat{B}c_{12} - \tilde{z}\hat{B}c_{22} & -\tilde{y}\hat{C} \end{bmatrix} \begin{pmatrix} d_{21} \\ d_{22} \end{pmatrix} = \begin{pmatrix} 1 \\ 0 \end{pmatrix}. \quad (26)$$

Remark 4.1—The system (25)–(26) is a rather imposing set of equations. Despite the rather simple forms for $\mathbf{c}_1$ and $\mathbf{c}_2$, these fail to yield any simple expressions in terms of A , ..., D and σ . Nevertheless, they can be solved numerically so that the sign of the Poincaré-Lyapunov constant can be determined (sections 4.1, 4.2).

Remark 4.2—At $\gamma = 0$ with $\sigma = \tau^* \sigma_1$, the factor τ^* can be eliminated from equation (25) so that the hats may be dropped.

4.1. Projection onto the center manifold

Next we write $\mathbf{u}_t$ as the sum of it's projection onto the subspace spanned by $\{s_1, s_2\}$ and the space transverse to this. Let

$$\mathbf{u}_t(\theta) = y_1(t)\mathbf{s}_1(\theta) + y_2(t)\mathbf{s}_2(\theta) + \mathbf{w}(t)(\theta) \quad \text{with} \quad \mathbf{w} := \mathbf{u}_t - y_1\mathbf{s}_1 - y_2\mathbf{s}_2.$$

The coefficients y_i are defined by the orthogonality type condition

$$y_1(t) = \langle \mathbf{n}_1, \mathbf{u}_t \rangle|_{\theta=0} \quad \text{and} \quad y_2(t) = \langle \mathbf{n}_2, \mathbf{u}_t \rangle|_{\theta=0}.$$

These equations along with (22) can be used to deduce equations for y_i . Continuing to denote d/dt with a dot, differentiating the first equation (at $\theta = 0$) above produces

$$\begin{aligned} \dot{y}_1 &= \langle \mathbf{n}_1, \dot{\mathbf{u}}_t \rangle = \langle \mathbf{n}_1, \mathbf{L}\mathbf{u}_t + \mathcal{F}(\mathbf{u}_t) \rangle \\ &= \langle \mathbf{L}^* \mathbf{n}_1, \mathbf{u}_t \rangle + \langle \mathbf{n}_1, \mathcal{F}(\mathbf{u}_t) \rangle \\ &= \sigma y_2 + \mathbf{d}_1^* \mathcal{F}(\mathbf{u}_t)(0) \end{aligned} \quad (27)$$

A similar computation results in an equation for y_2 and ultimately $\mathbf{w}$. We arrive at the *canonical* [25, 27] form of the system

$$\dot{y}_1 = \sigma y_2 + \mathbf{d}_1^* \mathcal{F}(\mathbf{u}_t)(0) \quad (28)$$

$$\dot{y}_2 = -\sigma y_1 + \mathbf{d}_2^* \mathcal{F}(\mathbf{u}_t)(0) \quad (29)$$

$$\begin{aligned} \dot{\mathbf{w}} &= \mathbf{L}\mathbf{u}_t + \mathcal{F}(\mathbf{u}_t) - \sigma y_2 \mathbf{s}_1 + \sigma y_1 \mathbf{s}_2 - \mathbf{d}_1^* \mathcal{F}(\mathbf{u}_t)(0) \mathbf{s}_1 - \mathbf{d}_2^* \mathcal{F}(\mathbf{u}_t)(0) \mathbf{s}_2 \\ &= \mathbf{L}\mathbf{w} + \mathcal{F}(\mathbf{u}_t) - [\mathbf{d}_1^* \mathcal{F}(\mathbf{u}_t)(0)] \mathbf{s}_1 - [\mathbf{d}_2^* \mathcal{F}(\mathbf{u}_t)(0)] \mathbf{s}_2. \end{aligned} \quad (30)$$

We will substitute $\mathbf{u}_t = y_1 \mathbf{s}_1 + y_2 \mathbf{s}_2 + \mathbf{w}$ into this system. It is useful to observe that

$$\mathbf{u}_t(\theta) = y_1(t) \left[\cos(\sigma\theta) \begin{pmatrix} 1 \\ c_{12} \end{pmatrix} - \sin(\sigma\theta) \begin{pmatrix} 0 \\ c_{22} \end{pmatrix} \right] + y_2(t) \left[\sin(\sigma\theta) \begin{pmatrix} 1 \\ c_{12} \end{pmatrix} + \cos(\sigma\theta) \begin{pmatrix} 0 \\ c_{22} \end{pmatrix} \right] + \begin{pmatrix} w_1 \\ w_2 \end{pmatrix}. \quad (31)$$

From the values of c_{12} , c_{22} found previously, and in light of the relations (13)–(14), it can be shown that

$$c_{12}\cos(\sigma)+c_{22}\sin(\sigma)=\frac{|A|}{B}=\frac{|\hat{A}|}{\hat{B}}, \quad \text{and} \quad -c_{12}\sin(\sigma)+c_{22}\cos(\sigma)=\frac{\sigma_1}{B}=\frac{\sigma}{\hat{B}}.$$

