PROBLEM-1

Bistability in cell signaling: How to make continuous processes discontinuous, and reversible processes irreversible

W. Xiong and J. E. Ferrell Jr., Xiong,
Chaos (2001) 11(1), 227-236

Consider the nonlinear ODE of the form

$$\frac{dx}{dt} = F(x_1, x_{2,\ldots\ldots,} x_n; s)$$

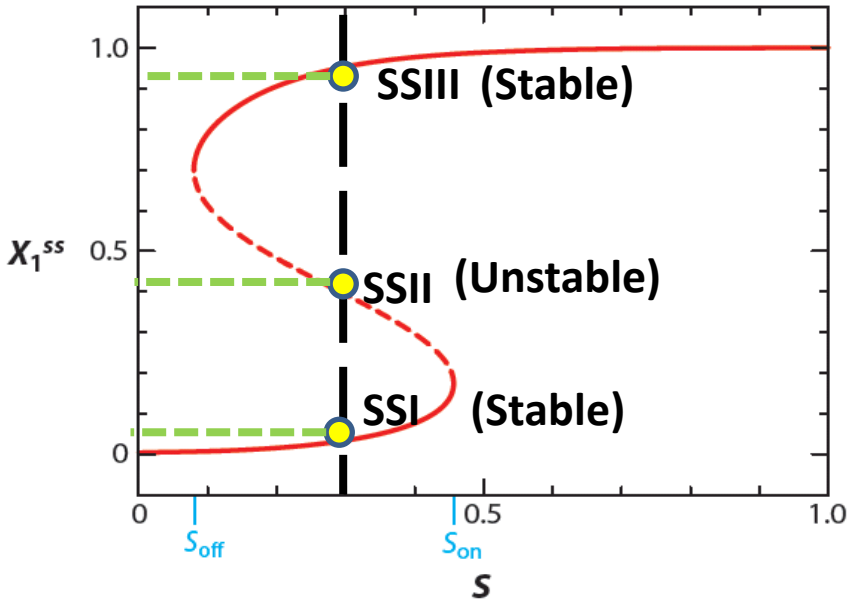
S is the Parameter

$$F(x_1, x_{2,\ldots\ldots,} x_n; s) = 0$$

Steady state equation

Bifurcation diagram :a plot of the asymptotic state of a variable (e.g., steady state) as it depends on the value of a parameter in the differential equations.

Bifurcation Diagram:

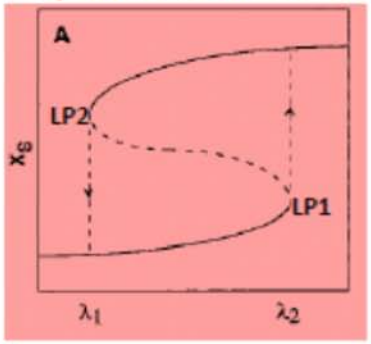


Bistability: A reaction network with two coexisting stable steady states, separated by an unstable steady state.

Classification of Multistability

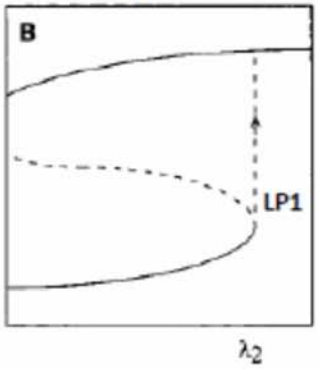
Reversible Transitions

Hysteresis
(Also called SN bif.)

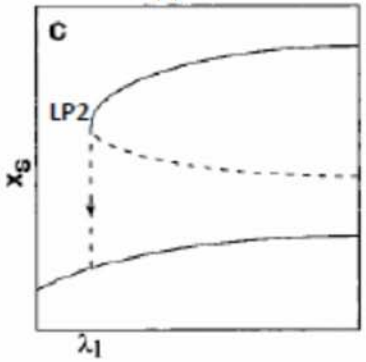


Irreversible Transitions

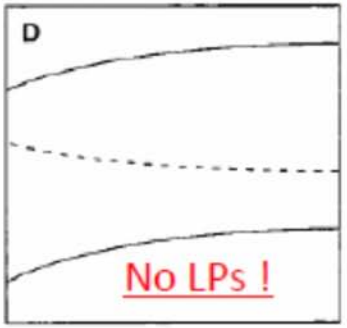
Type-1



Type-2



LP moved to infinity

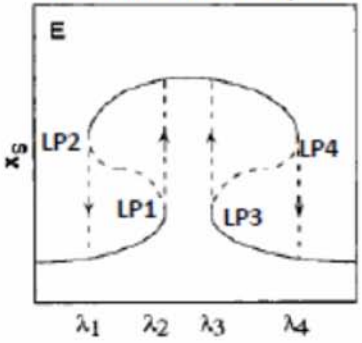


Notations used:

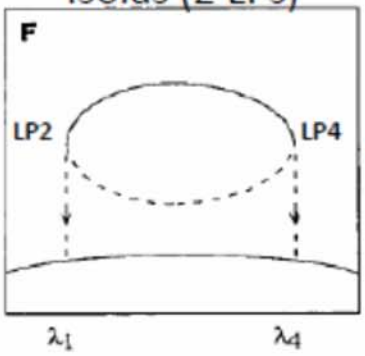
LP = limit points

SN = Saddle-Node.

Mushrooms (4 LPs)



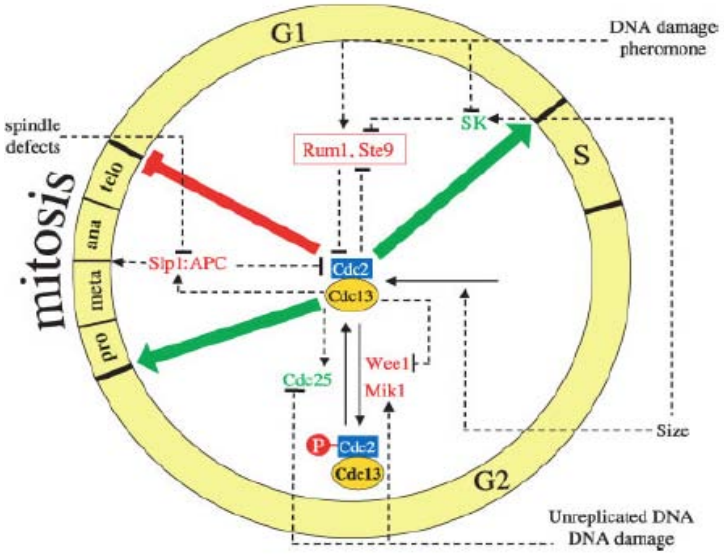
Isolas (2 LPs)

