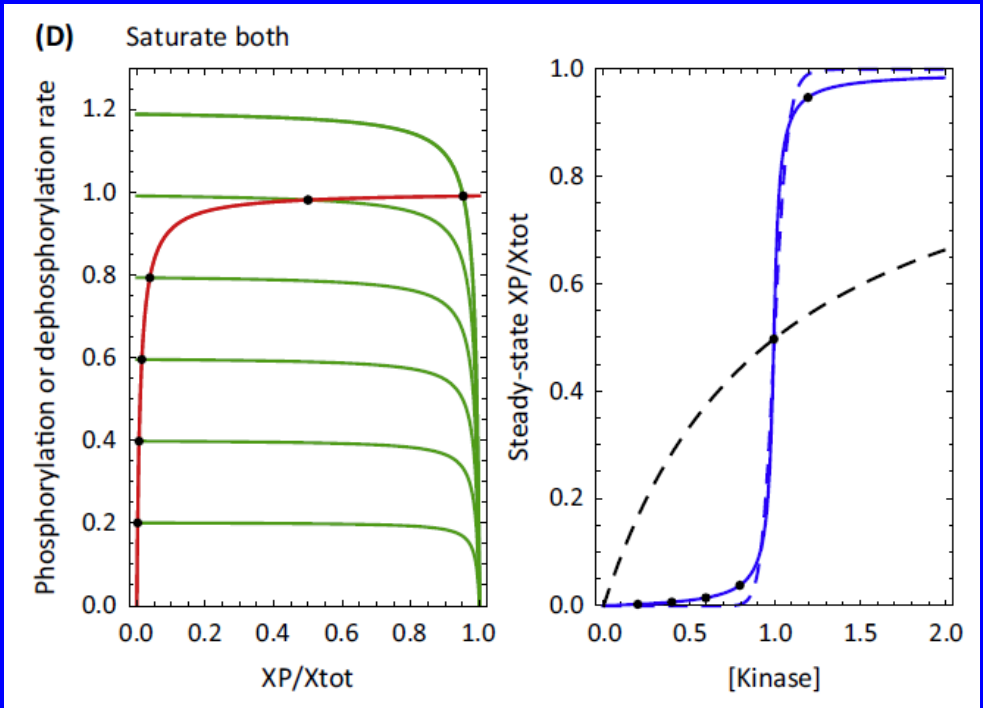
Review: mechanisms of ultrasensitivity

- Zero-order ultrasensitivity

Both the kinase and phosphatase are required to be close to saturation.



$$\frac{dXP}{dt} = k_1\, kinase \frac{X_{tot} - XP}{K_{m1} + X_{tot} - XP} - k_{-1}\, p'ase \frac{XP}{K_{m2} + XP}$$

Comments on Assignment 2

1) One loop

$$\frac{dOUT}{dt} = k_{out\_on} * A * (1 - OUT) - k_{out\_off} * OUT + k_{out\_min}$$

$$\frac{dA}{dt} = [stimulus * \frac{OUT^n}{OUT^n + ec_{50}^n} * (1 - A) - A + k_{min}] * \tau_A$$

2) Two loops

$$\frac{dOUT}{dt} = k_{out\_on} * (A + B) * (1 - OUT) - k_{out\_off} * OUT + k_{out\_min}$$

$$\frac{dA}{dt} = [stimulus * \frac{OUT^n}{OUT^n + ec_{50}^n} * (1 - A) - A + k_{min}] * \tau_A$$

$$\frac{dB}{dt} = [stimulus * \frac{OUT^n}{OUT^n + ec_{50}^n} * (1 - B) - B + k_{min}] * \tau_B$$

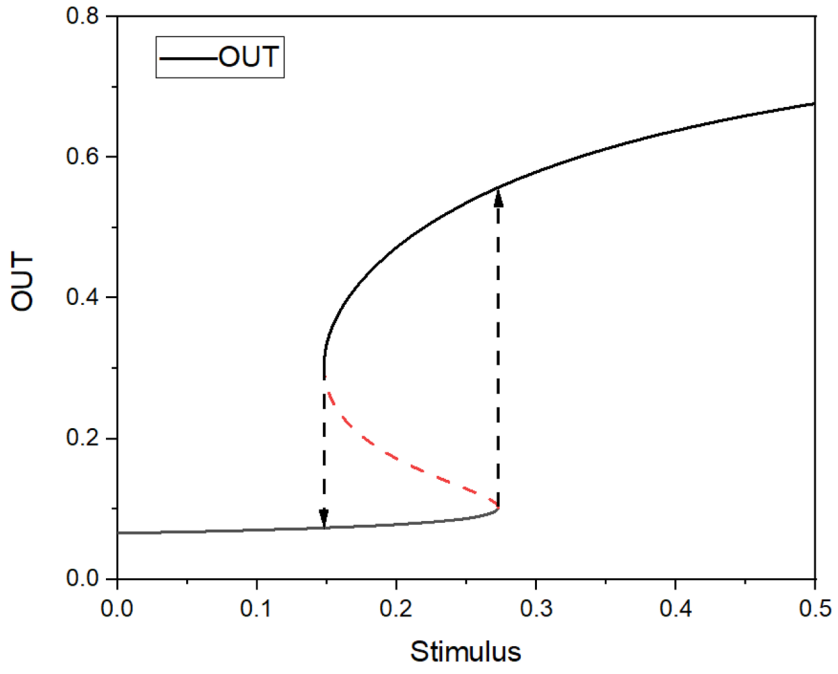
Plotting the bifurcation diagram of the one-loop and two-loop systems ([Out] versus Stimulus) in the following paper: Brandman, O., J. E. Ferrell, Jr., et al. (2005). Science **310**(5747): 496-498. Illustrating the history-(in)dependent properties of the system by plotting the dynamics of the system for "Stimulus" values at the bistable area with different initial values.

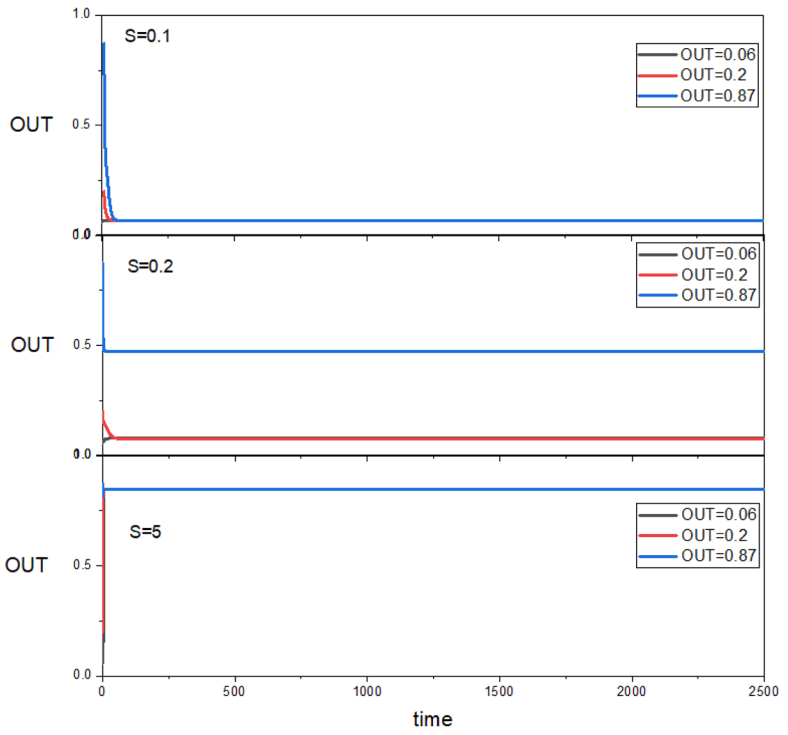
Comments on Assignment 2

Tools for bifurcation:

