Review: Mechanisms of generating ultrasensitivity

- Zero-order ultrasensitivity

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

Both enzymes are saturated

- Multistep ultrasensitivity

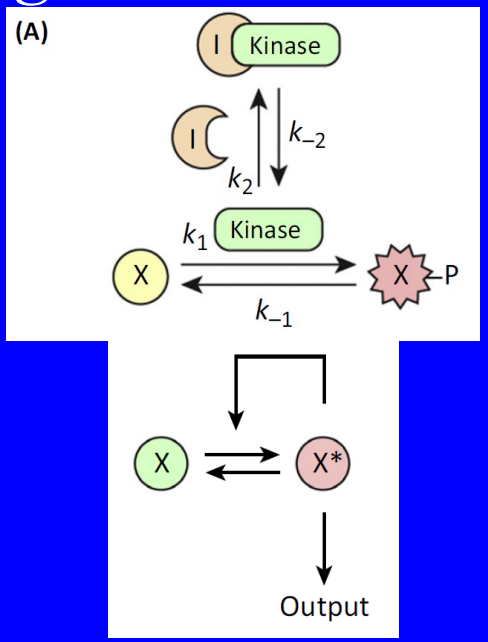
$$\frac{XP_n}{X_{tot}} = \frac{kinase^n}{(K_1 \cdots K_n) + (K_2 \cdots K_n) kinase + (K_3 \cdots K_n) kinase^2 + \ldots + kinase^n} \quad [VI]$$

Multisite phosphorylation, reciprocal regulation, and other forms of feed forward regulation

- Inhibitor ultrasensitivity

Stoichiometric inhibitors and substrate competition

- Positive feedback loops



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

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

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

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

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

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

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

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

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

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

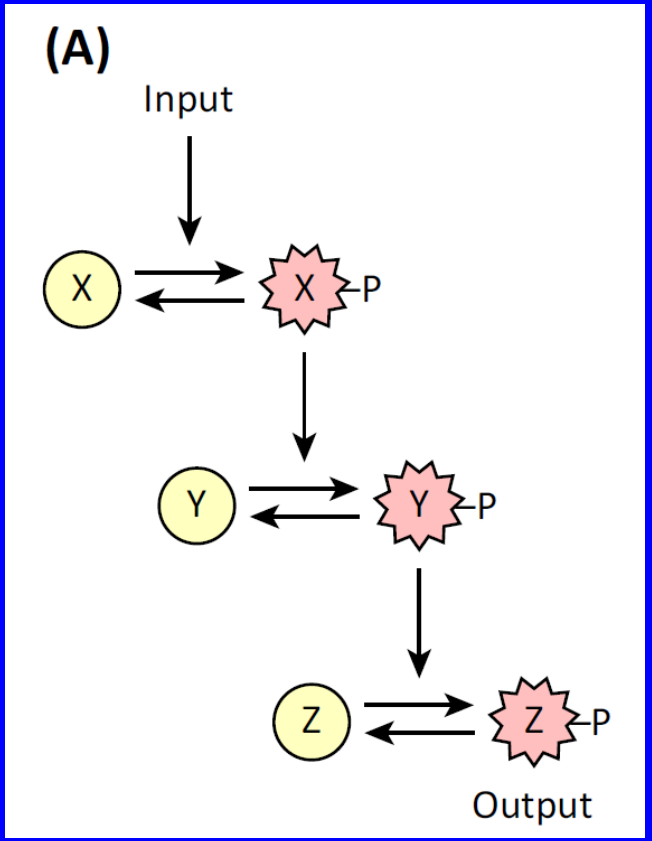
$a_1 = 0.004;$
$d_1 = 0.00016;$
$k_1 = 0.00016;$
$a_2 = 8;$
$d_2 = 0.32;$
$k_2 = 0.32;$
$a_3 = 1;$
$d_3 = 0.04;$
$k_3 = 0.04;$
$a_4 = 0.1;$
$d_4 = 0.004;$
$k_4 = 0.004;$
$p'_{asetot} = 1.$

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

2.3.3 Cascades, bistable switches, and oscillators

- Ultrasensitivity in signaling cascades

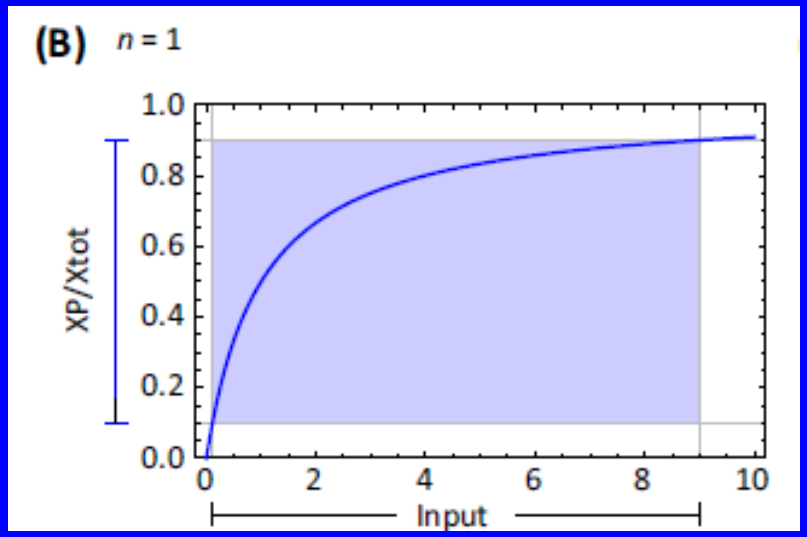
How would a signal change as it descended the cascade?



Michaelian cascades

First tier

$$Output = \frac{Input}{EC50 + Input}$$

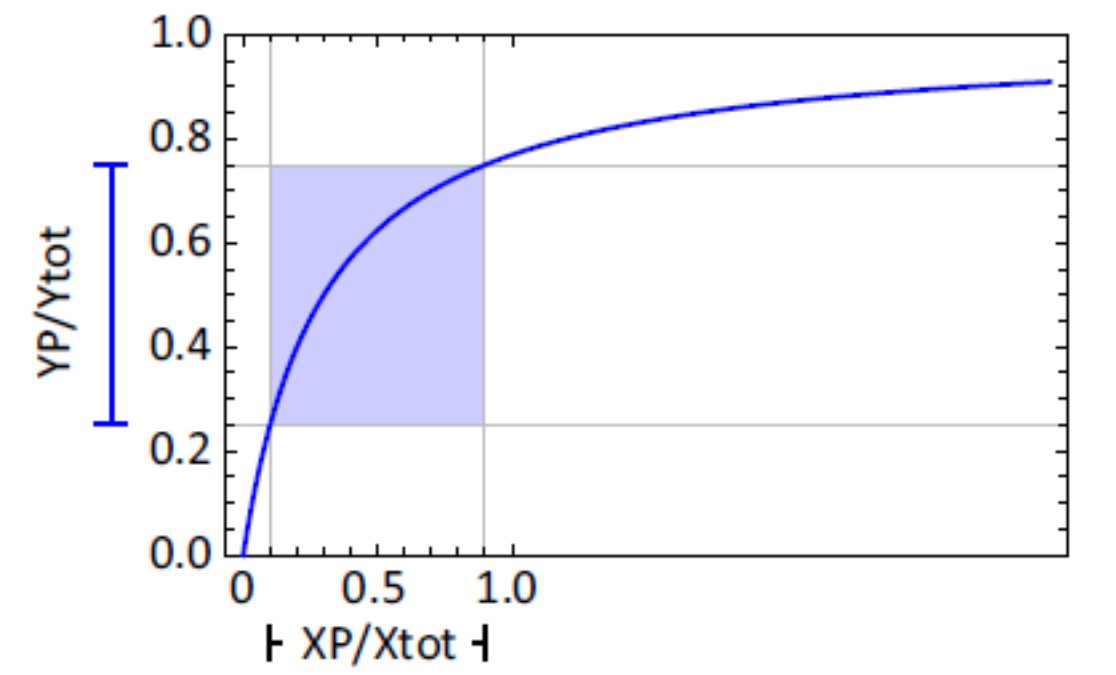


Supposing that initially we have an 81-fold change in input stimulus.

To maximize the difference in the output, we should center the EC50 at the geometric mean of the range of inputs.

*An 81-fold change in the input has yielded a **nine**-fold change in output !*

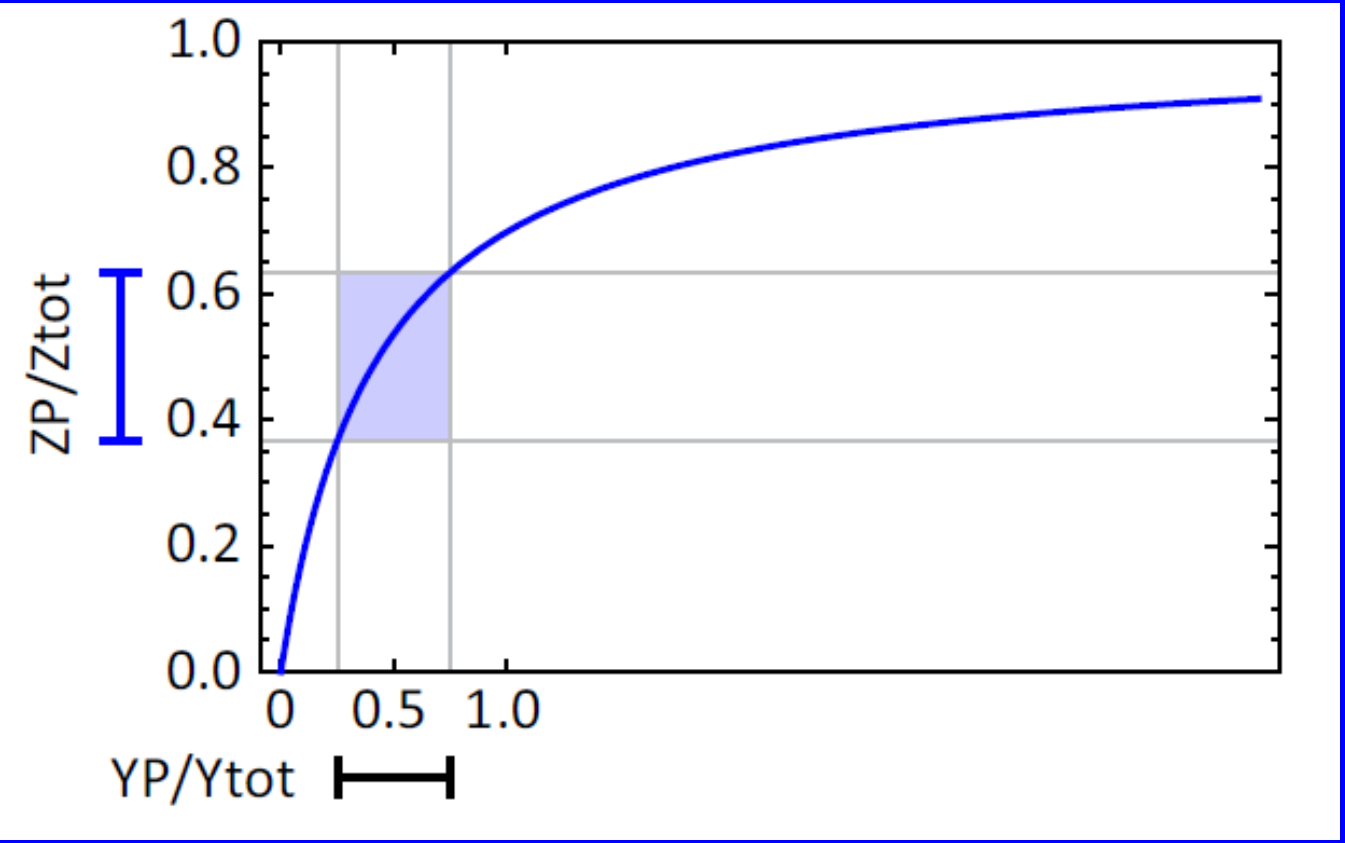
Second tier



A three-fold change in output !

$$Output = \frac{Input}{EC50 + Input}$$

Third tier



A $\sqrt{3}$ - fold change in output !