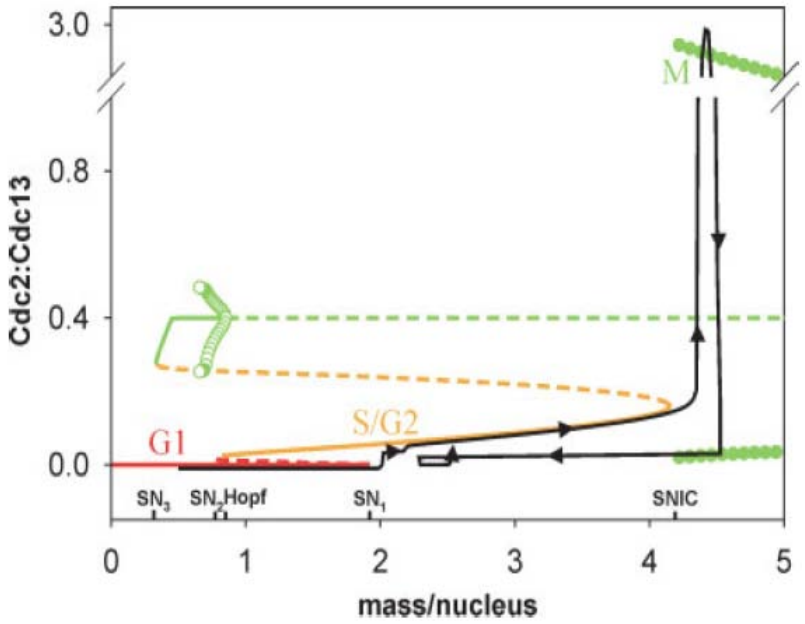


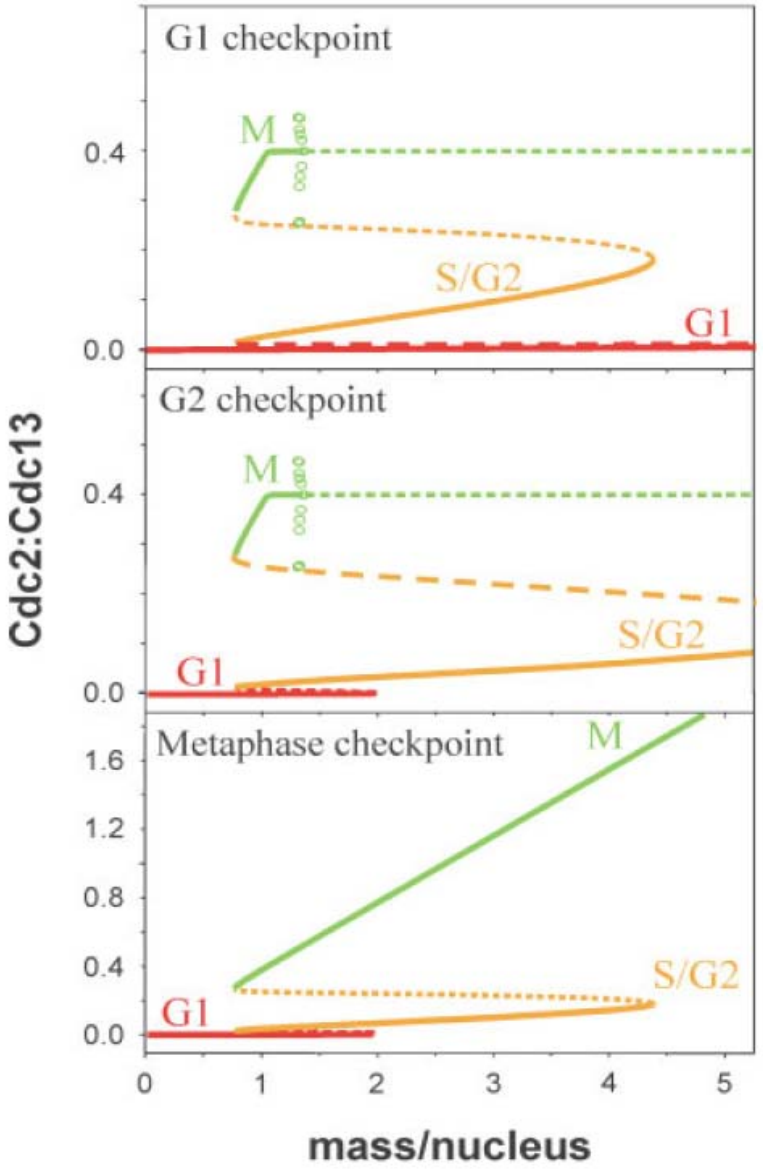
Special cases of Exotic Irreversible Transitions

Also, Tristability, saddle loop (has oscillations in SN bifurcations), SNIPER, etc. are special class of bifurcations.

Normal cell cycle and check points — bifurcation curves







The dynamics of cell cycle regulation, John J. Tyson, Attila Csikasz-Nagy, and Bela Novak, BioEssays 24:1095–1109

Oocyte maturation

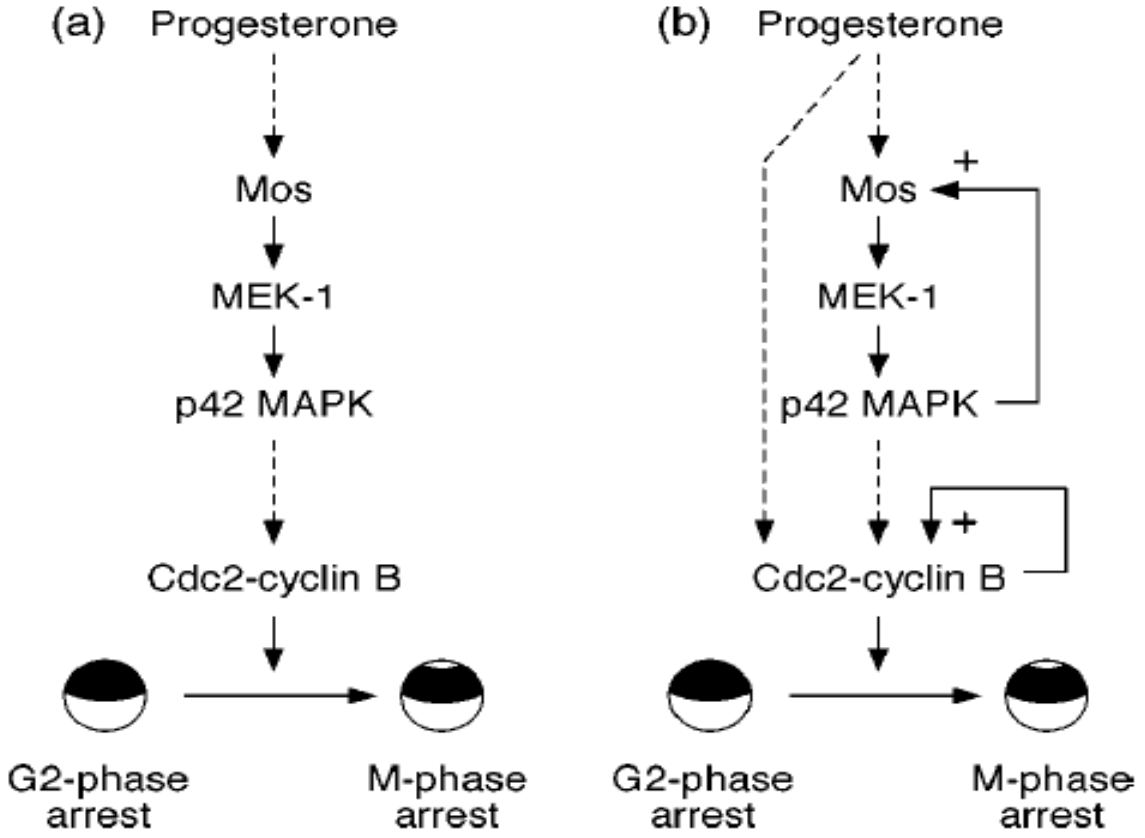


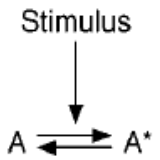
FIG. 1. Schematic view of *Xenopus* oocyte maturation. Oocytes begin in a stable G2-arrest state. The maturation-inducing agent progesterone brings about the activation of the MAP kinase cascade enzymes Mos, MEK-1, and p42 MAPK. The cascade promotes the activation of Cdc2-cyclin B complexes. Active Cdc2-cyclin B causes the oocyte to leave its G2-arrest state, enter meiosis I, and then arrest in metaphase of meiosis II. (a) Simple linear depiction of the signal transduction system that regulates maturation. (b) A more realistic depiction, including the positive feedback loops that are hypothesized to be critical for producing the all-or-none character of oocyte maturation.