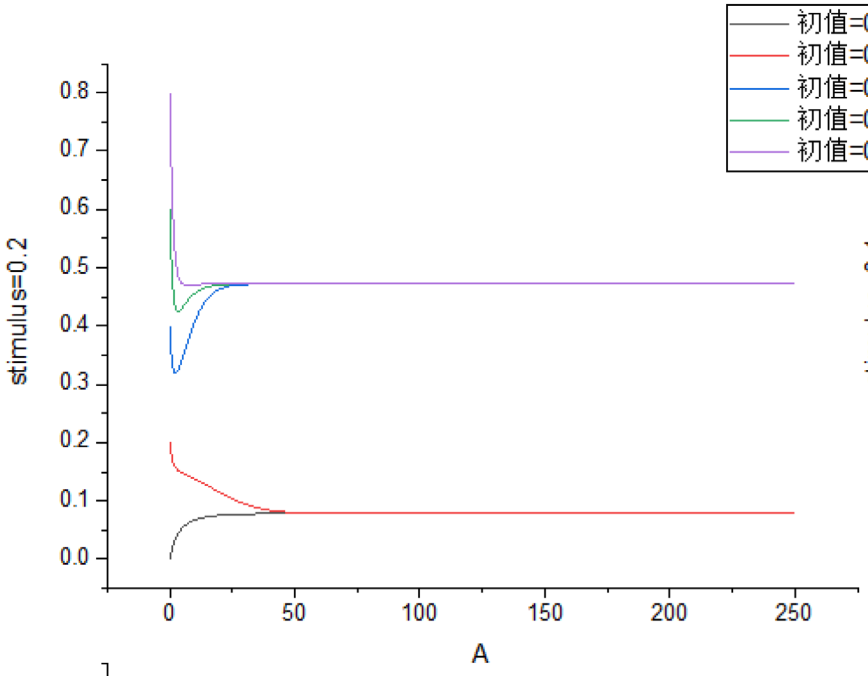
Oscill8, **XPP/XPPAUT**, Winpp, Matcont

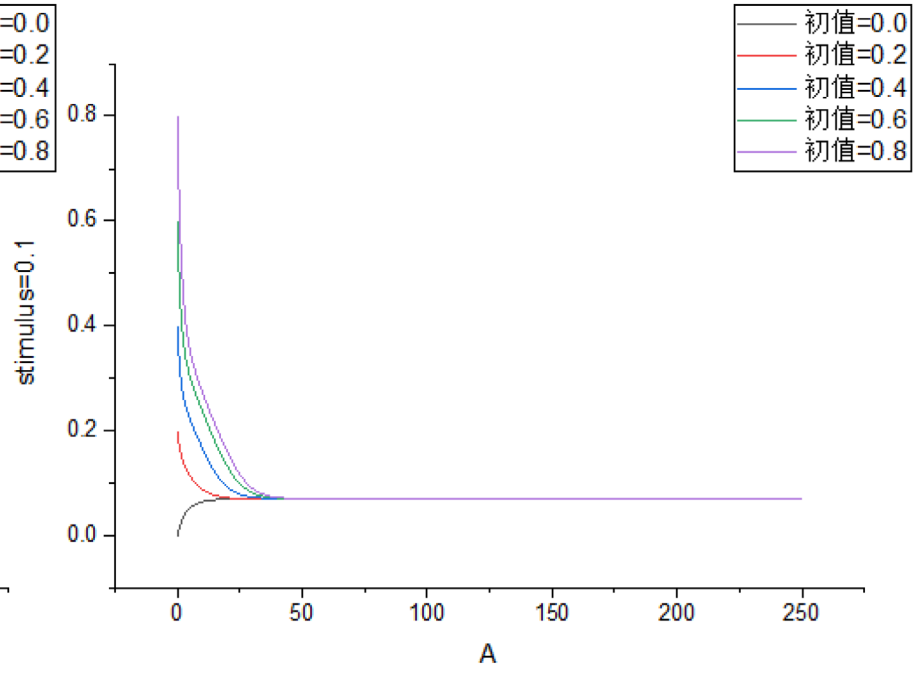
One loop

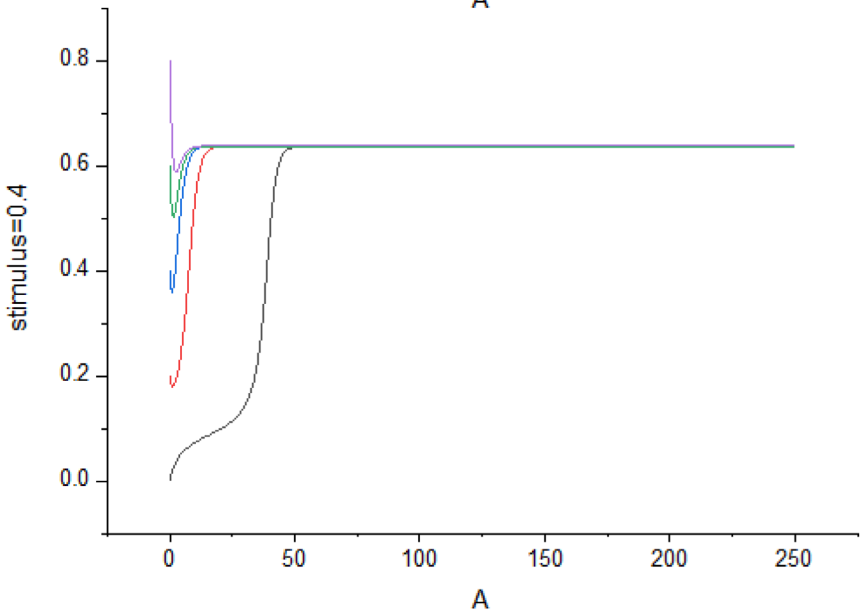




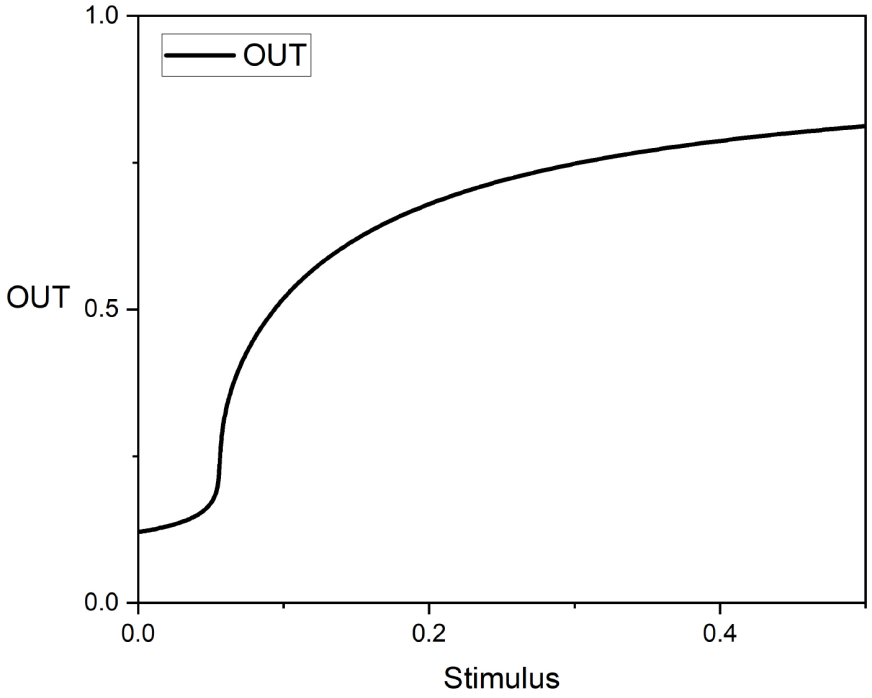
One loop (双稳态)

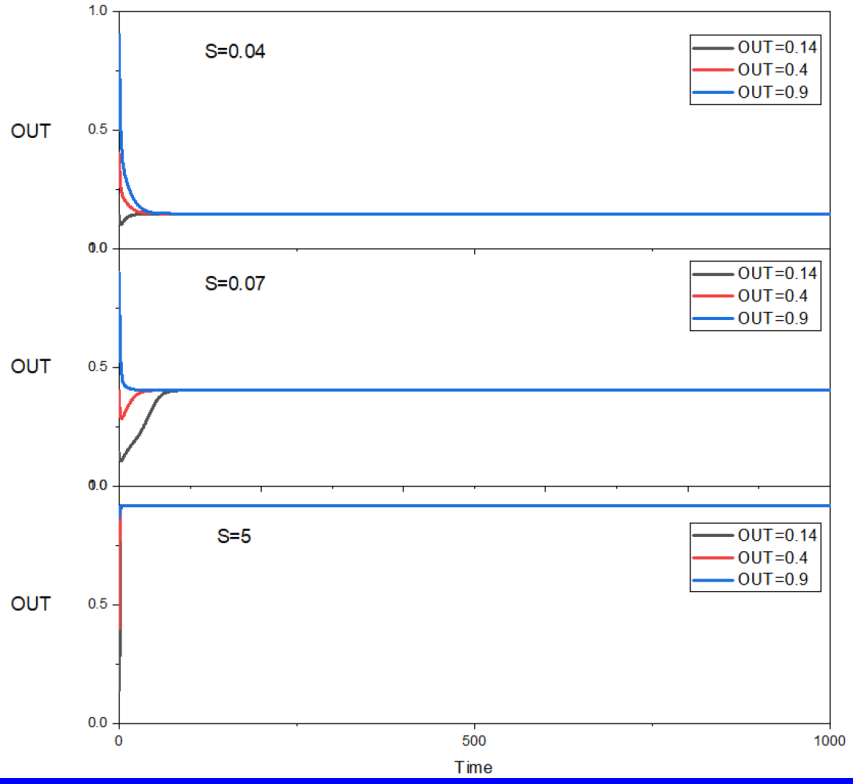

初值=0.0
初值=0.2
初值=0.4
初值=0.6
初值=0.8
stimulus=0.2
A


初值=0.0
初值=0.2
初值=0.4
初值=0.6
初值=0.8
stimulus=0.1
A


初值=0.0
初值=0.2
初值=0.4
初值=0.6
初值=0.8
stimulus=0.4
A

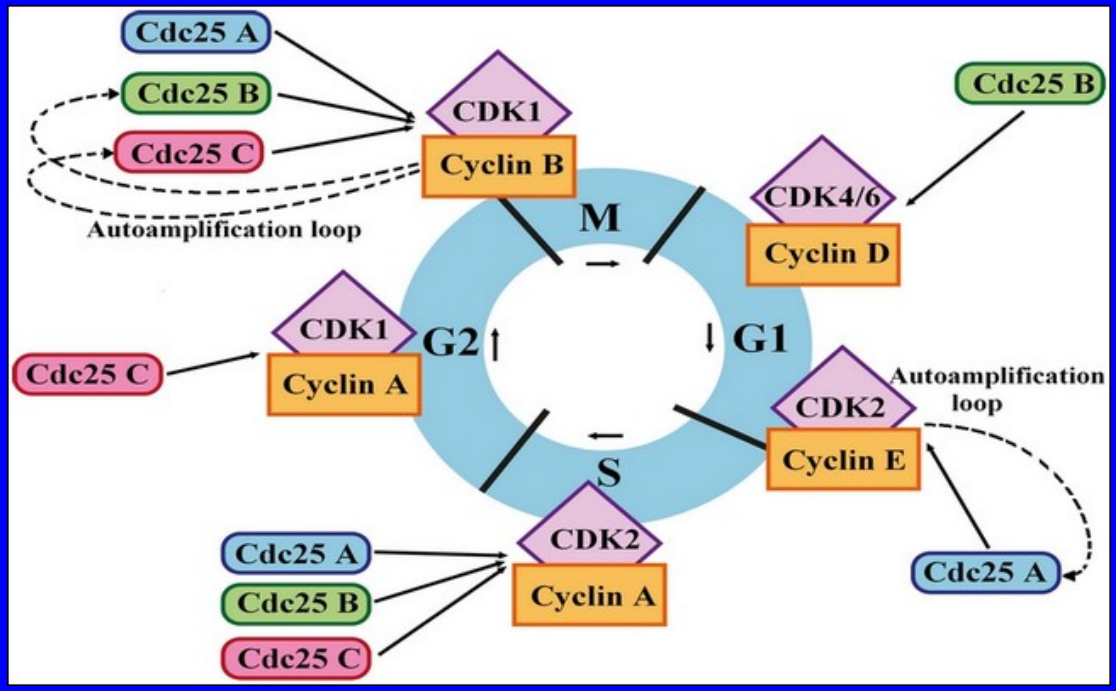
Two loops


OUT
1.0
0.5
0.0
OUT
0.0
0.2
0.4
Stimulus


S=0.04
S=0.07
S=5
OUT=0.14
OUT=0.4
OUT=0.9
OUT
1.0
0.5
0.0
0
500
1000
Time

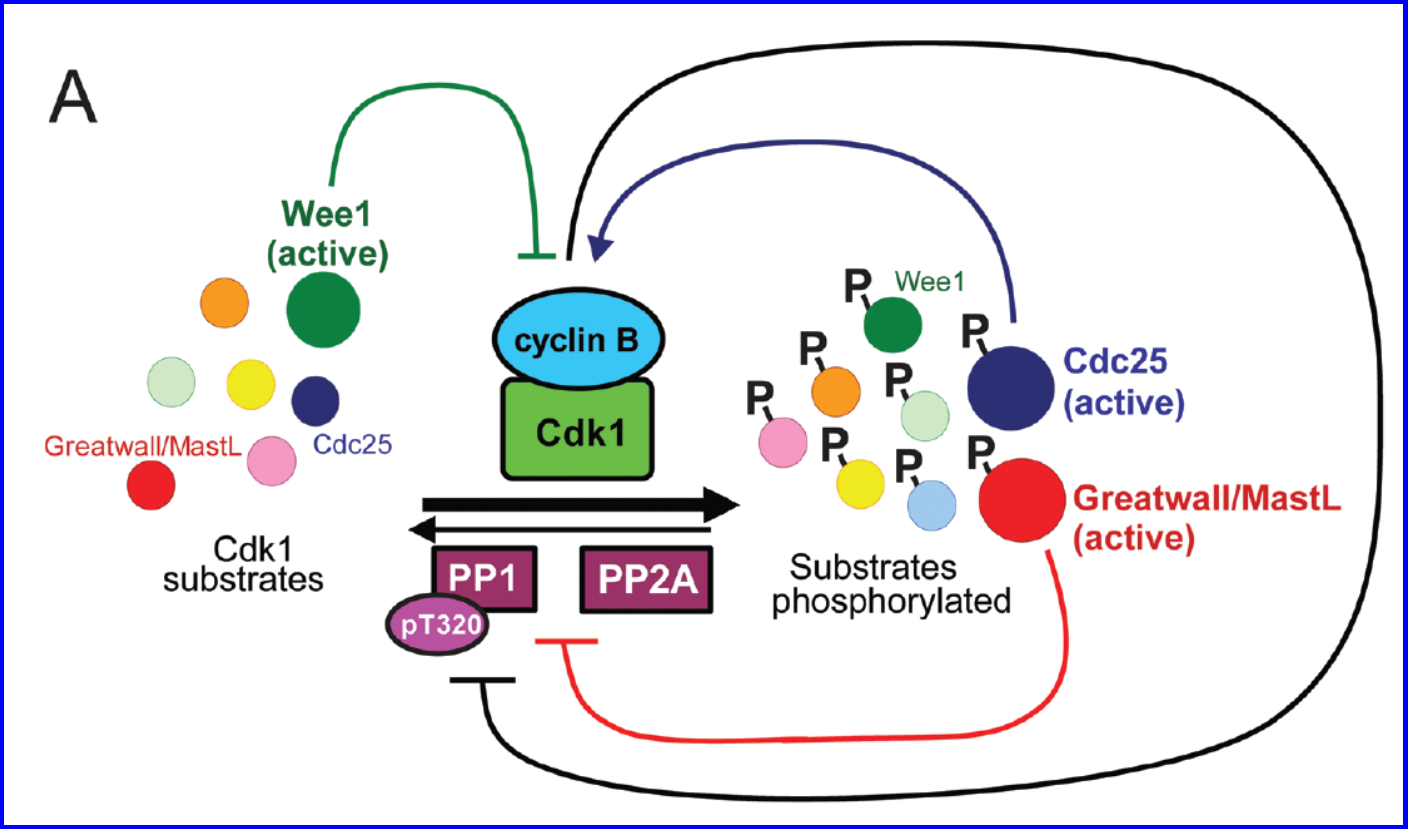
2.2.3.2 Multisite phosphorylation, stoichiometric inhibitors, and positive feedback