The change fold is decreasing in the cascade !

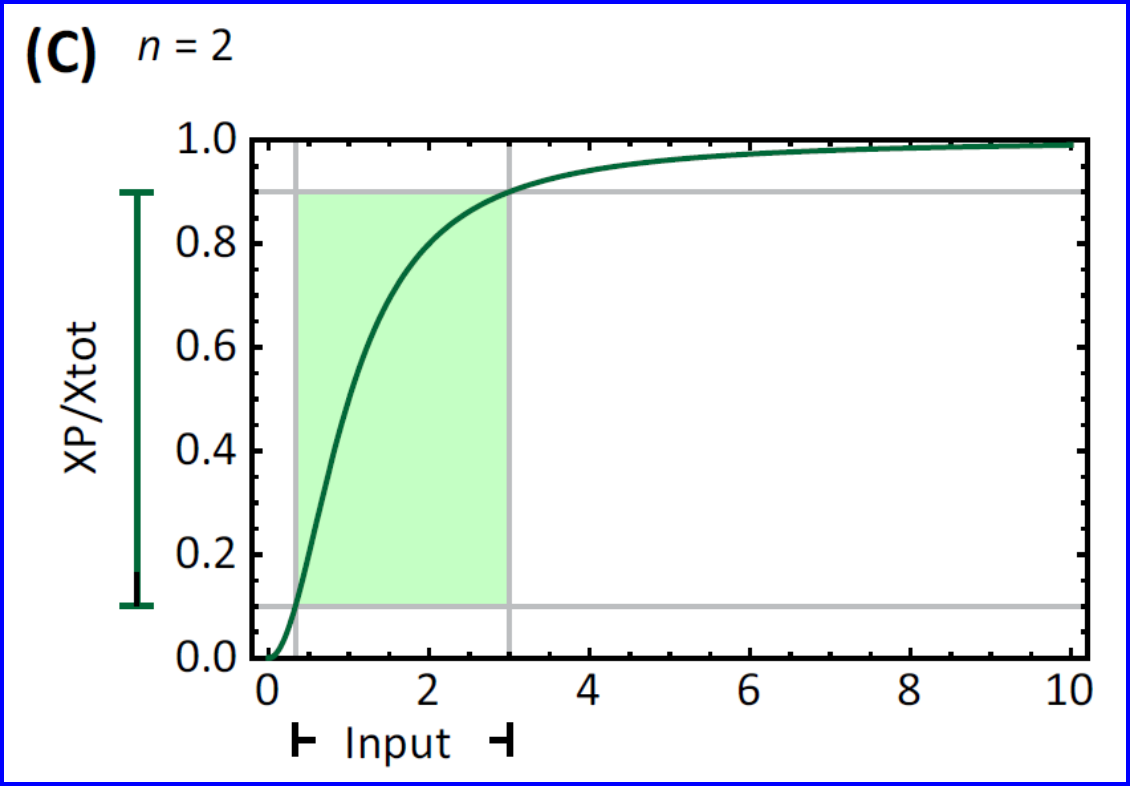
Ultrasensitive cascades

Each level's steady-state response to its upstream activator is ultrasensitive, described by a Hill function:

$$Output = \frac{Input^n}{EC50^n + Input^n}$$

Initially let us assume that the Hill exponent is two, and again center each input geometrically on its *EC50* to maximize the change in output.

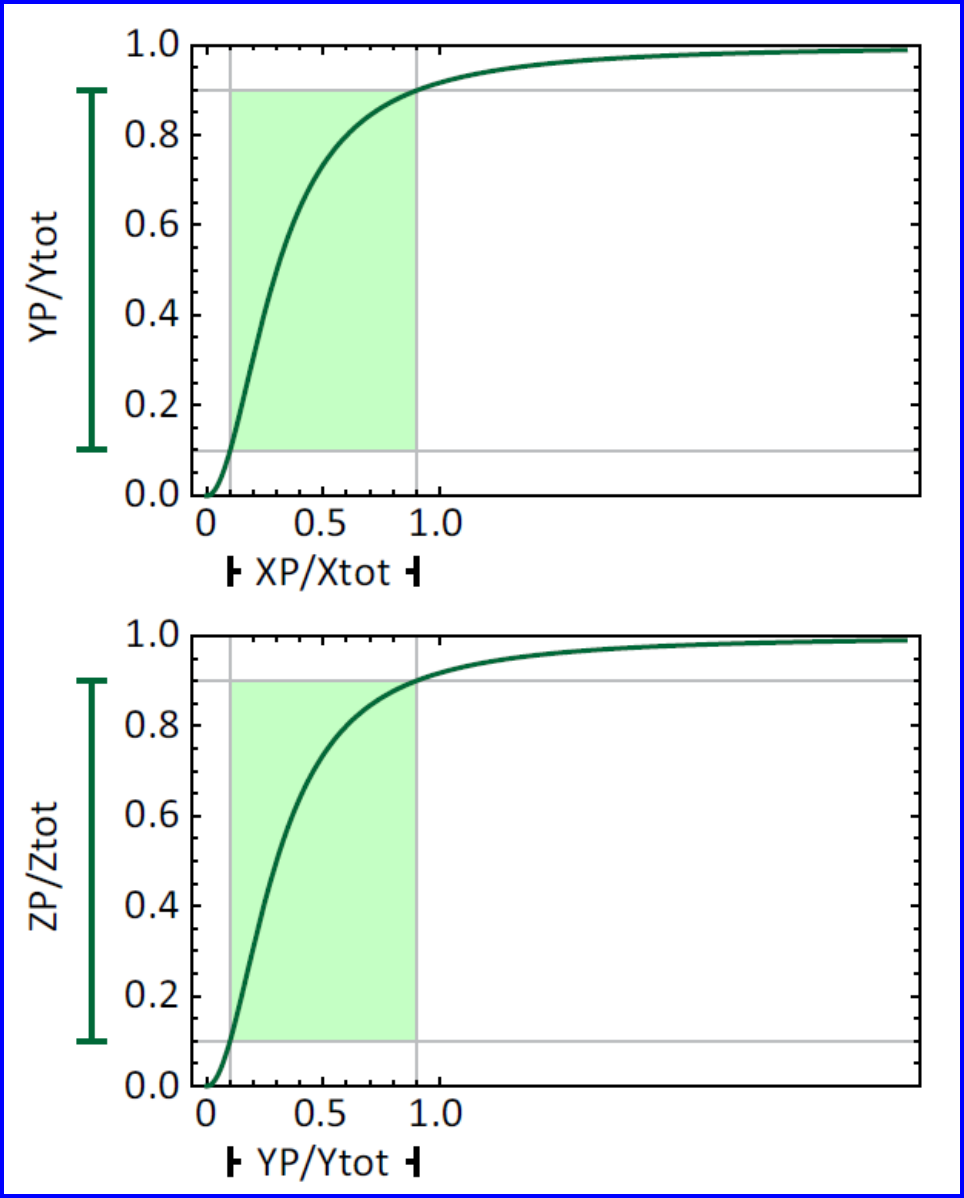
First tier

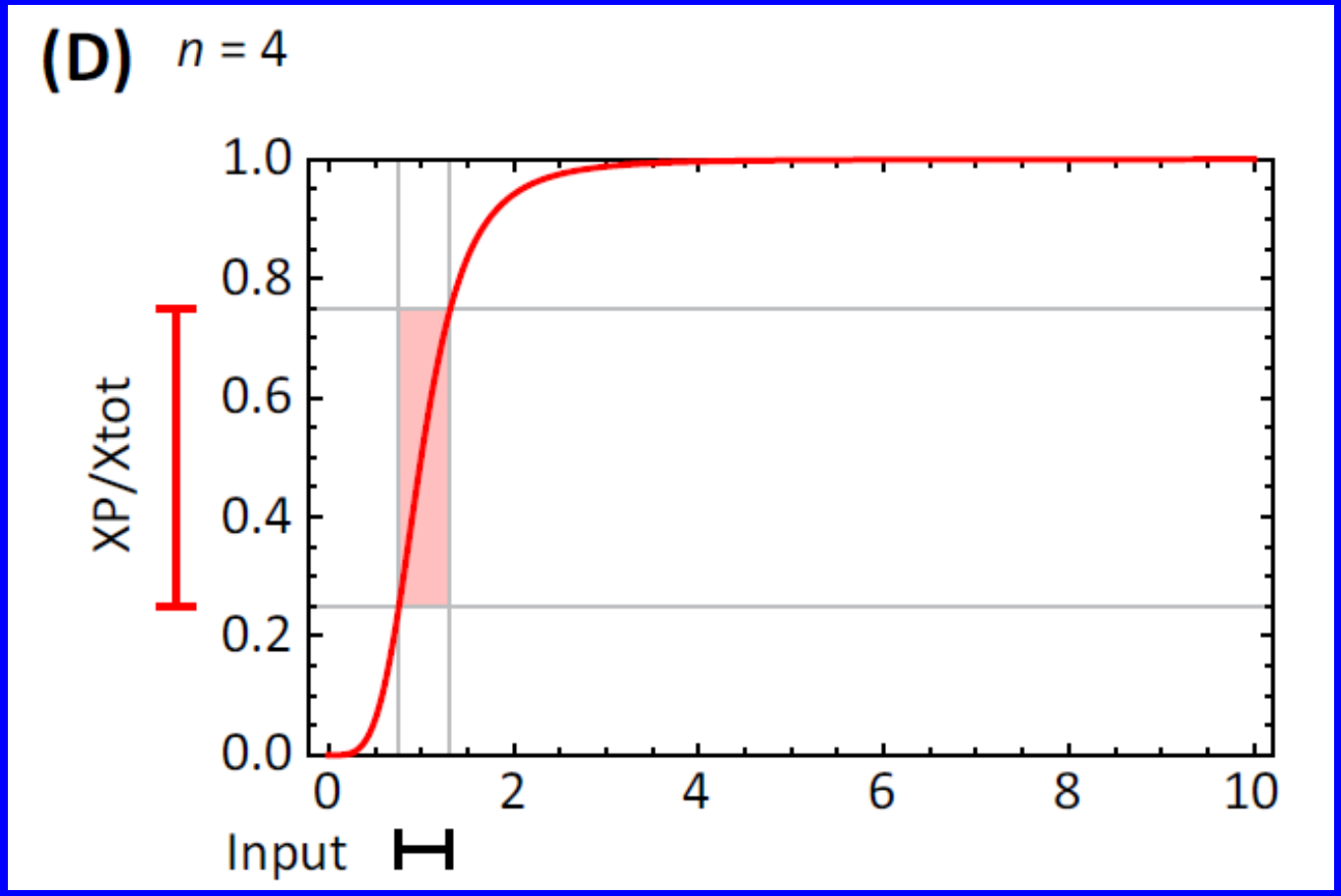


A nine-fold change in input produces a nine-fold change in XP!