Circuit, model building, steady state analysis and graphical methods

Circuit

(a)

Michaelian system
No feedback



$$A \underset{k_{-1}I}{\overset{k_1 S}{\rightleftarrows}} A^*.$$

ODE with mass conservation

$$\frac{dA^*}{dt} = k_1 S[A] = k_1 S[A_{tot}] - k_1 S[A^*],$$

(production of A*)

$$\frac{dA}{dt} = k_{-1} I[A^*]$$ (Loss of A*)

$$A_{tot} = A + A^*$$

Steady-state analysis

$$k_1 S[A_{tot}] - k_1 S[A^*] = k_{-1} I[A^*]$$

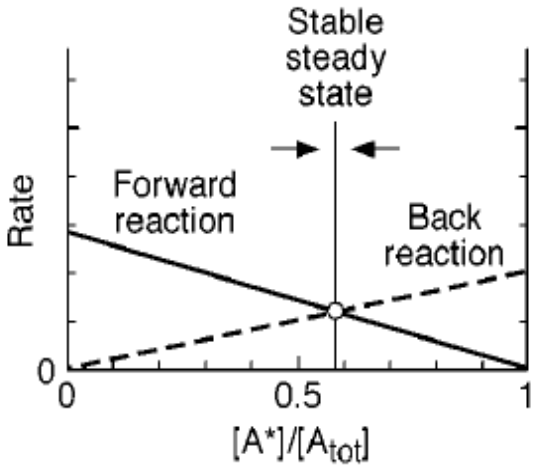
$$\frac{[A^*]}{[A_{tot}]} = \frac{S}{k_{-1} I / k_1 + S}$$

$$\frac{[A^*]}{[A_{tot}]} = \frac{S}{\mathrm{EC50} + S}$$

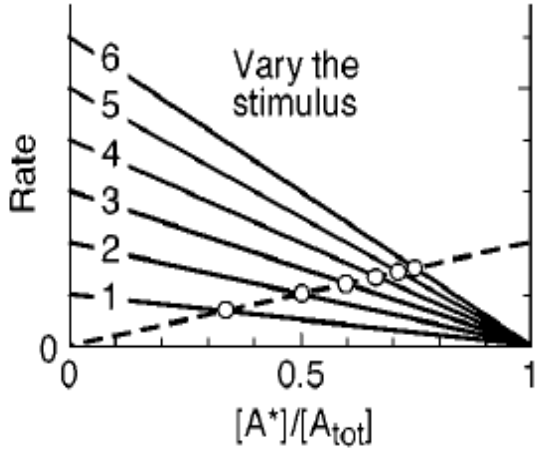
A/A* = unphosphos/Phos
S = stimulus (some kinase)
I = inhibitor (some phosphatase)

EC50 -- >the concentration or activity of *S* *required to produce* a 50% maximal response

(b) Rate balance plot

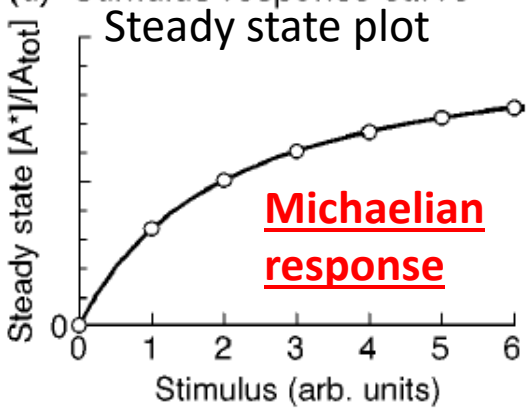


(c) Rate balance plot

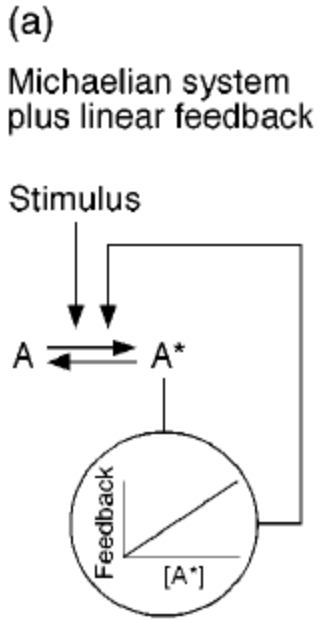


(d) Stimulus-response curve

Steady state plot



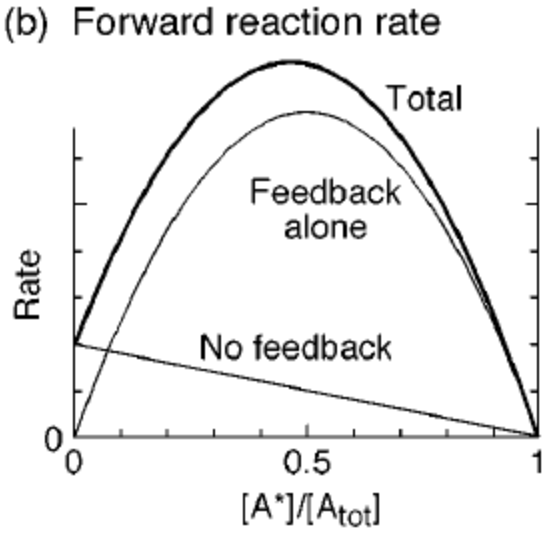
Michaelian system with linear feedback

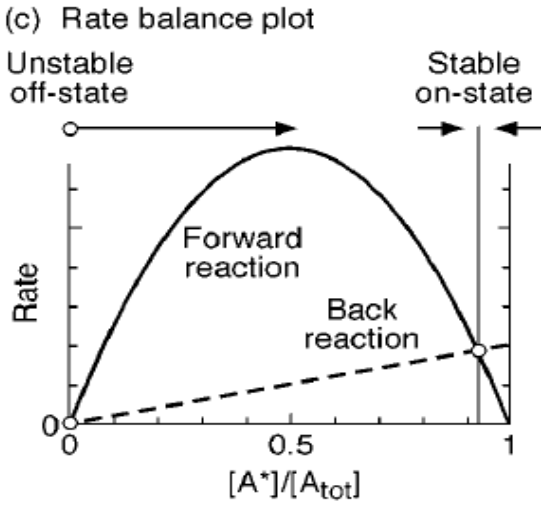


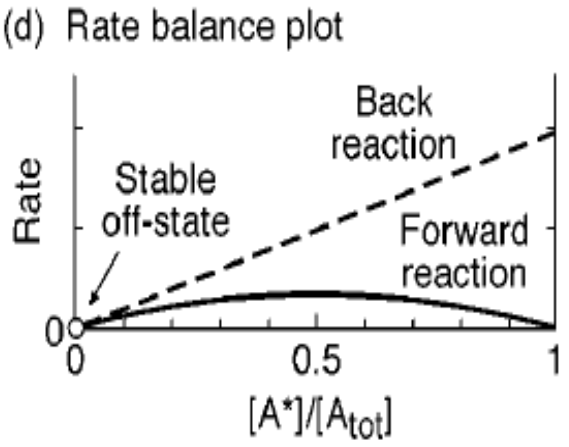
$$\frac{d[A^*]}{dt} = \text{basal rate} + \text{feedback rate}$$

$$= k_1 S[A] + k_2[A^*][A]$$

$$= (k_1 S + k_2[A^*])([A_{tot}] - [A^*]).$$







Nonlinear feedback (positive) and multistability

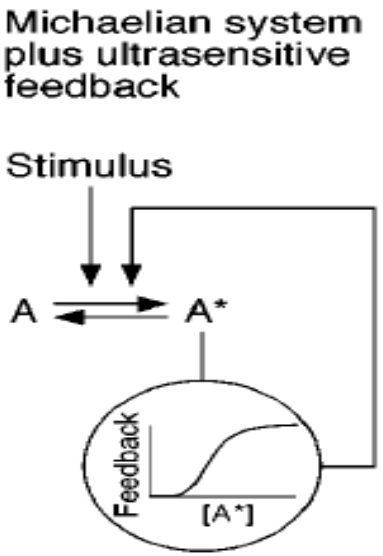
Questions to be answered by derivation and MATLAB simulations:

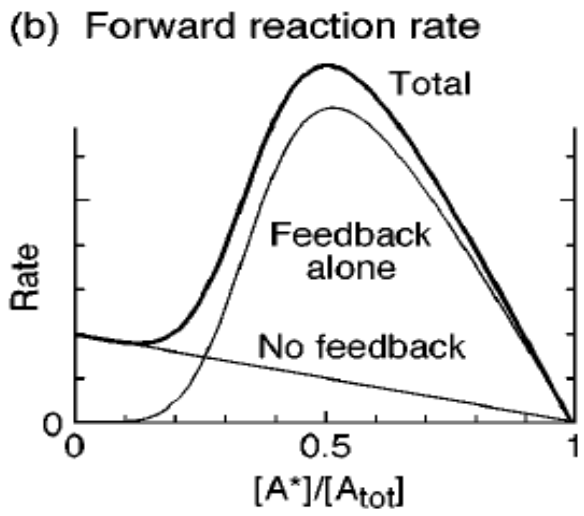
Assignment-1

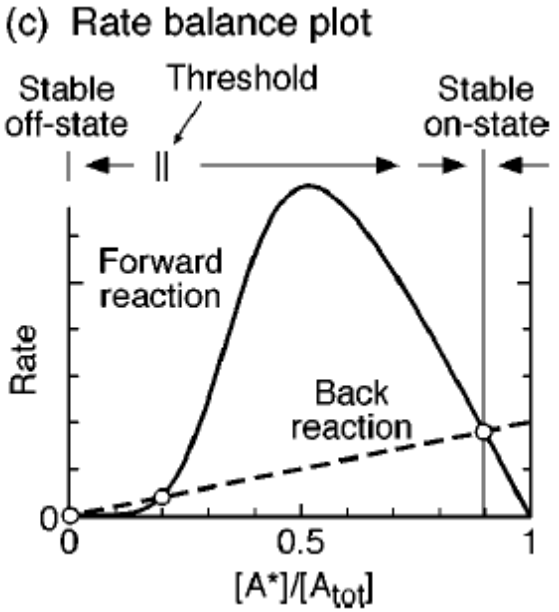
- What are the ODE and steady state equations for the circuit below ?

