A delay differential equation model of activation of endothelial nitric oxide synthase

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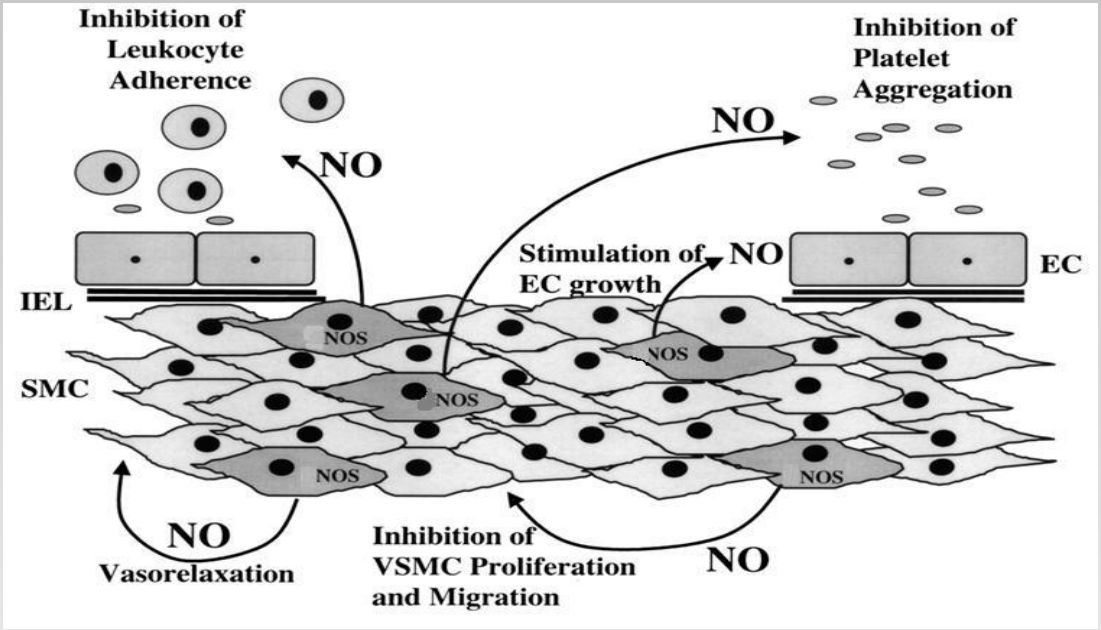
Outline

Nitric Oxide

Nitric Oxide (NO) is a radical that serves as a signaling molecule for cell-to-cell communication and intracellular communication.

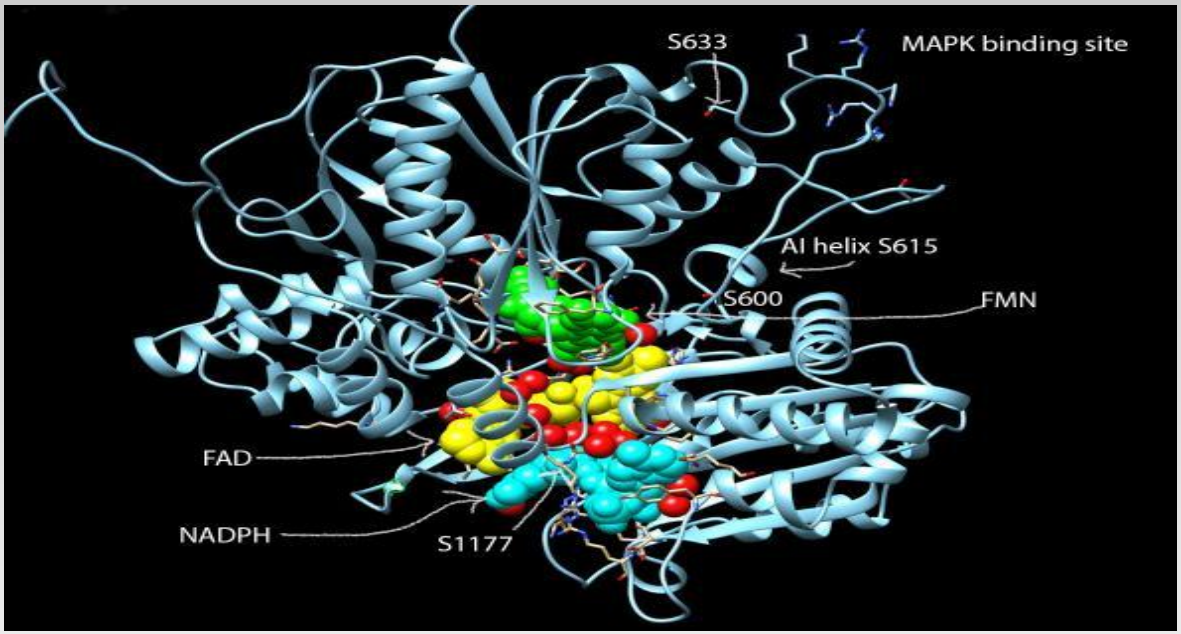


In the vasculature, it serves several critical functions including

- signaling smooth muscle cell relaxation and vasodilation,
- reducing platelet aggregation/adhesion at the endothelial layer, and
- inhibiting leukocyte chemotaxis and adhesion at the endothelium.

