A preliminary model of phosphorylation states of endothelial nitric oxide synthase

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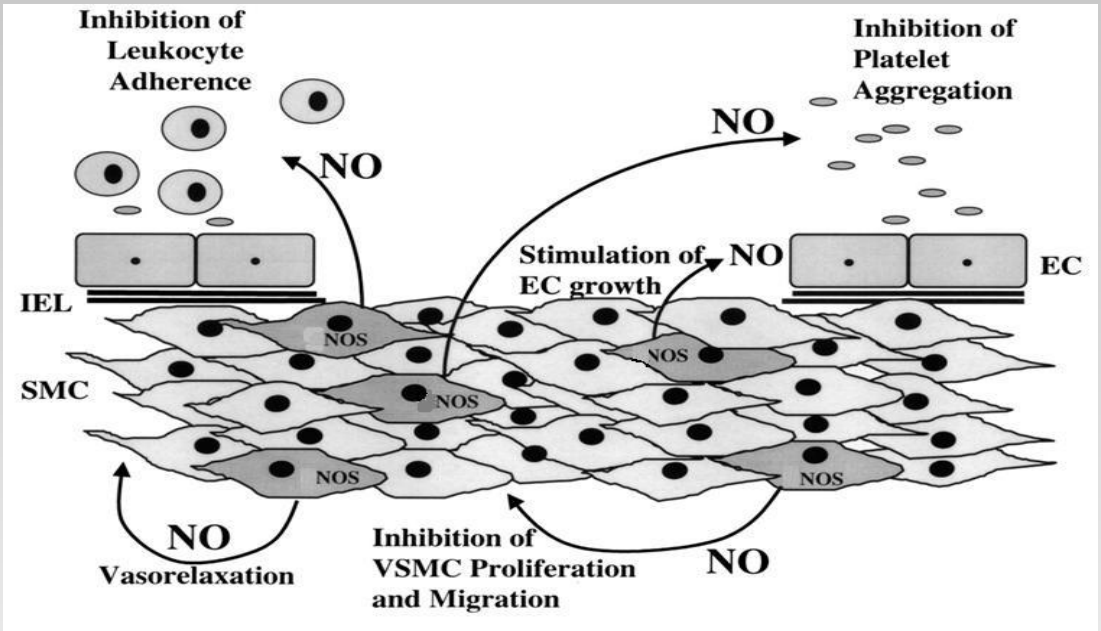
Outline

Nitric Oxide

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

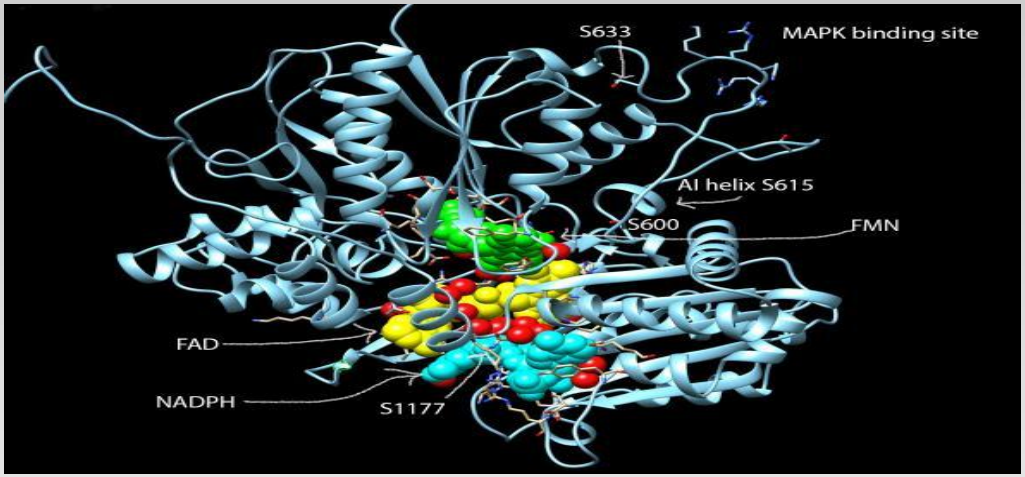


In the vasculature, it serves several critical functions including

- 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: eNOS may be activated or inhibited by various kinases via phosphorylation. Many of the binding sites on eNOS to specific kinases have been identified.

eNOS dysfunction is associated with vascular disease—atherosclerosis. Inability of endothelial cells to synthesize and release NO may decrease NO sensitivity as well as increase O_2 productivity.