Combined with (31), the components of $\mathbf{u}_t$ in our center manifold reduction will take the forms

$$\begin{aligned} \mathbf{u}_t(0) &= \begin{pmatrix} y_1+w_1(0) \\ c_{12}y_1+c_{22}y_2+w_2(0) \end{pmatrix}, \quad \text{and} \\ \mathbf{u}_t(-1) &= \begin{pmatrix} \cos(\sigma)y_1-\sin(\sigma)y_2+w_1(-1) \\ \frac{|\hat{A}|}{\hat{B}}y_1+\frac{\sigma}{\hat{B}}y_2+w_2(-1) \end{pmatrix}. \end{aligned}$$

The nonlinear part of (28) is then $\mathbf{d}_1 \cdot \vec{\Lambda}$ and that of (29) is $\mathbf{d}_2 \cdot \vec{\Lambda}$ where

$$\vec{\Lambda} := \left[\hat{F} \left(y_1+w_1(0), \frac{|\hat{A}|}{\hat{B}}y_1+\frac{\sigma}{\hat{B}}y_2+w_2(-1) \right), \hat{G} (\cos\sigma y_1-\sin\sigma y_2+w_1(-1), c_{12}y_1+c_{22}y_2+w_2(0)) \right]^T.$$

Using (21), these can be expressed in terms of at least degree three in y_i . A determination of the direction of the bifurcation requires a Taylor expansion of $\vec{\Lambda}$ of at most degree three, and for this, it is sufficient to express $\mathbf{w}$ in a truncated expansion of degree two [26]. Thus we put

$$\mathbf{w}(\theta) = \frac{1}{2}\mathbf{h}_1(\theta)y_1^2 + \mathbf{h}_2(\theta)y_1y_2 + \frac{1}{2}\mathbf{h}_3(\theta)y_2^2.$$

Differentiating with respect to t , substituting (28)–(29), and collecting terms produces

$$\begin{aligned} \dot{\mathbf{w}} &= \mathbf{h}_1y_1\dot{y}_1 + \mathbf{h}_2(\dot{y}_1y_2 + y_1\dot{y}_2) + \mathbf{h}_3y_2\dot{y}_2 \\ &= (\sigma y_2 + \mathbf{d}_2 \cdot \vec{\Lambda})(\mathbf{h}_1y_1 + \mathbf{h}_2y_2) + (-\sigma y_1 + \mathbf{d}_2 \cdot \vec{\Lambda})(\mathbf{h}_2y_1 + \mathbf{h}_3y_2) \\ &= -\sigma\mathbf{h}_2y_1^2 + \sigma(\mathbf{h}_1 - \mathbf{h}_3)y_1y_2 + \sigma\mathbf{h}_2y_2^2 + \text{h. o. t} \end{aligned} \quad (32)$$

The terms h.o.t. are of degree three or higher. We compare this expression with equation (30) letting prime indicate differentiation with respect to θ

$$\begin{aligned} \dot{\mathbf{w}} &= \mathbf{L}w + \mathcal{F}(\mathbf{u}_t) - [\mathbf{d}_1^* \mathcal{F}(\mathbf{u}_t)(0)] \mathbf{s}_1 - [\mathbf{d}_2^* \mathcal{F}(\mathbf{u}_t)(0)] \mathbf{s}_2 \\ &= \begin{cases} \frac{1}{2}\mathbf{h}_1'(\theta)y_1^2 + \mathbf{h}_2'(\theta)y_1y_2 + \frac{1}{2}\mathbf{h}_3'(\theta)y_2^2, & -1 \leq \theta < 0 \\ \hat{\Psi}\mathbf{w}(-1) - \hat{\Phi}\mathbf{w}(0), & \theta = 0 \end{cases} + \begin{cases} \mathbf{0}, & -1 \leq \theta < 0 \\ \vec{\Lambda}, & \theta = 0 \end{cases} - \mathbf{d}_1 \cdot \vec{\Lambda} \mathbf{s}_1(\theta) - \mathbf{d}_2 \cdot \vec{\Lambda} \mathbf{s}_2(\theta). \end{aligned}$$

We may express the components of $\vec{\Lambda}$ in the following way

$$\begin{aligned}\vec{\Lambda} &= \begin{pmatrix} \Lambda_{20}^{(1)} \\ \Lambda_{20}^{(2)} \end{pmatrix} y_1^2 + \begin{pmatrix} \Lambda_{11}^{(1)} \\ \Lambda_{11}^{(2)} \end{pmatrix} y_1 y_2 + \begin{pmatrix} \Lambda_{02}^{(1)} \\ \Lambda_{02}^{(2)} \end{pmatrix} y_2^2 + \text{h. o. t.} \\ &=: \vec{\Lambda}_{20} y_1^2 + \vec{\Lambda}_{11} y_1 y_2 + \vec{\Lambda}_{02} y_2^2 + \text{h. o. t.}\end{aligned}\quad (33)$$

Each of the coefficients appearing in this formulation depend on the derivatives in equation (21) as well as c_{12} , c_{22} , A/B , σ/B , $\cos \sigma$, and $\sin \sigma$. The neglected terms (h.o.t.) can be written using a similar notational convention, and this will be done for third degree terms.

