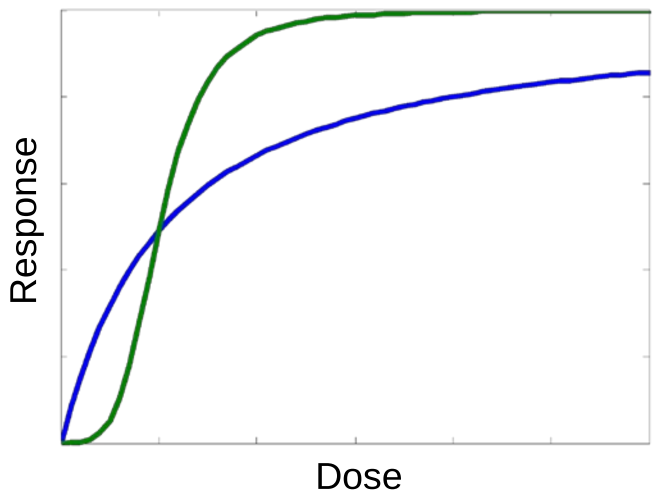
Understanding the Biochemical Origin of Sigmoidal Dose Responses: Ultrasensitive Response Motifs



September 5, 2017

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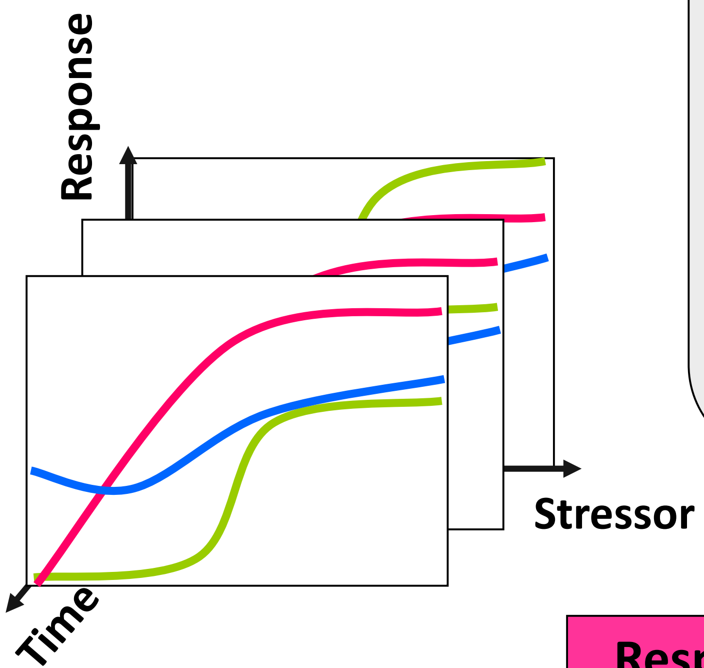
Sigmoidal Dose Response

1. Sigmoidal response is a common type of dose response observed in biological systems
2. These sigmoidal curves are often approximated empirically, among others, by Hill equations
3. Steep sigmoidal responses can mediate switch-like, threshold effects
4. Cooperativity is the often-cited mechanism for sigmoidal responses
5. Diverse biochemical mechanisms other than cooperativity can generate sigmoidal responses
6. As chemical toxicity testing is increasingly based on in vitro cell assays, understanding the mechanisms becomes ever important

Outline

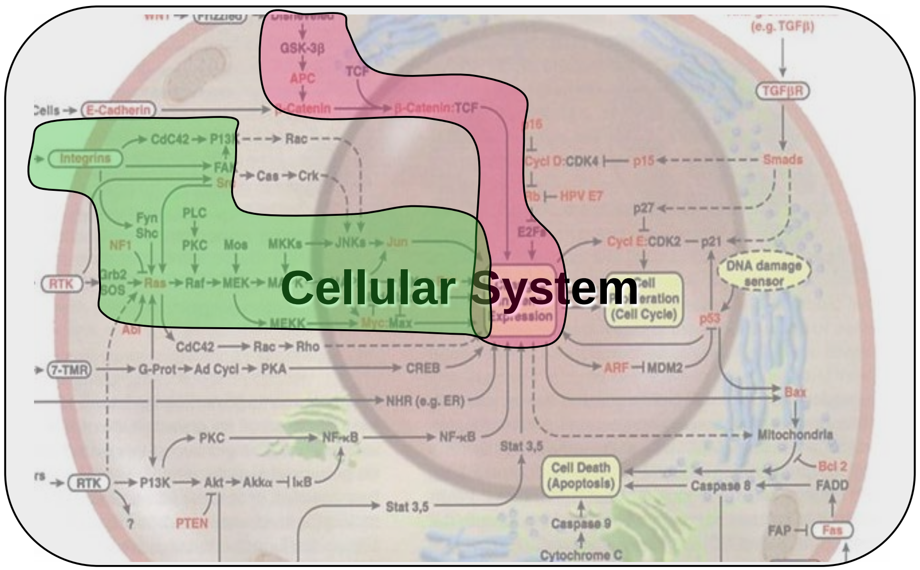
1. Sigmoidal dose response, Hill function, and ultrasensitivity
2. Common ultrasensitive response motifs (URM)
3. MAPK: an example of URM combination
4. Role of signal amplification thru URM in cellular dynamics