A Study of Control in eNOS

A KSU research team is studying the time course of signaling events (with an eye toward control/feedback mechanisms).

They have shown that eNOS response to external signals (e.g. bradykinin) can oscillate between active and inactive states and resulting signals.

These may be modified or influenced by perturbations in insulin, glucose, and growth factors.

John Salerno, professor of Biotechnology
Carol Chrestensen, associate professor of Biochemistry

Kennesaw State University

Simple Feedback Model

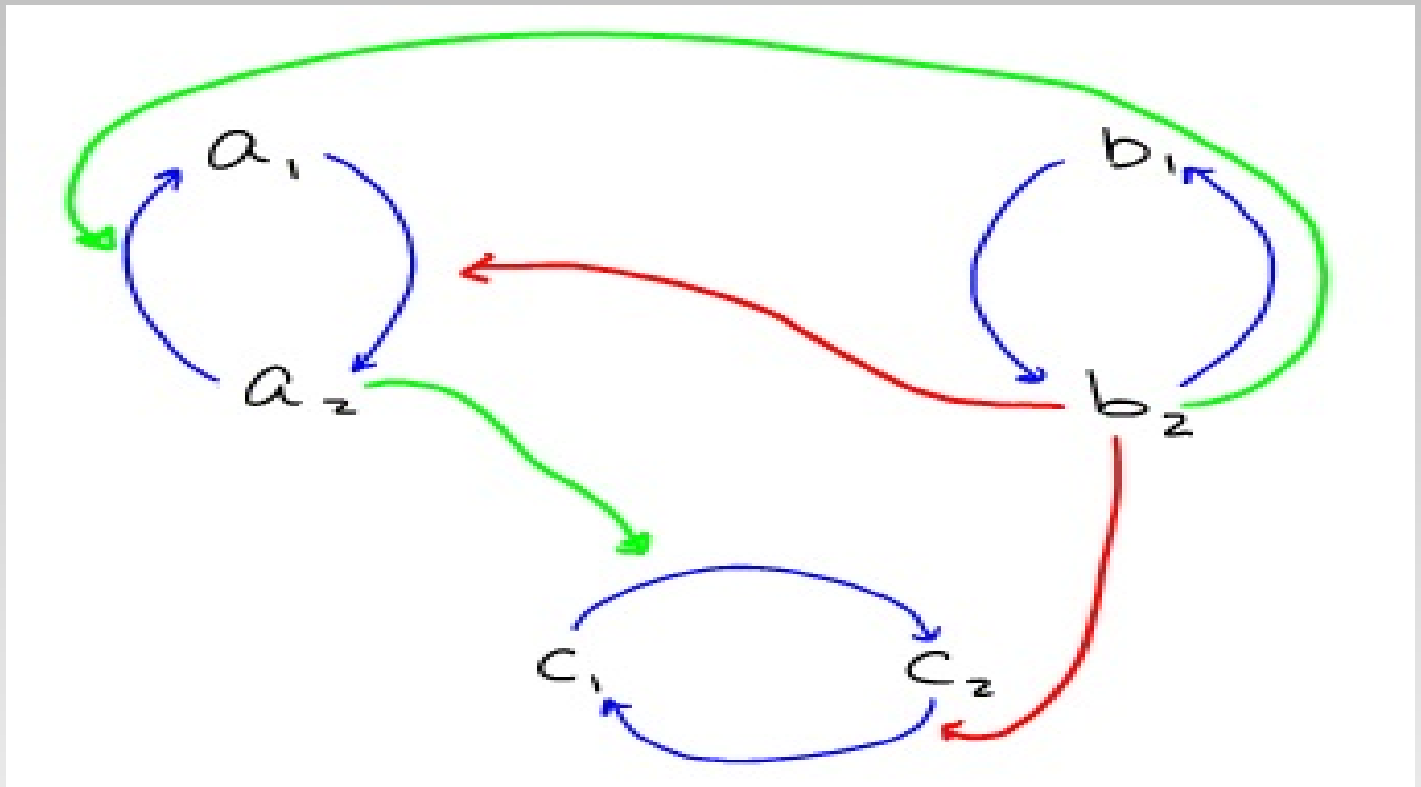
Species: **A** a kinase with concentration of inactive and active form a_1 and a_2 $(a_1 + a_2 = const)$