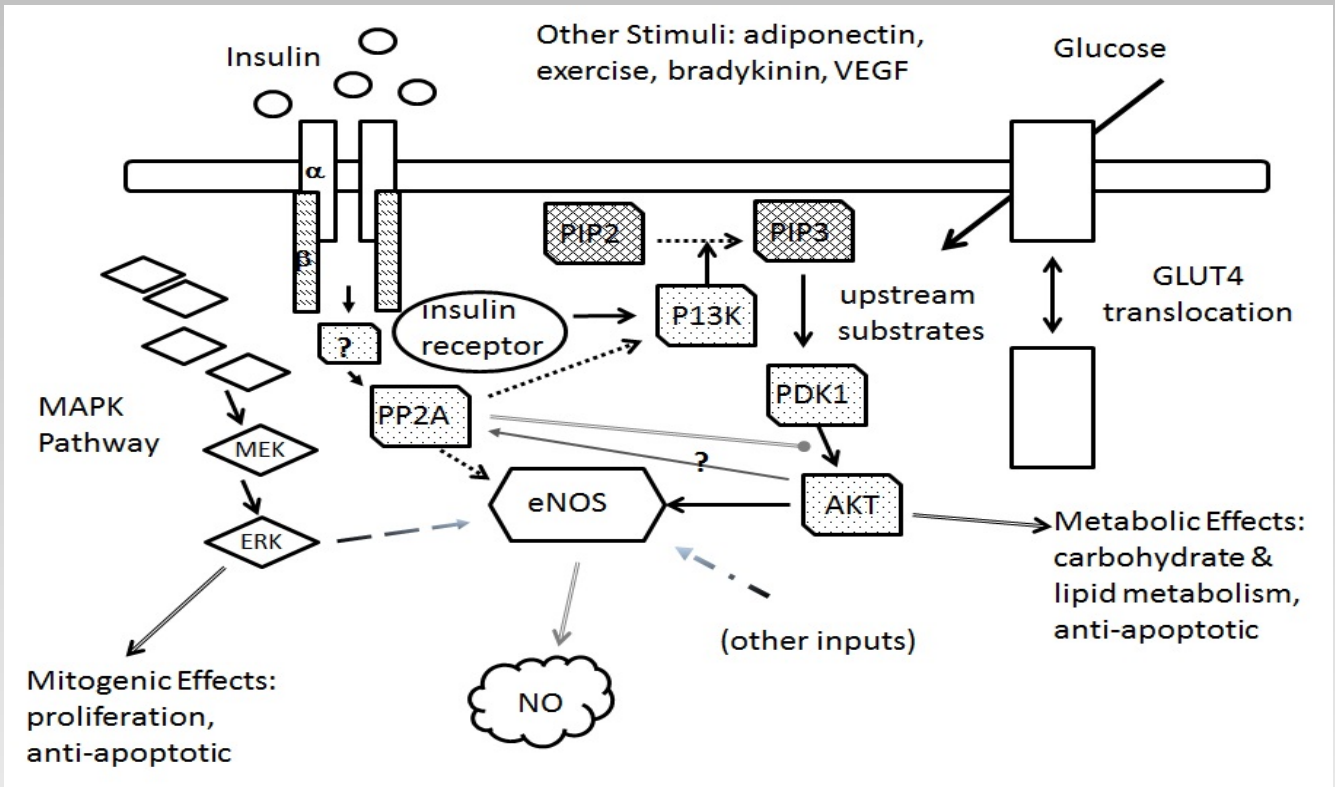
What is eNOS?

Endothelial nitric oxide synthase (eNOS) is the primary enzyme in the vasculature responsible for NO production and use by endothelial cells for regulation of vascular tone.



3D Model of eNOS: Many of the binding sites on eNOS to specific agonists have been identified.

Signal Cascade



A sequence of signals may be stimulated for which eNOS is downstream of a cascade involving various kinases and phosphatases.

A Study of Control in eNOS

Studies of enzyme activation via phosphorylation have shown that oscillatory behavior, such as cycles of NO production, may occur.

- ERK (extracellular signal-related kinase): Liu *et al.* (2011) & Albeck *et al.* (2013)

- eNOS: Wang *et al.* (2009) & the KSU team Chrestensen and Salerno (2014-17)

Options for mathematical modeling include

- A multi-species cascade (i.e. large system of ODEs) or
- Inclusion of time delay (small system of DDEs)