- a. Multisite phosphorylation of Cdc25C

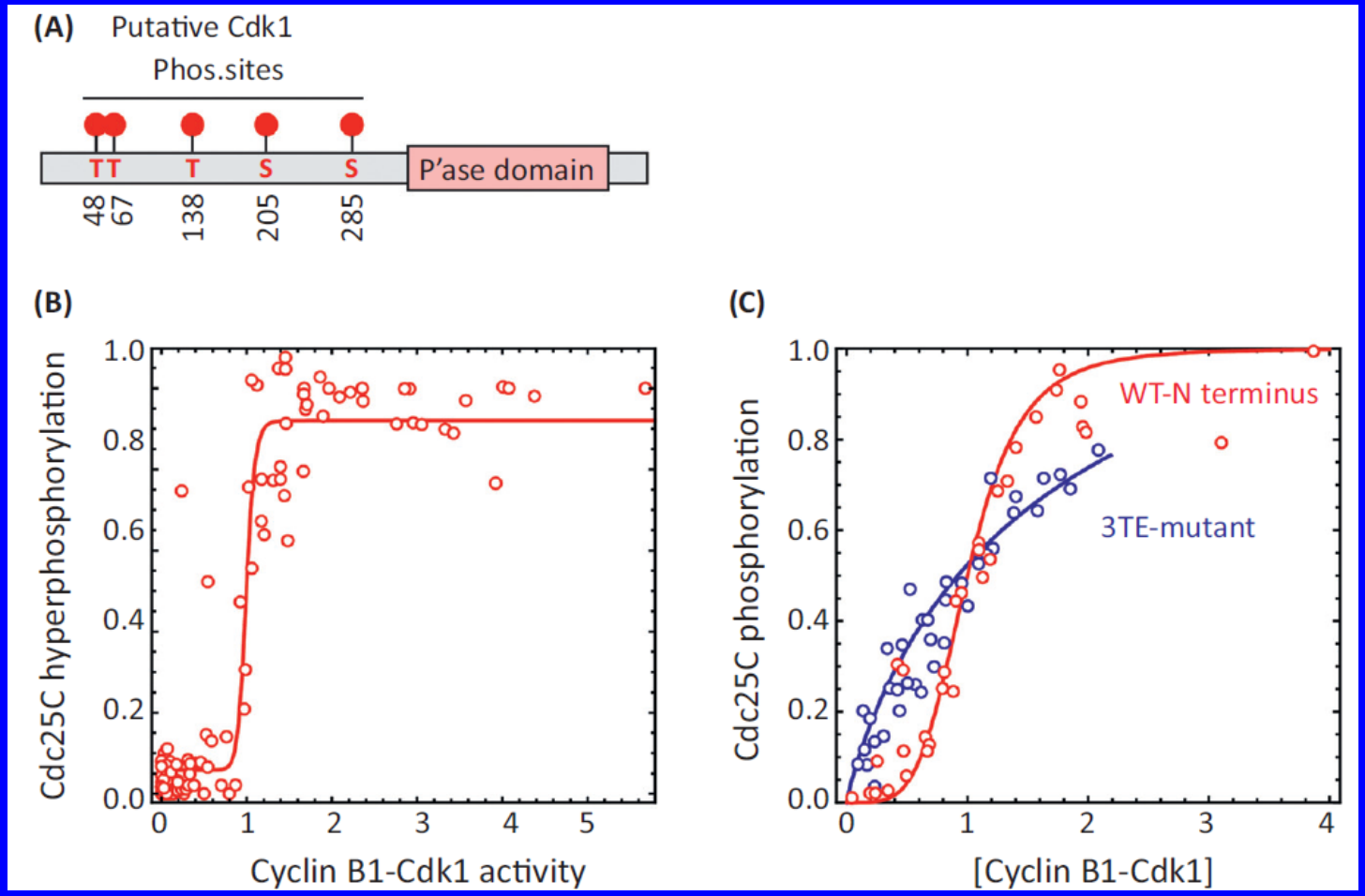


The roles of CDKs in cell cycle progression

Positive feedback between Cdc25c and Cdk1



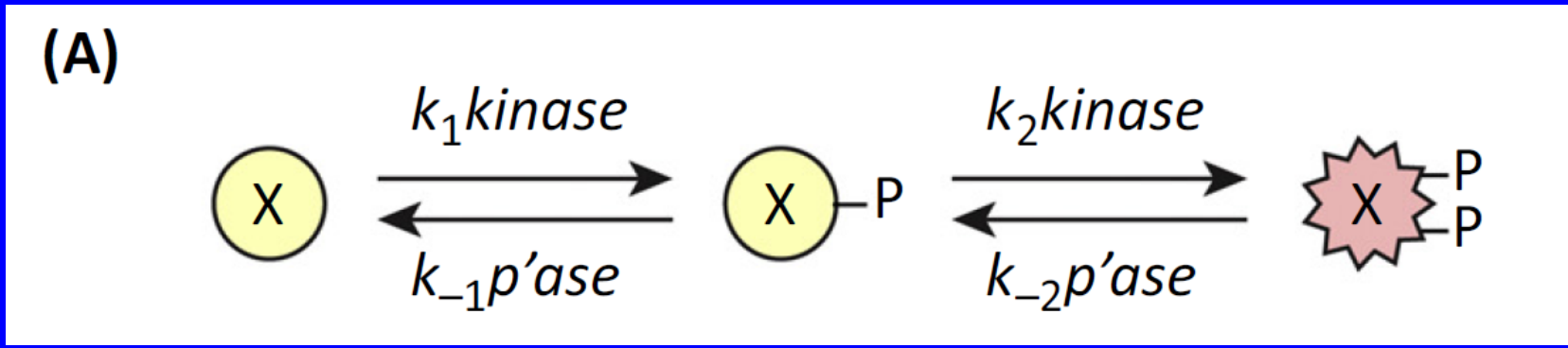
Potapova TA, Sivakumar S, Flynn JN, Li R, Gorbsky GJ. Molecular Biology of the Cell. 2011; **22**(8): 1191-206.



Multisite phosphorylation of Cdc25C by Cdk1.

Ferrell JE, Jr, Ha SH.. Trends Biochem Sci. 2014; **39**(11): 556-69.

Ultrasensitivity from multisite phosphorylation



$$\frac{dX}{dt} = -k_1\,kinase \cdot X + k_{-1}\,p'ase \cdot XP \qquad [I]$$

$$\frac{dXP}{dt} = k_1\,kinase \cdot X - k_{-1}\,p'ase \cdot XP - k_2\,kinase \cdot XP + k_{-2}\,p'ase \cdot XPP \qquad [II]$$

$$\frac{dXPP}{dt} = k_2\,kinase \cdot XP - k_{-2}\,p'ase \cdot XPP \qquad [III]$$

$$X_{tot} = X + XP + XPP$$

The output of the system is ***XPP***; the steady state concentration of XPP (denoted XPP_{ss}),as a fraction of X_{tot} is given by:

$$\frac{XPP_{ss}}{X_{tot}} = \frac{kinase^2}{\frac{k_{-1}k_{-2}\,p'ase^2}{k_1 k_2} + \frac{k_{-2}\,p'ase}{k_2}\,kinase + kinase^2} \qquad [IV]$$

Note that if we took the two steps individually, the EC_{50} values for the first and second reactions would be given by $\frac{k_{-1}p'_{ase}}{k_1}$ and $\frac{k_{-2}p'_{ase}}{k_2}$, respectively. We can write Equation IV as:

$$\frac{XPP_{ss}}{X_{tot}} = \frac{kinase^2}{K_1 K_2 + K_2\,kinase + kinase^2} \qquad [V]$$

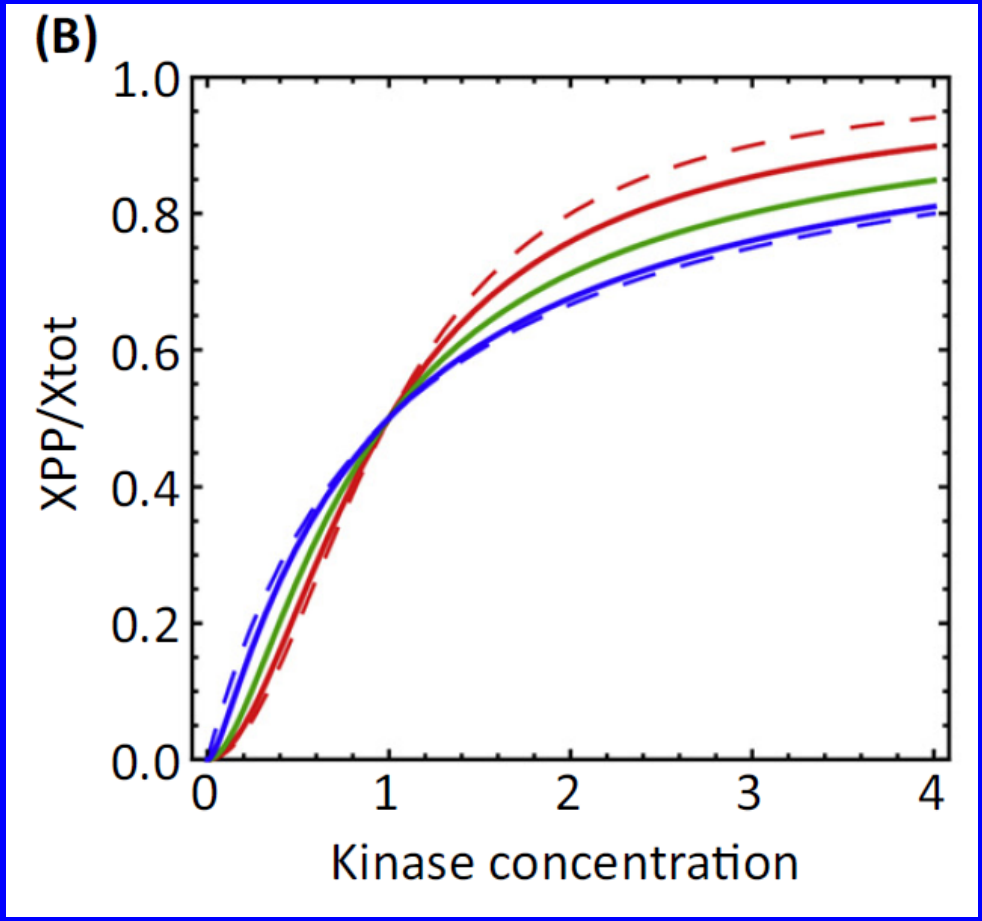
For multisite phosphorylation with n phosphorylation sites, the steady state level of the fully n-phosphorylated substrate is given by:

$$\frac{XP_n}{X_{tot}} = \frac{kinase^n}{(K_1 \cdots K_n) + (K_2 \cdots K_n)kinase + (K_3 \cdots K_n)kinase^2 + \ldots + kinase^n} \quad \text{[VI]}$$

Steady state responses in dual phosphorylation

$K_2 \ll K_1 \Rightarrow$ Hill curve with a Hill exponent of 2

$K1 \ll K2 \Rightarrow$ a Michalian response curve



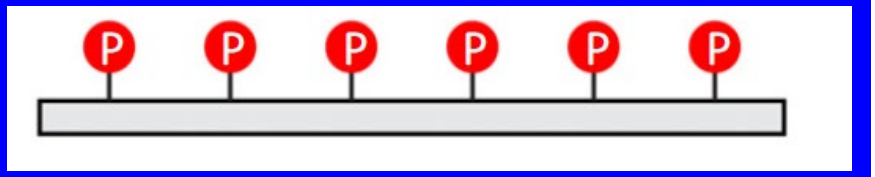
Further interpretation

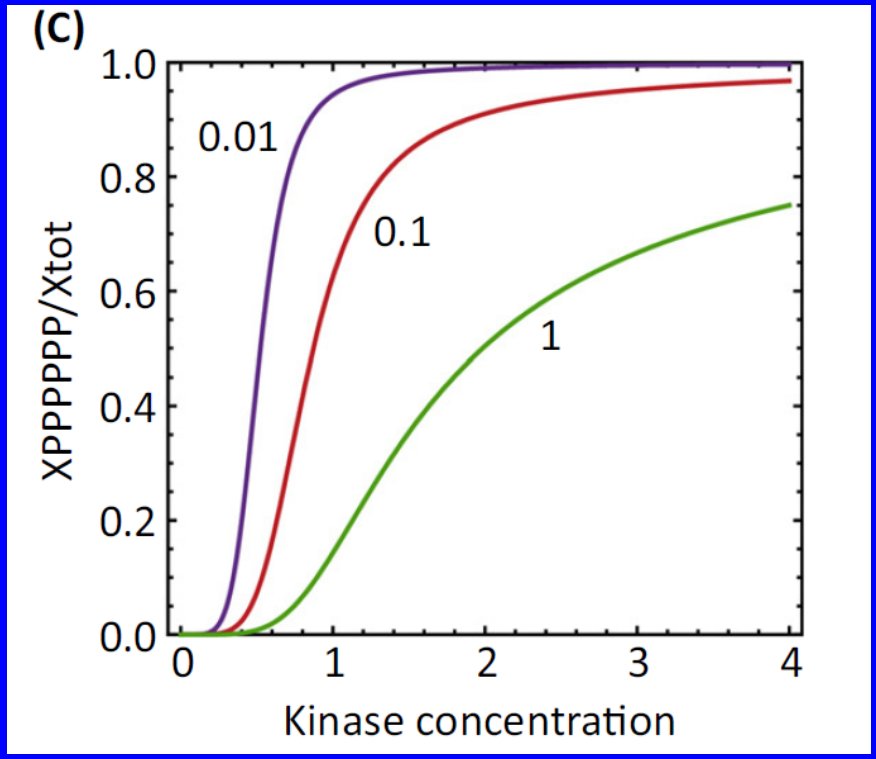
The condition for high ultrasensitivity is that K_2 is substantially smaller than K_1, which means that the second phosphorylation is more favorable than the first.