- How do you generate forward reaction rate and the rate balance plot?

- What is the nature of forward and backward reaction rates?



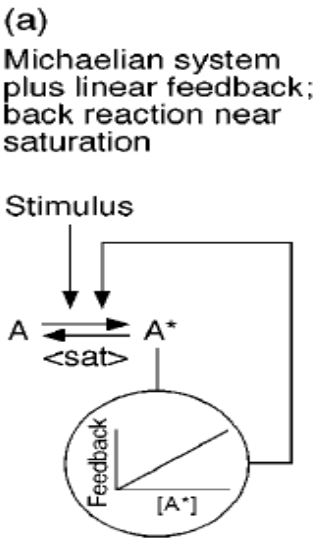


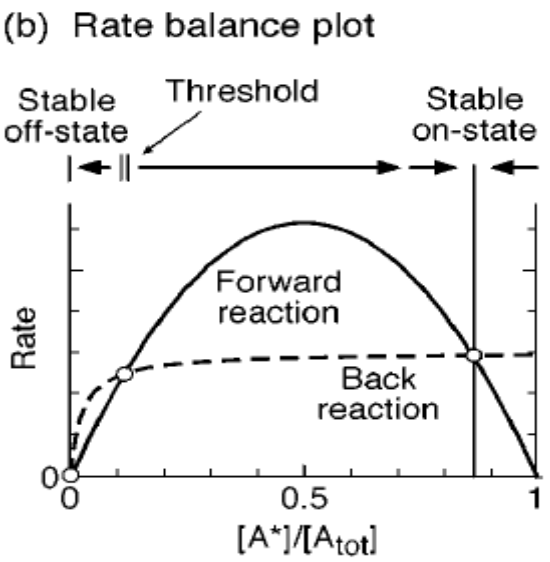


Questions to be answered by derivation and MATLAB simulations:

Assignment-2

- What are the ODE and steady state equations for the figure below?
- How do you generate forward reaction rate and the rate balance plot in the figure below?
- How does saturation in backward reaction of plays a role in multistability? What is the nature of forward reaction?

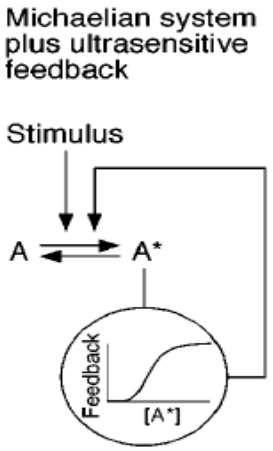


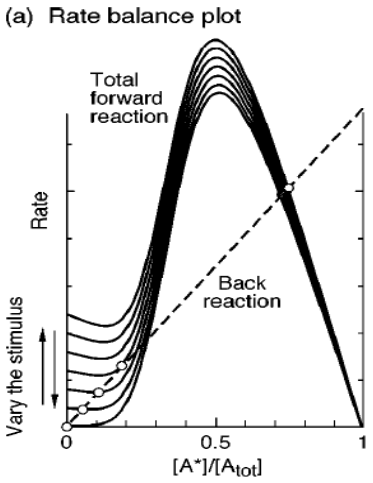


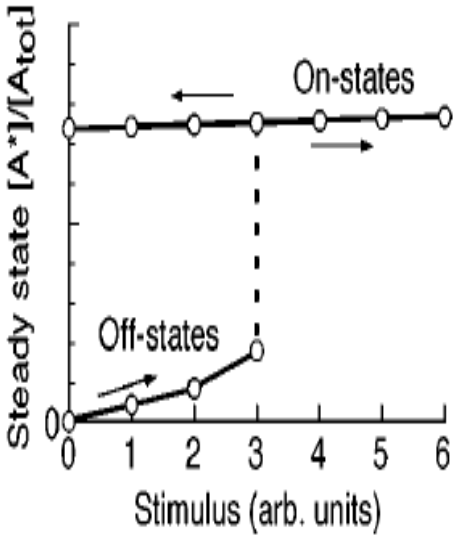
Questions to be answered by derivation and MATLAB simulations:

Assignment-3

- What are the ODE and steady state equations for the figure below?
- How do you generate forward reaction rate and the rate balance plot in the figure below?
- What do you understand by hysteresis , memory and irreversible transitions?







Question in synthetic biology

Does bistability occur in oocyte maturation? IF so, what are the experimental evidence?

Dimensionless analysis- Example

$$\frac{dN}{dt} = rN(1-\frac{N}{K})$$ (There are two parameters r and K)

Reduce the ODE to the following equation with no parameters

$$\frac{dy}{dt} = y(1-y)$$

Solution

$$\frac{1}{k}\frac{dN}{dt} = \frac{r}{K}N(1-\frac{N}{K})$$

$$\frac{d\left(\frac{N}{K}\right)}{dt} = r\frac{N}{K}(1-\frac{N}{K})$$

Put $y = \frac{N}{K}$ and this gives

$$\frac{dy}{dt} = ry(1-y)$$

Now put $\tau = rt$ (τ is dimensionless)

$$\frac{dy}{d\tau} = y(1-y)$$

Another way -- Scaling and dimensionless

$$\frac{dN}{dt} = rN(1-\frac{N}{K})$$ (There are two parameters r and K)

Reduce the ODE to the following equation with no parameters

$$\frac{dy}{dt} = y(1-y)$$

$$x = \frac{N}{x_0} \text{ and } \tau = \frac{t}{t_0} \text{ (This is scaling)}$$

$$dx = \frac{dN}{x_0} \text{ and } d\tau = \frac{dt}{t_0}$$

The equation $\frac{dN}{dt} = rN(1-\frac{N}{K})$ then becomes

$$\frac{x_0}{t_0}\frac{dx}{d\tau} = rx_0x(1-\frac{xx_0}{K})$$

On simplification this becomes

$$\frac{dx}{d\tau} = t_0rx(1-\frac{xx_0}{K})$$

Put $x_0 = K$ and $t_0 = \frac{1}{r}$, we get

$$\frac{dx}{d\tau} = x(1-x)$$

Problem –2 in dimensionless analysis

$$\frac{dx}{dt} = I - rx$$ (There are two parameters I and r)

Reduce this ODE to

$$\frac{dy}{dt} = 1\text{-}y$$

Assignment-4:

$$\frac{dA^*}{dt} = k_1 s(A_T - A^*) - k_{-1} I A^*$$ (There are three parameters)

Reduce this ODE equation to

$$\frac{dx}{d\tau} = \varepsilon(1 - x) - x$$ (With only one parameter)

Also

(a) identify the Steady states (b) perform the stability analysis around fixed points

Assignment-5: Reduce the following ODE to dimensionless equation

$$\frac{dA^*}{dt} = (k_1 s + k_2[A^*])([A_T] - [A^*])$$

Problem-2
Another model for G2-M transition

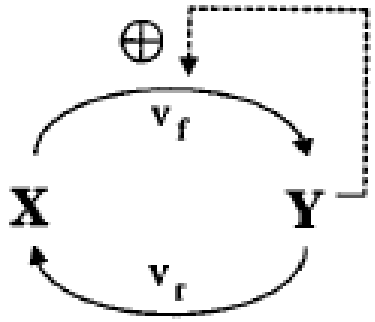
Instabilities in phosphorylation-dephosphorylation cascades and cell cycle checkpoints