Simple Feedback Model

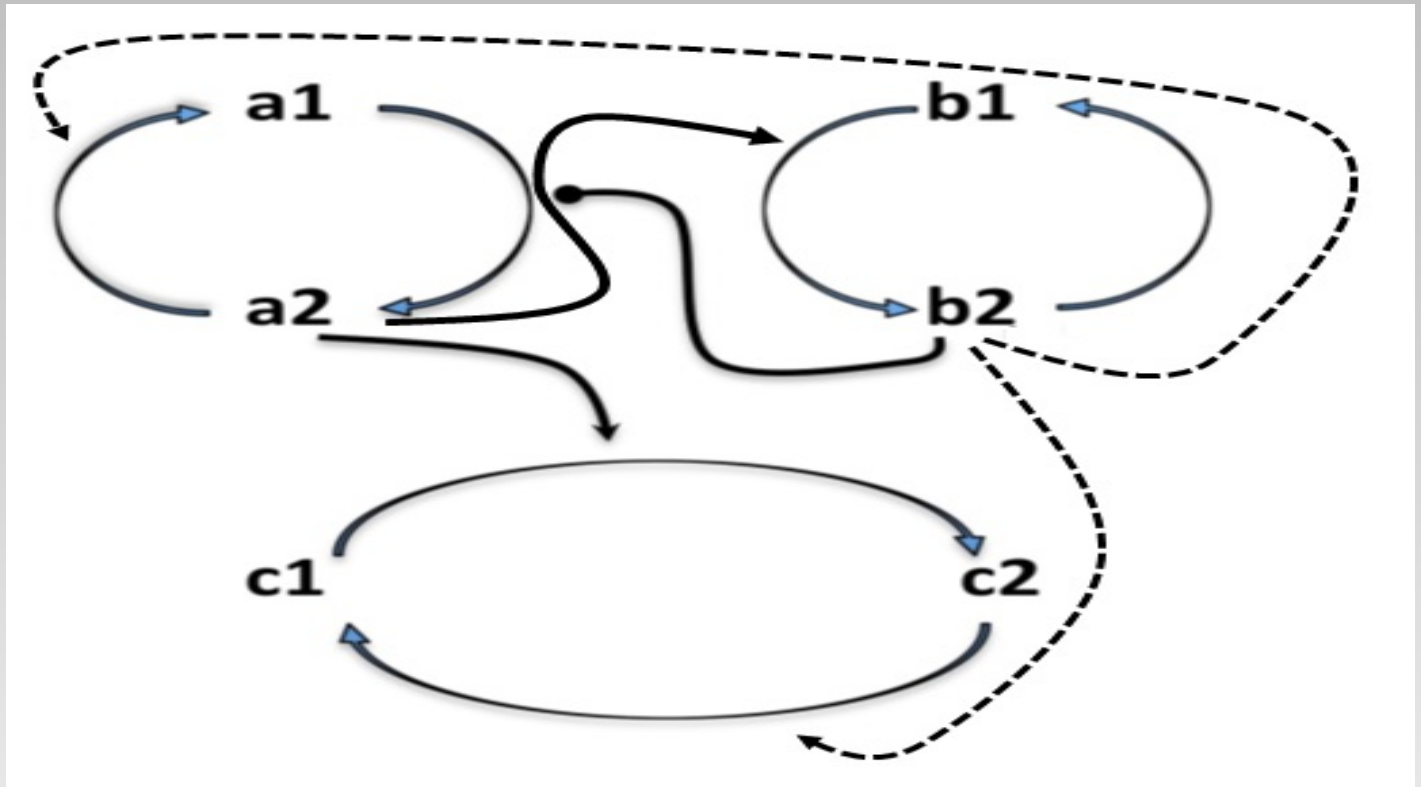
Species: **A** a kinase with concentration of inactive and active form a_1 and a_2 ($a_1 + a_2 = const$)

B a phosphatase with concentrations of inactive and active form b_1 and b_2 ($b_1 + b_2 = const$)

C an enzyme with concentrations of inactive and active form c_1 and c_2 ($c_1 + c_2 = const$). Species C is phosphorylated by a_2 and dephosphorylated by b_2

The interactions are assumed to be Michaelis Menton kinetics and includes a steady state partition for the kinase (e.g. for two substrates).

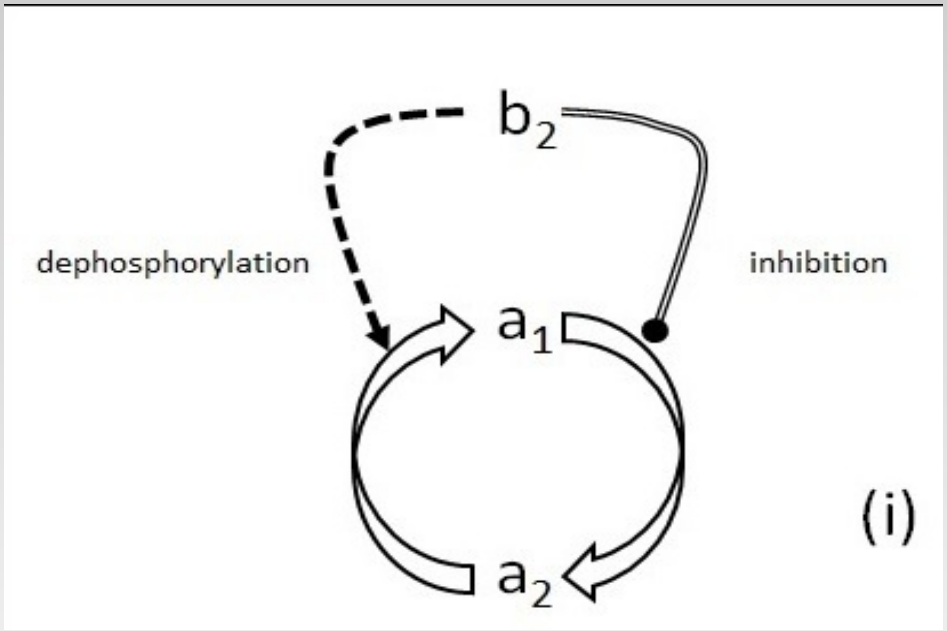
A Simple Feedback Model



Feedback network schematic for a one kinase (a), one phosphatase (b), and one passive species (c) model. Inactive states are indicated by subscript 1 and active by 2. Solid arrows denote phosphorylation, the solid blunted line denotes inhibition of phosphorylation, and dashed arrows represents dephosphorylation.