This defines a boundary value problem for the functions $\mathbf{h}_i$. To facilitate finding the solution we introduce some convenient notation. Let

$$S_{ij} = \begin{pmatrix} \Lambda_{ij}^{(1)} & 0 & \Lambda_{ij}^{(2)} & 0 \\ 0 & \Lambda_{ij}^{(1)} & 0 & \Lambda_{ij}^{(2)} \end{pmatrix},$$

$$\mathbf{p} = \begin{pmatrix} d_{11} \\ d_{11}c_{12} + d_{21}c_{22} \\ d_{12} \\ d_{12}c_{12} + d_{22}c_{22} \end{pmatrix}, \quad \text{and} \quad \mathbf{q} = \begin{pmatrix} d_{21} \\ -d_{11}c_{22} + d_{21}c_{12} \\ d_{22} \\ -d_{12}c_{22} + d_{22}c_{12} \end{pmatrix}.$$

Define the vectors with values in $\mathbb{R}^6$

$$\mathbf{H} = \begin{pmatrix} \mathbf{h}_1 \\ \mathbf{h}_2 \\ \mathbf{h}_3 \end{pmatrix}, \quad \mathbf{P} = \begin{pmatrix} 2S_{20}\mathbf{p} \\ S_{11}\mathbf{p} \\ 2S_{02}\mathbf{p} \end{pmatrix}, \quad \mathbf{Q} = \begin{pmatrix} 2S_{20}\mathbf{q} \\ S_{11}\mathbf{q} \\ 2S_{02}\mathbf{q} \end{pmatrix}, \quad \text{and} \quad \mathbf{R} = \begin{pmatrix} 2\vec{\Lambda}_{20} \\ \vec{\Lambda}_{11} \\ 2\vec{\Lambda}_{02} \end{pmatrix}.$$

And let T be the 6×6 matrix (of 2×2 blocks) defined by

$$\sigma T = \sigma \begin{pmatrix} O & -2I & O \\ I & O & -I \\ O & 2I & O \end{pmatrix}.$$

Matching the right hand sides of (30) and (32) produces the system of equations

$$\mathbf{H}'(\theta) = \sigma T \mathbf{H} + \mathbf{P} \cos(\sigma\theta) + \mathbf{Q} \sin(\sigma\theta). \quad (34)$$

subject to the boundary conditions

$$\begin{pmatrix} \hat{\Psi} & O & O \\ O & \hat{\Psi} & O \\ O & O & \hat{\Psi} \end{pmatrix} \mathbf{H}(-1) - \begin{pmatrix} \hat{\Phi} & O & O \\ O & \hat{\Phi} & O \\ O & O & \hat{\Phi} \end{pmatrix} \mathbf{H}(0) - \sigma T \mathbf{H}(0) = \mathbf{P} - \mathbf{R}. \quad (35)$$

Using the method of undetermined coefficients, we find that the general solution of (34) is

$$\mathbf{H}(\theta) = e^{\sigma \theta T} \mathbf{K} + \mathbf{X} \cos(\sigma \theta) + \mathbf{Y} \sin(\sigma \theta), \quad \text{where} \quad \sigma \begin{pmatrix} -T & I_6 \\ -I_6 & -T \end{pmatrix} \begin{pmatrix} \mathbf{X} \\ \mathbf{Y} \end{pmatrix} = \begin{pmatrix} \mathbf{P} \\ \mathbf{Q} \end{pmatrix}. \quad (36)$$

The constant of integration $\mathbf{K}$ is found by imposing (35). Calling the first two block matrices on the left side of (35) $\hat{\Psi}_6$ and $\hat{\Phi}_6$, the equation for $\mathbf{K}$ is

$$\begin{aligned} [\hat{\Psi}_6 e^{-\sigma T} - \hat{\Phi}_6 - \sigma T] \mathbf{K} &= \mathbf{P} - \mathbf{R} - \hat{\Psi}_6 (\cos \sigma \mathbf{X} - \sin \sigma \mathbf{Y}) + (\hat{\Phi}_6 + \sigma T) \mathbf{X} \\ &= U \mathbf{P} + V \mathbf{Q} - \mathbf{R}, \end{aligned}$$

where

$$U = \begin{bmatrix} J_1 & 2J_2 & 2J_1 \\ -J_2 & -J_1 & J_2 \\ 2J_1 & -2J_2 & J_1 \end{bmatrix}, \quad \text{and} \quad V = \begin{bmatrix} J_2 & -2J_1 & 2J_2 \\ J_1 & -J_2 & -J_1 \\ 2J_2 & 2J_1 & J_2 \end{bmatrix},$$

with

$$J_1 = \frac{1}{3\sigma} (\sigma I + \sin \sigma \hat{\Psi}), \quad \text{and} \quad J_2 = \frac{1}{3\sigma} (\cos \sigma \hat{\Psi} - \hat{\Phi}).$$