Since the less favorable phosphorylation nevertheless happens first, one can regard the first phosphorylation as causing the subsequent phosphorylation to become more favorable.

Steady state responses in multistep phosphorylation

Different K_6 for the curves



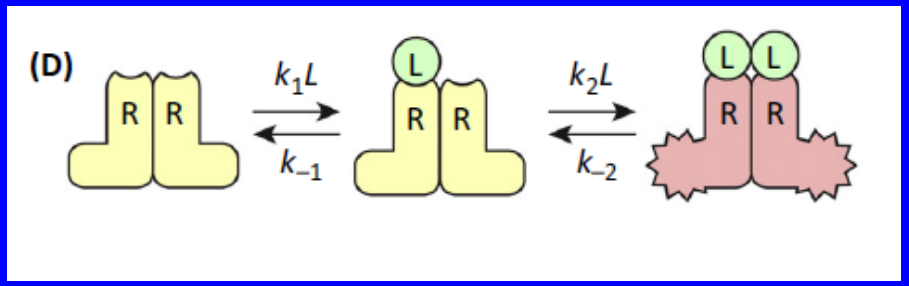


If one of the last phosphorylations is more favorable than the previous ones, it can make the response curve substantially more ultrasensitive

Cooperative receptor

The above mechanism is analogous to the situation with a cooperative receptor, where the binding of a first ligand makes the binding of the second ligand more energetically favorable.

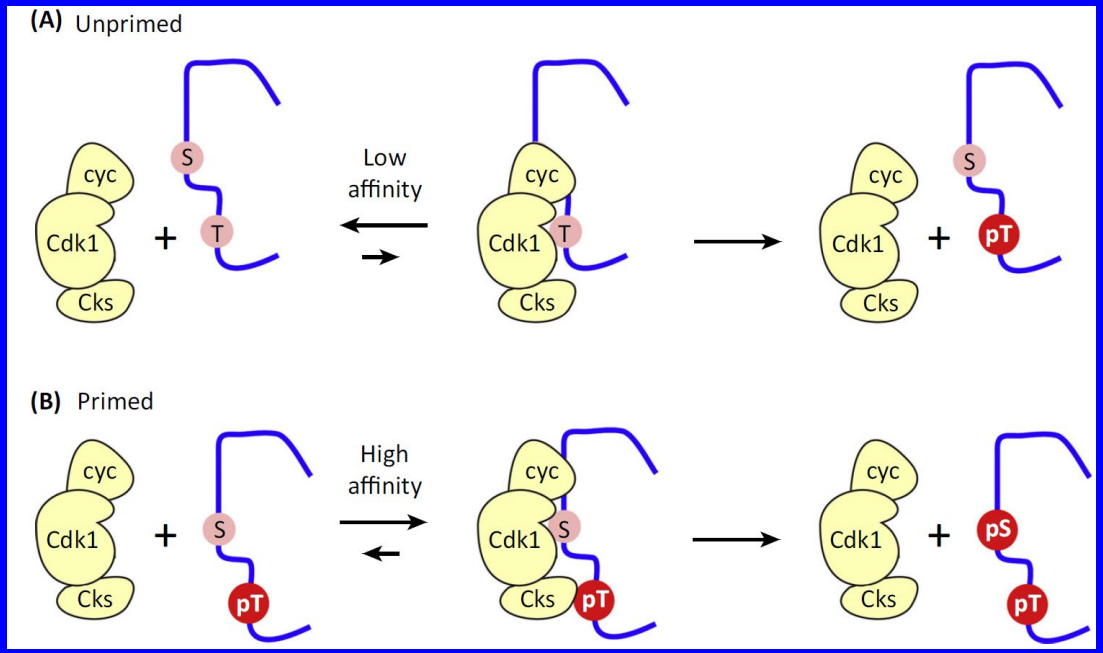
KNF style (sequential) activation of a dimeric receptor by the binding of two ligands.



$$\frac{R_2L_2}{R_{tot}} = \frac{L^2}{K_1K_2 + K_2L + L^2}$$

Priming and cooperativity in multisite phosphorylation

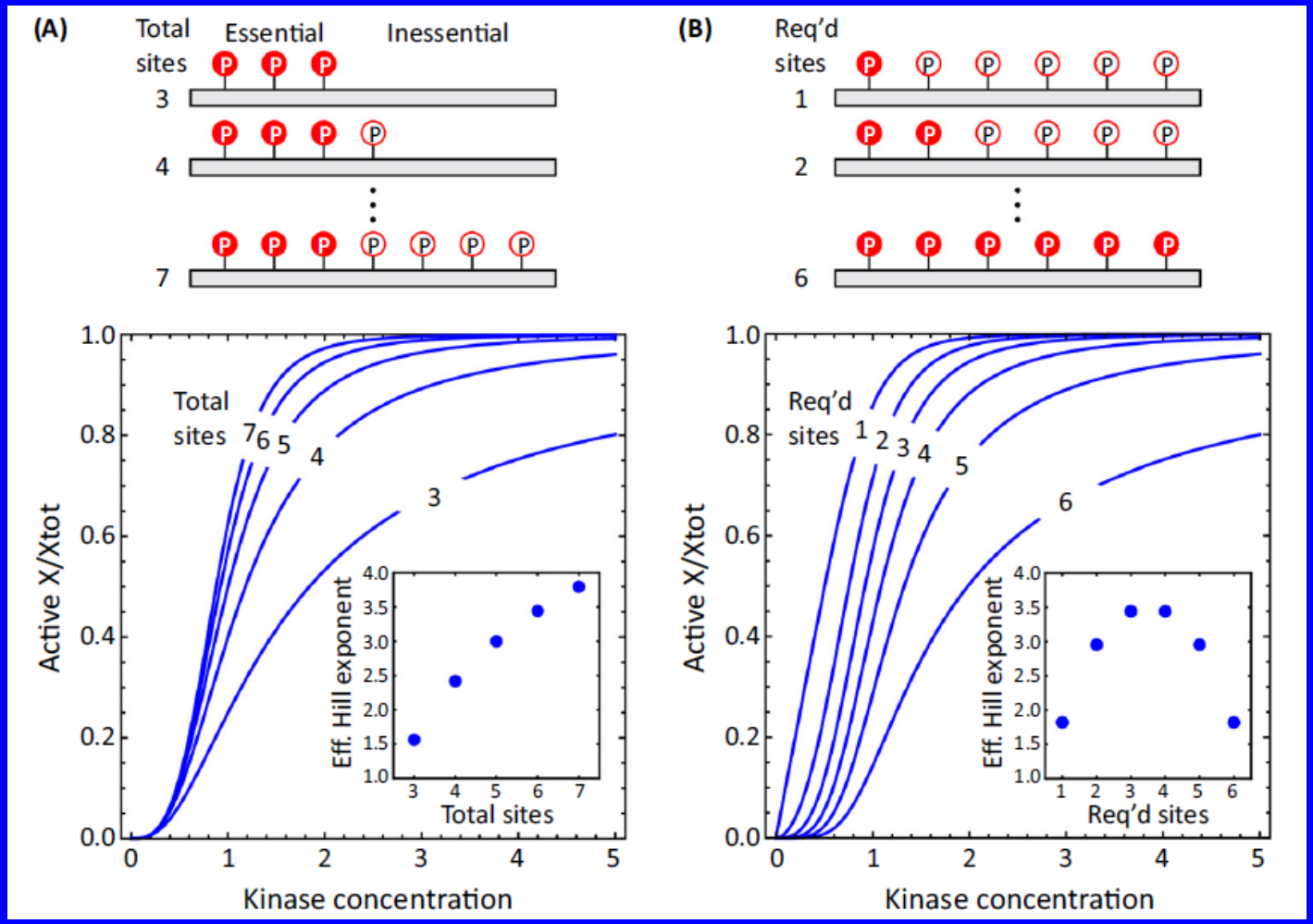
Cooperativity in multisite phosphorylation from priming.



The result is cooperative phosphorylation of the second site – the priming can be thought of as a positive allosteric interaction – and an increase in the overall ultrasensitivity of the second site's response .

Extra phosphorylation sites and ultrasensitivity

If we wish to generate the maximum amount of ultrasensitivity, how many sites should we require to be phosphorylated for activation of X?



It is best to require that only approximately half of the sites be phosphorylated for activation.

This phenomenon may be particularly important in cases where the function of a protein depends on the amount of phosphorylation sites, rather than the exact identities of the sites phosphorylated. For example, the phosphorylation of eight clustered Cdk1 sites near the N terminus of the protein is sufficient to cause Ste5 to dissociate from the plasma membrane, and it appears that (to a first approximation) phosphorylation of any four of the eight sites is sufficient to half-maximally knock Ste5 off the membrane .

Saturation and competition in multisite phosphorylation

We have considered multisite phosphorylation as though each step is described by mass action kinetics, and the intermediary enzyme–substrate complexes are ignored in concentration. What happens if we use saturatable kinetics and explicitly consider the complexes?

Substrate competition in multisite phosphorylation

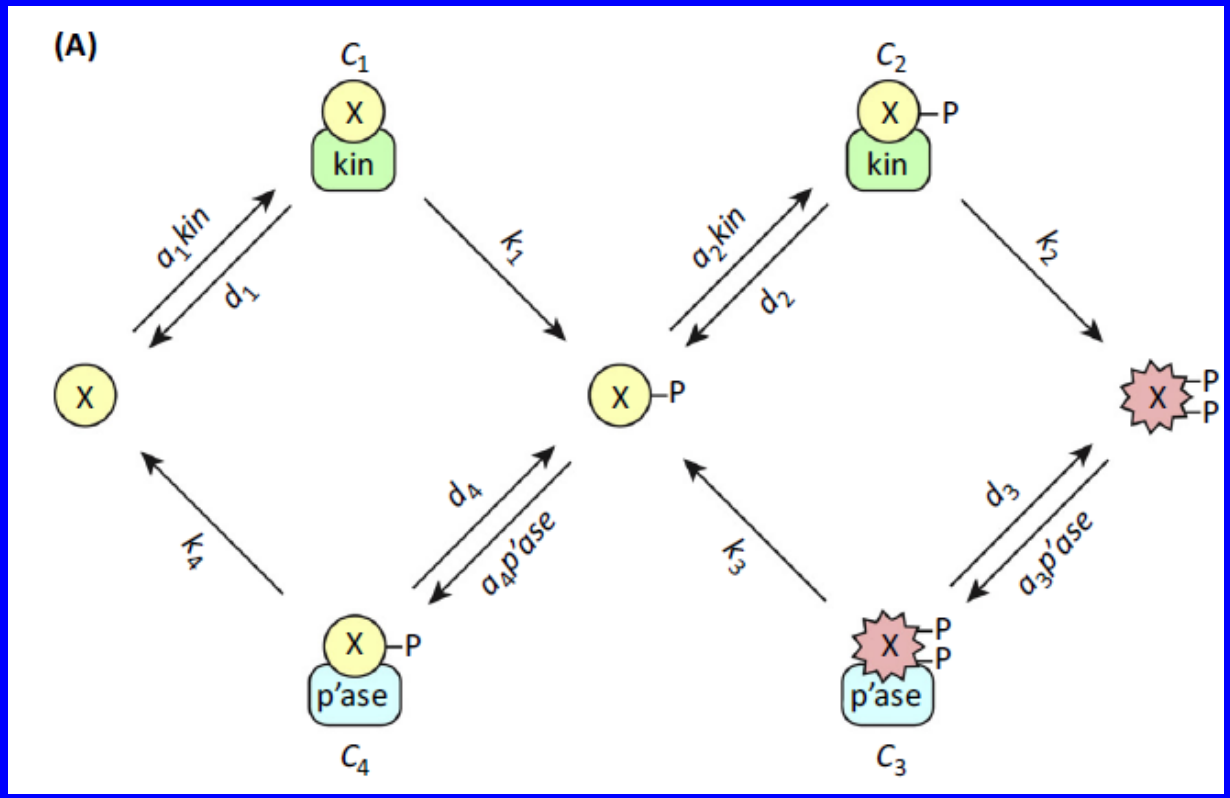
Obviously, saturating the phosphorylation and dephosphorylation reactions can add additional zero-order ultrasensitivity to the overall response.

It is surprising that the system undergoes a qualitative change from monostable to bistable while considering substrate competition.

Markevich NI, Hoek JB, Kholodenko BN.J Cell Biol. 2004; **164**(3): 353-9.

Implicit positive feedback from competition for converting enzymes

A dual phosphorylation mechanism that includes intermediary complexes



Ferrell JE, Jr, Ha SH. Trends in Biochemical Sciences. 2014; **39**(11): 556-69.