Simple Feedback Model

$$\frac{da_1}{dt} = -K_{a1} f_p(b_2) \frac{a_1}{K_{an} + a_1} - K_{a2} g_p(b_2) \frac{a_1}{K_{am} + a_1} + K_{a3} b_2 \frac{a_2}{K_{ao} + a_2} + K_{a4} \frac{a_2}{K_{ap} + a_2} \tag{1}$$



Phosphatase inhibits activation and directly deactivates kinase.

Simple Feedback Model

$$\frac{da_1}{dt} = -f_p(b_2)\frac{K_{a1}a_1}{K_{an}+a_1} - g_p(b_2)\frac{K_{a2}a_1}{K_{am}+a_1} + +b_2\frac{K_{a3}a_2}{K_{ao}+a_2} + \frac{K_{a4}a_2}{K_{ap}+a_2}$$

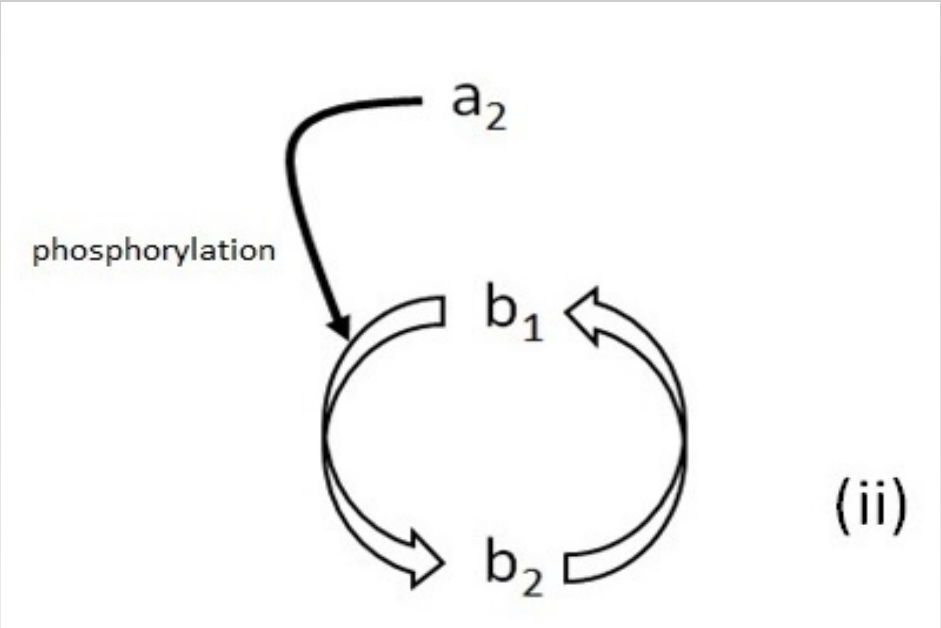
$$\frac{da_2}{dt} = -\frac{da_1}{dt}$$

$$f(x) = \frac{r_1}{x + r_1 + r_2}, \quad g(x) = 1 - f(x)$$

where r_1 and r_2 are rate constants for kinase activation and deactivation.

Simple Feedback Model

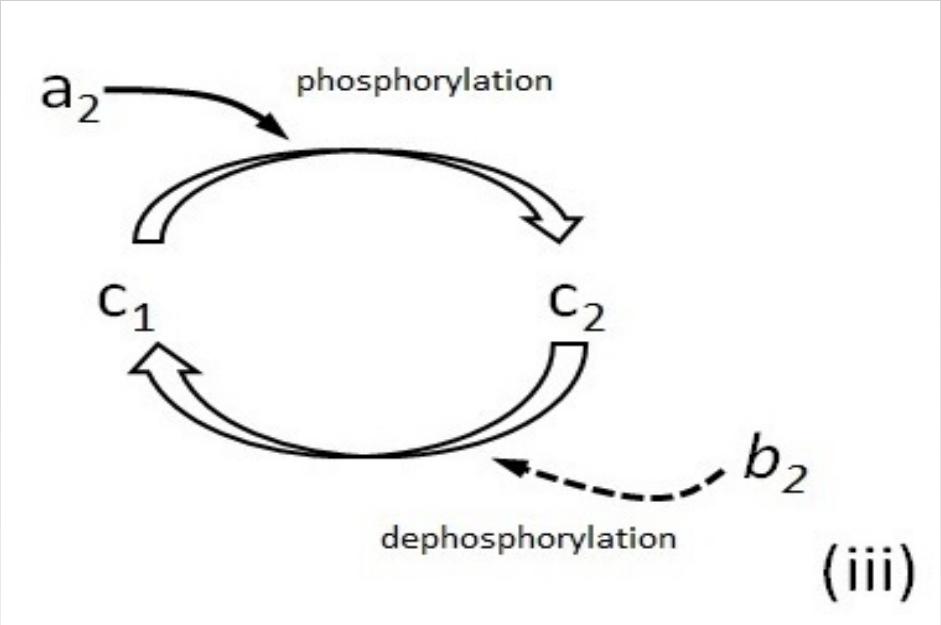
$$\frac{db_1}{dt} = -K_{b1}\frac{b_1}{K_{bn} + b_1} - K_{b2}a_2\frac{b_1}{K_{bm} + b_1} + K_{b3}\frac{b_2}{K_{bo} + b_2} \tag{2}$$



Kinase acts to phosphorylate the phosphatase.