With $\mathbf{H}$ determined, the values of $\mathbf{w}(0)$ and $\mathbf{w}(-1)$ are

$$\begin{aligned} \mathbf{w}_1(0) &= \frac{1}{2} H_1(0) y_1^2 + H_3(0) y_1 y_2 + \frac{1}{2} H_5(0) y_2^2, \\ \mathbf{w}_2(0) &= \frac{1}{2} H_2(0) y_1^2 + H_4(0) y_1 y_2 + \frac{1}{2} H_6(0) y_2^2, \\ \mathbf{w}_1(-1) &= \frac{1}{2} H_1(-1) y_1^2 + H_3(-1) y_1 y_2 + \frac{1}{2} H_5(-1) y_2^2, \quad \text{and} \\ \mathbf{w}_2(-1) &= \frac{1}{2} H_2(-1) y_1^2 + H_4(-1) y_1 y_2 + \frac{1}{2} H_6(-1) y_2^2. \end{aligned} \quad (37)$$

Here, the components of $\mathbf{H}$ are

$$H_i(0)=K_i+X_i, \quad \text{and} \quad H_i(-1)=(e^{-\sigma T}\mathbf{K})_i+\cos\sigma X_i-\sin\sigma Y_i.$$

Returning to the evolution on the two dimensional center manifold, we can state equations (28)–(29) in Poincaré normal form. Let⁷

$$\varepsilon_{ij}=\mathbf{d}_1 \cdot \vec{\Lambda}_{ij} \quad \text{and} \quad \zeta_{ij}=\mathbf{d}_2 \cdot \vec{\Lambda}_{ij}.$$

Then $y_{1,2}$ satisfy

$$\dot{y}_1=\sigma y_2+\varepsilon_{20}y_1^2+\varepsilon_{11}y_1y_2+\varepsilon_{02}y_2^2+\varepsilon_{30}y_1^3+\varepsilon_{21}y_1^2y_2+\varepsilon_{12}y_1y_2^2+\varepsilon_{03}y_2^3, \quad (38)$$

$$\dot{y}_2=-\sigma y_1+\zeta_{20}y_1^2+\zeta_{11}y_1y_2+\zeta_{02}y_2^2+\zeta_{30}y_1^3+\zeta_{21}y_1^2y_2+\zeta_{12}y_1y_2^2+\zeta_{03}y_2^3. \quad (39)$$

The direction of the bifurcation is determined by the sign of the Lyapunov-Poincaré coefficient which is given by Bautin's formula [28]

$$\Delta=\frac{1}{8\sigma}((\varepsilon_{20}+\varepsilon_{02})(-\varepsilon_{11}+\zeta_{20}-\zeta_{02})+(\zeta_{20}+\zeta_{02})(\varepsilon_{20}-\varepsilon_{02}+\zeta_{11})) \\ +\frac{1}{8}(3\varepsilon_{30}+\varepsilon_{12}+\zeta_{21}+3\zeta_{03}).$$

From (16),

$$\nu:=\operatorname{Re}\left(\frac{d\omega}{d\tau}\right)\Big|_{\tau^*}=\frac{2\sigma_1^2(A^2+D^2+2\sigma_1^2)}{(|A|+|D|+2\tau^*(|AD|-\sigma_1^2))^2+4\sigma_1^2(1+(|A|+|D|)\tau^*)^2}>0$$

With the sign of ν unambiguous, the direction of a generic Hopf bifurcation as well as the stability of resulting periodic solutions is determined by the sign of Δ . If $\Delta < 0$, the bifurcation is supercritical with a stable periodic solution arising for $\tau > \tau^*$. The amplitude $\mathcal{A}$ and frequency f_{rq} of the periodic solution in this case satisfy [26, 25]

$$\mathcal{A}=\sqrt{-\frac{\nu\gamma}{\Delta}}+O(\gamma), \quad f_{rq}=\frac{\sigma_1}{2\pi}+o(1), \quad \text{as } \gamma \rightarrow 0^+, \quad \text{where } \gamma=\tau-\tau^*.$$

If $\Delta > 0$, the bifurcation is subcritical with an unstable periodic orbit existing for $\tau < \tau^*$.

⁷The components of $\vec{\Lambda}$ can be found listed in the appendix.

One would hope to obtain criteria readily checked by reference to two or three straightforward ratios of parameters. And models of some phenomena lend themselves to such results (see for example [29, 27] as well as applications in [30, 28]). Unfortunately, even the simple one kinase-phosphatase model considered here still contains sixteen rate and Michaelis constants upon which the critical time delay and frequency depend. Despite its complexity, we have obtained conditions that may be verified, if only numerically, with parameters as they become known.

4.2. Numerical results

For illustration, returning to the test parameters in table 1, we compute the values of

$$\Delta = -17.3, \quad \text{and} \quad \nu = 0.0851.$$

As the previous numerical simulation suggests, the bifurcation in this case is indeed supercritical. The expected amplitude of periodic solutions is approximately

$$\mathcal{A} \approx 0.07 \sqrt{\tau - \tau^*}, \quad \text{for} \quad 0 < \tau - \tau^* \ll 1$$

This gives

$$\begin{pmatrix} y_1(t) \\ y_1(t) \\ u(t) \\ v(t) \end{pmatrix} \approx \begin{pmatrix} \mathcal{A} \cos(\sigma t) \\ -\mathcal{A} \sin(\sigma t) \\ \mathcal{A} \cos(\sigma t) \\ c_{12}\mathcal{A} \cos(\sigma t) - c_{22}\mathcal{A} \sin(\sigma t) \end{pmatrix}, \quad \text{so that}$$