Biochemical circuits/networks mediate cellular responses



Physical/chemical stressor

Perturbation

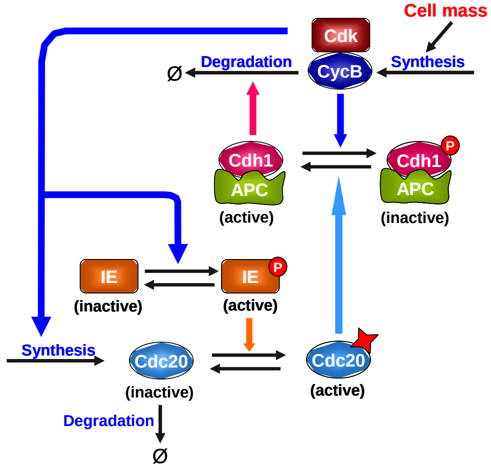


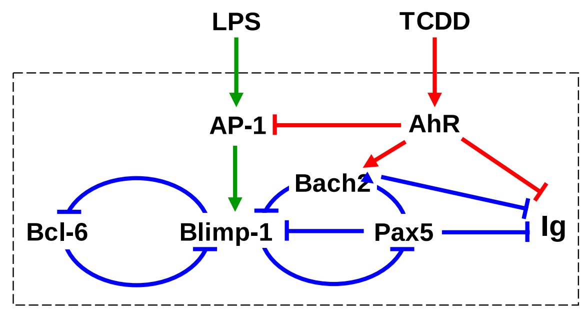
Response X | **Response Y** | **Response Z**

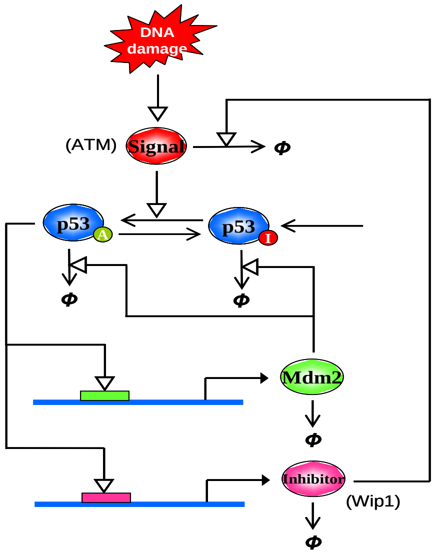
(**Response:** metabolism, gene expression, proliferation, apoptosis, carcinogenesis……)

Bottom-up approach to understanding molecular circuits

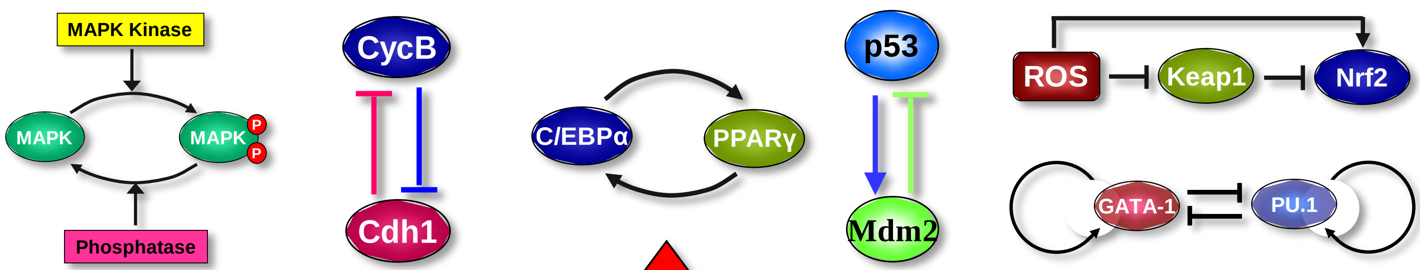
Circuits / networks



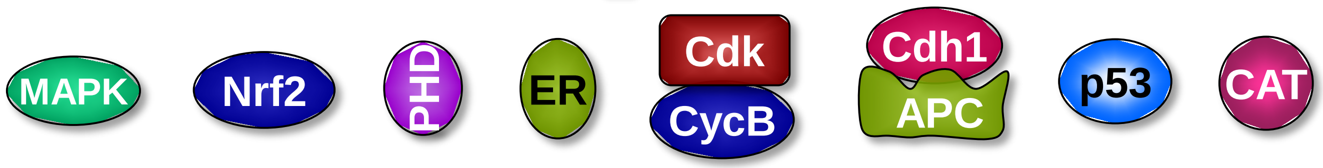




Network motifs



Part list

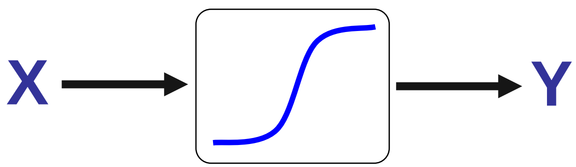


Network Motif

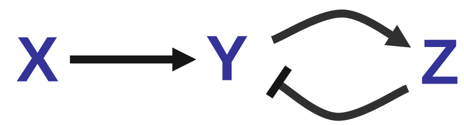
Network motifs are relatively simple building blocks that frequently appear in complex molecular circuits and possess specific signaling properties.

Important common motifs:

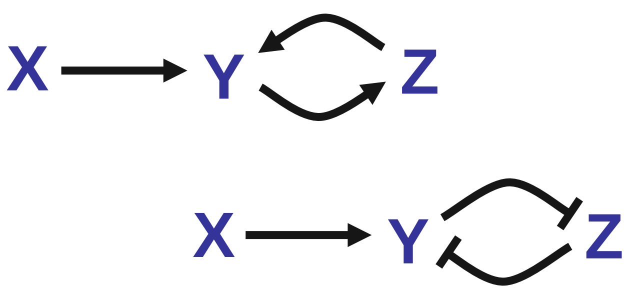
I. Ultrasensitive motif



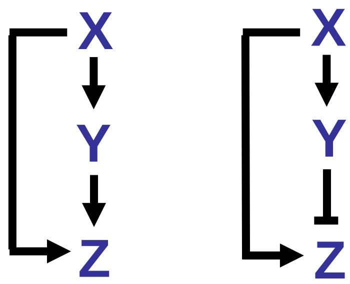
III. Negative feedback motif



II. Positive or double-negative feedback motif

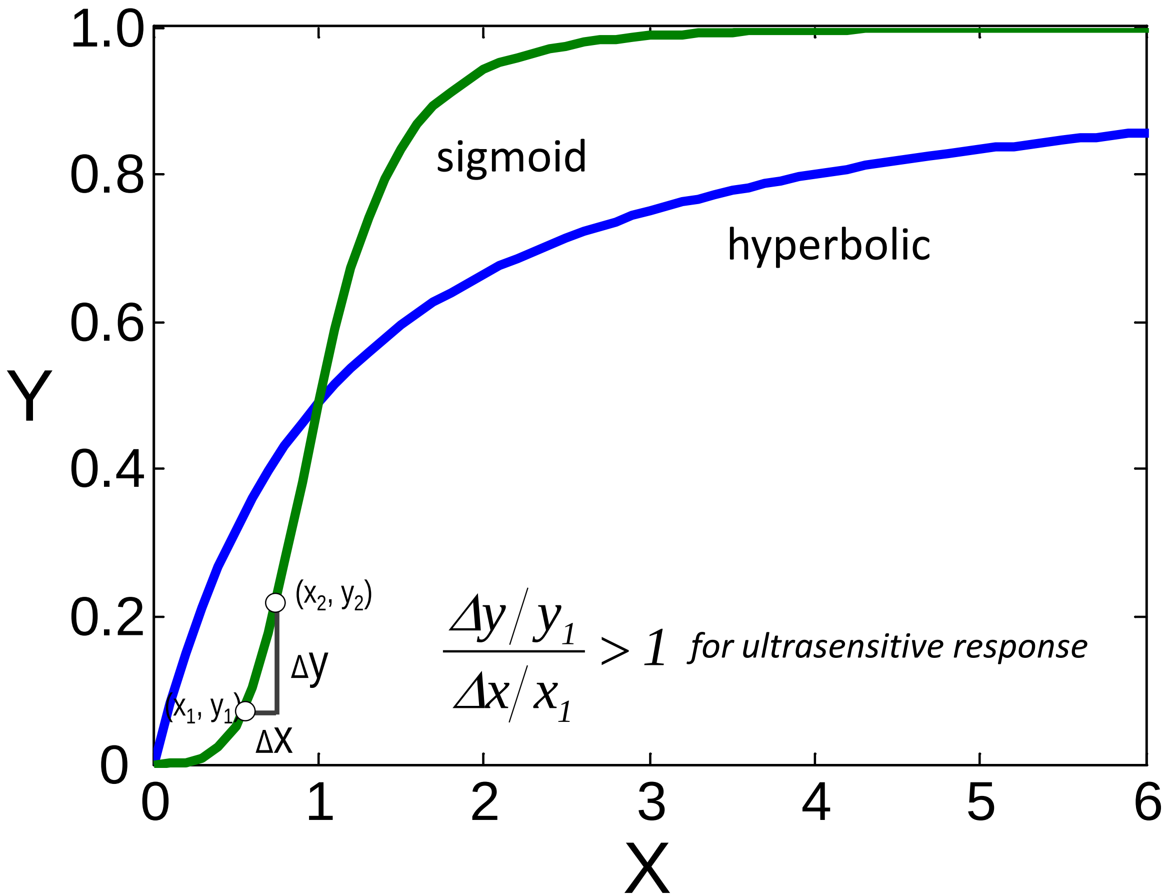


IV. Feedforward motif



Ultrasensitive response motifs

Ultrasensitivity refers to a (steady-state) stimulus-response that is significantly steeper than the hyperbolic, Michaelis-Menten form such that it appears globally as a sigmoid curve on a **linear** scale.



- Ultrasensitive response allows amplification of **percentage** change locally.
- The steepness of the globally sigmoid curve is usually approximated by Hill function.

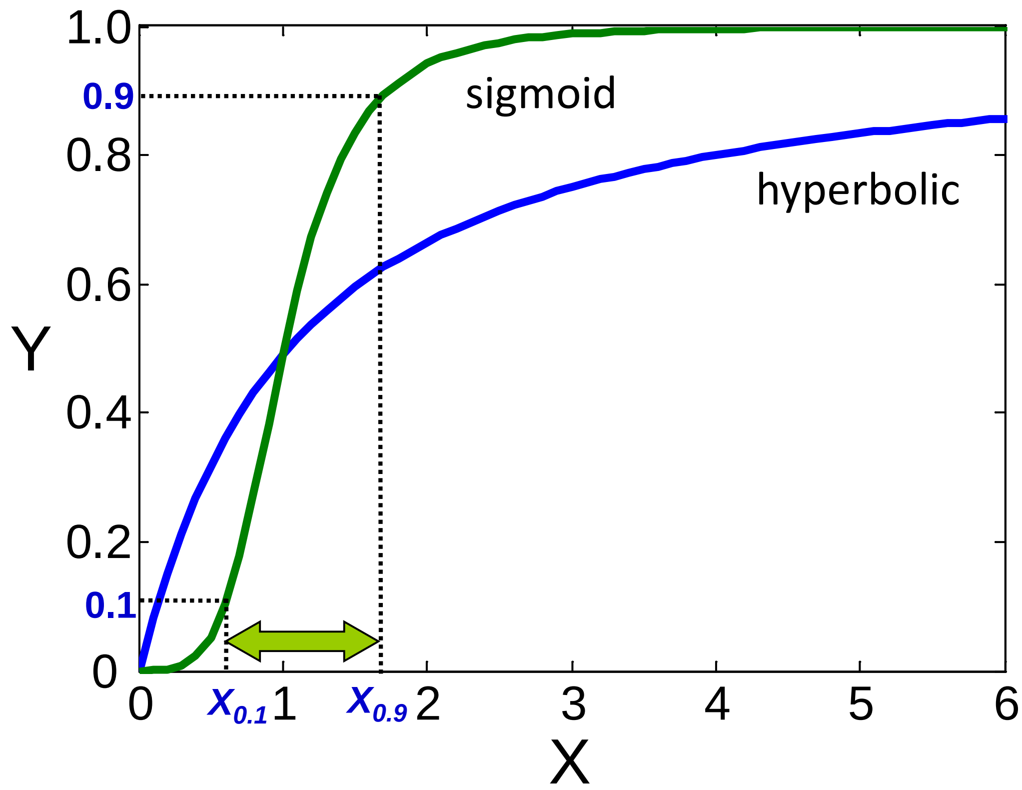
Hill Function

Hill Function

$$Y = \frac{X^n}{K^n + X^n}$$

Hill coefficient

$$n = \frac{\ln 81}{\ln \dfrac{X_{0.9}}{X_{0.1}}}$$



- The Hill coefficient measures globally how steep the sigmoid curve is by using the Michaelis-Menten formalism as reference.
- An ultrasensitive response, when approximated by Hill function, has n significantly greater than 1.