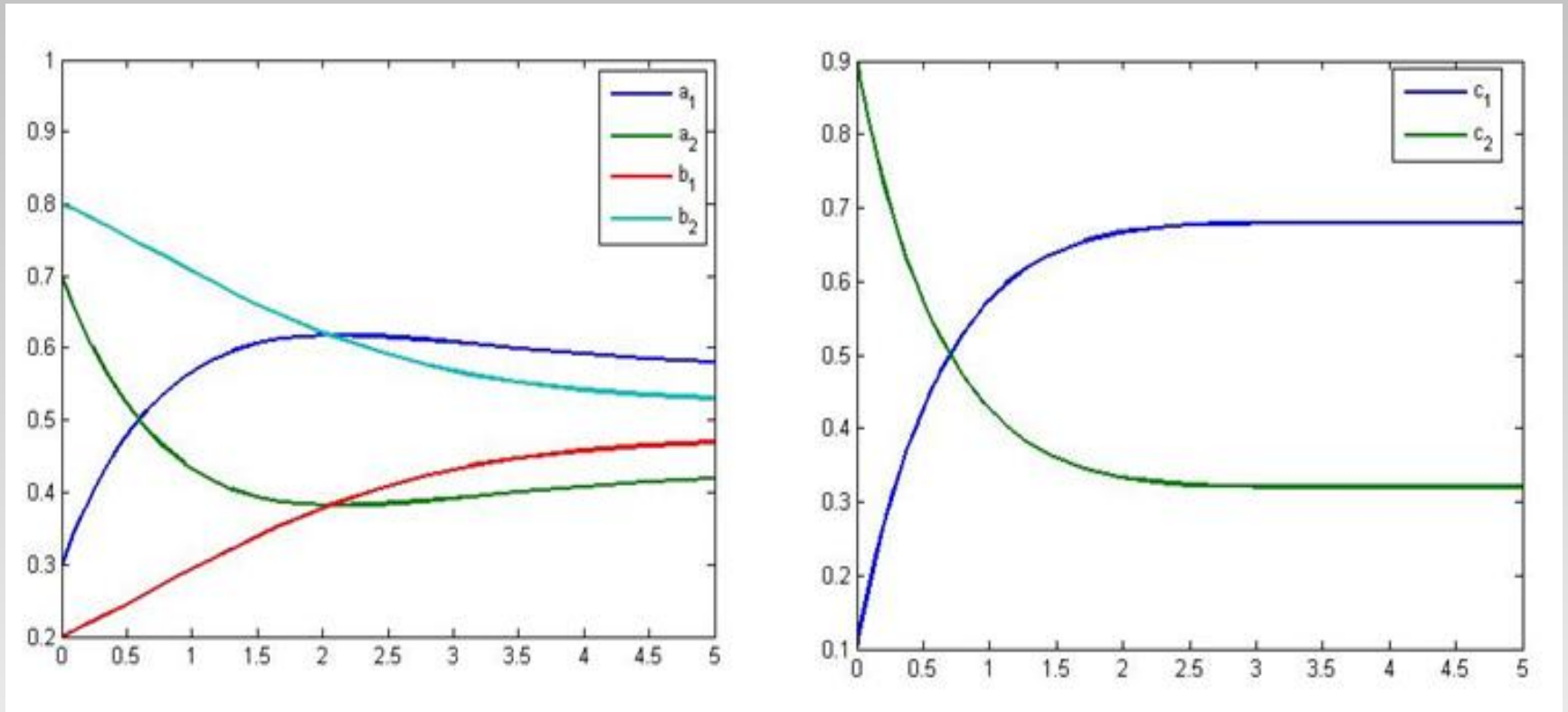
Simple Feedback Model

$$\frac{dc_1}{dt} = -K_{c1}\frac{c_1}{K_{cn}+c_1} - K_{c2}a_2\frac{c_1}{K_{cm}+c_1} + K_{c3}b_2\frac{c_2}{K_{co}+c_2} + K_{c4}\frac{c_2}{K_{cp}+c_2} \qquad (3)$$



Kinase and Phosphatase activate or deactivate a site on eNOS.

Simple Feedback Model



For a wide range of parameter values, the system exhibits simple decay to equilibrium.