BD Aguda

Oncogene (1999) 18, 2846 - 2851

PD cycle with feedback and identification of transcritical bifurcation under different mathematical structure

Assignment-6



$x \xrightarrow{f} y$

$y \xrightarrow{r} x$

$E_{tot} = x + y$ (mass conservation)

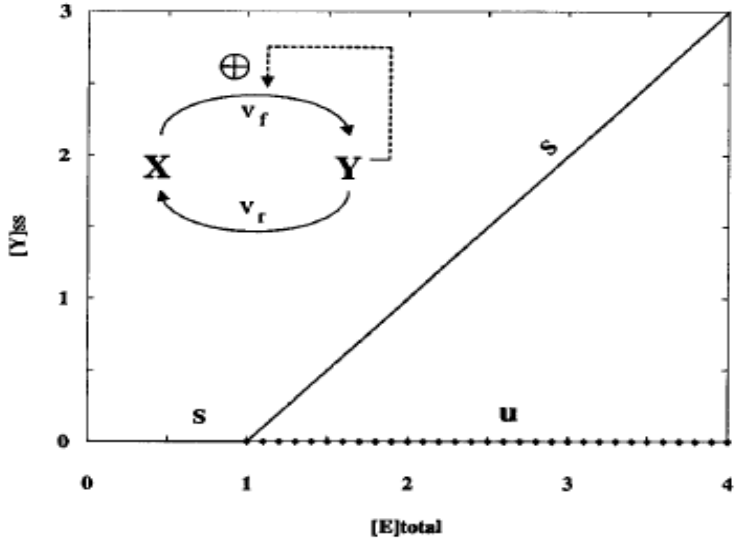
$$\frac{dy}{dt} = v_f(x,y) - v_r(x,y) = f(y)$$

$v_f = k_f xy$ and $v_r = k_r y$

Show that $\lambda = \frac{df}{dy} = (\frac{dv_f}{dy} + \frac{dv_r}{dx}) - (\frac{dv_f}{dx} + \frac{dv_r}{dy})$

Also identify the condition for transcritical bifurcation and the parameter that brings transcritical bifurcation

Reduce it to the normal form.

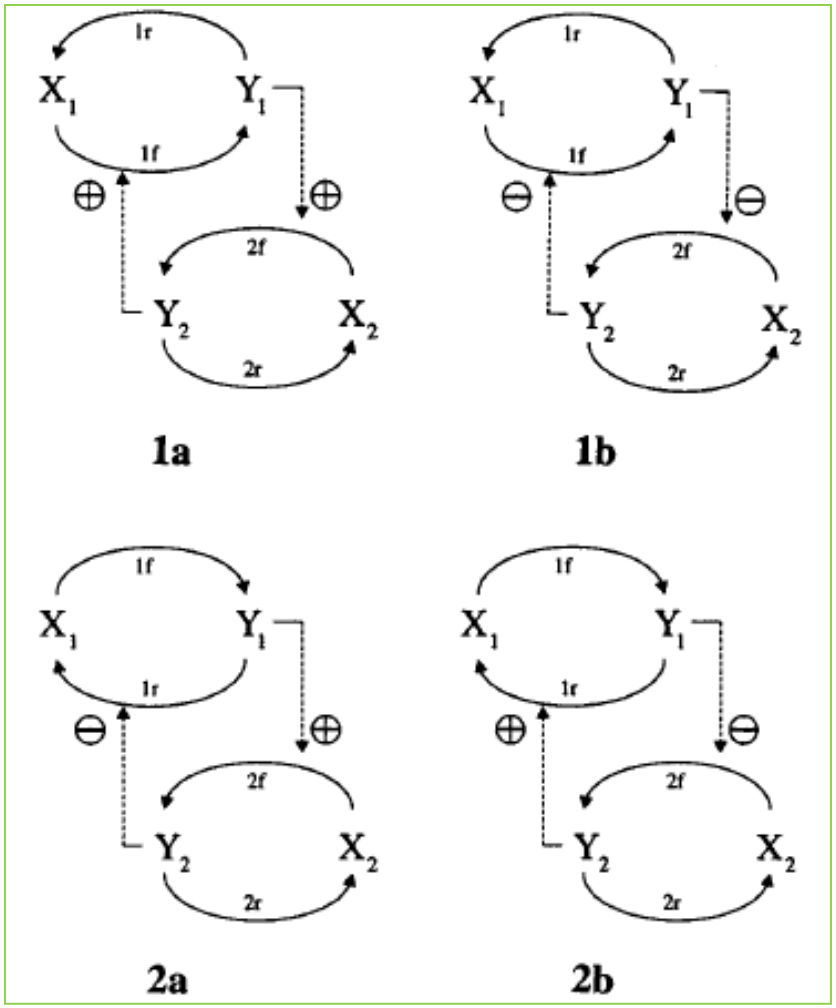


If suppose that Michealis-Menten kinetics is considered and the rates of forward and backward reactions are given

$$v_f = \frac{k_f yx}{k_{mf} + x} \text{ and } v_r = \frac{k_r y}{k_{mr} + y}$$

What is $\frac{dy_1}{dt}$ and $\frac{dy_2}{dt}$?

PD cycle with feedback in cascade and identification of transcritical bifurcation



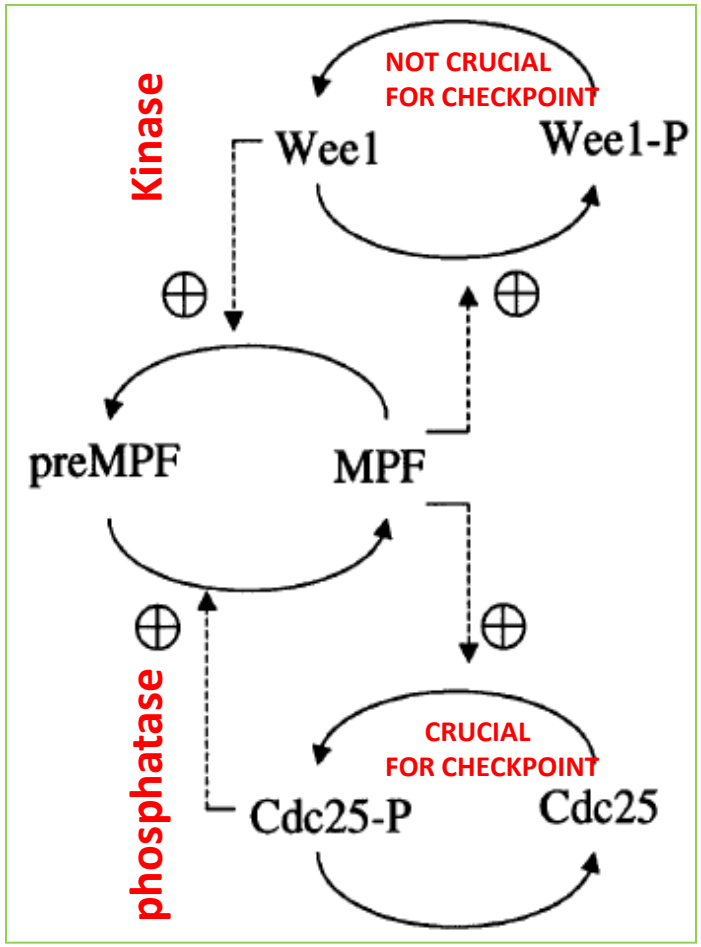
Four cases of unstable coupling between two PD cycles.

The (+) symbol indicates that species Yi catalyzes the reaction while (-) means inhibition

Assignment-7

Which cascade cycle will give rise to transcritical bifurcation?
All 4 or only 1(a) and 1(b)?

G2-M Checkpoint through transcritical bifurcation



PD cycles involved in the regulation of the mitosis promoting factor (MPF) in the cell cycle.

PreMPF is the tyrosine-phosphorylated inactive form of MPF.

Wee1 is a kinase and Cdc25 is a phosphatase.

Assignment -8

(1) What is the G2-M checkpoint in terms of dynamics

(2) And how can you show the checkpoint in terms of codimension-1 bifurcation.