The other two tiers

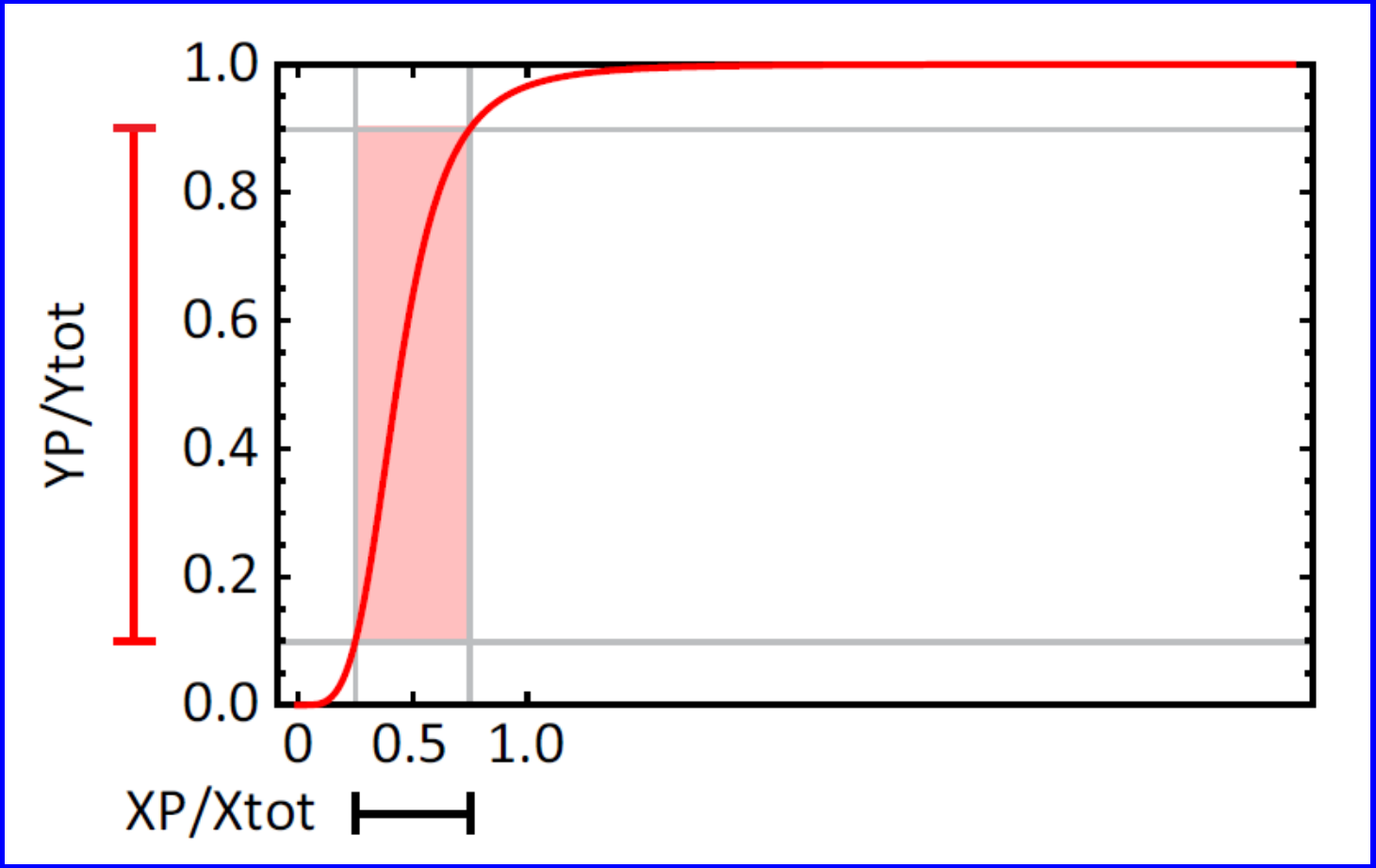
A nine-fold change in XP and YP produces a nine-fold change in YP and ZP, respectively.





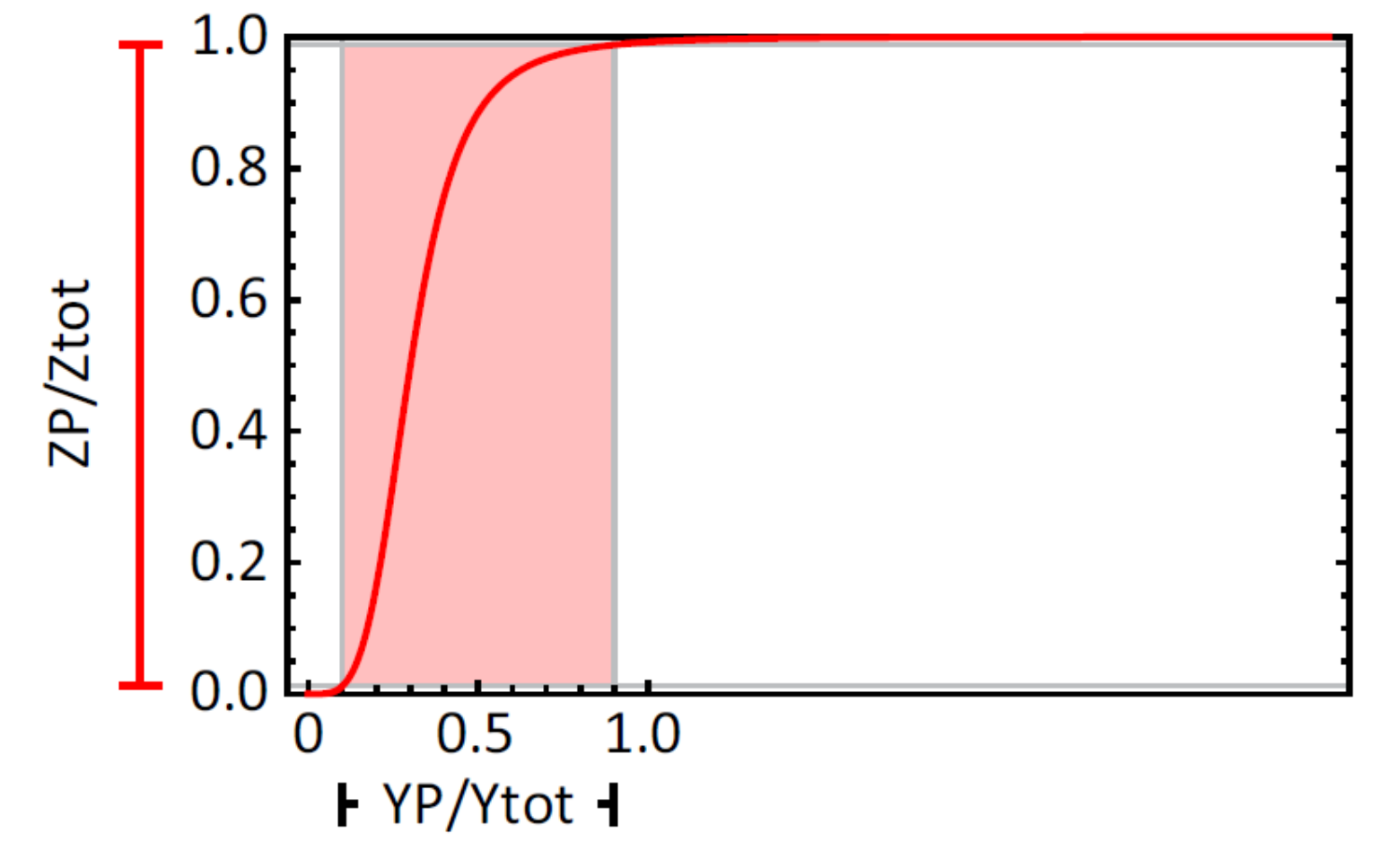
a $\sqrt{3}$ -fold change in input is translated into a three-fold change in XP!

Second tier



A nine-fold change in YP!

Third tier



An 81-fold change in ZP!

General rule

In general, if the input to the Hill function ranges between *Input1 = EC50/a* and *Input2 = EC50*a*, with *a > 1*, so that the ratio of the inputs is:

$$\frac{Input_2}{Input_1} = a^2, \quad \Rightarrow \quad \frac{Output_2}{Output_1} = a^n$$

The fold-change in the output is bigger than the fold change in the input if n > 2, and smaller if n < 2. Therefore, an ultrasensitive signaling cascade can convert a modest change in input signal into a highly switch-like output.

Sensitivity amplification from signaling cascades

Local sensitivity

$$S_{local} = \lim_{\Delta input \to 0} \frac{\Delta output}{output} / \frac{\Delta input}{input} = \frac{d \ln output}{d \ln input}$$

Local sensitivity function for the whole cascade

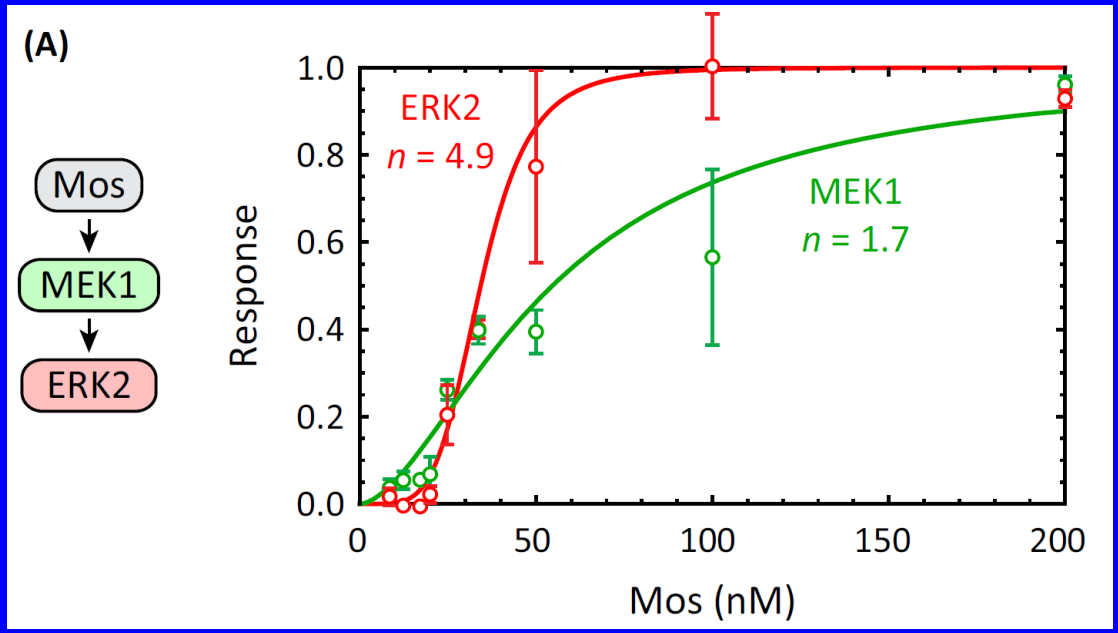
$$S_{local} = \frac{d \ln ZP}{d \ln input}$$