Hill Function

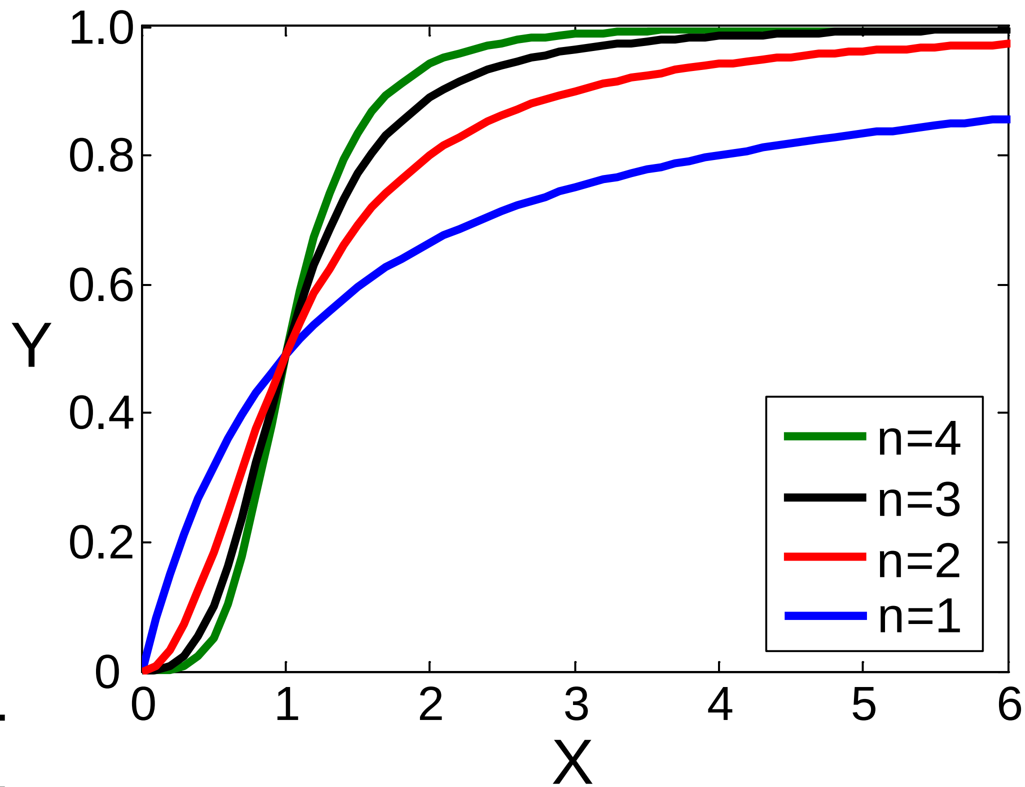
Hill Function

$$Y = \frac{X^n}{K^n + X^n}$$

Hill coefficient

$$n = \frac{\ln 81}{\ln \frac{X_{0.9}}{X_{0.1}}}$$

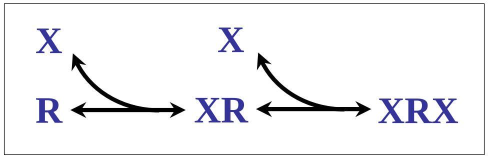
$X_{0.9}/X_{0.1}$	n
81	1
9	2
4.33	3
3	4



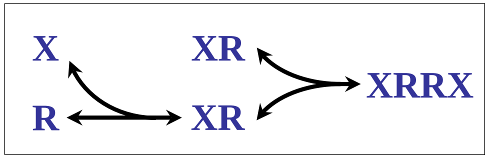
- The higher the Hill coefficient (n), the steeper the sigmoid curve.
- When n=1, the Hill function reduces to the Michaelis-Menten function.