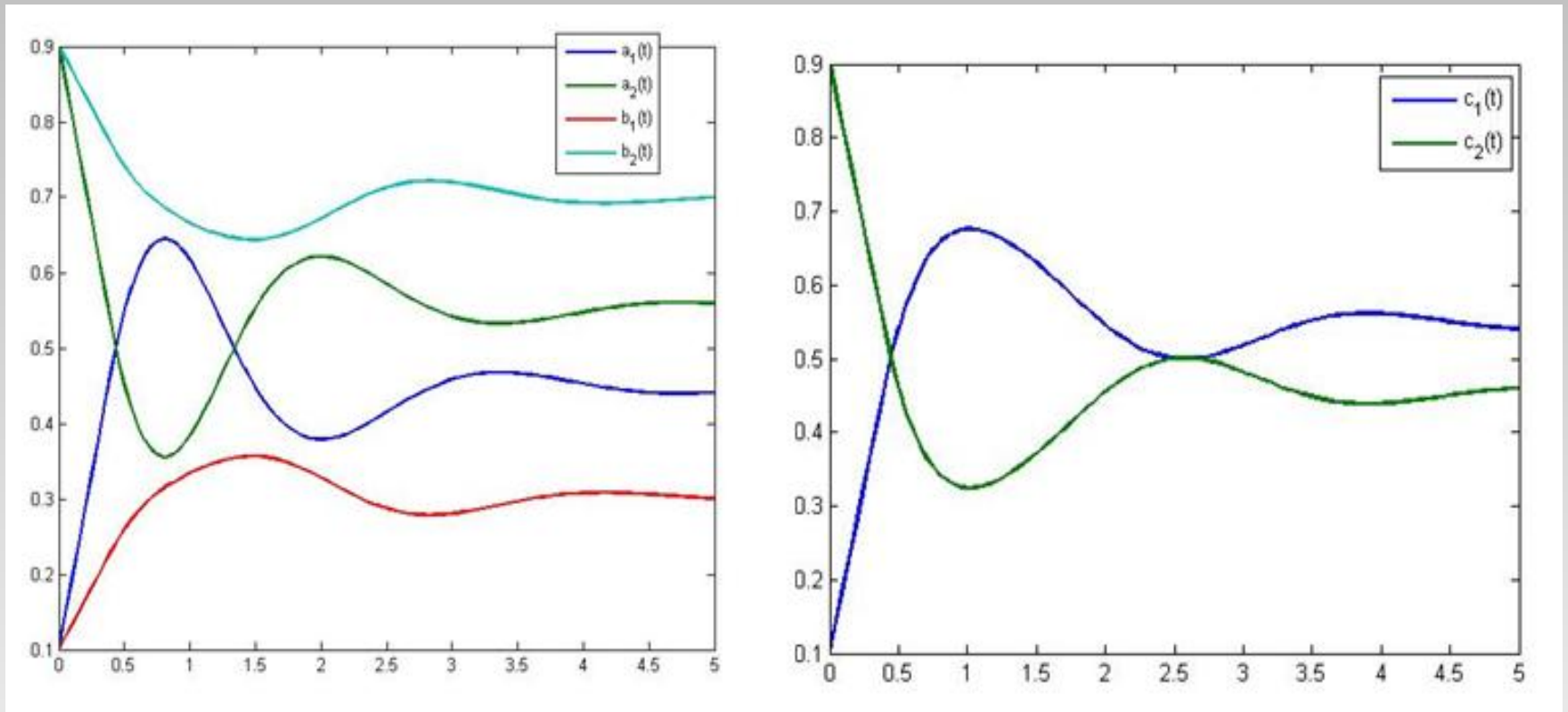
Feedback Model with Delay

We may introduce time delays to account for various features—e.g. formation of protein complexes, diffusion, or interactions of unspecified intermediaries. We replace equations (1) and (2) with

$$\begin{aligned}\frac{da_1}{dt} &= -K_{a1} f_p(b_2(t-\tau_b))\frac{a_1}{K_{an}+a_1} - K_{a2} g_p(b_2(t-\tau_b))\frac{a_1}{K_{am}+a_1} + \\ &+ K_{a3} b_2(t-\tau_{b'})\frac{a_2}{K_{ao}+a_2} + K_{a4}\frac{a_2}{K_{ap}+a_2} \qquad (4)\end{aligned}$$

$$\frac{db_1}{dt} = -K_{b1}\frac{b_1}{K_{bn}+b_1} - K_{b2} a_2(t-\tau_a)\frac{b_1}{K_{bm}+b_1} + K_{b3}\frac{b_2}{K_{bo}+b_2} \qquad (5)$$

Feedback Model with Delay



Introduction of delay results in oscillatory behavior similar to that observed experimentally.

Steady State Analysis: Single Delay Time τ

Let $a := a_1$ and $b := b_1$. And perturb off of the long time state (a_e, b_e) letting

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

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

$$\frac{d\mathbf{u}}{dt} = \Psi \mathbf{u}(t - \tau) - \Phi \mathbf{u} \tag{6}$$

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

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

The parameters $A, D < 0$, $C > 0$, and B has undetermined sign.

A Sufficiency Theorem

Theorem 1:

If $\sqrt{|BC|} < \frac{\sqrt{3}}{2} \min(|A|, |D|)$, then every solution $\mathbf{u}$ of (6) decays to zero as $t \to \infty$.

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Remark 1: The proof entails consideration of the transcendental characteristic equation.

$$\det\left(e^{-\omega\tau}\Psi - \Phi - \omega I\right) = 0$$

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Remark 2: In the absence of delay ($\tau = 0$), the equilibrium is asymptotically stable provided $BC < AD$ and this condition holds necessarily if $B < 0$.

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Remark 3: The characteristic equation admits a pure imaginary eigenvalue, $\omega = \sigma_1 i$, if and only if $|BC| > |AD|$. Hence we can seek conditions under which a Hopf bifurcation occurs taking τ as a bifurcation parameter.

Existence of Hopf Bifurcation

Theorem 2:

Suppose the system (4)–(5) has a stable equilibrium (a_e, b_e) solution in the absence of delay ($\tau = 0$), and let (6) be the linearization of the system about this equilibrium. Then there exists a critical delay τ^* for which the equilibrium state changes stability provided $|BC| > |AD|$.

It can be shown that for any such set of parameter values A, B, C, D, that

$$\nu := \text{Re}\left(\frac{d\omega}{d\tau}\right)\Big|_{\tau^*} > 0.$$

Hence a generic Hopf bifuraction may occur.