By the chain role, we have

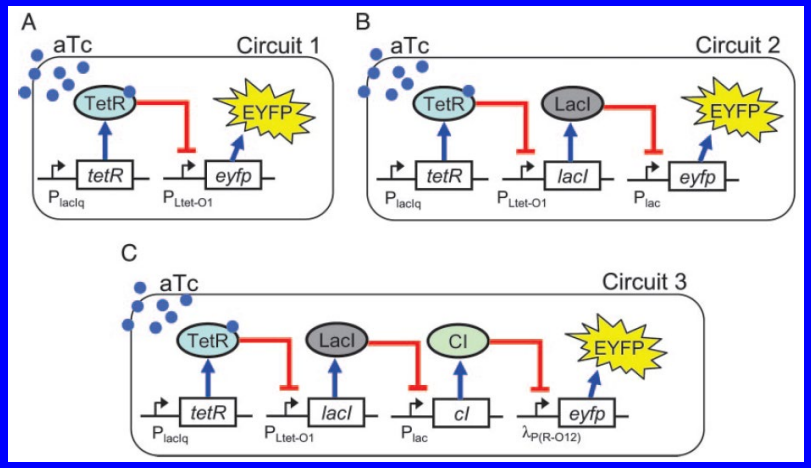
$$\frac{d \ln ZP}{d \ln input} = \frac{d \ln ZP}{d \ln YP} \frac{d \ln Y P}{d \ln XP} \frac{d \ln XP}{d \ln input}$$

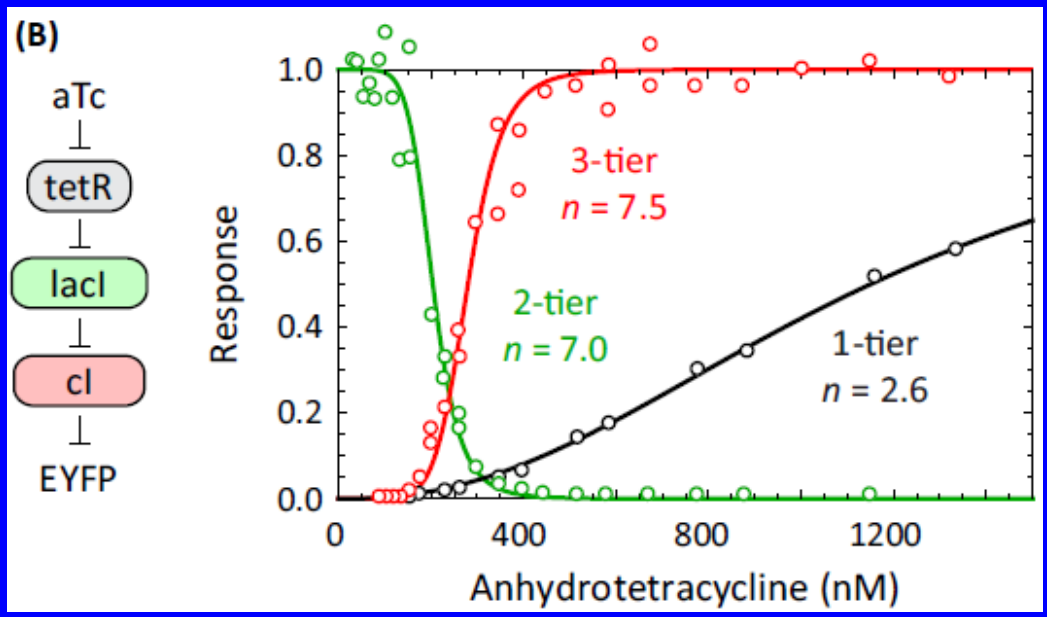
Ultrasensitivity amplification from signaling cascades : experimental evidence

A physiological protein kinase cascade. Steady-state response of the mitogen-activated protein kinase kinase MEK1 and ERK2 to recombinant bacterially-expressed Mos in Xenopus oocyte extracts.



Huang, C.-Y. F. and J. E. Ferrell, Jr. (1996). PNAS **93**(19): 10078-10083.





A synthetic transcriptional cascade. The input is ATC and the output is EYFP production. In the circuits, ATC regulates EYFP transcription and translation through one intermediary (tetR), two (tetR regulating lacI), or three (tetR regulating lacI which regulates cI) .

Hooshangi, S., S. Thiberge, et al. (2005). PNAS **102**(10): 3581-3586.

Ultrasensitivity in bistability

A highly ultrasensitive response can approach a step function in a monostable switch with no built-in memory.

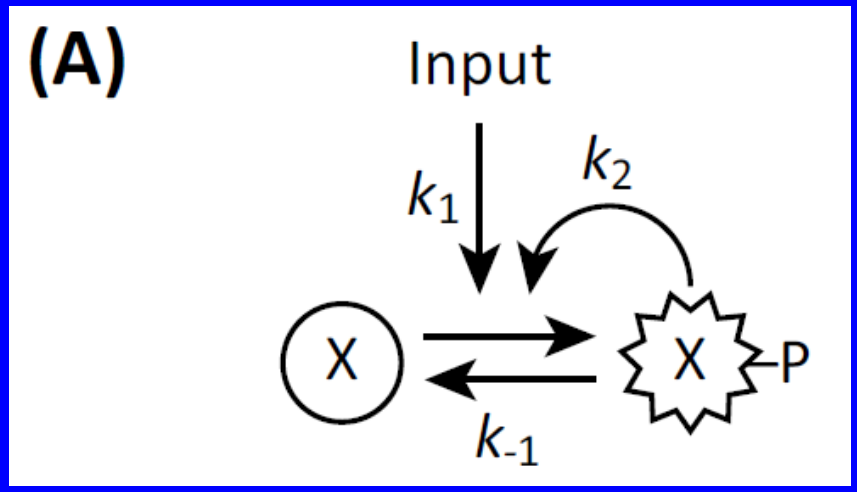
Example: doorbell buzzer

Systems with positive feedback loops (either implicit or explicit) can function as a bistable switch with hysteresis or irreversibility built into the system.

Example: toggle switch, like a light switch. Flip the switch and the light turns on and stays on indefinitely.

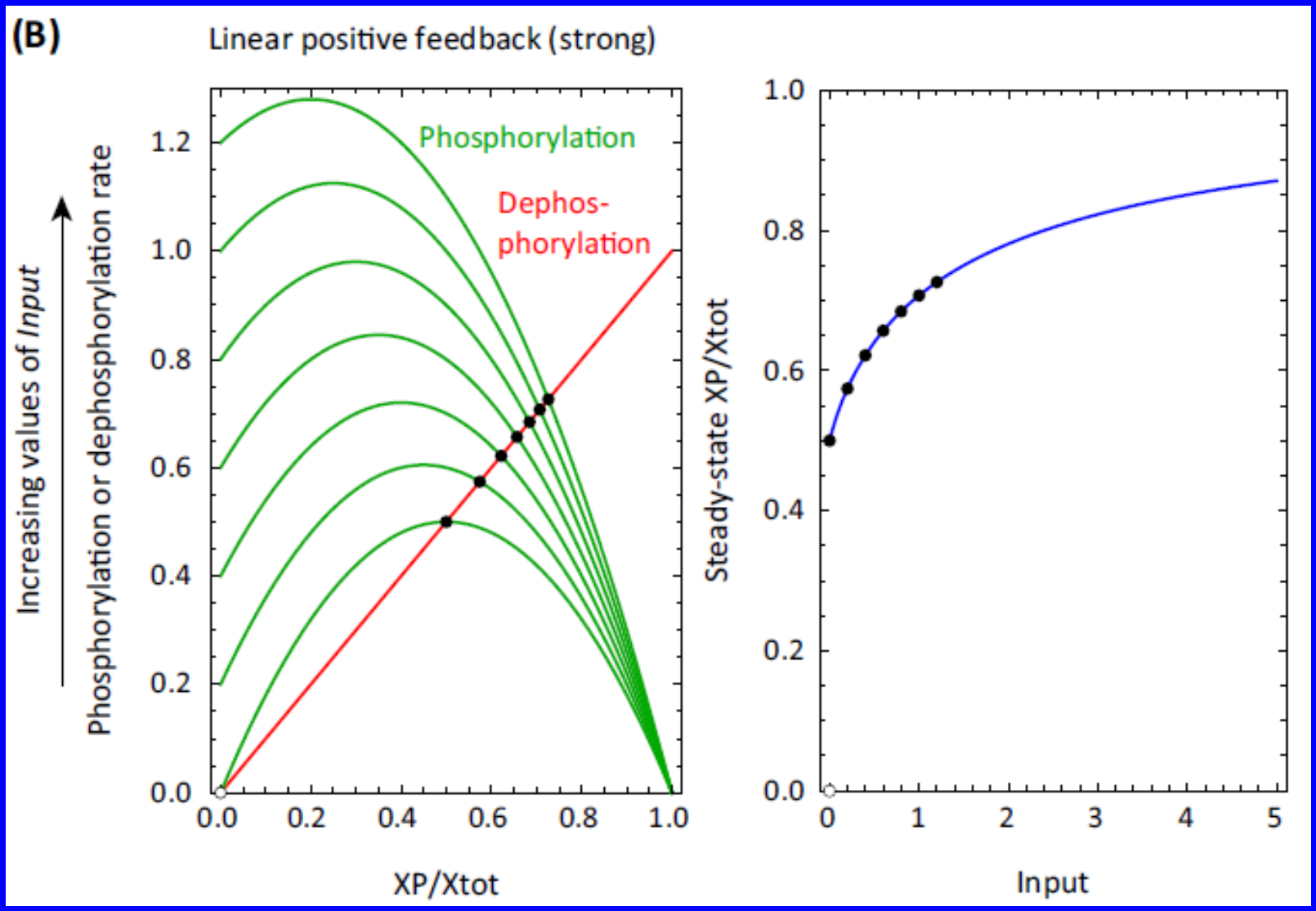
Bistability is not an inevitable consequence of positive feedback! Ultrasensitivity is required.