Common ultrasensitive motifs and the biochemical basis of sigmoidal responses

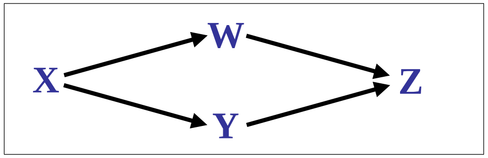
(1) Positive cooperative binding



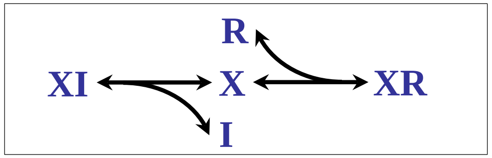
(2) Homo-multimerization



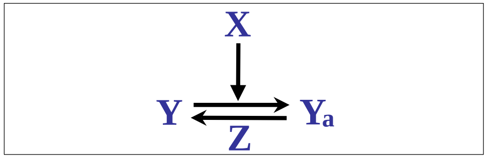
(3) Multi-step signaling



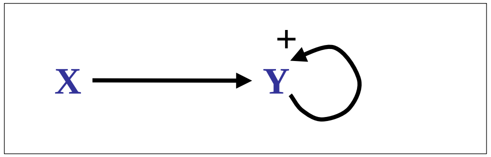
(4) Molecular titration



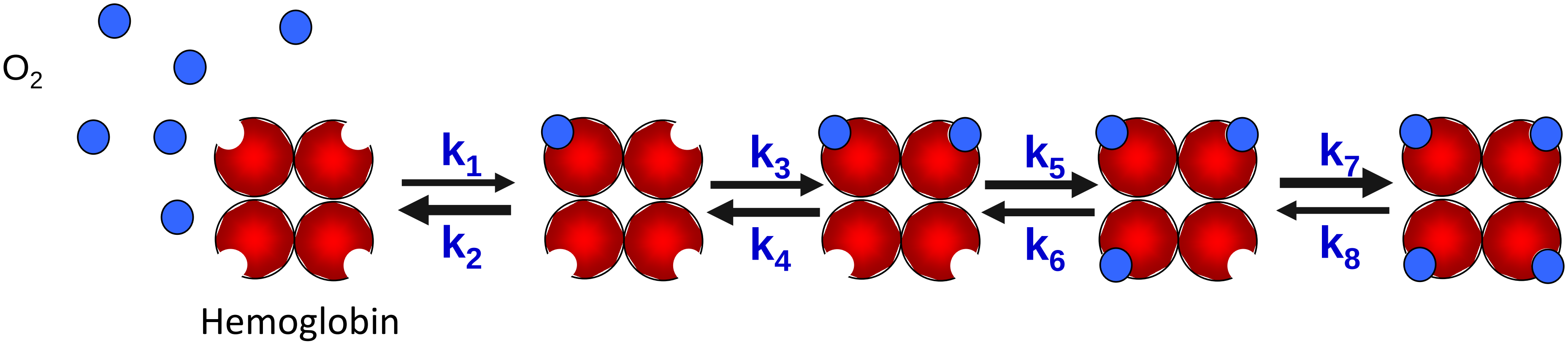
(5) Zero-order covalent modification cycle



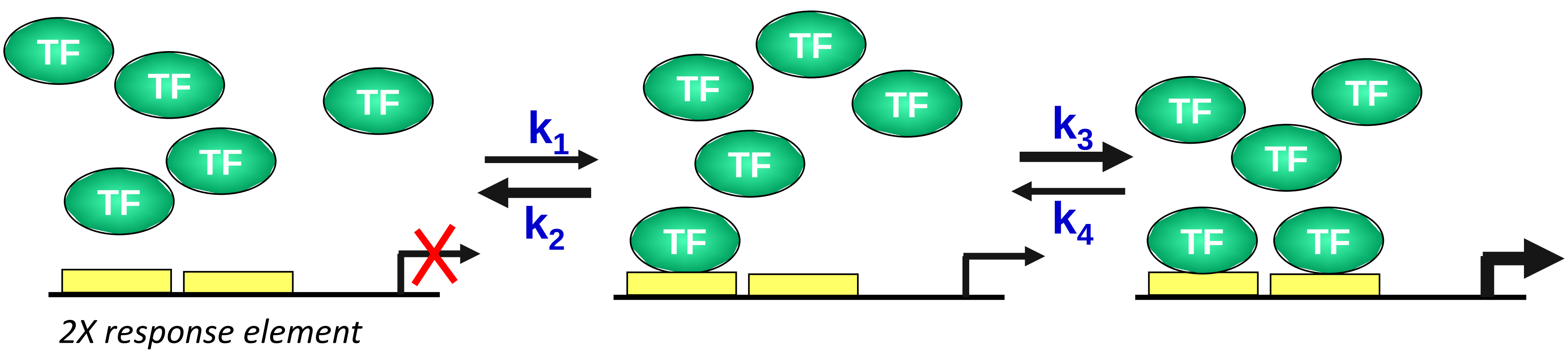
(6) Positive feedback



(1) Positive cooperative binding



** Positive cooperative binding occurs when* $\frac{k8}{k7} < \frac{k6}{k5} < \frac{k4}{k3} < \frac{k2}{k1}$



** Positive cooperative binding occurs when* $\frac{k4}{k3} < \frac{k2}{k1}$

(1) Positive cooperative binding

$$X + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} XR + X \underset{k_4}{\overset{k_3}{\rightleftharpoons}} X_2R + X \underset{k_6}{\overset{k_5}{\rightleftharpoons}} X_3R + X \underset{k_8}{\overset{k_7}{\rightleftharpoons}} X_4R$$

Approximated by Hill function

$$[R_{bound}] = \frac{R_{total}[X]^n}{K^n + [X]^n}$$

n: Hill coefficient

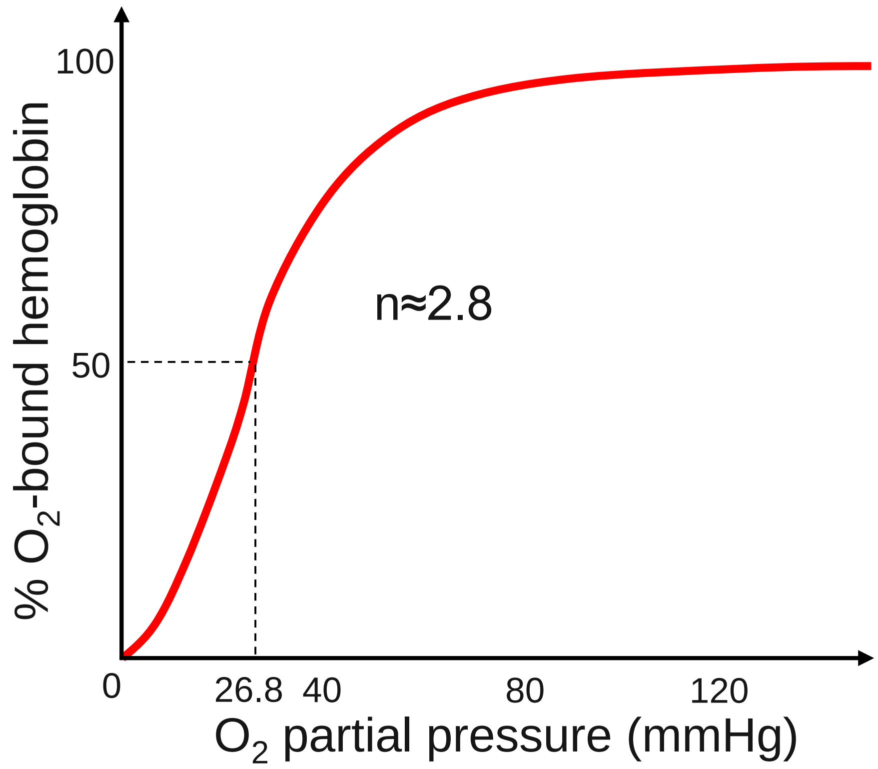
- *When* $\frac{k8}{k7} = \frac{k6}{k5} = \frac{k4}{k3} = \frac{k2}{k1}$