Recalling the original change of variables in which t was replaced with $t(\tau^* + \gamma)$ with $0 < \gamma \ll 1$ (note that this has no effect on the numerical value of the period since using the $t = \tau s$ notation $\sigma s = \sigma_1 \tau (t/\tau) = \sigma_1 t$), the solutions near equilibrium are

$$\begin{aligned} a(t) &\approx a_e + \mathcal{A} \cos(\sigma_1 t), \\ b(t) &\approx b_e + \mathcal{A} (c_{12} \cos(\sigma_1 t) - c_{22} \sin(\sigma_1 t)). \end{aligned}$$

The amplitude of oscillations for $b(t)$ in the neighborhood of the equilibrium and for the parameter values taken here is

$$\mathcal{A} \sqrt{c_{12}^2 + c_{22}^2} \approx 1.97 \mathcal{A}.$$

This roughly 2:1 relation between amplitudes of oscillations in a and b (similarly u and v) is apparent in figure 5 (4).

The amplitude of oscillation for the target species c can likewise be determined—relative to those of a . Letting $c := c_1$, normalizing so that $c_2 = 1 - c_1$, and taking the aforementioned nondimensionalization (so that the coefficient K_{c1} is replaced with 1 in the resulting equations), equation (5) becomes

$$\frac{dc}{dt} = -\phi_{c,n}(c) - K_{c2}(1-a)\phi_{c,m}(c) + K_{c3}(1-b)\phi_{c,o}(1-c) + K_{c4}\phi_{c,p}(1-c) \quad (40)$$

Defining w via $c = c_e + w(t)$, we find the linear equation in w

$$\frac{dw}{dt} + Ew = K_{c2}\phi_{c,m}(c_e)u - K_{c3}\phi_{c,o}(1-c_e)v = \rho_1 \mathcal{A} \cos(\sigma_1 t) + \rho_2 \mathcal{A} \sin(\sigma_1 t) \quad (41)$$

where

$$\begin{aligned} E &= \phi'_{c,n}(c_e) + K_{c2}(1-a_e)\phi'_{c,m}(c_e) + K_{c3}(1-b_e)\phi'_{c,o}(1-c_e) + K_{c4}\phi'_{c,p}(1-c_e), \\ \rho_1 &= K_{c2}\phi_{c,m}(c_e) - K_{c3}\phi_{c,o}(1-c_e)c_{12}, \quad \text{and} \\ \rho_2 &= K_{c3}\phi_{c,o}(1-c_e)c_{22}. \end{aligned}$$

Ignoring the transient state, we obtain the particular solution

$$w_p = \eta_1 \mathcal{A} \cos(\sigma_1 t) + \eta_2 \mathcal{A} \sin(\sigma_1 t)$$

where

$$\eta_1 = \frac{-\rho_1 E - \rho_2 \sigma_1}{E^2 + \sigma_1^2}, \quad \text{and} \quad \eta_2 = \frac{\rho_1 \sigma_1 - \rho_2 E}{E^2 + \sigma_1^2}.$$

The amplitude of the resulting oscillations about the equilibrium are therefore

$$\mathcal{A} \sqrt{\frac{\rho_1^2 + \rho_2^2}{E^2 + \sigma_1^2}}.$$

Supplementing the parameters listed in table 1 with the additional parameters for equation (40) in table 2, the equilibrium is calculated as $c_e = 0.8806$. And, for this set of parameters, the amplitude of oscillations for c is approximately $1.19\mathcal{A}$. The long-time solutions of the full (a, b, c) , nonlinear system for τ below and above the critical value is presented in figure 5.

5. Discussion

In a recent review article on PP2A [21], AKT can be seen as an example for our model. In this example, AKT is **A**, PP2A is **B**, and eNOS is **C**. When active, AKT phosphorylates and activates eNOS. This is our a_2 action on **C**. AKT is a substrate of PP2A (causing inhibition and reversion to a_1). Phosphatidylinositol 3-kinase (PI3K), an activator of phosphoinositide-dependent kinase-1 (PDK1)—the kinase responsible for direct activation of AKT—is also dephosphorylated by PP2A resulting in less PI3K which acts to decrease conversion of a_1 to a_2 (the first two terms in equation (1) including the steady state partition). While we have no direct evidence of PP2A acting on PDK1, this model allows for that possibility by the fractionation of this term. Further PP2A acts to dephosphorylate and inhibit eNOS which is modeled by our b_2 action on **C**. While we have no evidence in this example of PP2A being activated directly by AKT, there are examples of kinases directly activating respective phosphatases (our a_2 action on **B**). PKA for example, another eNOS activating kinase (on the same sites as AKT), has been shown to directly activate PP2A [31]. The inclusion of a single kinase species here has admitted limitations. There is much that we still do not know about how phosphatases are regulated, two excellent reviews [20, 19] discuss their importance and how much we still have to learn about these important signaling molecules.