Bistability in a simple positive feedback model

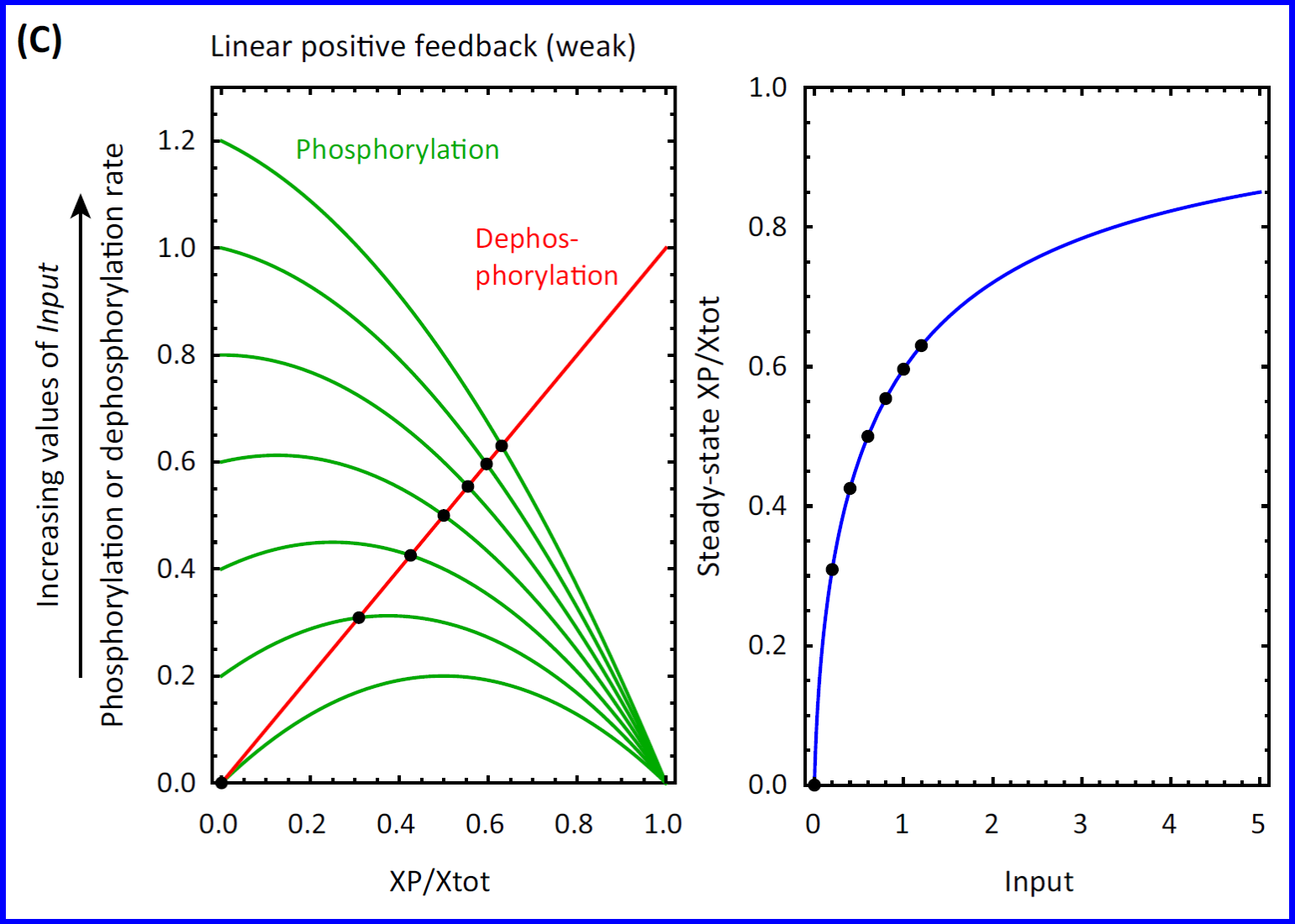


Mass action kinetics

$$\frac{dXP}{dt} = (k_1 Input + k_2 XP)(X_{tot} - XP) - k_{-1} XP$$

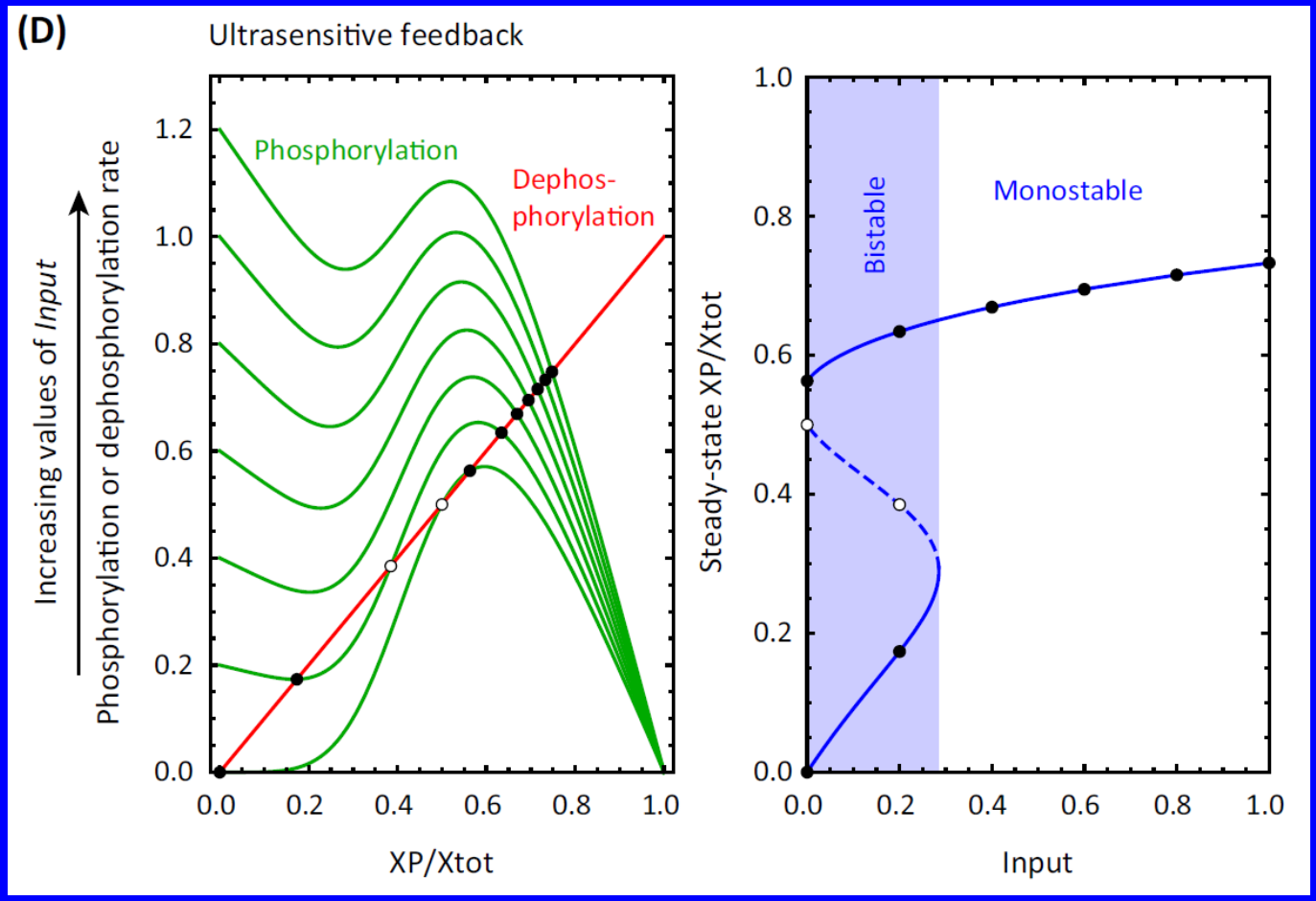


(B) $k_1 = 1,\ k_{-1} = 1,\ k_2 = 2,\ f[XP] = XP;$

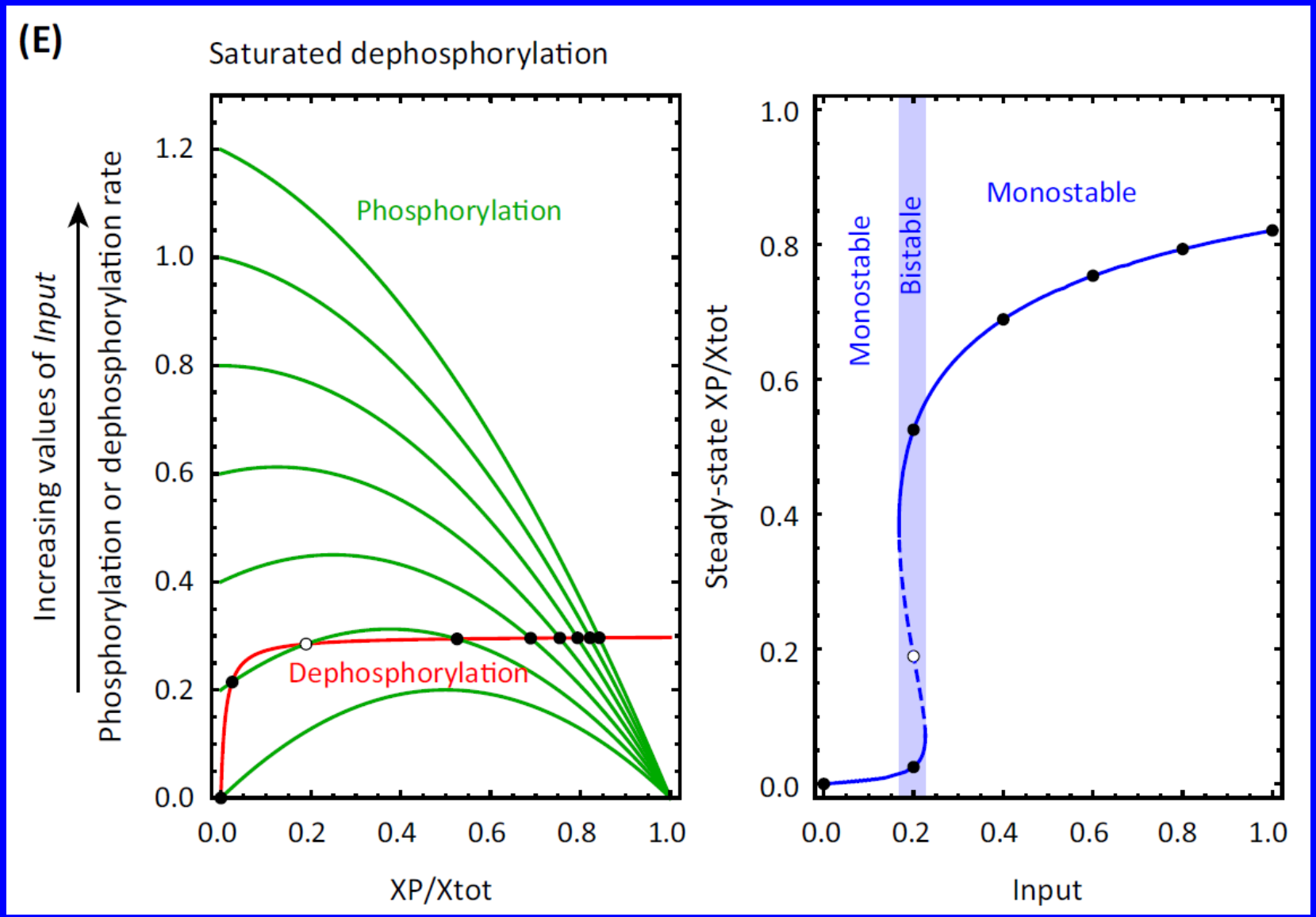


(C) $k_1 = 1,\ k_{-1} = 1,\ k_2 = 0.8,\ f[XP] = XP;$

$$\frac{dXP}{dt} = \left(k_1 Input + k_2 \frac{XP^n}{K^n + XP^n} \right)(X_{tot} - XP) - k_{-1} XP$$