  n=1

- *When* $\frac{k8}{k7} < \frac{k6}{k5} < \frac{k4}{k3} < \frac{k2}{k1}$

  the affinity for subsequent binding is greater than that for previous binding,

  n=1~4



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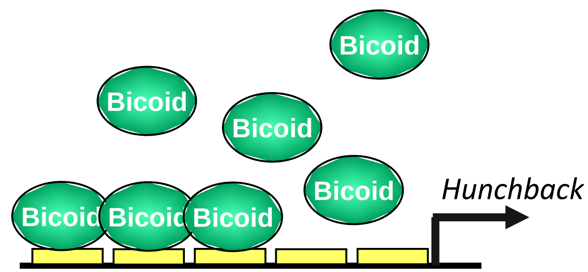
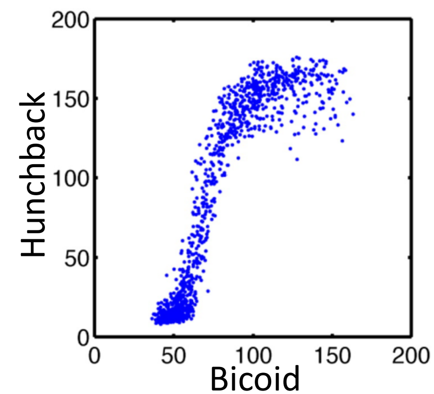
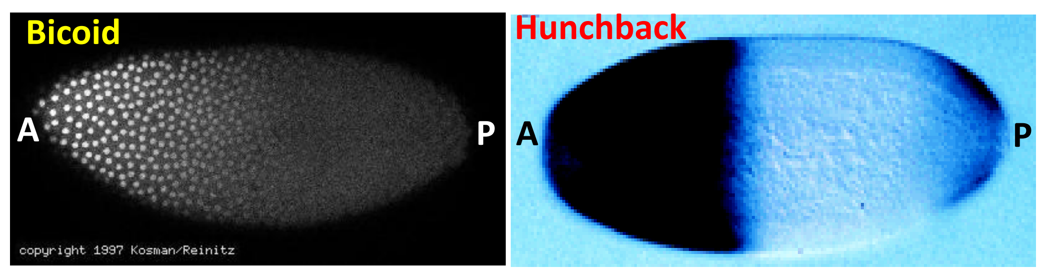
Drosophila **embryo anterior-posterior axis patterning by morphogen Bicoid**