Mathematically, even in the highly reduced model considered here, the results arise as rather complicated conditions on model parameters. Despite this, several criteria have been presented that do allow for numerical verification if measurements, or more reliable numerical ranges, of parameter values become available. Parameter fitting, between the mathematical model and experimentation, may provide numerical ranges for some reaction rate and Michaelis constants (in light of ongoing *in vitro* and cellular studies currently in process by co-author Chrestensen). In addition to existence of oscillatory behavior, some notable characteristics such as amplitude and frequency may be illuminated by the results. In particular, the results suggest that the amplitude of oscillations in the eNOS target site may be predicted relative to that of the kinase (or phosphatase) species. While it remains to coordinate the mathematical model with laboratory findings, the model is readily expanded to include intermediate species, the potential for feedback from the target species eNOS (if evidence of this is found), as well as distinct time delays in the existing reactions, and these are the subject of ongoing study.

Acknowledgments

This work was supported in part by the National Institutes of Health under award number R15GM110634 to Carol A. Chrestensen.

References

1. Ignarro LJ, Cirino G, Casini A, Napoli C. Nitric oxide as a signaling molecule in the vascular system: an overview. *J Cardiovasc Pharm.* 1999; 6:879–886. DOI: 10.1097/00005344-199912000-00016
2. Ignarro L. Nitric oxide as a unique signaling molecule in the vascular system: A historical review. *J Physiol Pharmacol.* 2002; 53(4):503–514. [PubMed: 12512688]

3. Förstermann U, Münzel T. Endothelial nitric oxide synthase in vascular disease: From marvel to menace. *Circulation*. 2006; 113:1708–1714. DOI: 10.1161/CIRCULATIONAHA.105.602532 [PubMed: 16585403]
4. Farkas K, Sárman B, Jermendy G, Somogyi A. Endothelial nitric oxide in diabetes mellitus: too much or not enough? *Diabetes Nutr Metab*. 2000; 13(5):287–297. [PubMed: 11105972]
5. Cannon RO. Role of nitric oxide in cardiovascular disease: focus on the endothelium. *Clin Chem*. 1998; 44(8B):1809–1819. [PubMed: 9702990]
6. Ghosh DK, Salerno JC. Nitric oxide synthases: Domain structure and alignment in enzyme function and control. *Front Biosci*. 2003; 8:193–209. DOI: 10.2741/959
7. Sessa WC. eNOS at a glance. *J Cell Sci*. 2004; 117:2427–2429. <http://jcs.biologists.org/content/117/12/2427.abstract>. [PubMed: 15159447]
8. Mount PF, Kemp BE, Power DA. Regulation of endothelial and myocardial NO synthesis by multi-site eNOS phosphorylation. *J Mol Cell Cardiol*.
9. Liu P, Kevrekidis IG, Shvartsman SY. Substrate-dependent control of ERK phosphorylation can lead to oscillations. *Biophys J*. 2011; 101:2572–2581. DOI: 10.1016/j.bpj.2011.10.025 [PubMed: 22261044]
10. Albeck JG, Mills GB, Brugge JS. Frequency-modulated pulses of ERK activity transmit quantitative proliferation signals. *Mol Cell*. 2013; 49:249–261. DOI: 10.1016/j.molcel.2012.11.002 [PubMed: 23219535]
11. Wang H, Wang AX, Liu Z, Chai W, Barrett EJ. The trafficking/interaction of eNOS and caveolin-1 induced by insulin modulates endothelial nitric oxide production. *Mol Endocrinol*. 2009; 23(10): 1613–1623. DOI: 10.1210/me.2009-0115 [PubMed: 19608646]
12. Vital-Lopez FG, Varshney A, Maranas CD, Armaou A. Implication of dynamics in signal transduction and targeted disruption analyses of signaling networks. *Comput Chem Eng*. 2008; 32:2065–2071. DOI: 10.1016/j.compchemeng.2007.10.022
13. Heinrich R, Neel BG, Rapoport TA. Mathematical models of protein kinase signal transduction. *Mol Cell*. 2002; 9:957–970. DOI: 10.1016/S1097-2765(02)00528-2 [PubMed: 12049733]
14. Calder WKM, Gilbert D. When kinases meet mathematics: the systems biology of MAPK signalling. *FEBS Letters*. 2005; 579:1891–1895. DOI: 10.1016/j.febslet.2005.02.002 [PubMed: 15763569]
15. Salerno J, Ghosh D, Razdan R, Helms K, Brown C, McMurphy J, Rye E, Chrestensen C. Endothelial nitric oxide synthase is regulated by erk phosphorylation at ser602. *Biosci Rep*. 2014; 34:535–545. DOI: 10.1042/BSR20140015
16. Church JE, Qian J, Kumar S, Black SM, Venema RC, Papapetropoulos A, Fulton DJR. Inhibition of endothelial nitric oxide synthase by the lipid phosphatase pten. *Vascul Pharmacol*. 2010; 52(5–6):191–198. DOI: 10.1016/j.vph.2009.11.007 [PubMed: 19962452]
17. Salerno JC, Ghosh DK. Space, time and nitric oxide—neuronal nitric oxide synthase generates signal pulses. *FEBS J*. 2009; 276:6677–6688. DOI: 10.1111/j.1742-4658.2009.07382.x [PubMed: 19843161]
18. Cleland WW. Partition analysis and the concept of net rate constants as tools in enzyme kinetics. *Biochemistry*. 1975; 14:3220–3224. DOI: 10.1021/bi00685a029 [PubMed: 1148201]
19. Bononi A, Agnoletto C, Marchi ED, Marchi S, Patergnani S, Bonora M, Giorgi C, Missiroli S, Poletti F, Rimessi A, Pinton P. Protein kinases and phosphatases in the control of cell fate. *Enzyme Research*. 2011; 2011:1–26. DOI: 10.4061/2011/329098
20. Brautigan DL. Protein ser/ thr phosphatases the ugly ducklings of cell signalling. *FEBS J*. 2013; 280:324–345. DOI: 10.1111/j.1742-4658.2012.08609.x [PubMed: 22519956]
21. Wlodarchak N, Xing Y. PP2A as a master regulator of the cell cycle. *Crit Rev Biochem Mol Biol*. 2016; :1–23. DOI: 10.3109/10409238.2016.1143913
22. Saccoa F, Perfetto L, Castagnolia L, Cesarenia G. The human phosphatase interactome: An intricate family portrait. *FEBS Letters*. 2012; 586:2732–2739. DOI: 10.1016/j.febslet.2012.05.008 [PubMed: 22626554]
23. Feng X, Sun T, Bei Y, Ding S, Zheng W, Lu Y, Shen P. S-nitrosylation of ERK inhibits ERK phosphorylation and induces apoptosis. *Scientific Reports*. 2013; 3:1–6. art. no. 1814. DOI: 10.1038/srep01814