(D) $k_1 = 1, k_{-1} = 1, k_2 = 2,\ f[XP] = \frac{XP^5}{0.5^5 + XP^5}$;



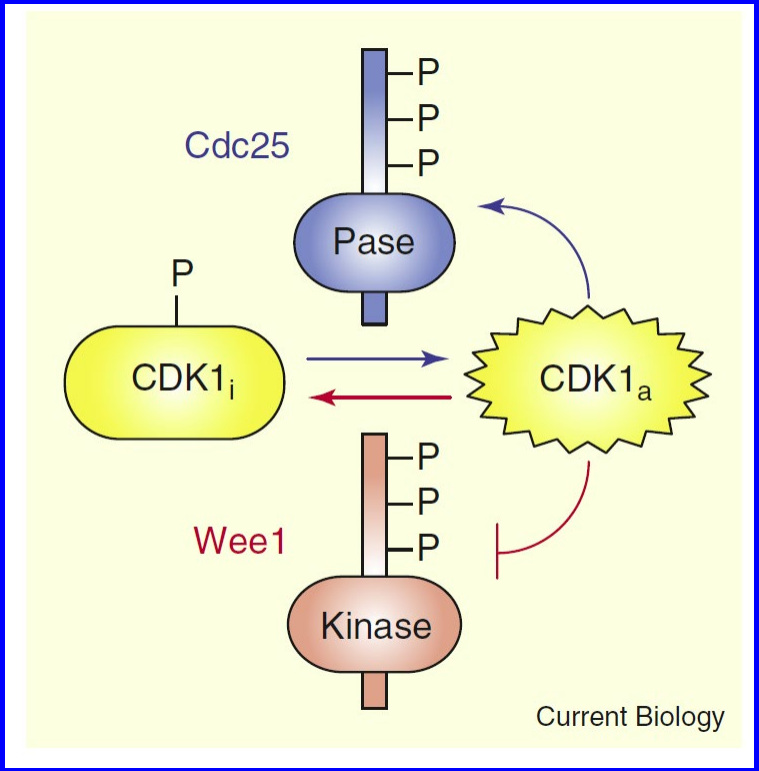
(E) $k_1 = 1, k_{-1} = 0.3, k_2 = 0.8, K = 0.01,\ f[XP] = XP,$

$dephosphorylation\ rate = \frac{XP}{K+XP}.$

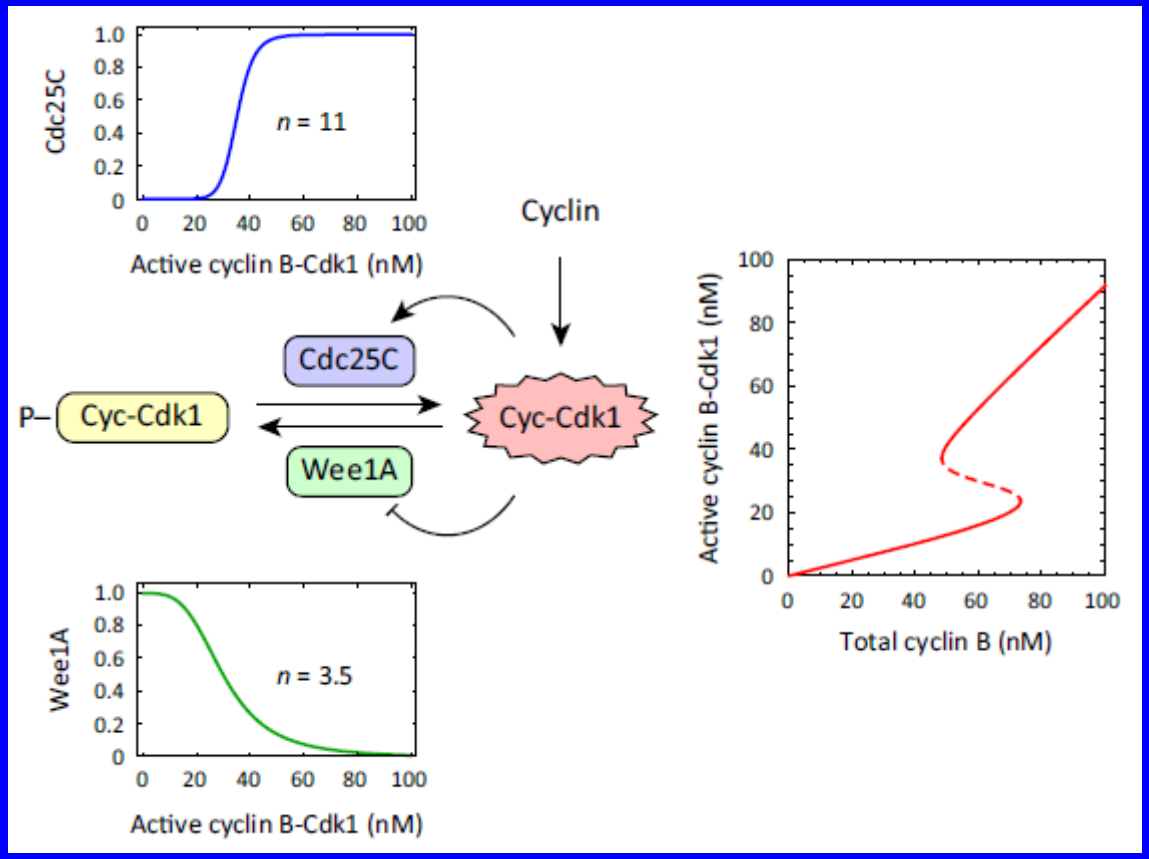
Whether known bistable switches are actually built out of components with highly ultrasensitive responses?

One well-studied bistable switch is the Cdk1–Cdc25C–Wee1A system, a circuit that regulates mitotic entry in eukaryotic cells.

The system consists of a positive feedback loop of CycB–Cdk1 and Cdc25C, and a double-negative feedback loop of Cdk1 and Wee1A.

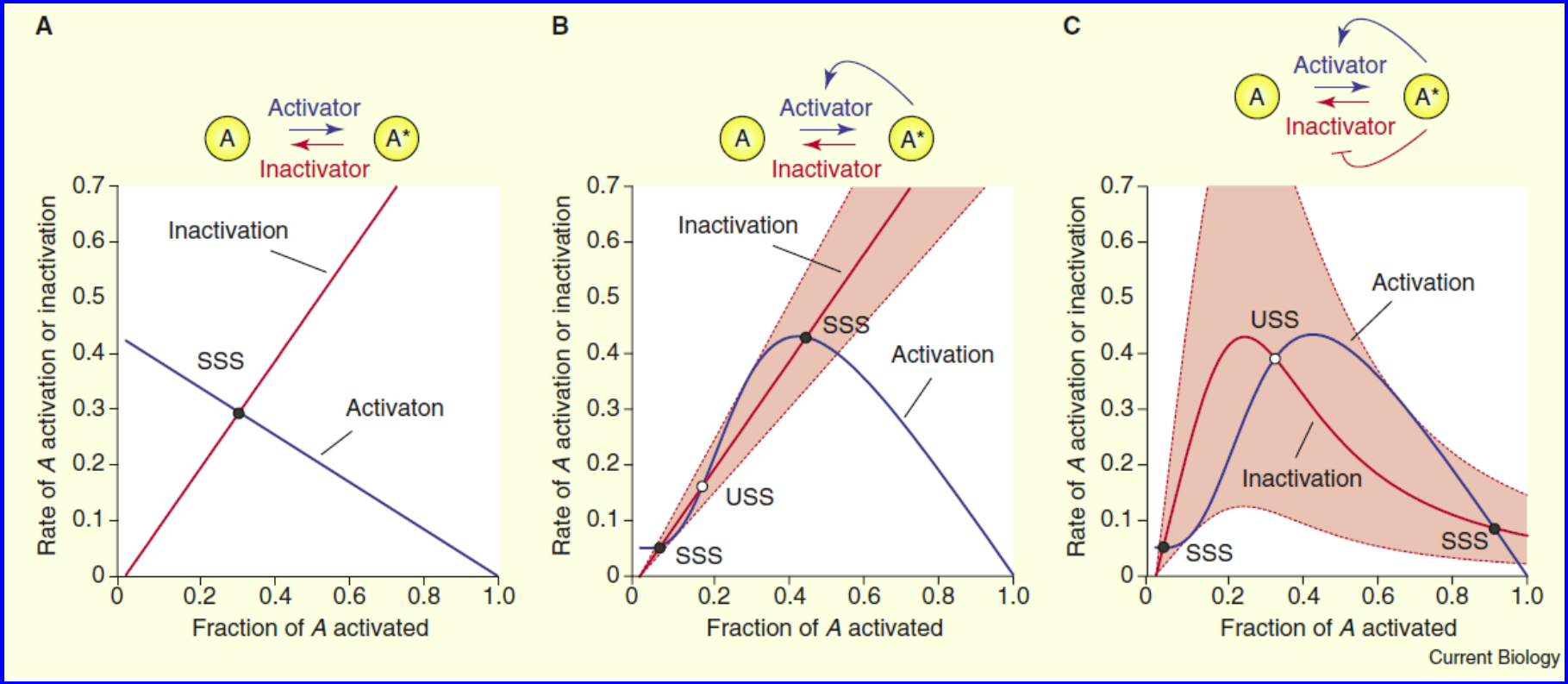


Experimental studies have shown that the steady-state response of Cdc25C to cyclin B–Cdk1 is highly ultrasensitive, with a Hill exponent of 11 and the response of the Cdk1-inhibitory kinase Wee1A exhibits a Hill coefficient of 3.5