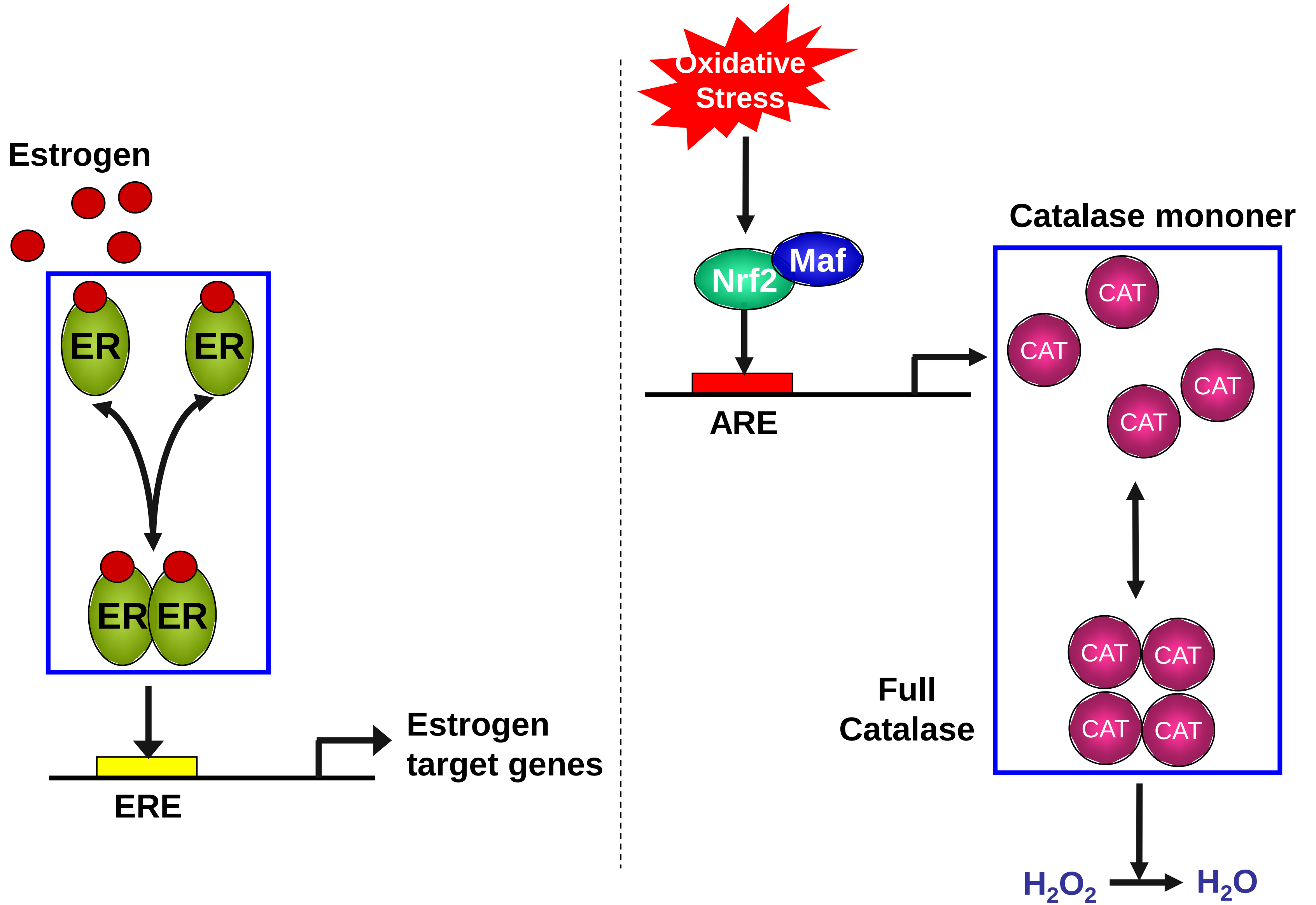






Adapted from Schaeffer et al, PNAS 1999 and Gregor et al, Cell 2007

(2) Homo-multimerization



(2) Homo-multimerization

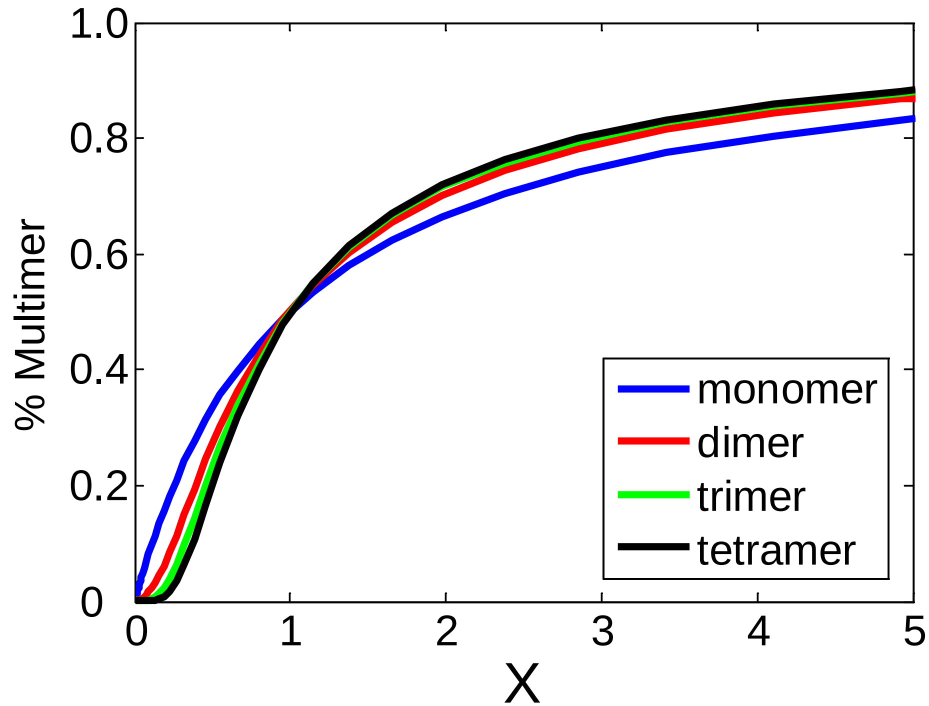
$$X + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \boxed{XR + XR \underset{k_4}{\overset{k_3}{\rightleftharpoons}} X_2R_2}$$