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



A three species model with a kinase (A), phosphatase (B), and a third species (C) such as serine. Each species has an inactive and an active form.

Simple Feedback Model

$$\frac{da_1}{dt} = -\frac{U_1 a_1}{K_{a_n} + a_1} f(b_2) - \frac{U_2 a_1}{K_{a_m} + a_1} g(b_2) + \frac{U_3 a_2}{K_{a_o} + a_2} b_2 + \frac{U_4 a_2}{K_{a_p} + a_2} \quad (1)$$

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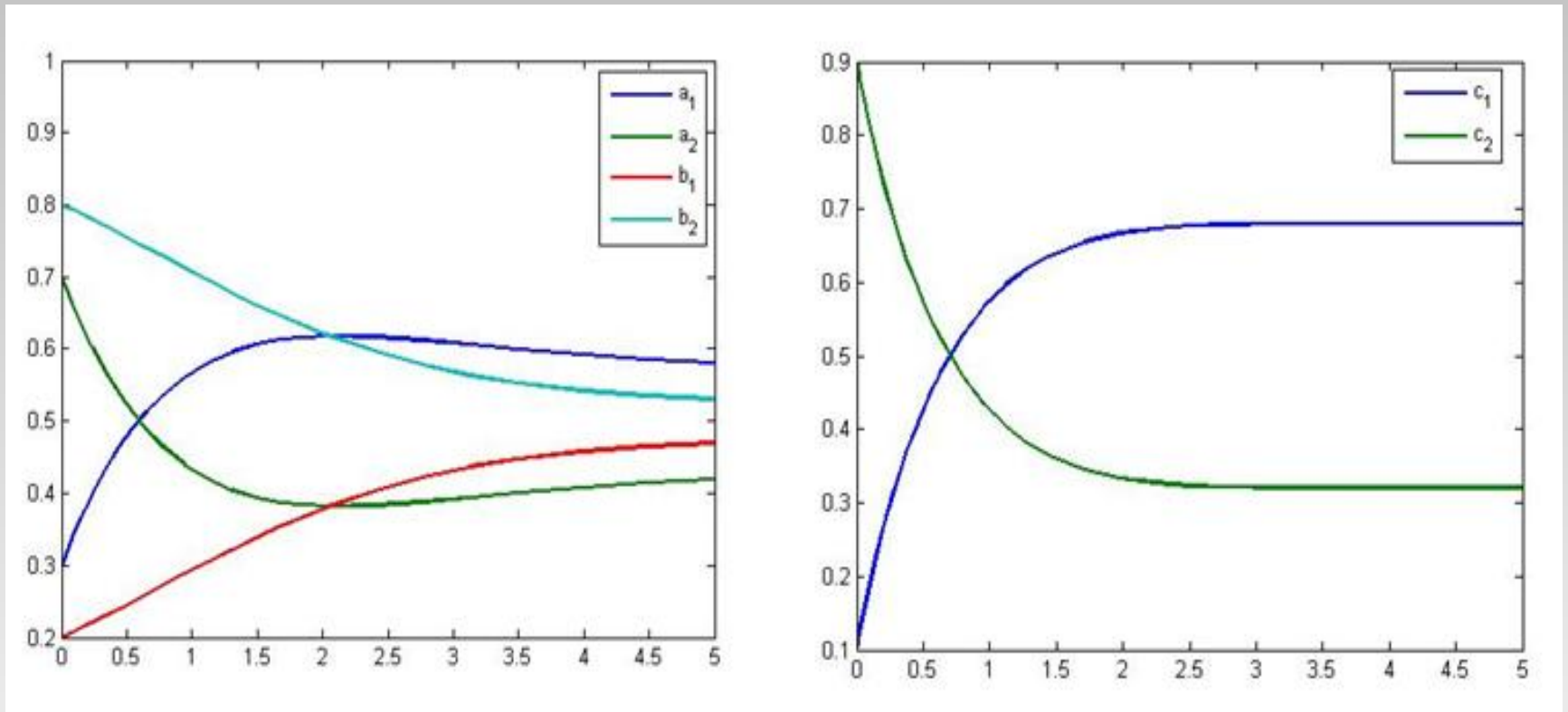
$$\frac{da_2}{dt} = -\frac{da_1}{dt}$$