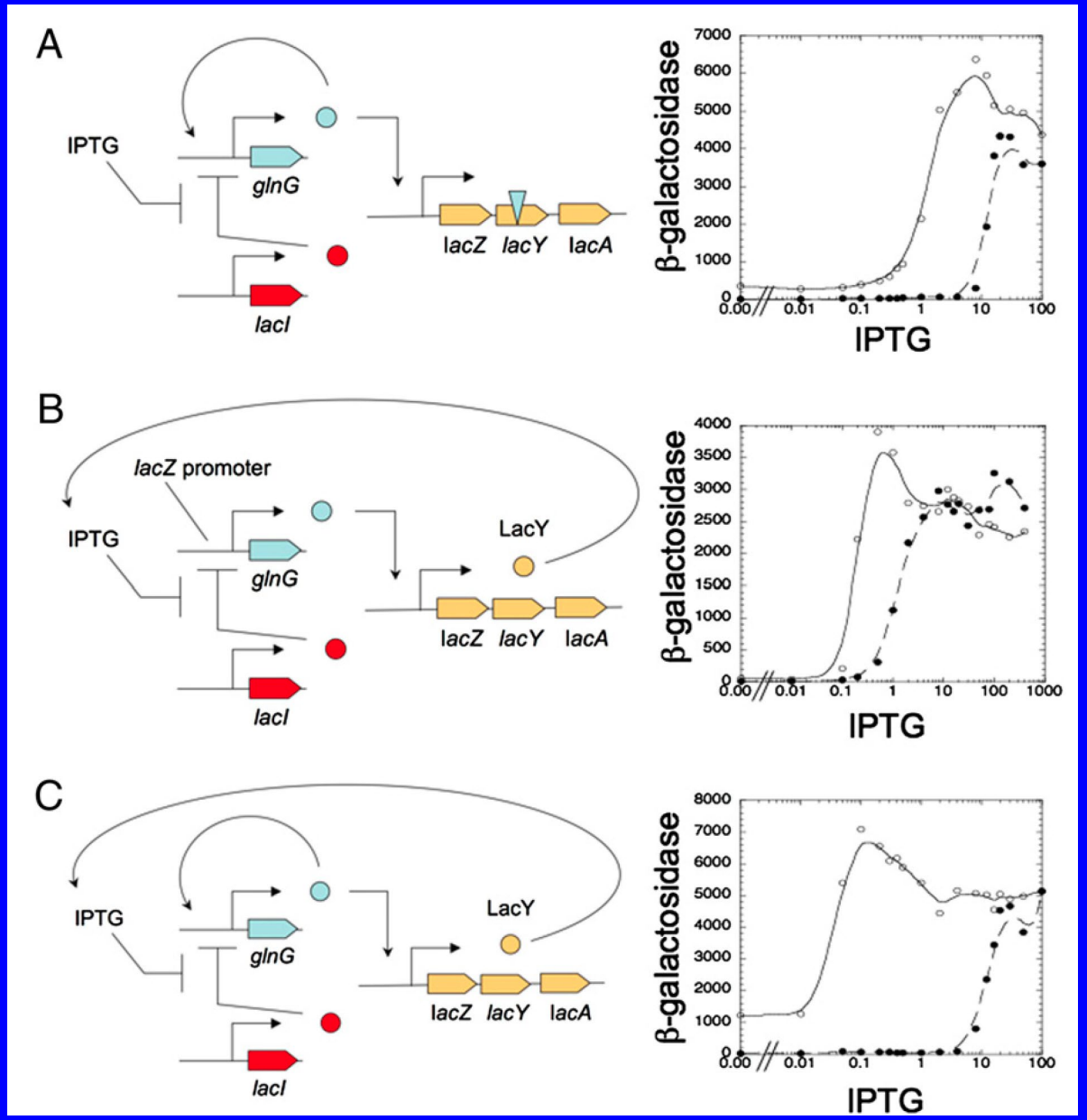
Kim, S. Y. and J. E. Ferrell Jr (2007). Cell **128**(6): 1133-1145.

Feedback regulation of opposing enzymes yields robust bistable responses



(A) No feedback loops. (B) One feedback loop. (C) Two feedback loops.

Ferrell Jr, J. E. (2008). Current Biology **18**(6): R244-R245.



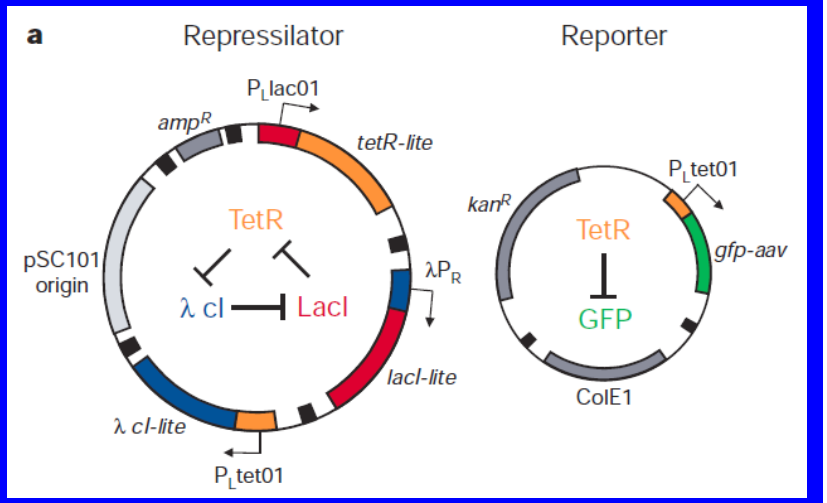
Chang, D.-E., S. Leung, et al. (2010). PNAS **107**(1): 175-180.

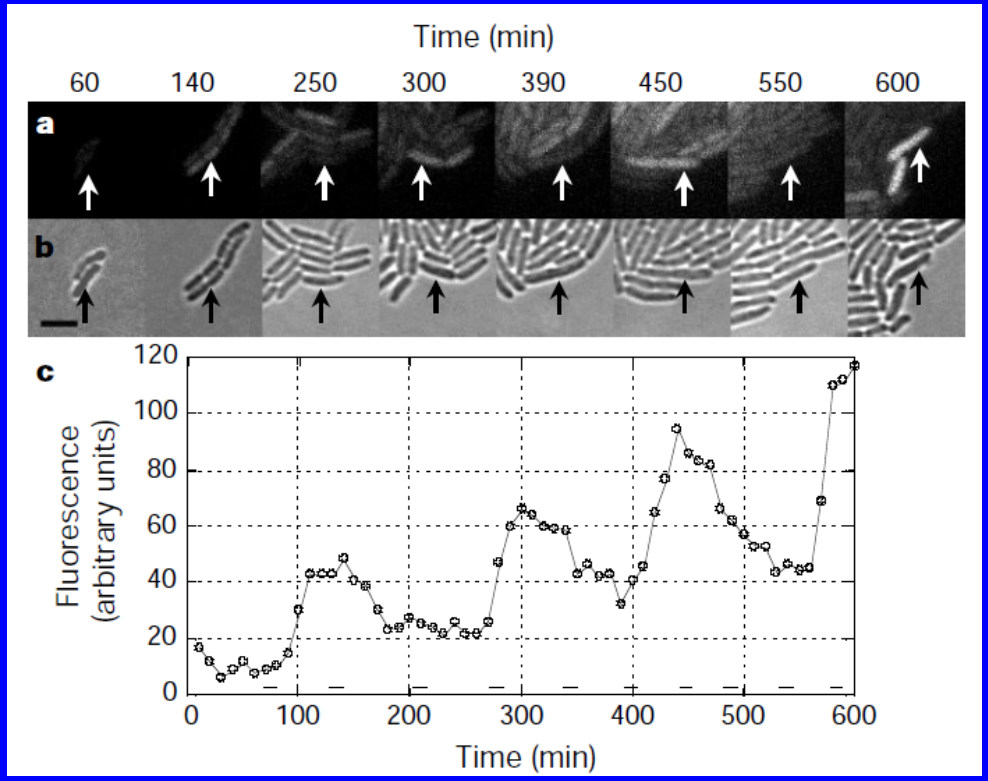
Ultrasensitivity in oscillations

Limit cycle oscillations approach the same dynamical pattern of behavior – the same frequency and amplitude, plus or minus a phase shift – regardless of the initial conditions, as contrasted with harmonic oscillators, where the amplitude of the oscillations varies with the initial conditions.

Ultrasensitive components not only promote switch-like steady state behaviors (above), but also oscillatory dynamical behaviors.

A synthetic oscillatory network of transcriptional regulators

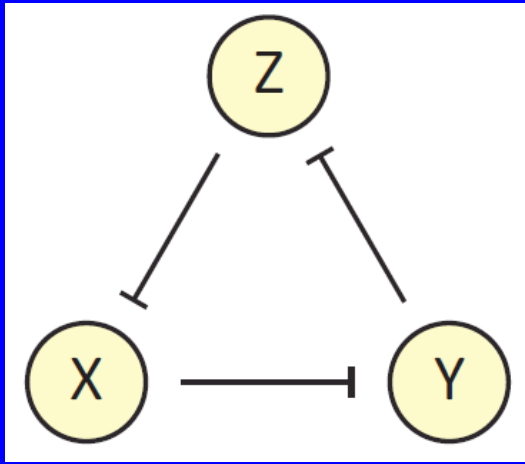




Elowitz, M. B. and S. Leibler (2000). Nature **403**(6767): 335-338.

A simple protein oscillator circuit

(A)