Substrate inhibition from competition for converting enzymes

The situation here can be thought of as a specific example of substrate inhibition, where substrate X inhibits the production of XPP by the kinase through competition, and substrate XPP inhibits the production of X by the phosphatase through competition. In general, substrate inhibition can increase ultrasensitivity and generate bistability.

$$\frac{dX}{dt} = d_1 C_1 + k_4 C_4 - a_1 X \cdot kinase \quad [I]$$

$$\frac{dXP}{dt} = k_1 C_1 + k_3 C_3 + d_2 C_2 + d_4 C_4 - a_2 XP \cdot kinase - a_4 XP \cdot p'ase \quad [II]$$

$$\frac{dXPP}{dt} = k_2 C_2 + d_3 C_3 - a_3 XPP \cdot p'ase \quad [III]$$

$$\frac{dC_1}{dt} = a_1 X \cdot kinase - (d_1 + k_1) C_1 \quad [IV]$$

$$\frac{dC_2}{dt} = a_2 XP \cdot kinase - (d_2 + k_2) C_2 \quad [V]$$

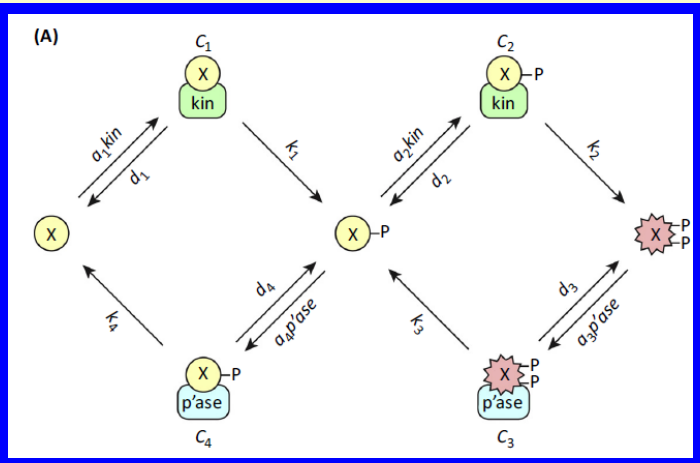
$$\frac{dC_3}{dt} = a_3 XPP \cdot p'ase - (d_3 + k_3) C_3 \quad [VI]$$

$$\frac{dC_4}{dt} = a_4 XP \cdot p'ase - (d_4 + k_4) C_4 \quad [VII]$$

$$X_{tot} = X + XP + XPP + C_1 + C_2 + C_3 + C_4 \quad [VIII]$$

$$kinase_{tot} = kinase + C_1 + C_2 \quad [IX]$$

$$p'ase_{tot} = p'ase + C_3 + C_4 \quad [X]$$



$a_1 = 0.004;$

$d_1 = 0.00016;$

$k_1 = 0.00016;$

$a_2 = 8;$

$d_2 = 0.32;$

$k_2 = 0.32;$

$a_3 = 1;$

$d_3 = 0.04;$

$k_3 = 0.04;$

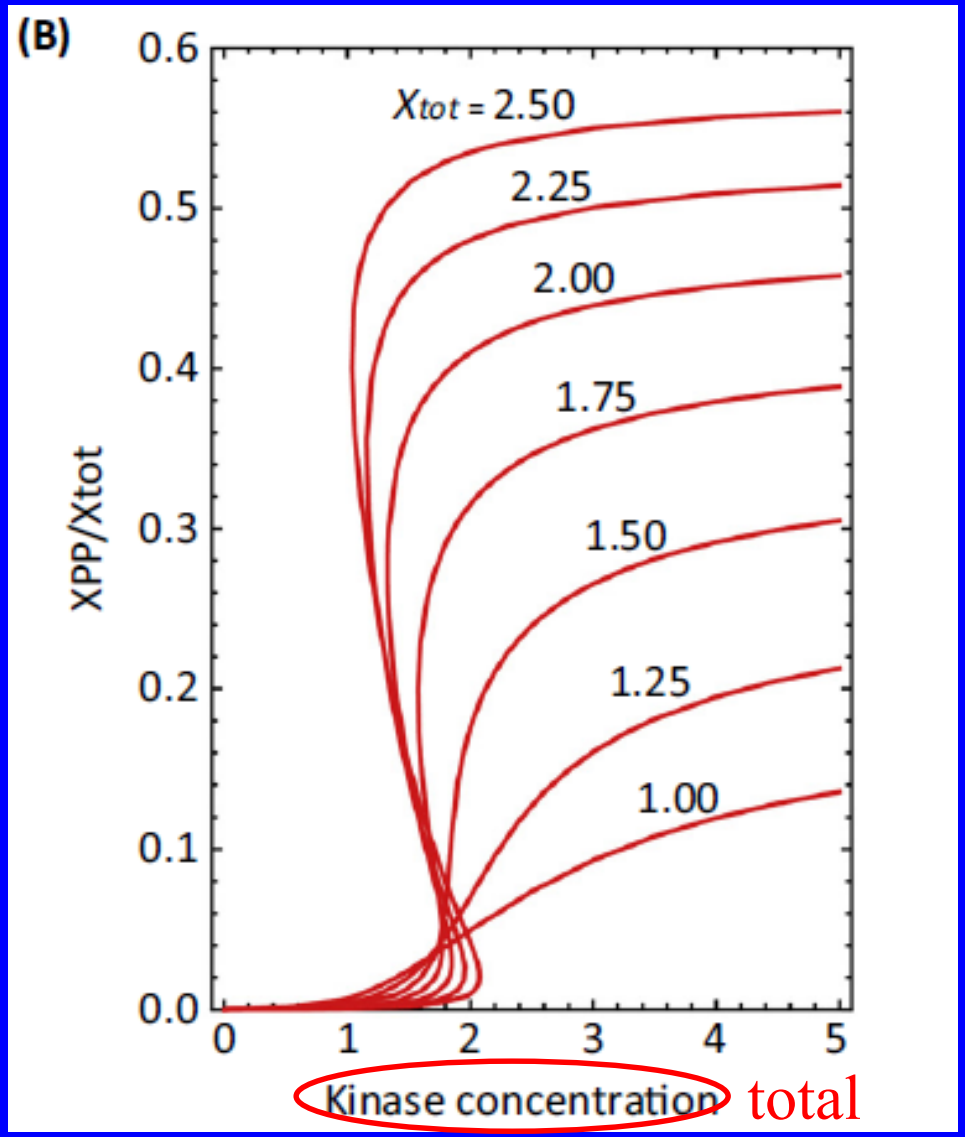
$a_4 = 0.1;$

$d_4 = 0.004;$

$k_4 = 0.004;$

$p'_{asetot} = 1.$

Bistable responses emerge when the enzymes are saturated and the substrate concentration is high

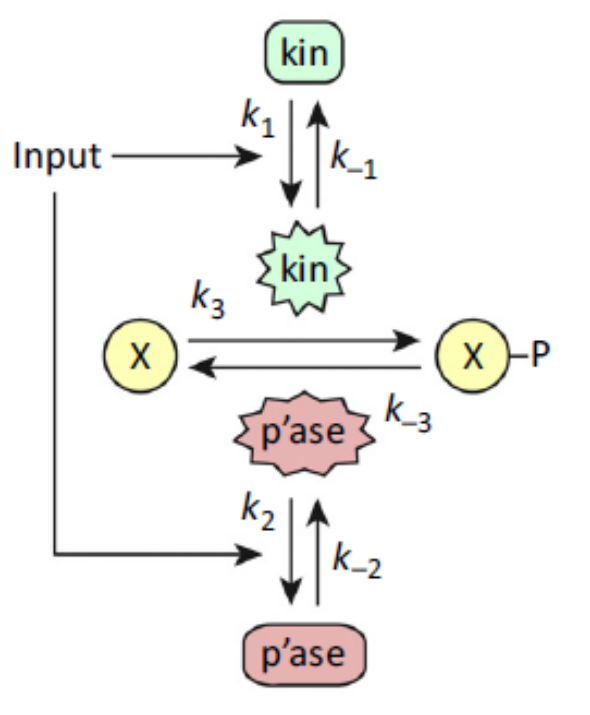


The existence of mulsistability by implicit loop

Thus far, the documented instances of multistability in cellular regulation have always involved explicit positive or double negative feedback loops, rather than (or in addition to) these implicit loops. That said, it is hard to believe that nature does not utilize thes clever mechanism somewhere.

Maybe implicit loops can be utilized in synthetic circuits to produce multistability.

Reciprocal regulation



(A) Schematic depiction of an input that activates a kinase and inactivates an opposing phosphatase.

An example is the control of Cdk1 by the DNA damage checkpoint proteins Chk1 and Chk2, which activate the Cdk1-inactivating kinase Wee1 and inactivate the Cdk1-activating phosphatase cell division cycle protein 25C (Cdc25).

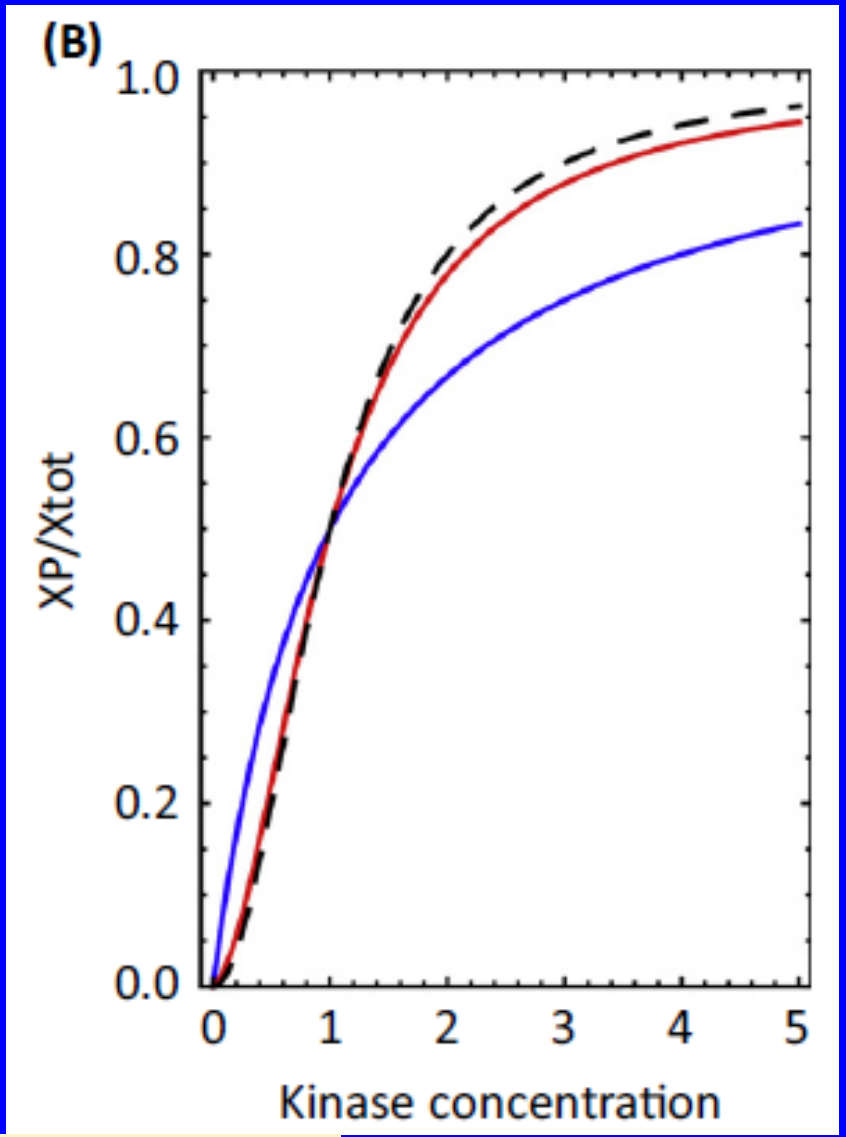
$$0 = k_1(1 - kinase_{act})input - k_{-1}kinase_{act}$$

$$0 = k_2\, p'ase_{act}\, input - k_{-2}(1 - p'ase_{act})$$

$$\frac{dXP}{dt} = k_3 kinase_{act}(X_{tot} - XP) - k_{-3}\, p'ase_{act} \cdot XP$$

$$\frac{XP}{X_{tot}} = \frac{K_2 input + input^2}{K_1K_2K_3 + (K_2 + K_2K_3)input + input^2}$$