24. Driver, R. Ordinary and Delay Differential Equations. Springer-Verlag; New York: 1977. (Print) 978-1-4684-9467-9 (Online)
25. Hale, JK.; Lunel, SMV. Introduction to Functional Differential Equations. Springer-Verlag; New York: 1993.
26. Diekmann, O.; van Gils, SA.; Lunel, SMV.; Walther, H-O. Delay Equations Functional-, Complex-, and Nonlinear Analysis. Springer-Verlag; New York: 1995.
27. Kalmár-Nagy T, Stépán G, Moon FC. Subcritical Hopf bifurcation in the delay equation model for machine tool vibrations. Nonlinear Dynam. 2001; 26:121–142. DOI: 10.1023/A:1012990608060
28. Stépán, G. Retarded dynamical systems: Stability and characteristic functions, Pitman Research dois in Mathematics. Vol. 210. Longman; Essex: 1989.
29. Yu W, Cao J. Hopf bifurcation and stability of periodic solutions for van der Pol equation with time delay. Nonlinear Analysis. 2005; 62:141–165. DOI: 10.1016/j.na.2005.03.017
30. Stech HW. Hopf bifurcation calculations for functional differential equations. J of Math Anal and App. 1985; 109:472–491. DOI: 10.1016/0022-247X(85)90163-5
31. Ahn J, McAvoy T, Rakhilin SV, Nishi A, Greengard P, Nairn A. Protein kinase a activates protein phosphatase 2a by phosphorylation of the b56delta subunit. Proc Natl Acad Sci U S A. 2007; 104:2979–2984. DOI: 10.1073/pnas.0611532104 [PubMed: 17301223]

Appendix: Components of the vector $\tilde{\Lambda}$

For ease of notation, let us label the coefficients in (21) as F_i and G_i , $i = 1, \dots, 7$ in the order in which they appear from left to right—i.e. $F_1 = \frac{1}{2}F_{11}$, $F_2 = F_{12}$, $G_3 = \frac{1}{2}G_{22}$ and so forth. Set $\alpha = |\hat{A}|/\hat{B}$ and $\beta = \sigma/\hat{B}$. Together with this short hand, coefficients for the second degree terms are

$$\begin{aligned}
 \Lambda_{20}^{(1)} &= \alpha^2 F_3 + \alpha F_2 + F_1 \\
 \Lambda_{20}^{(2)} &= \cos \sigma c_{12} G_2 + c_{12}^2 G_3 \\
 \Lambda_{11}^{(1)} &= 2\alpha \beta F_3 + \beta F_2 \\
 \Lambda_{11}^{(2)} &= \cos \sigma c_{22} G_2 - \sin \sigma c_{12} G_2 + 2c_{12} c_{22} G_3 \\
 \Lambda_{02}^{(1)} &= \beta^2 F_3 \\
 \Lambda_{02}^{(2)} &= -\sin \sigma c_{22} G_2 + c_{22}^2 G_3
 \end{aligned}$$

The coefficients for the third degree terms are necessarily more complicated. With the observation that $G_1 = G_4 = G_5 = 0$, the remaining coefficients are