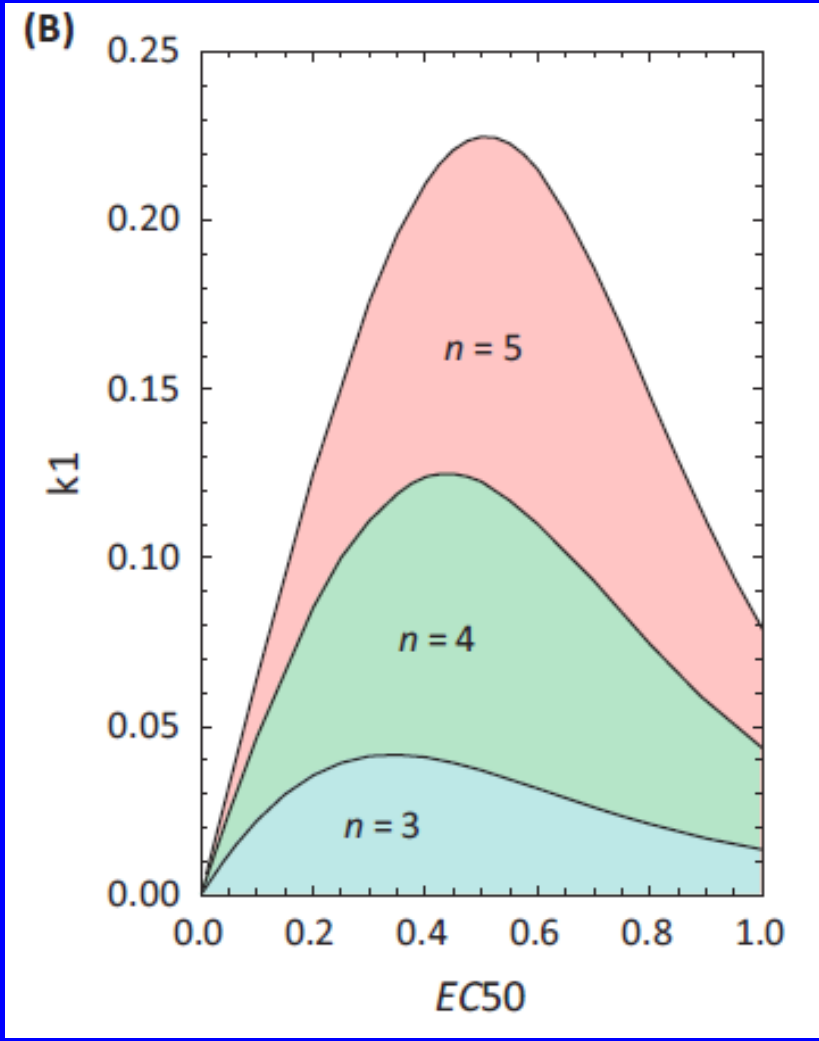


$$\frac{dX}{dt} = k_1(X_{tot} - X) - k_{-1}X\frac{Z^n}{EC50^n + Z^n}$$

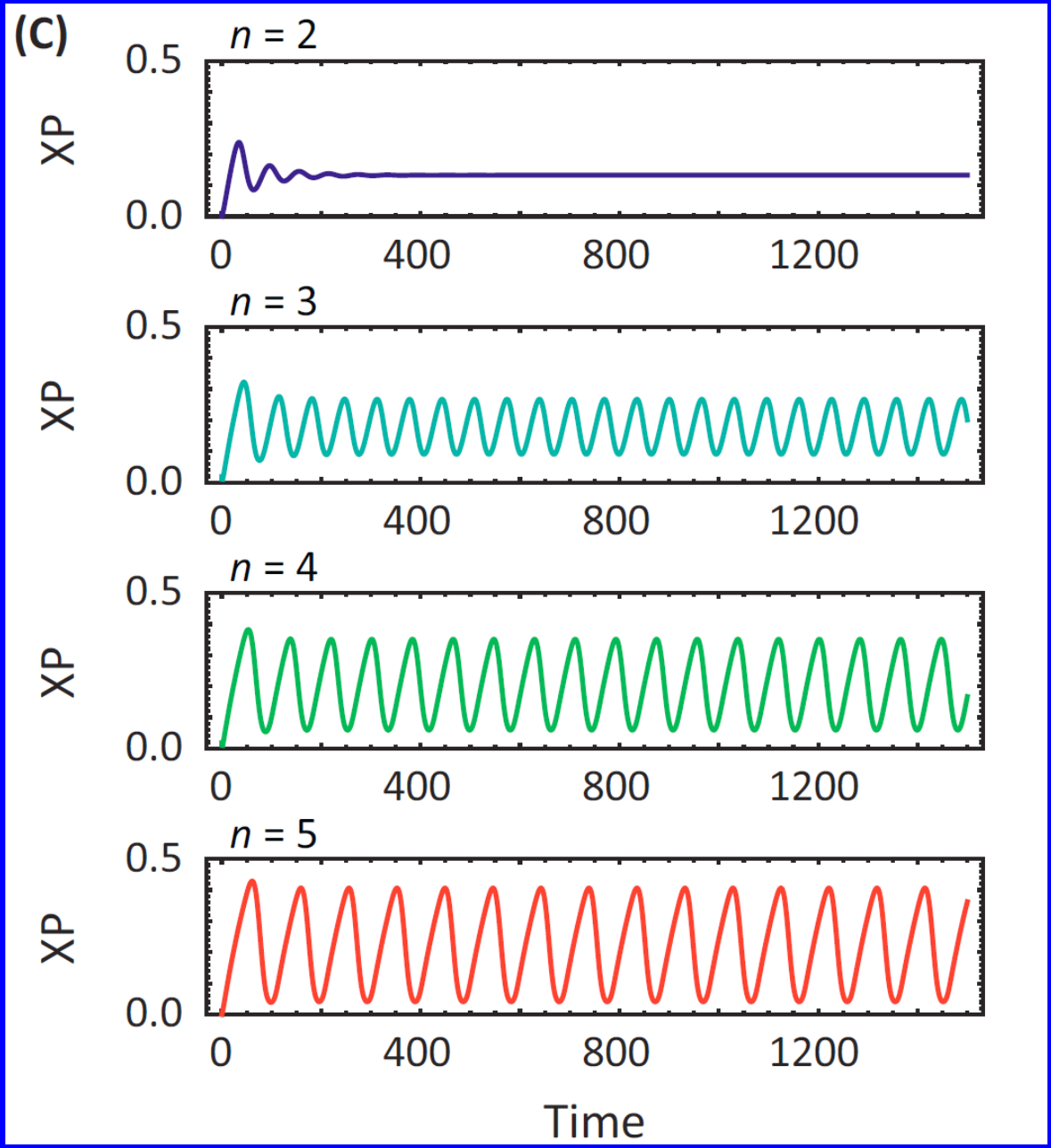
$$\frac{dY}{dt} = k_1(Y_{tot} - Y) - k_{-1}Y\frac{X^n}{EC50^n + X^n}$$

$$\frac{dZ}{dt} = k_1(Z_{tot} - Z) - k_{-1}Z\frac{Y^n}{EC50^n + Y^n}$$

(B) Values from the model that yield limit cycle oscillations.



(C) Time courses of XP oscillations for different assumed values of n. The amplitude increases with n.



There are other more complicated ways to construct an oscillator circuit. The circuit could contain more than three components and, in general, the longer the loop, the greater the chances for oscillations, and *there is a trade-off between how long the loop is and how much ultrasensitivity is required for oscillations*.

Alternatively, the circuit could include a bistable trigger and behave like a relaxation oscillator, or it could be a long positive feedback loop interlinked with shorter negative feedback loops, or the model of the circuit could include explicit time delays

Summary

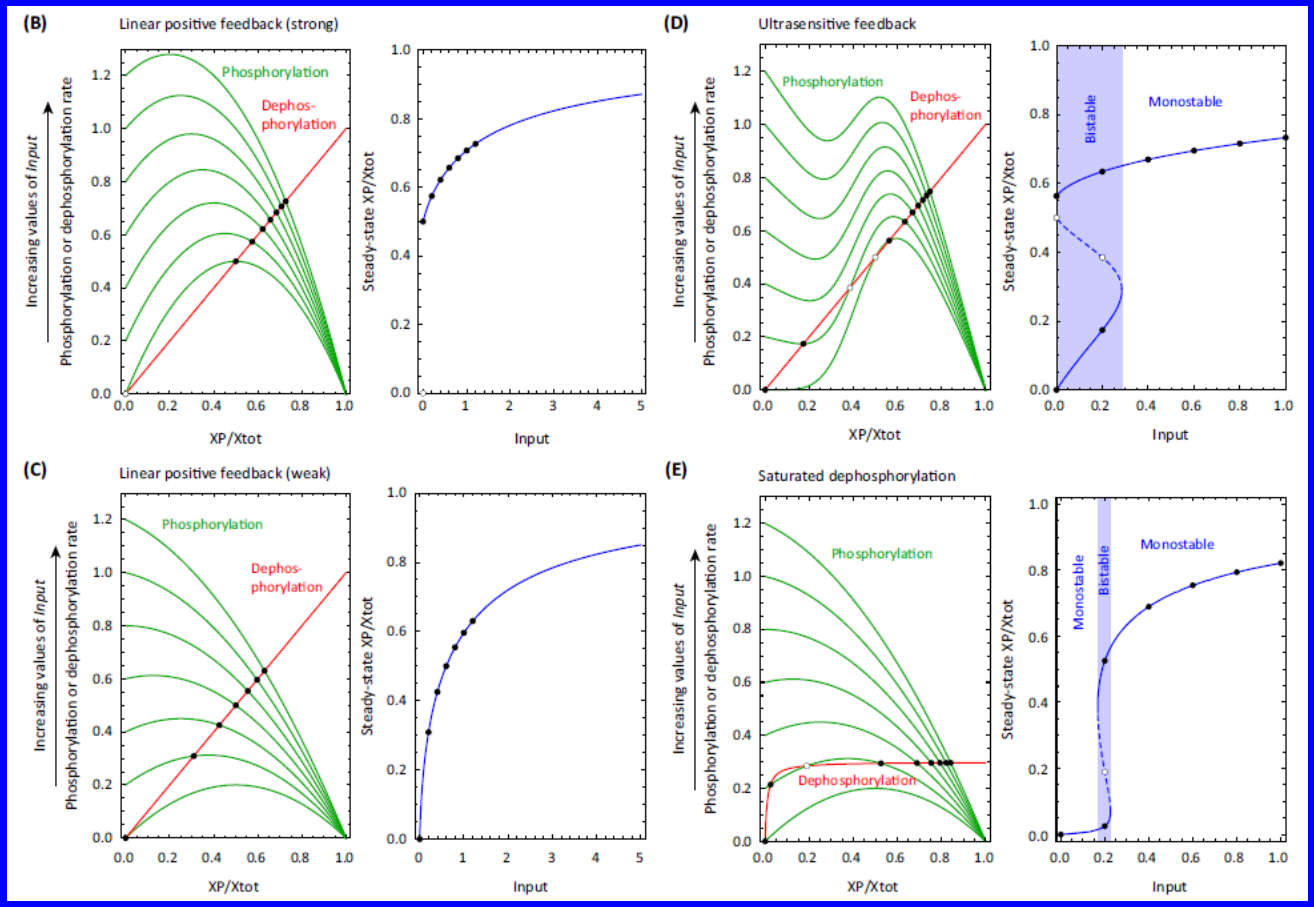
Four classes of distinct mechanisms can generate ultrasensitive responses: zero-order ultrasensitivity, multisite phosphorylatiom, inhibitor ultrasensitivity, positive feedback loops.

Ultrasensitive responses can be critical for the generation of oscillations and bistability.

Ultrasensitivity has become an important brick in the wall of systems biology, and a highly useful ingredient for the engineering of synthetic biology circuits.

Assignment

Reproducing Figs3B-E in the literature : Ferrell Jr, J. E. and S. H. Ha (2014). Trends in Biochemical Sciences **39**(12): 612-618.



Model and parameters

Mass action kinetics:

$$\frac{dXP}{dt} = (k_1 Input + k_2 XP)(X_{tot} - XP) - k_{-1} XP$$

Hill function:

$$\frac{dXP}{dt} = \left(k_1 Input + k_2 \frac{XP^n}{K^n + XP^n}\right)(X_{tot} - XP) - k_{-1} XP$$

Mass action kinetics with saturated dephophorylation:

$$\frac{dXP}{dt} = (k_1 Input + k_2 XP)(X_{tot} - XP) - k_{-1} \frac{XP}{K + XP}$$

(B) $k_1 = 1$, $k_{-1} = 1$, $k_2 = 2$, $f[XP] = XP$; (C) $k_1 = 1$, $k_{-1} = 1$, $k_2 = 0.8$, $f[XP] = XP$;
(D) $k_1 = 1, k_{-1} = 1, k_2 = 2$, $f[XP] = \frac{XP^5}{0.5^5 + XP^5}$; and
(E) $k_1 = 1, k_{-1} = 0.3, k_2 = 0.8, K = 0.01$, $f[XP] = XP$, *dephosphorylation rate* $= \frac{XP}{K+XP}$.
In all cases the green curves correspond to *Input* = 0, 0.2, 0.4, 0.6, 0.8, 1, and 1.2 (from bottom to top).