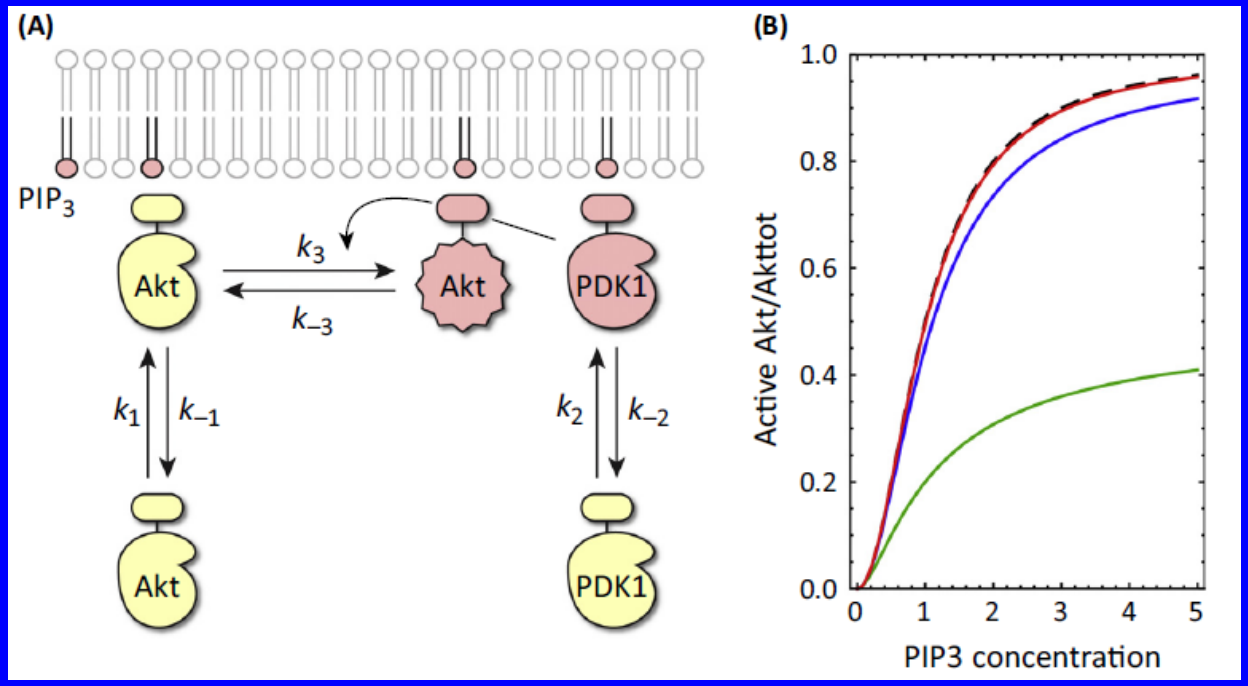
Ultrasensitivity from reciprocal regulation, based upon Equation VI in Box 5. The blue curve assumes that all of the K values equal 1; it is a Michaelian response. The red curve assumes that $K_1 = 10$, $K_2 = 0.1$, and $K_3 = 1$. The broken black curve is a Hill curve with a Hill exponent of 2.



$$\frac{XP}{X_{tot}} = \frac{input^2}{K_1K_2K_3 + input^2}$$

Feed-forward regulation

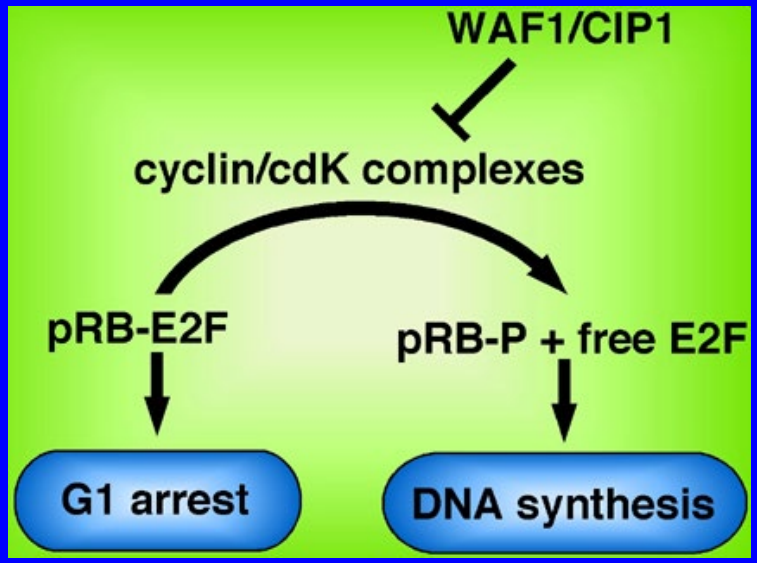
In fact, the dual phosphorylation mechanism described above is one type of feed-forward regulation. The kinase contributes to the activation of X both directly and indirectly.



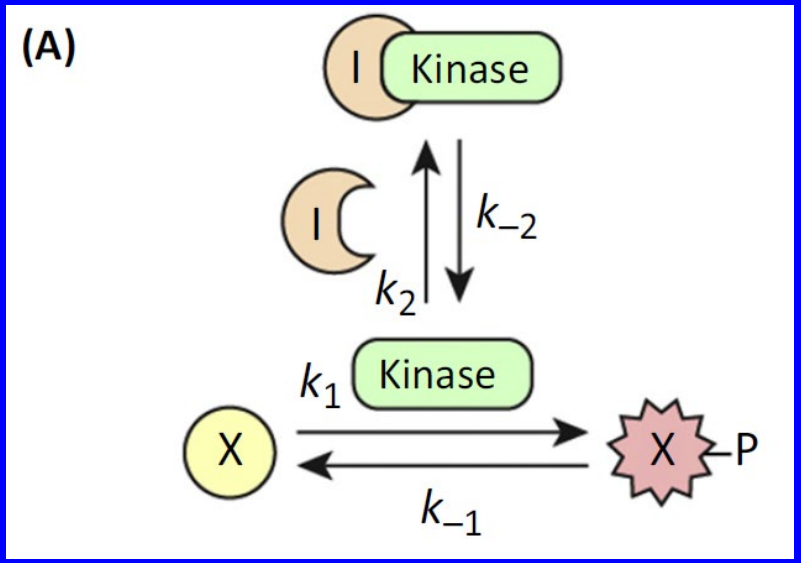
$$\frac{Akt_{act}}{Akt_{tot}} = \frac{PIP_3^2}{K_1K_2K_3 + (K_1K_3 + K_2K_3)PIP_3 + (1 + K_3)PIP_3^2}$$

Stoichiometric inhibitors and inhibitor ultrasensitivity

Inhibition of CDKs by p21



Schematic depiction of the inhibition of substrate (X) phosphorylation by a high affinity stoichiometric inhibitor (I) of the kinase



$$\frac{dXP}{dt} = k_1 kinase(X_{tot} - XP) - k_{-1} XP$$

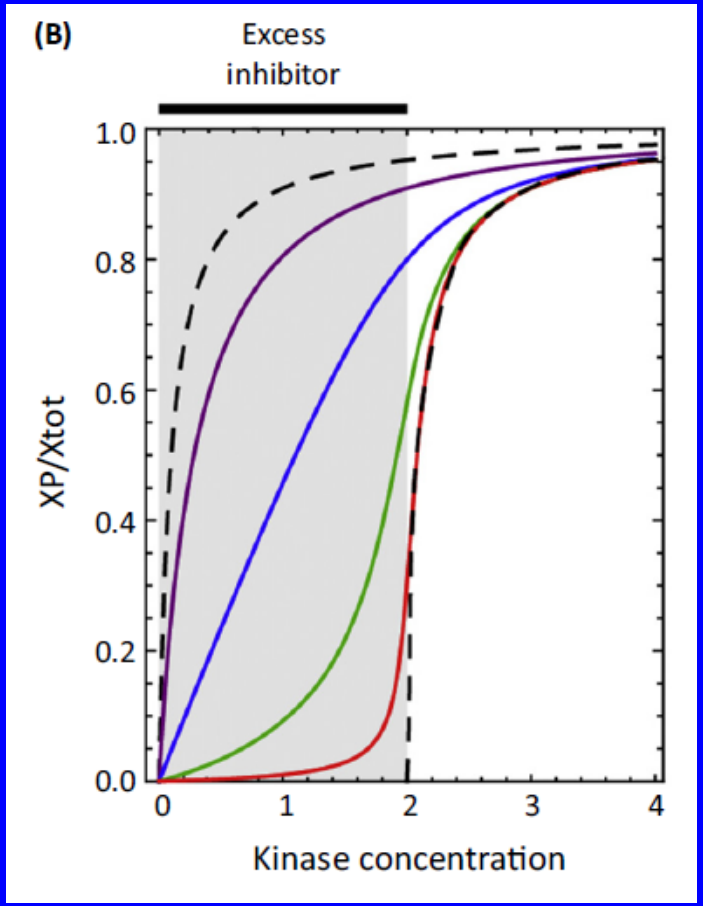
$$\frac{dkinase}{dt} = -k_2 kinase \cdot I + k_{-2} complex$$

$$I_{tot} = I + complex$$

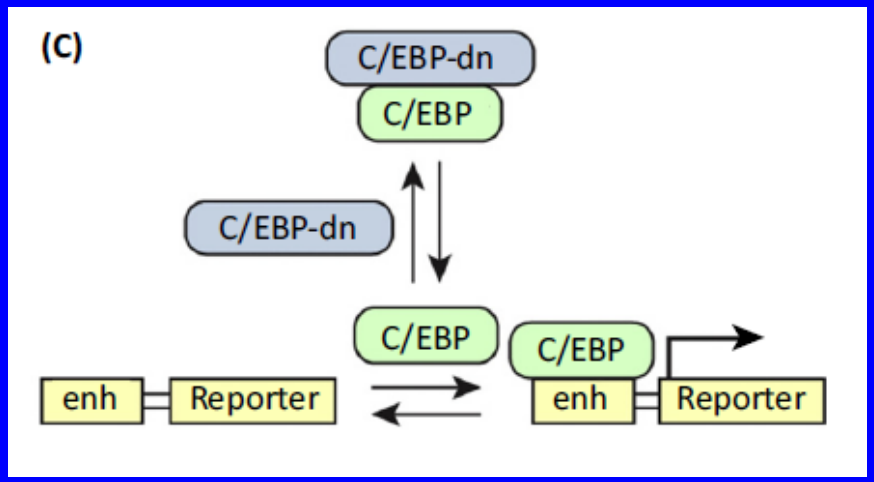
$$kinase_{tot} = kinase + complex$$

$$\frac{XP}{X_{tot}} = \frac{K_1 I_{tot} + K_1 K_2 - K_1 kinase_{tot} + 2K_2 kinase_{tot} - K_1 \sqrt{I_{tot}^2 + 2\left(K_2 - kinase_{tot}\right) I_{tot} + \left(K_2 + kinase_{tot}\right)^2}}{2K_1 I_{tot} - 2\left(K_1 - K_2\right)\left(K_1 + kinase_{tot}\right)}$$

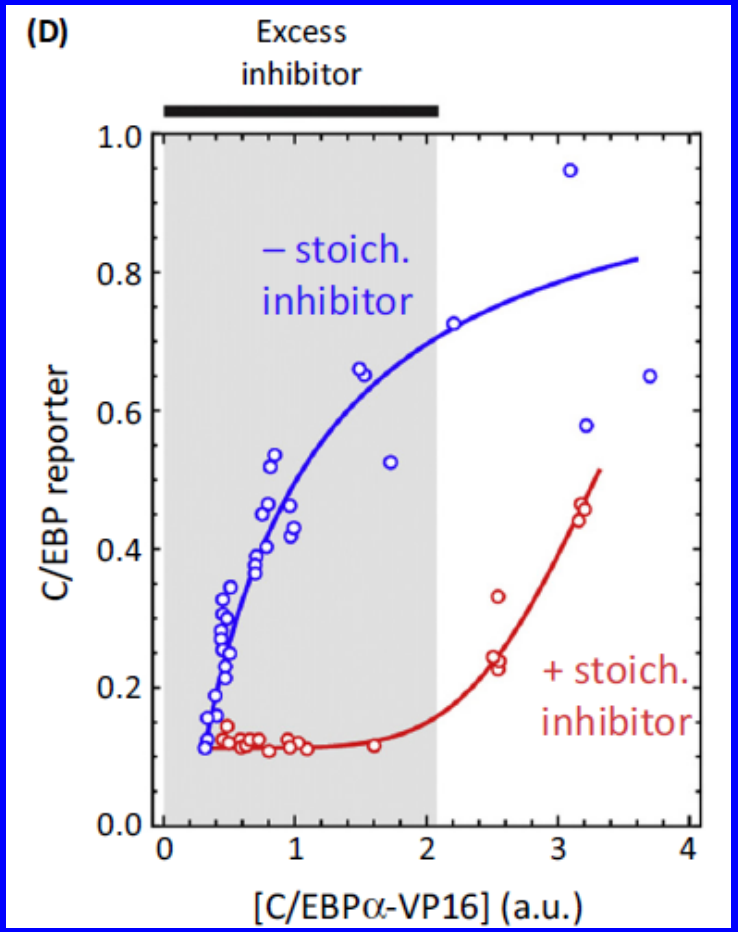
Inhibitor ultrasensitivity. The curves correspond to $X_{tot} = 1$, $I_{tot} = 2$, $K_1 = 0.1$, and $K_2 = 1$ (purple), 0.1 (blue), 0.01 (green), or 0.001 (red). The red curve has an effective Hill exponent of 9.7. The broken black curves show responses assuming no inhibitor (left) or two units of inhibitor with a K_2 value approaching zero (right).