$$\begin{aligned}
 \Lambda_{30}^{(1)} &= F_4 + \frac{1}{2}\alpha F_2 H_1(0) + \alpha F_3 H_2(-1) + \frac{1}{2}F_2 H_2(-1) + F_1 H_1(0) + \alpha F_5 + \alpha^2 F_6 + \alpha F_7, \\
 \Lambda_{03}^{(1)} &= \frac{1}{2}\beta F_2 H_5(0) + \beta F_3 H_6(-1) + \beta^3 F_7 \\
 \Lambda_{21}^{(1)} &= F_2 H_4(-1) + 2F_1 H_3(0) + \beta F_5 + 2\alpha \beta F_6 + 3\beta \alpha^2 F_7 + \alpha F_2 H_3(0) + \frac{1}{2}\beta F_2 H_1(0) + \beta F_3 H_2(-1) + 2\alpha F_3 H_4(-1), \\
 \Lambda_{12}^{(1)} &= F_1 H_5(0) + \beta F_2 H_3(0) + 2\beta F_3 H_4(-1) + \alpha F_3 H_6(-1) + 3\alpha \beta^2 F_7 + \frac{1}{2}\alpha F_2 H_5(0) + \beta^2 F_6 + \frac{1}{2}F_2 H_6(-1), \\
 \Lambda_{30}^{(2)} &= c_{12}^2 \cos \sigma G_6 + \frac{1}{2} \cos \sigma G_2 H_2(0) + \frac{1}{2} c_{12} G_2 H_1(-1) + c_{12} G_3 H_2(0) + c_{12}^3 G_7, \\
 \Lambda_{03}^{(2)} &= -c_{22}^2 \sin \sigma G_6 - \frac{1}{2} \sin \sigma G_2 H_6(0) + \frac{1}{2} c_{22} G_2 H_5(-1) + c_{22} G_3 H_6(0) + c_{22}^3 G_7, \\
 \Lambda_{21}^{(2)} &= c_{12} G_2 H_3(-1) + \frac{1}{2} c_{22} G_2 H_1(-1) + c_{22} G_3 H_2(0) + 2c_{12} G_3 H_4(0) + 3c_{12}^2 c_{22} G_7 + \cos \sigma G_2 H_4(0) - \frac{1}{2} \sin \sigma G_2 H_2(0) - c_{12}^2 \sin \sigma G_6 + \\
 \text{and} \\
 \Lambda_{12}^{(2)} &= c_{22} G_2 H_3(-1) + \frac{1}{2} c_{12} G_2 H_5(-1) + c_{12} G_3 H_6(0) + 2c_{22} G_3 H_4(0) + 3c_{12} c_{22}^2 G_7 + c_{22}^2 \cos \sigma G_6 + \frac{1}{2} \cos \sigma G_2 H_6(0) - \sin \sigma G_2 H_4(0) -
 \end{aligned}$$

Highlights

- We model activation by phosphorylation of endothelial nitric oxide (NO) synthase.
- Stability analysis of a delay equation model of NO regulation is performed.
- Sufficient conditions for a Hopf bifurcation are stated.
- Features of oscillating solutions are obtained via center manifold reduction.

Figure 1.

Various stimuli initiate signaling cascades involving a system of kinases, phosphatases, and eNOS; changing NO production and ultimately impacting metabolism and growth. Phosphatidylinositol bisphosphate/trisphosphate (PIP2/3) at the plasma membrane serve as substrates for signal enzymes such as phosphatidylinositide 3-kinase (PI3K), phosphoinositide-dependent kinase-1 (PDK1), and protein kinase B (AKT). Phosphatase involvement in these signaling cascades is less well understood. However, protein phosphatase 2 (PPA2) is known to dephosphorylate eNOS.

Figure 2.

An abstract schematic of the feedback network for the one kinase–one phosphatase–eNOS model is depicted. (i) The active phosphatase b_2 directly inactivates the kinase ($a_2 \rightarrow a_1$) through dephosphorylation (dashed arrow), as well as inhibiting phosphorylation of the kinase ($a_1 \rightarrow a_2$) (blunted arrow). (ii) The active kinase directly phosphorylates the phosphatase (solid arrow) converting the inactive into the active form ($b_1 \rightarrow b_2$). (iii) The active kinase a_2 phosphorylates eNOS ($c_1 \rightarrow c_2$), and the active phosphatase dephosphorylates eNOS ($c_2 \rightarrow c_1$). (iv) The complete, interactive network is shown.

Figure 3. Long-time solution of the linear system (9) as a function of delay time τ for the parameter values in table 1. The stability of the origin is seen to change as τ passes the numerically determined critical value $\tau^* = 1.8054$.

Figure 4.

Solutions to the linearized system for τ below ($\tau = 1.0$) and above ($\tau = 1.81$) the critical value. The left shows u and v plotted against t . The right side shows the (u, v) plane. The bifurcation appears to change the stable spiral toward the origin into an isolated closed orbit indicating a likely supercritical Hopf bifurcation.

Figure 5. Long-time solutions to the full system for τ below (left) and above (right) the critical value. (The values on the time axis are arbitrary and do not represent any particular units.)

Table 1

Sample Parameter Values

$K_{a1} = 1$	$K_{b1} = 1$	$K_{an} = 0.5$	$K_{bn} = 1$
$K_{a2} = 0.1$	$K_{b2} = 1$	$K_{am} = 0.5$	$K_{bm} = 0.1$
$K_{a3} = 2$	$K_{b3} = 0.1$	$K_{ao} = 0.05$	$K_{bo} = 0.02$
$K_{a4} = 0.05$	$r_1 = 0.5$	$K_{ap} = 0.5$	$r_2 = 0.01$

Table 2

Additional Sample Parameter Values

$K_{c2} = 1$	$K_{c3} = 1$	$K_{c4} = 0.1$	
$K_{cn} = 1$	$K_{cm} = 0.5$	$K_{co} = 0.5$	$K_{cp} = 0.5$