Numerical Results

Sample Parameter Values

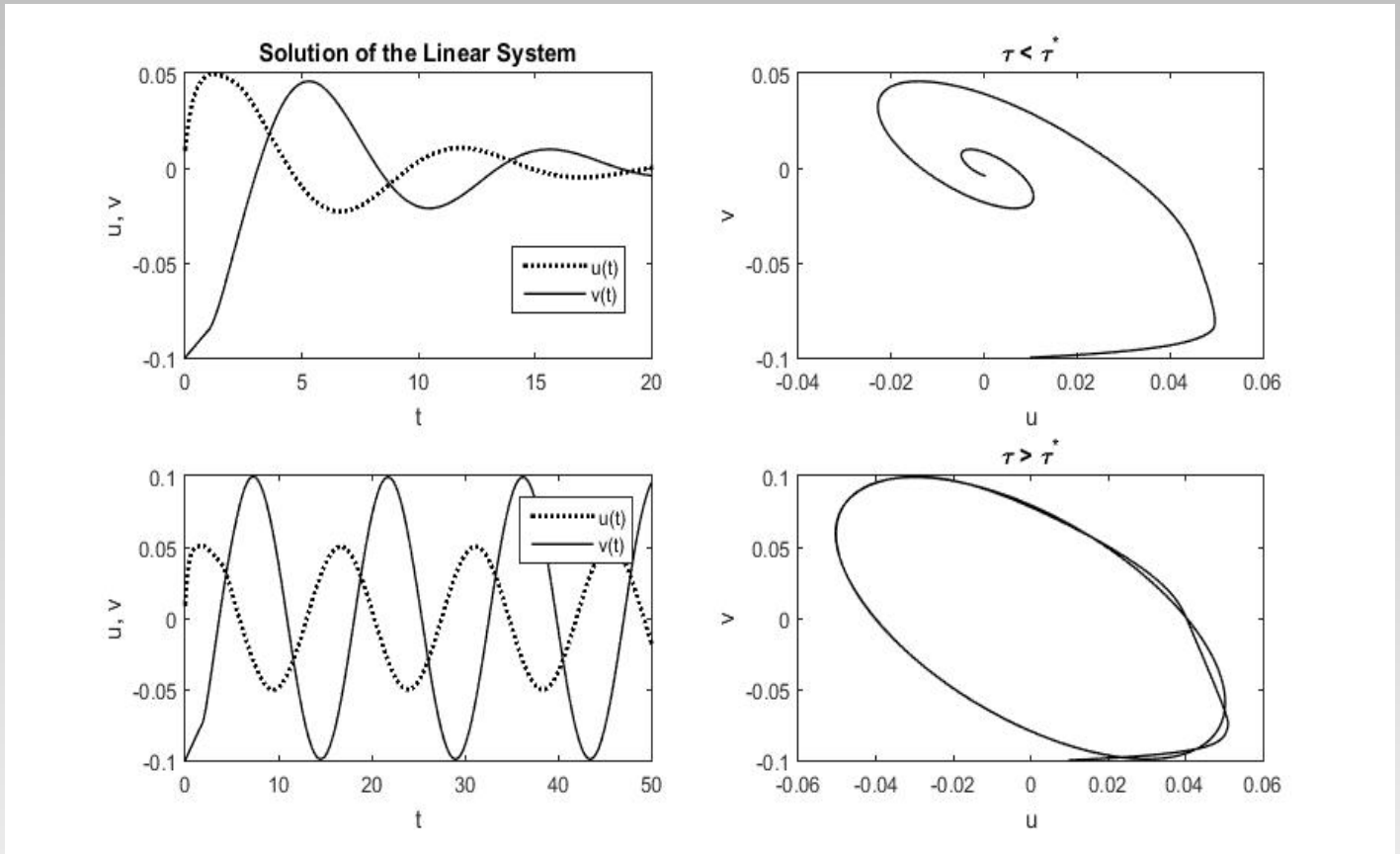
$K_{a1} = 1$ $K_{a2} = 0.1$ $K_{a3} = 2$ $K_{a4} = 0.05$	$K_{b1} = 1$ $K_{b2} = 1$ $K_{b3} = 0.1$ $r_1 = 0.5$	$K_{an} = 0.5$ $K_{am} = 0.5$ $K_{ao} = 0.05$ $K_{ap} = 0.5$	$K_{bn} = 1$ $K_{bm} = 0.1$ $K_{bo} = 0.02$ $r_2 = 0.01$
$K_{c2} = 1$ $K_{cn} = 1$	$K_{c3} = 1$ $K_{cm} = 0.5$	$K_{c4} = 0.1$ $K_{co} = 0.5$	 $K_{cp} = 0.5$

For the parameter values in 1, we find

$$a_e = 0.9360, \quad b_e = 0.6451, \quad \sigma_1 = 0.4344, \quad \tau^* = 1.8054,$$
$$A = -2.9576, \quad B = -1.5153, \quad C = 0.8658, \quad D = -0.0627.$$