Engineering ultrasensitivity by Stoichiometric inhibitors



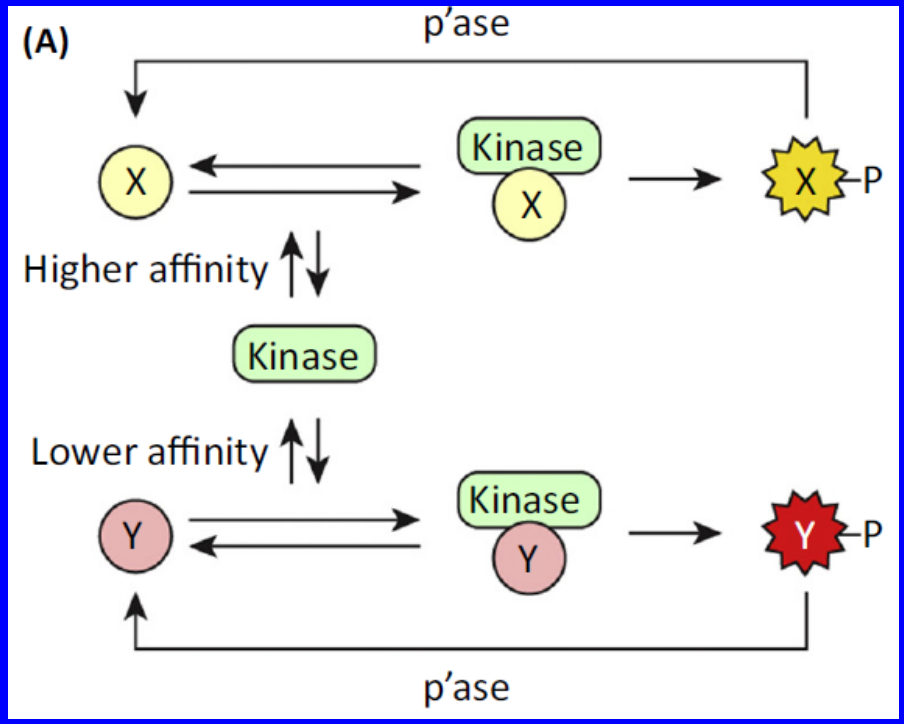
A high affinity stoichiometric inhibitor can add a threshold to the response of a synthetic C/EBPa reporter, resulting in ultrasensitive responses.



Buchler, N.E. and Cross, F.R. (2009) Mol. Syst. Biol. 5, 272

Competing substrates

A high-affinity substrate can, in principle, act as an inhibitor of lower affinity substrates by competing for access to the kinase.



A competing substrates mechanism was proposed to explain the ultrasensitive response of the Wee1 protein to cyclin B1–Cdk1 in Xenopus egg extracts.

Model

$$\frac{dX}{dt} = -a_1 kinase \cdot X + d_1 complex_1 + k_{-1} XP$$

$$\frac{dY}{dt} = -a_2 kinase \cdot Y + d_2 complex_2 + k_{-2} YP$$

$$\frac{dcomplex_1}{dt} = a_1 kinase \cdot X - d_1 complex_1 - k_1 complex_1$$

$$\frac{dcomplex_2}{dt} = a_2 kinase \cdot Y - d_2 complex_2 - k_2 complex_2$$

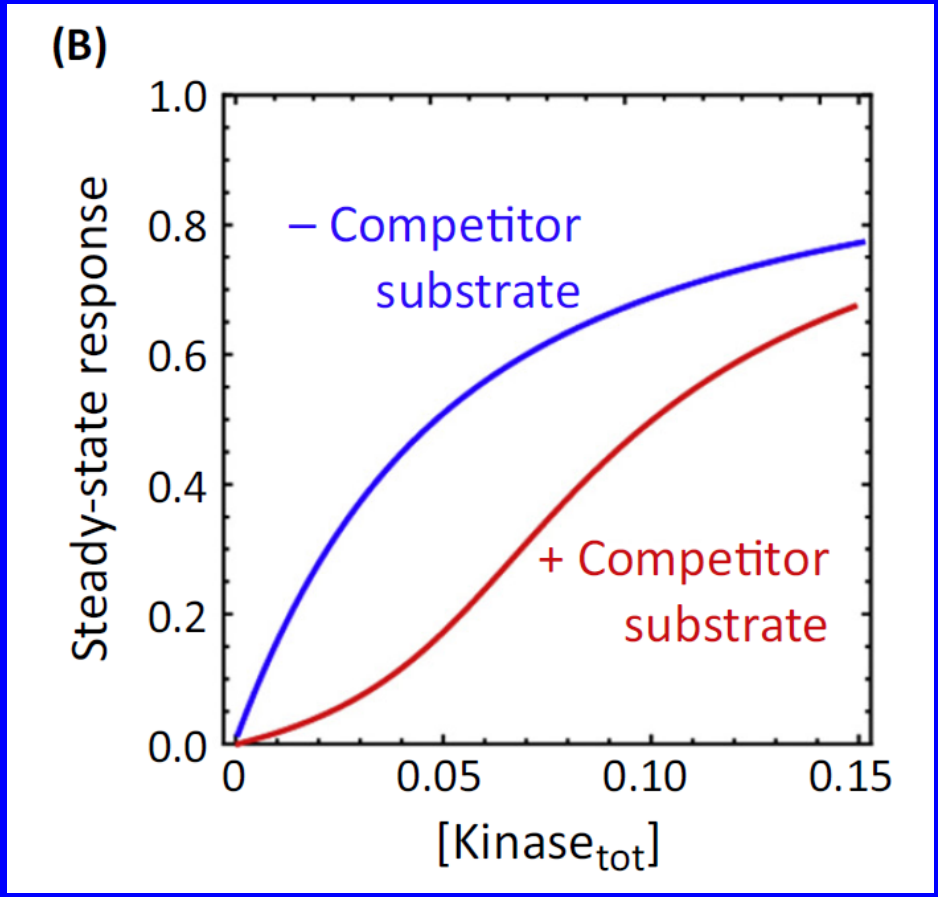
$$\frac{dXP}{dt} = k_1 complex_1 - k_{-1} XP$$

$$\frac{dYP}{dt} = k_2 complex_2 - k_{-2} YP$$

$$\frac{dkinase}{dt} = -a_1 kinase \cdot$$

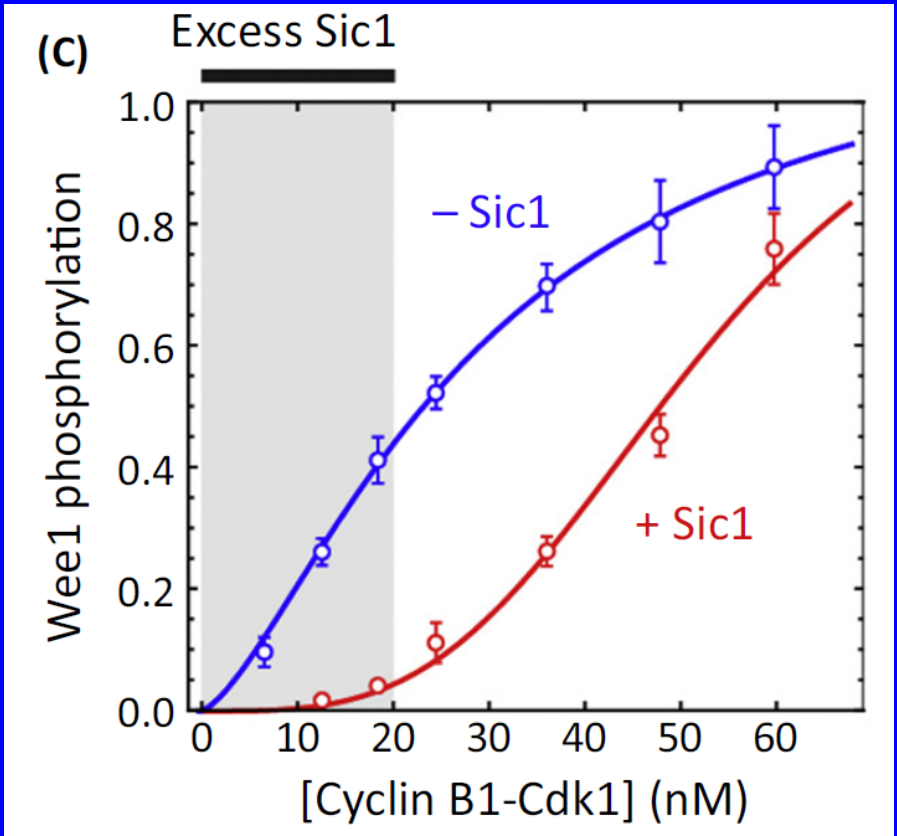
$$X + (d_1 + k_1) complex_1 - a_2 kinase \cdot Y + (d_2 + k_2) complex_2$$

Ultrasensitive response of substrate Y to the kinase in the presence (red) or absence (blue) of a high affinity competitor X



The high-affinity substrate (X) ties up the first increments of kinase. Only after this substrate is saturated would lower affinity substrates (Y) be phosphorylated.

Experimental demonstration of ultrasensitivity from substrate competition

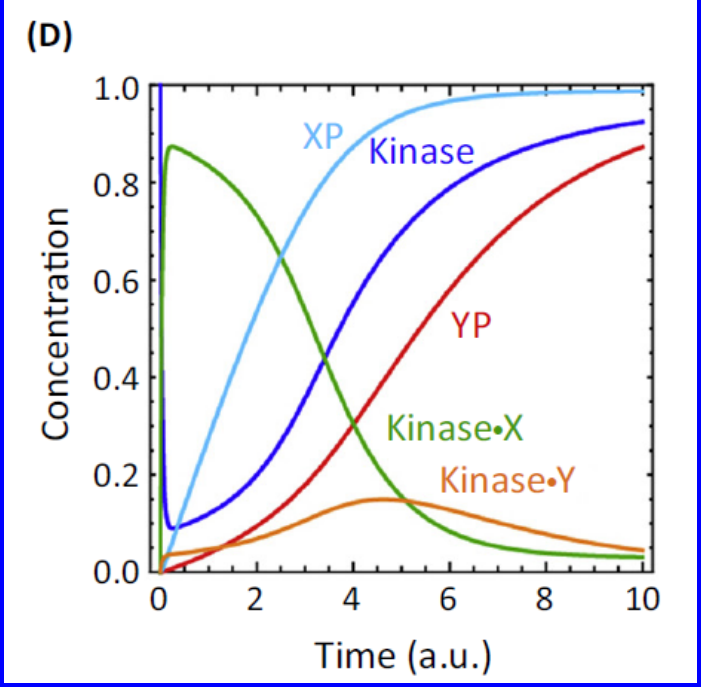


adding the high affinity Cdk1 substrate Sic1 – which is often regarded simply as a stoichiometric inhibitor but is also a Cdk1 substrate – to a reconstituted Cdk1/Wee1 system increases the ultrasensitivity of the Wee1 response

Dynamics of substrate competition

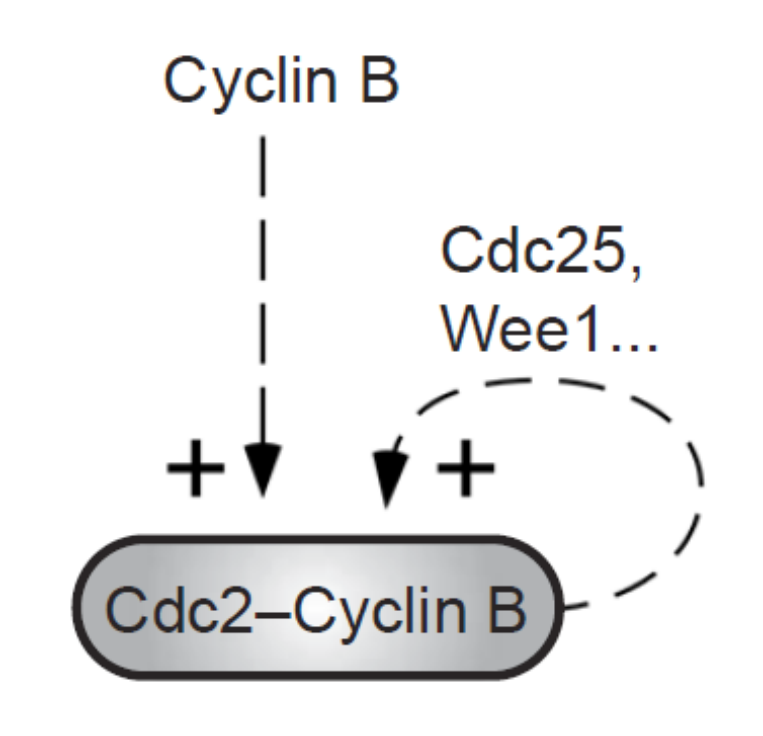
Temporal threshold from substrate competition