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

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

Simple Feedback Model



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 (3) with

$$\frac{da_1}{dt} = -\frac{U_1 a_1}{K_{a_n} + a_1} f(b_2(t-\tau_b)) - \frac{U_2 a_1}{K_{a_m} + a_1} g(b_2(t-\tau_b)) + \\ + \frac{U_3 a_2}{K_{a_o} + a_2} b_2(t-\tau_{b'}) + \frac{U_4 a_2}{K_{a_p} + a_2}$$

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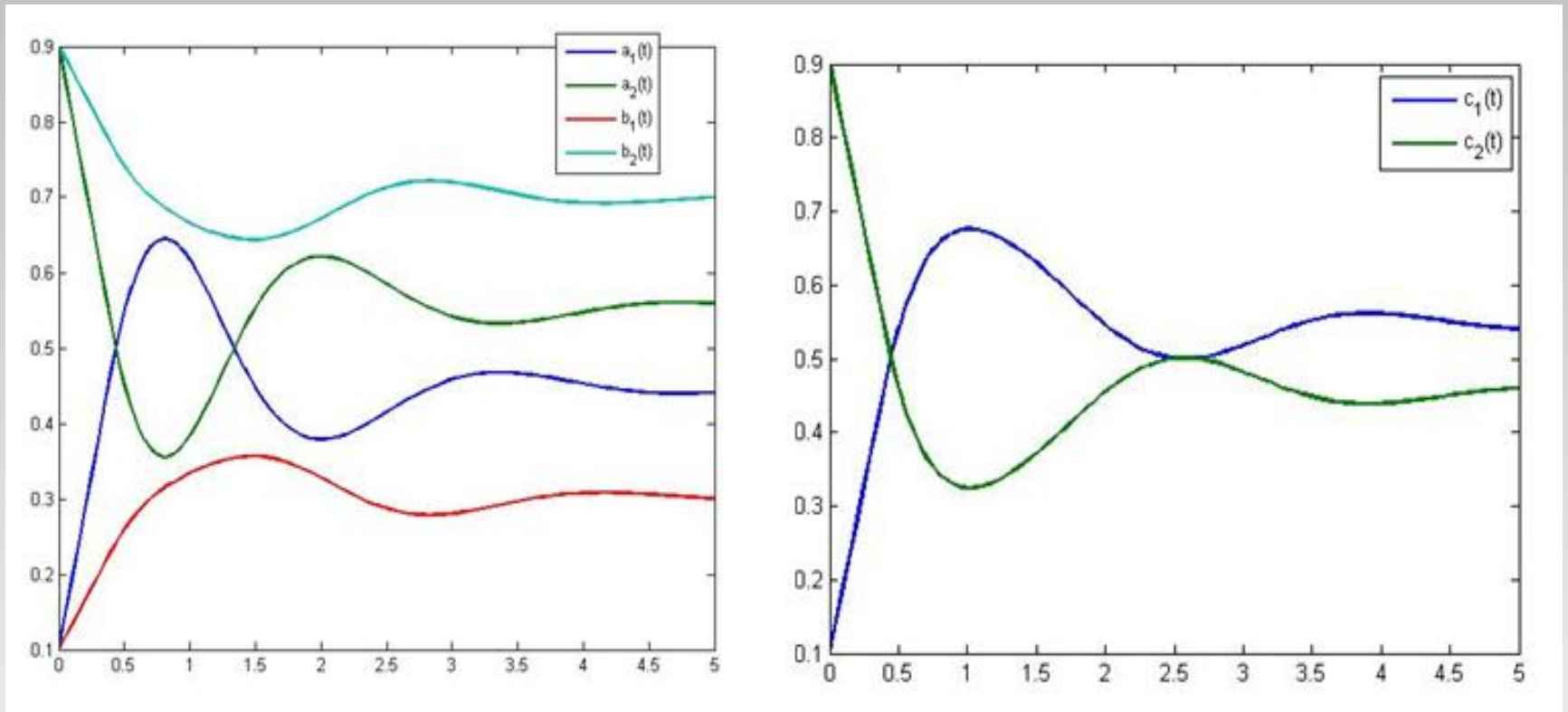
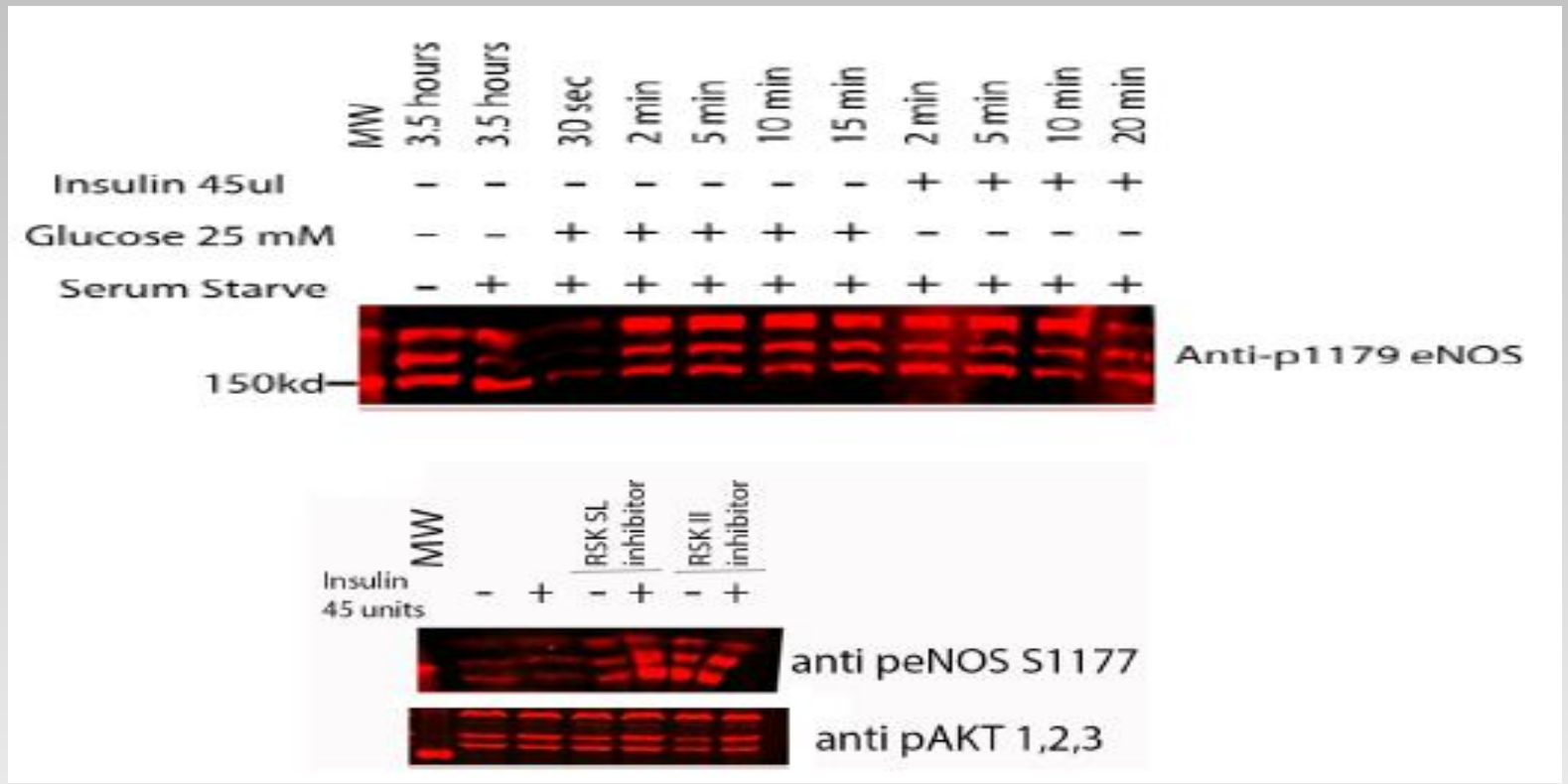