(3) Identify which if G2 phase and M phase in the bifurcation diagram

(4) Why Wee1 may not be the crucial target of the check point?

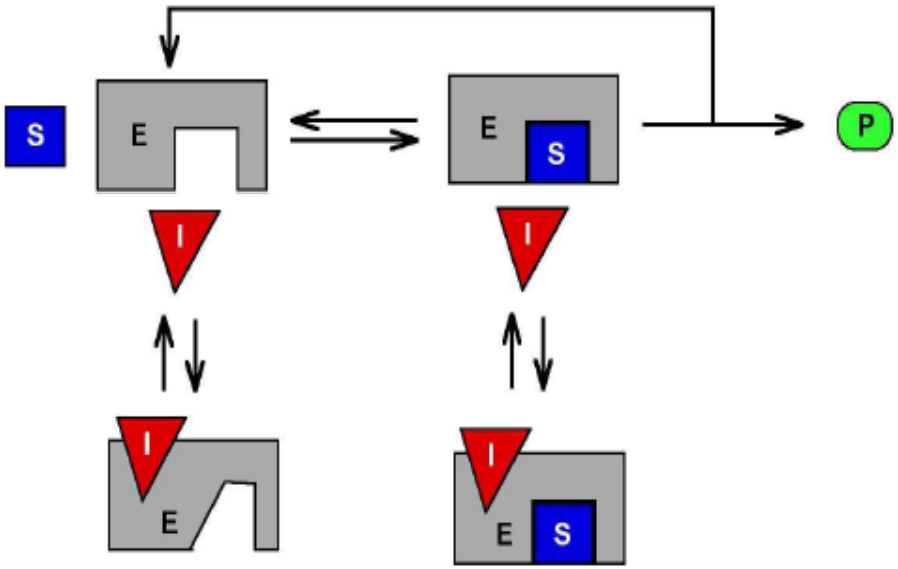
Allosteric Regulation

- The catalytic efficiency of an enzyme depends on the conformation of its active site.
- This conformation depends, in turn, on the overall configuration of the protein (its tertiary structure). This configuration, and hence the nature of the active site, can be altered by modifications to the chemical energy landscape of the protein, e.g. the position and strength of charges.
- These modifications can be made by molecules that bind the protein.
- This mode of enzyme regulation, called allosteric control, was proposed by Francois Jacob and Jacques Monod in 1961.

Allosteric Regulation - Different from competitive inhibition

- The term allostery (from Greek, allo: other, steros: solid, or shape) emphasizes the distinction from competitive inhibition – the regulating molecule need not bear any chemical resemblance to the substrate.
- Likewise, the site on the enzyme where the regulator binds (called the allosteric site) can be distinct from the active site in both position and chemical structure.
- Typically, the binding of an allosteric regulator to a protein invokes a transition between a **functional state** and a **non-functional state**.
- For enzymes, this is typically a transition between a catalytically active form and an inactive form.

Allosteric regulations - reaction and model



- Consider an enzyme that binds a single allosteric regulator. Assume that inhibitor has no effect on substrate binding.

- The reactions are

$$S + E \underset{k_{-1}}{\overset{K_1}{\longleftrightarrow}} ES \overset{k_2}{\longleftrightarrow} E + P$$

$$E + I \underset{k_{-i1}}{\overset{k_{i1}}{\longleftrightarrow}} EI$$

$$ES + I \underset{k_{-3}}{\overset{k_3}{\longleftrightarrow}} ESI$$

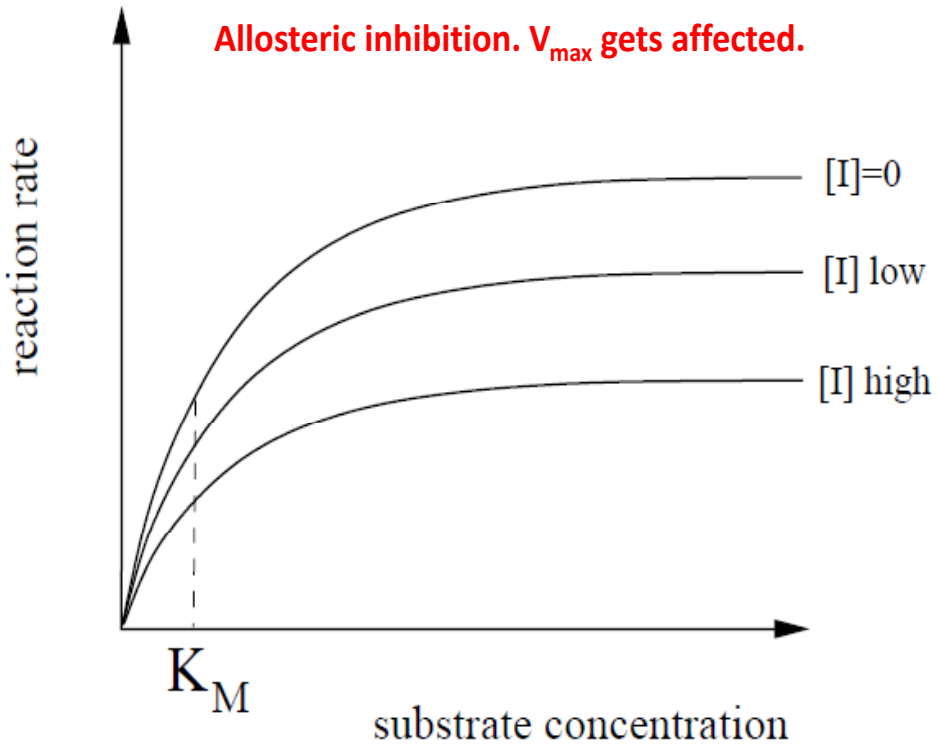
$$S + EI \underset{k_{-i2}}{\overset{k_{i2}}{\longleftrightarrow}} ESI$$

- The rate $S \rightarrow P$ is given as

$$\boxed{\frac{v_{max} S}{(1 + I/K_I)(K_M + S)}}$$

with $K_{I1,I2} = K_I$.

Allosteric regulations



- This allosteric inhibitor I reduces the limiting rate V_{max}, but does not affect the half-saturating concentration K_M.
- More generally, other allosteric inhibition schemes impact both V_{max} and K_M.