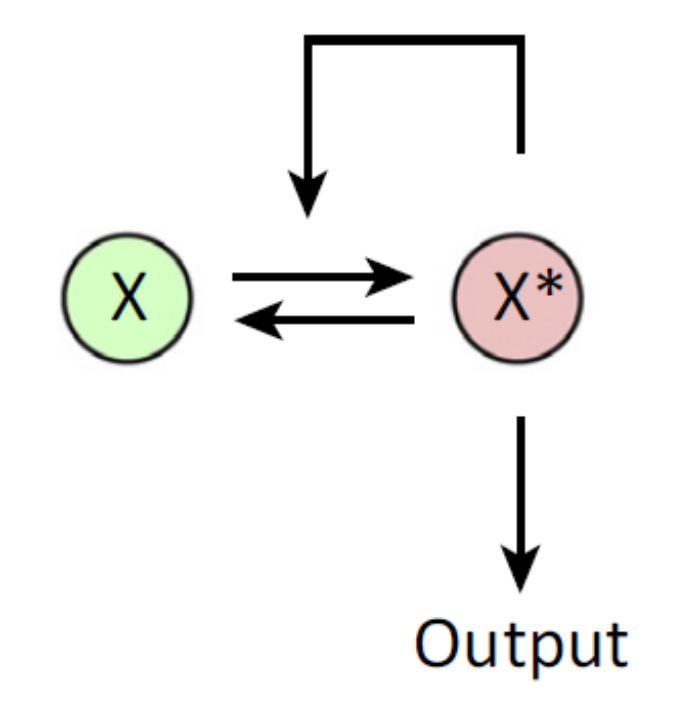
There is a time lag in the formation of complexes between the kinase and Y.



Temporal thresholds like this might allow master regulatory proteins that control the activity of hundreds of target proteins to generate distinct, temporally-ordered waves of substrate phosphorylation.

Ultrasensitivity from positive feedback





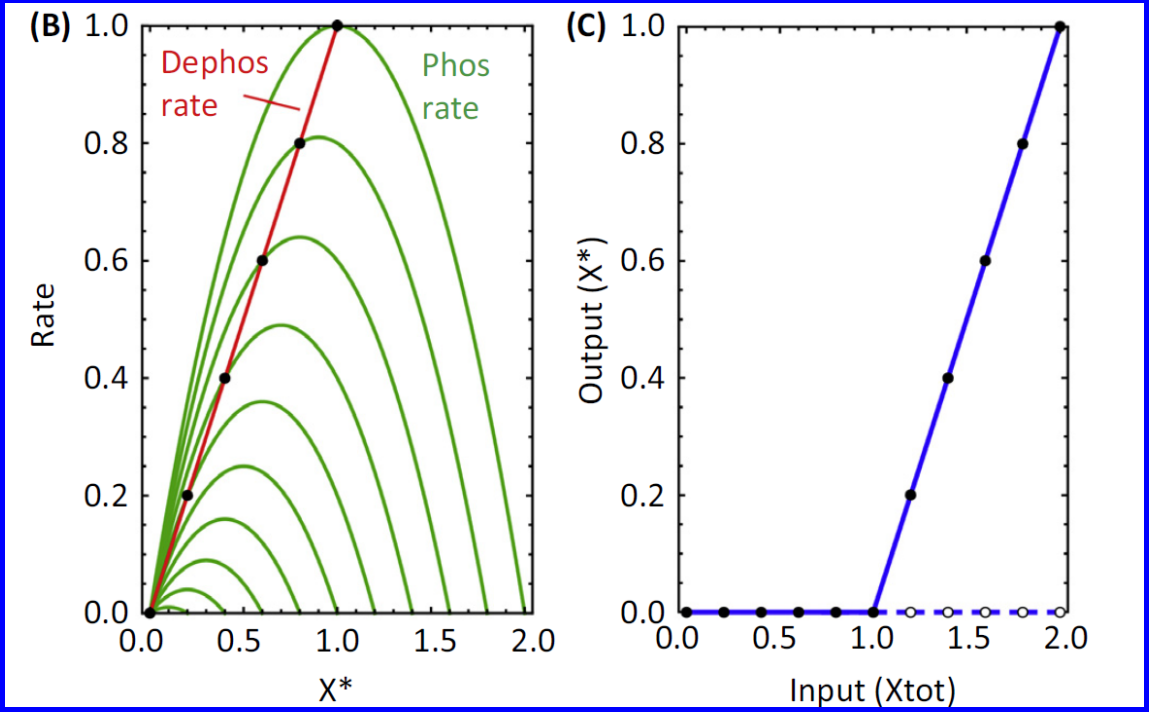
Cdk1 activates its activator (Cdc25C) and inhibits its inhibitor (Wee1A).

If the concentration of the X - X* complex is small compared to X and X*, then the system can be described by a single rate equation:

$$\frac{dX^*}{dt} = k_1(X_{tot} - X^*)X^* - k_{-1}X^*$$

$$X^* = 0 \; or \; X^* = X_{tot} - \frac{k_{-1}}{k_1}$$

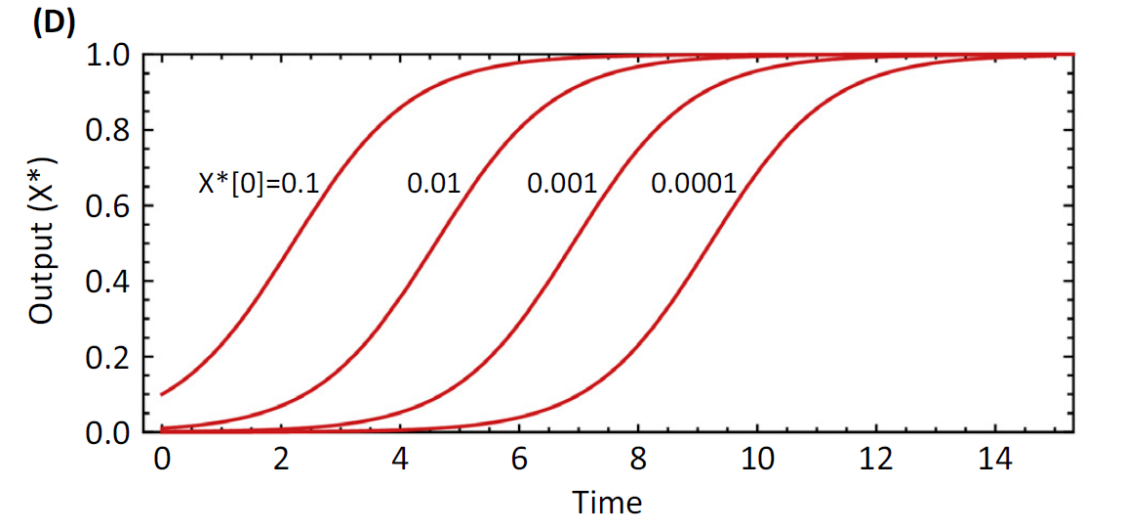
The system has a discrete threshold below which there is no output, and then above the threshold the output increases sharply with the input.



Transcritical bifurcation

Time lags in the response by positive feedback

Time course for a system responding to two units of input, assuming various initial values of X*.



If the system starts with X* exactly equal to zero, there will be no response to the input stimulus; the system just sits on its unstable steady state.

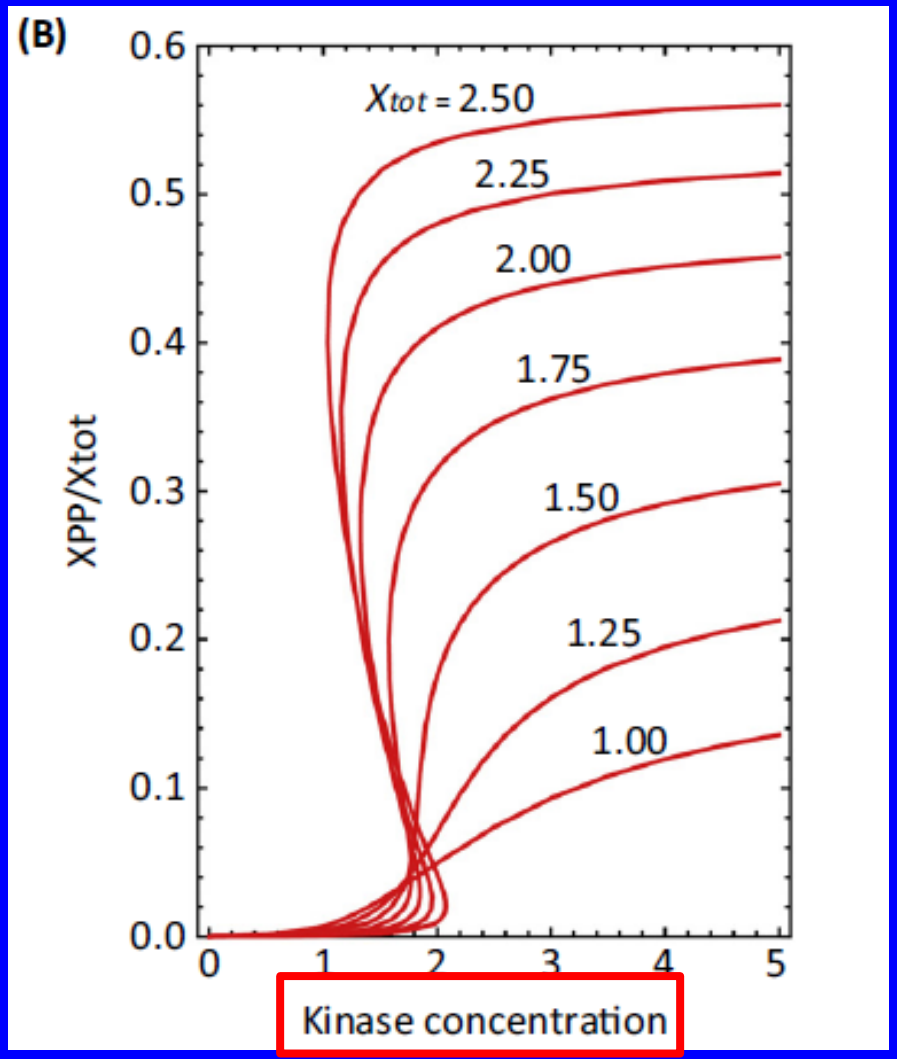
Summary

- Three mechanisms for ultrasensitivity: multisite phosphorylation, stoichiometric inhibitors, and positive feedback.

- There is a limited amount of experimental evidence to support the occurrence of several of these mechanisms.

Homework

Reproducing "Bistable response by Implicit positive feedback in multisite phosphorylation"



$kinase_{tot}$

$$\frac{dX}{dt} = d_1 C_1 + k_4 C_4 - a_1 X \cdot kinase \quad [I]$$

$$\frac{dXP}{dt} = k_1 C_1 + k_3 C_3 + d_2 C_2 + d_4 C_4 - a_2 XP \cdot kinase - a_4 XP \cdot p'ase \quad [II]$$

$$\frac{dXPP}{dt} = k_2 C_2 + d_3 C_3 - a_3 XPP \cdot p'ase \quad [III]$$

$$\frac{dC_1}{dt} = a_1 X \cdot kinase - (d_1 + k_1) C_1 \quad [IV]$$

$$\frac{dC_2}{dt} = a_2 XP \cdot kinase - (d_2 + k_2) C_2 \quad [V]$$

$$\frac{dC_3}{dt} = a_3 XPP \cdot p'ase - (d_3 + k_3) C_3 \quad [VI]$$

$$\frac{dC_4}{dt} = a_4 XP \cdot p'ase - (d_4 + k_4) C_4 \quad [VII]$$

$$X_{tot} = X + XP + XPP + C_1 + C_2 + C_3 + C_4 \quad [VIII]$$

$$kinase_{tot} = kinase + C_1 + C_2 \quad [IX]$$

$$p'ase_{tot} = p'ase + C_3 + C_4 \quad [X]$$

$a_1 = 0.004;$
$d_1 = 0.00016;$
$k_1 = 0.00016;$
$a_2 = 8;$
$d_2 = 0.32;$
$k_2 = 0.32;$
$a_3 = 1;$
$d_3 = 0.04;$
$k_3 = 0.04;$
$a_4 = 0.1;$
$d_4 = 0.004;$
$k_4 = 0.004;$
$p'_{asetot} = 1.$

Ferrell, J. E., Jr, et al. (2014). Trends in Biochemical Sciences **39**(11): 556-569.