Introduction of delay results in oscillatory behavior similar to that observed experimentally.

Observed Oscillations



Serum starved (top) and non-serum starved (bottom) bovine aortic endothelial cells are treated with insulin and/or glucose and probed for specific enzyme phosphorylation states. (Results from Dr. Chrestensen's lab.)

A Stability Analysis (w/o delay)

Normalizing, and assuming a single pair of Michaelis constants, the kinase-phosphatase system may be restated as

$$\frac{da}{dt} = -\frac{a}{K_a + a}\left[U_1 f(1-b) + U_2 g(1-b)\right] + \frac{1-a}{K_a + 1 - a}\left[U_3(1-b) + U_4\right] \quad (7)$$

$$\frac{db}{dt} = -\frac{b}{K_b + b}\left[V_1 + V_2(1-a)\right] + \frac{(1-b)V_3}{K_b + 1 - b} \quad (8)$$

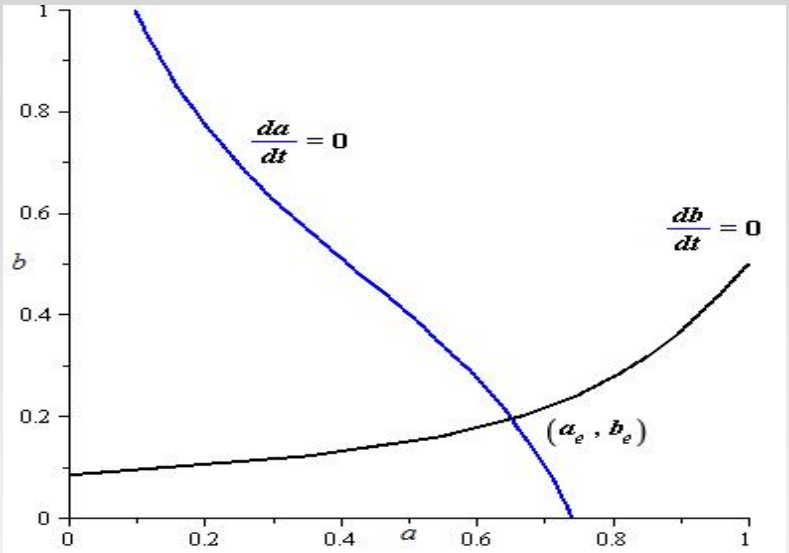
Here $a := a_1$ and $b := b_1$ are the concentrations of **inactive** kinase and phosphatase, respectively.

A stability result

Theorem:

There exists a non-zero (and non-unity) equilibrium state (a_e, b_e) that is linearly asymptotically stable with $0 < b_e < 1 - b_e$ and $1 - a_e < a_e < 1$ provided

$$U_1 + U_2 > U_3 + U_4, \quad \text{and} \quad V_1 > V_3.$$



Example of equilibrium with nonzero active kinase $1 - a_e$ and phosphatase $1 - b_e$.