Cooperativity

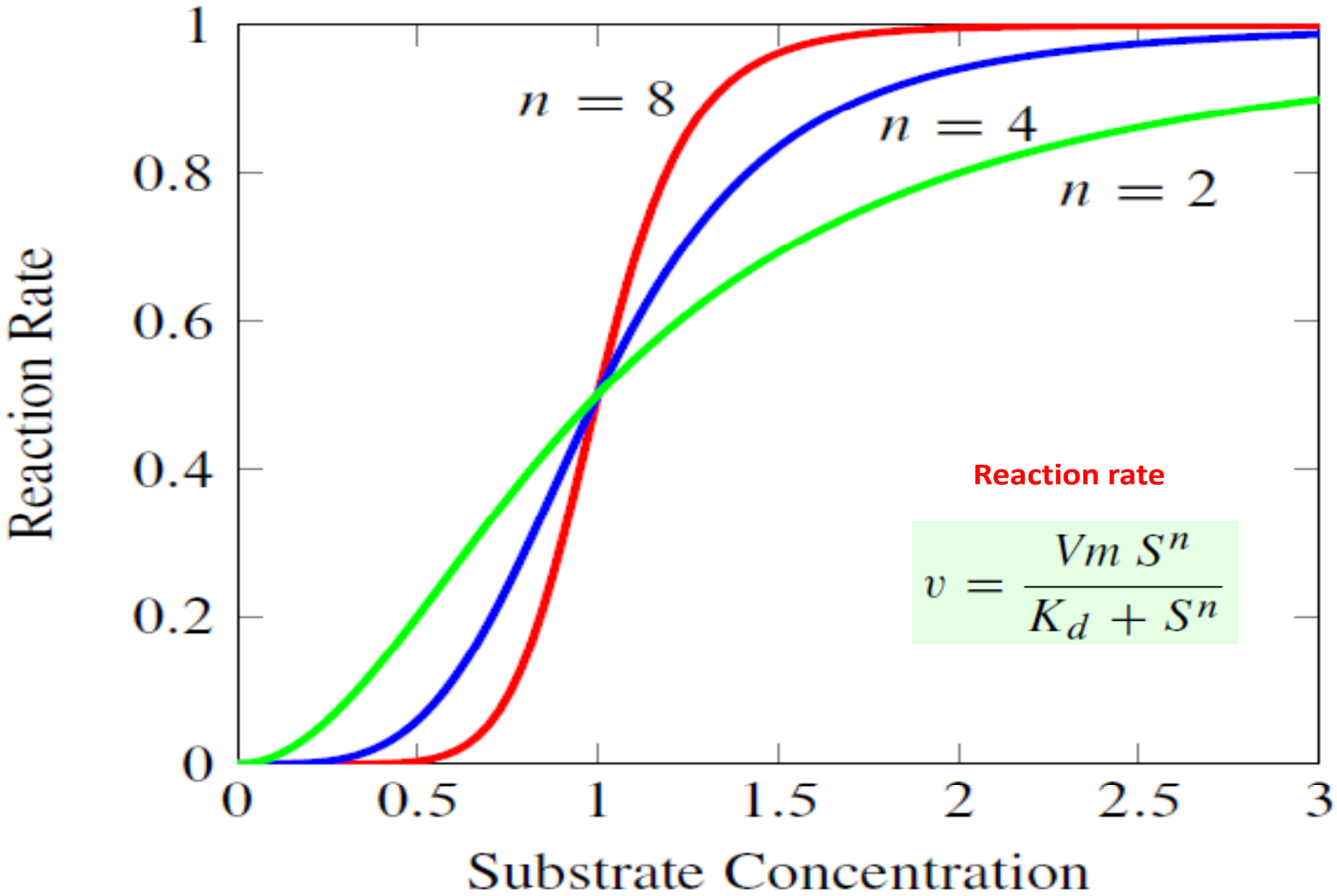
- The term cooperativity is used to describe potentially independent binding events that have a significant influence on one another, leading to nonlinear behaviors.
- Cooperativity exhibits sigmoidal behavior unlike the hyperbolic behavior seen in Michaelis-Menten kinetics.
- To address cooperativity, consider the binding of a molecule n ligands X bind to a protein P **simultaneously**. The reaction scheme is

$$P + nx \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} PX_n$$

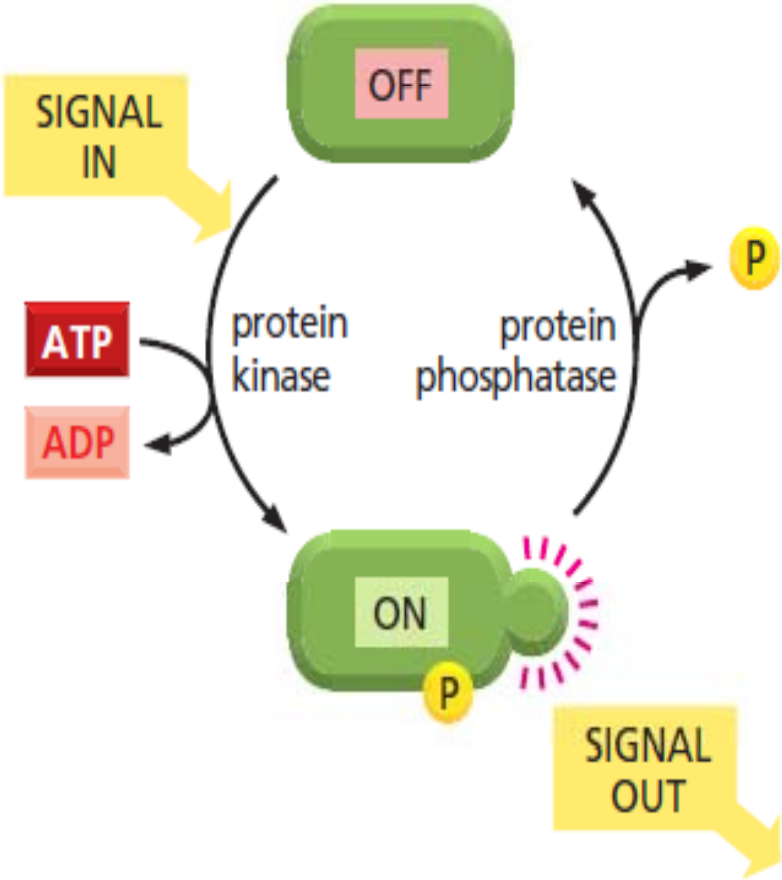
- At equilibrium, $K_a = \frac{k_1}{k_{-1}} = \frac{[PX_n]}{[P][x]^n}$, and $[P_{total}] = [P] + [PXn]$.

- The fractional saturation given by $Y = \frac{[PX_n]}{[P]_{total}} = \frac{x^n}{\frac{1}{k_a} + x^n} = \boxed{\frac{x^n}{K_d + x^n}}$

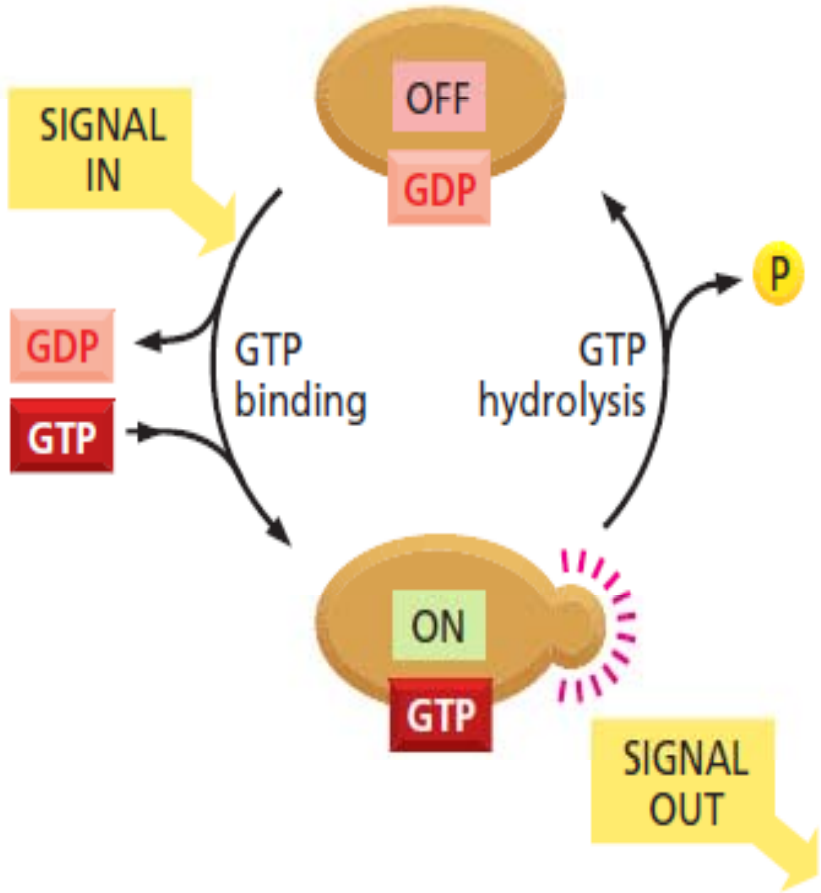
Sigmoidal plot of Hill's equation



Molecular switch in PdP and GTP reactions

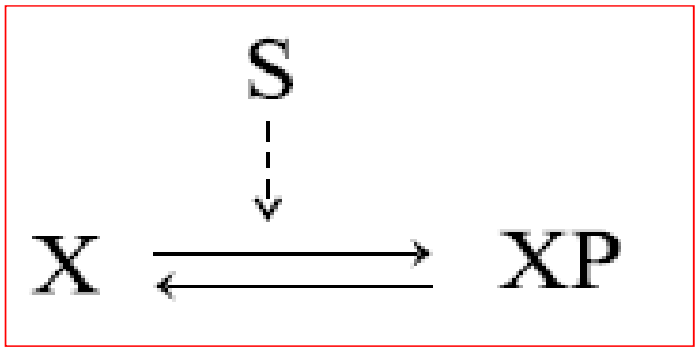


(A) SIGNALING BY PROTEIN PHOSPHORYLATION



(B) SIGNALING BY GTP-BINDING PROTEIN

Example of hyperbolic response - phosphorylation-dephosphorylation (PdP) reactions