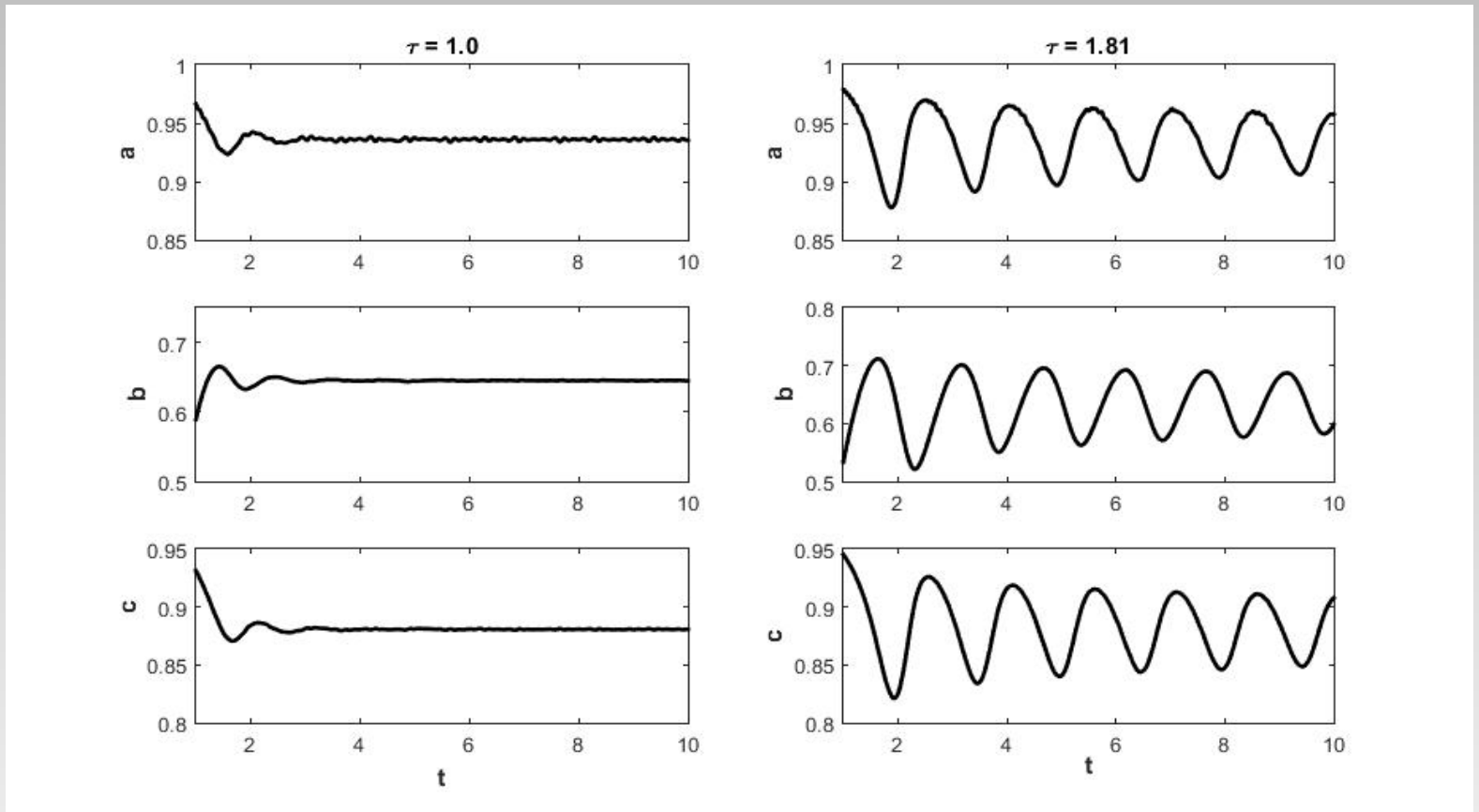
Taking $c := c_1 = c_e + w(t)$, and linearizing equation (3) we have $c_e = 0.8806$.

Critical Delay Parameter Value



Solution of the linear system (6) for delay time below, and slightly above the critical value τ^*.

Long Time Solutions $\tau < \tau^*$ and $\tau > \tau^*$



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.)

Qualities of Oscillatory Solutions

For the parameters values in table 1, the Lyapunov-Poincaré coefficient $\Delta < 0$. Hence there is a supercritical Hopf bifurcation with a stable periodic solution arising for $\tau > \tau^*$. We obtain the values

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

Characteristics of the resulting oscillatory solutions are determined. In particular, the amplitude $\mathcal{A}$ and frequency f_{rq} satisfy

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

Qualities of Oscillatory Solutions

The near equilibrium solutions are[1]

$$\begin{aligned} a(t) &\approx a_e + \mathcal{A}\cos(\sigma_1 t), \\ b(t) &\approx b_e + \mathcal{A}(c_{12}\cos(\sigma_1 t) - c_{22}\sin(\sigma_1 t)). \\ c(t) &\approx c_e + \mathcal{A}(\eta_1\cos(\sigma_1 t) + \eta_2\sin(\sigma_1 t)) \end{aligned}$$

The amplitude of oscillations for b and c can be stated relative to those of a. For this numerical illustration

- The amplitude of oscillations of b are $\approx 1.97\mathcal{A}$.
- The amplitude of oscillations of c are $\approx 1.19\mathcal{A}$.

[1] Parameters c_{12}, c_{22}, η_1, and η_2 depend on $A, \ldots, D$ and the eigenvalue σ_1

Summary

- With time delays, the simple system is able to capture observed dynamics while leaving much of the complex cascade unspecified.
- Unfortunately, even the highly reduced system does not yield one or two simple critical parameters.
- Despite this, several numerically testable conditions have been obtained.
- Notable characteristics, namely amplitude and frequency, are provided by the current analysis.
- In particular, this suggests that the amplitude of oscillations of the target site on eNOS can be predicted relative to that of the kinase species.

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