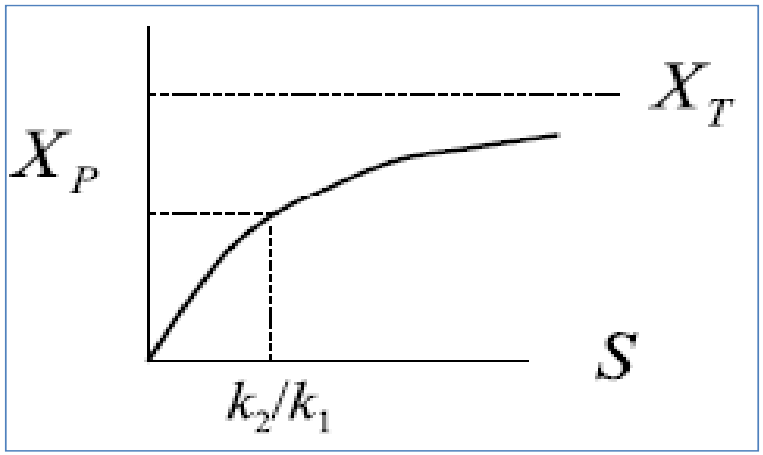
$$\frac{dX_P}{dt} = k_1 S(X_T - X_P) - k_2 X_P$$

$$X_T - X_P = X$$

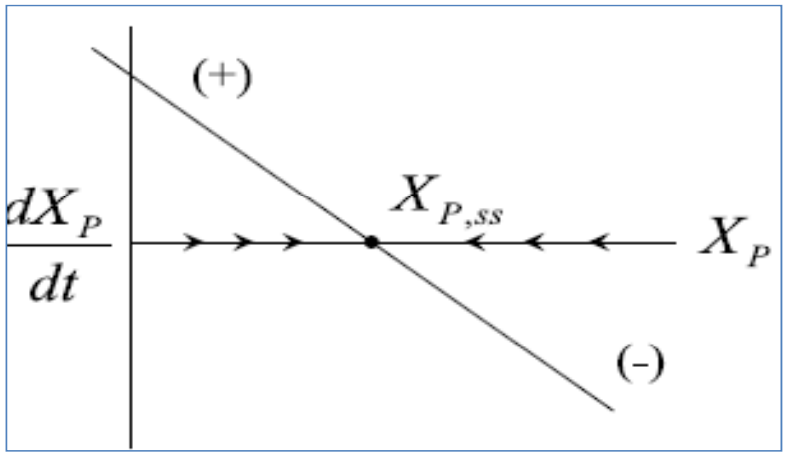
Solving $\frac{dX_P}{dt} = 0$

$$X_{P,ss} = \frac{X_T S}{(k_2/k_1)+S}$$

Signal - Response curve



Phase plot



Ultrasensitivity and Zero-order ultrasensitivity

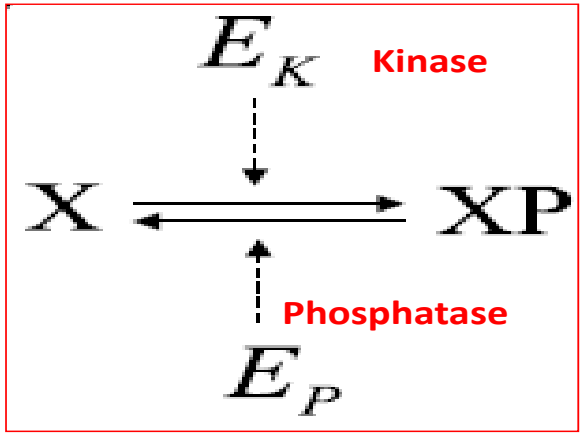
- **Definition of ultrasensitivity**: A modest change in the concentration of substrate of an enzyme, or a modest change in the activity of an enzyme, greatly changes the net rate of the reaction catalyzed by that enzyme.

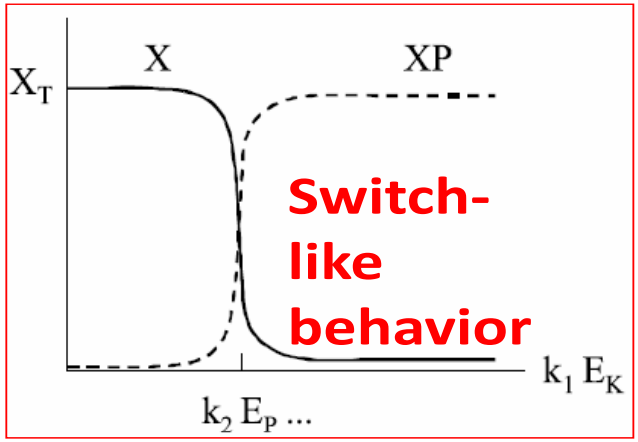
- **Zero-order ultrasensitivity** : Two enzymes catalyze the same biochemical transformation, one in the forward direction and the other in the reverse direction.

- Both enzymes must be nearly saturated with substrate, so that both the reverse and forward reactions are approximately zero-order.

- At steady state, the substrate concentrations are often such that the rates of both reactions are nearly equal.

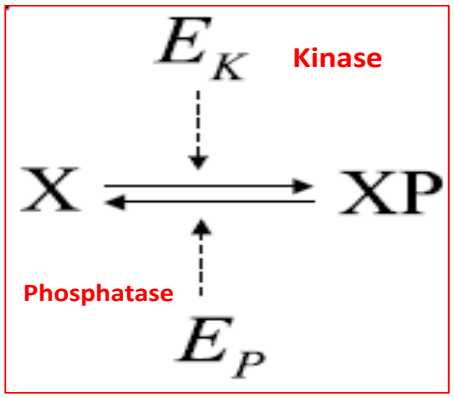
Zero-order ultrasensitivity - phosphorylation-dephosphorylation (PdP) reactions





- Now, suppose there is a small increase in the activity (*Vmax)*of the **enzyme catalyzing the forward reaction.** This unbalances the rates so that the forward reaction is faster.
- Since this enzyme is saturated with substrate, a large drop in the concentration of substrate is required to bring the rates back into balance.
- Thus, a small change in enzyme activity results in a large change in the ratio of substrate to product.

Zero-order ultrasensitivity - Model



$$\frac{dX}{dt} = -\frac{k_1 E_K X}{K_{m1} + X} + \frac{k_2 E_P (X_T - X)}{K_{m2} + X_T - X}$$

$X_T - X = X_P$ (Conservation relation)

At steady state

$$\frac{k_1 E_K X}{K_{m1} + X} = \frac{k_2 E_P (X_T - X)}{K_{m2} + X_T - X}$$

simplifying and scaling the relevant variables,

$$x = X/X_T,\ u_1 = k_1 E_K,\ u_2 = k_2 E_P, J_1 = K_{m1}/X_T,\ J_2 = K_{m2}/X_T$$

$$u_1 x(J_2 + 1 - x) = u_2(1 - x)(J_1 + x)$$

Solving this quadratic equation,

$$x = G(u_1, u_2, J_1, J_2) \qquad \textit{Goldbeter-Koshland} \text{ function, } G$$

$$G(u_1, u_2, J_1, J_2) = \frac{2u_1 J_2}{B + \sqrt{B^2 - 4(u_2 - u_1) u_1 J_2}}$$

$$B = u_2 - u_1 + u_2 J_1 + u_1 J_2$$

(i) CHECK !
(ii) How this expression is obtained?

How to determine the sigmoidal nature of GK function?

- Consider the scaled steady-state equation

$$\boxed{u_1 x(J_2 + 1 - x) = u_2(1 - x)(J_1 + x)}$$

- It is easier to think of u_1 as a function of x than x as a function of u_1.
- So $u_1 = u_2 \frac{J_1+x}{J_2+1-x} \frac{1-x}{x}$
- Zeros of u_1 are x = and x = - J_1.
- Vertical asymptotes are $x = 1 + J_2$ and $x = 0$.
- For $0 < J_1, J_2 << 1$, The curve should exhibit switch-like behavior.