$$\frac{d[X_2R_2]}{dt} = k_3[XR]^2 - k_4[X_2R_2]$$

at steady state

$$[X_2R_2] = \frac{k_3}{k_4}[XR]^2$$

and when X is at low concentrations, [XR] is approximately linear to [X], so…



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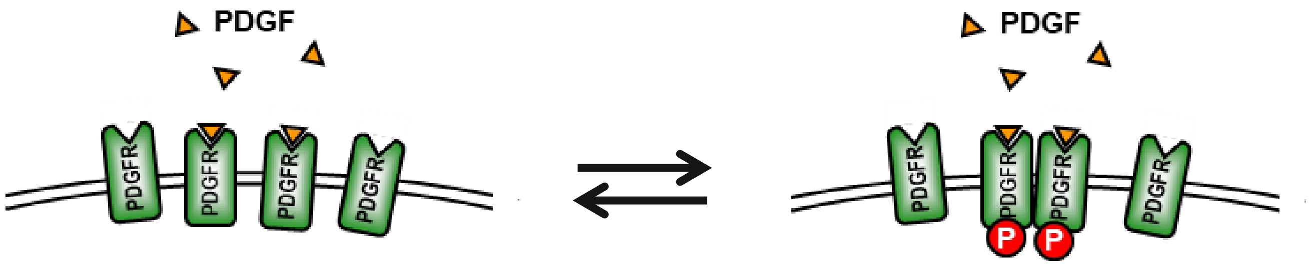
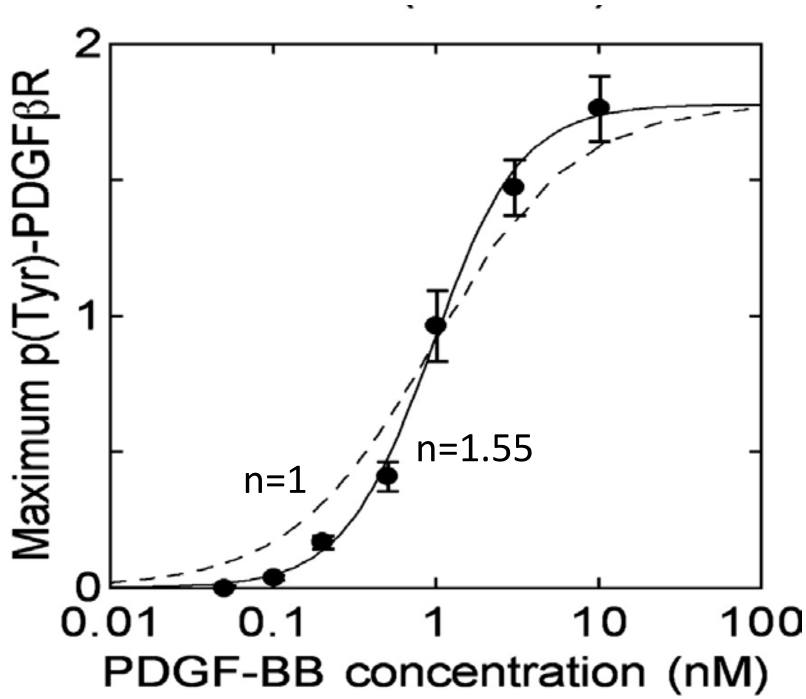
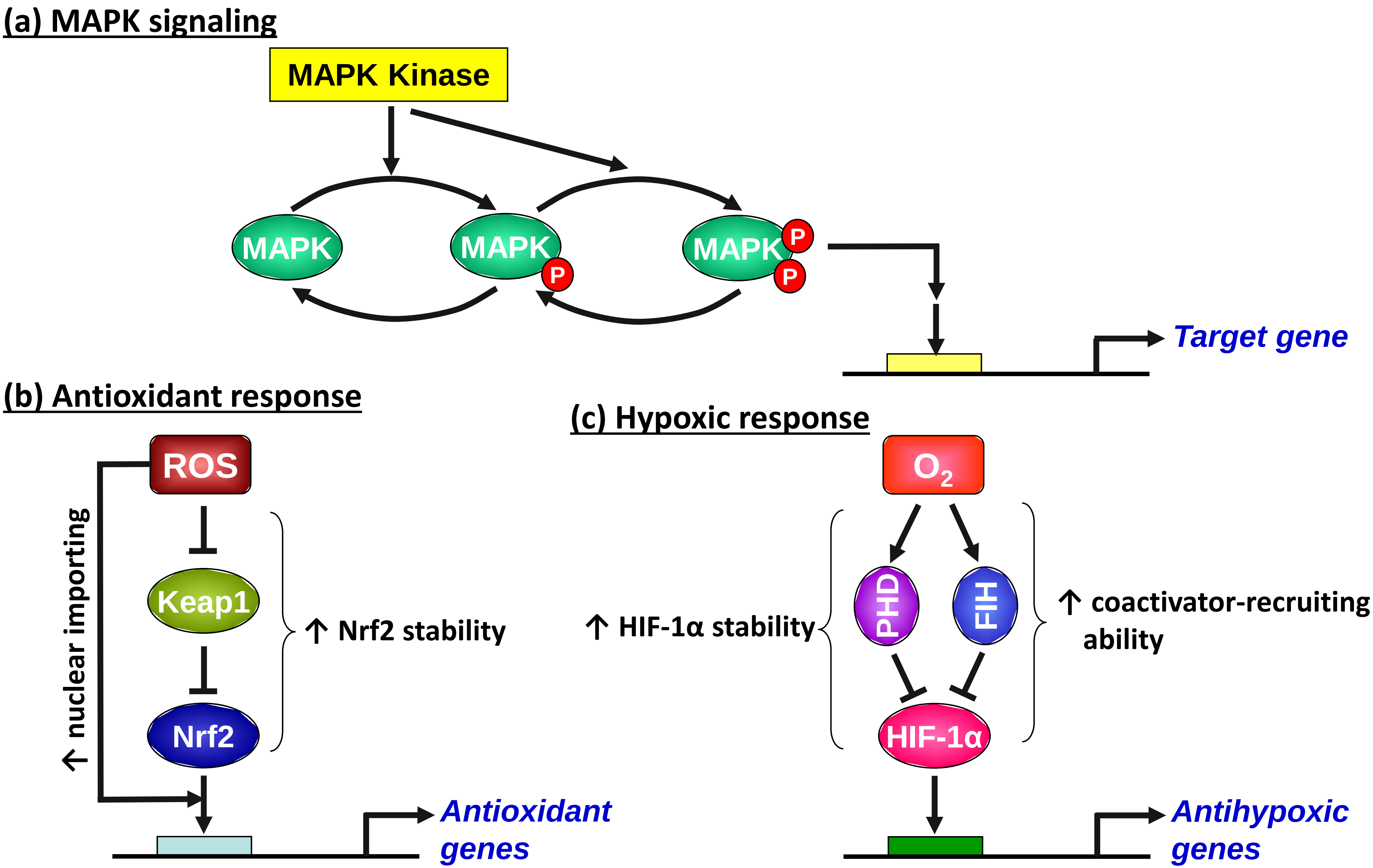


- Binging of PDGF receptor (PDGFR) by its ligand PDGF leads to receptor dimerization and autophosphorylation.
- Data shows results of tyrosine phosphorylation of PDGFβR in NIH 3T3 fibroblasts stimulated by human recombinant PDGF-BB as ligand.



Park CS et al, JBC 2003

(3) Multi-step signaling



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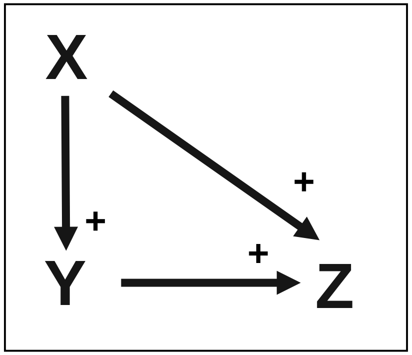
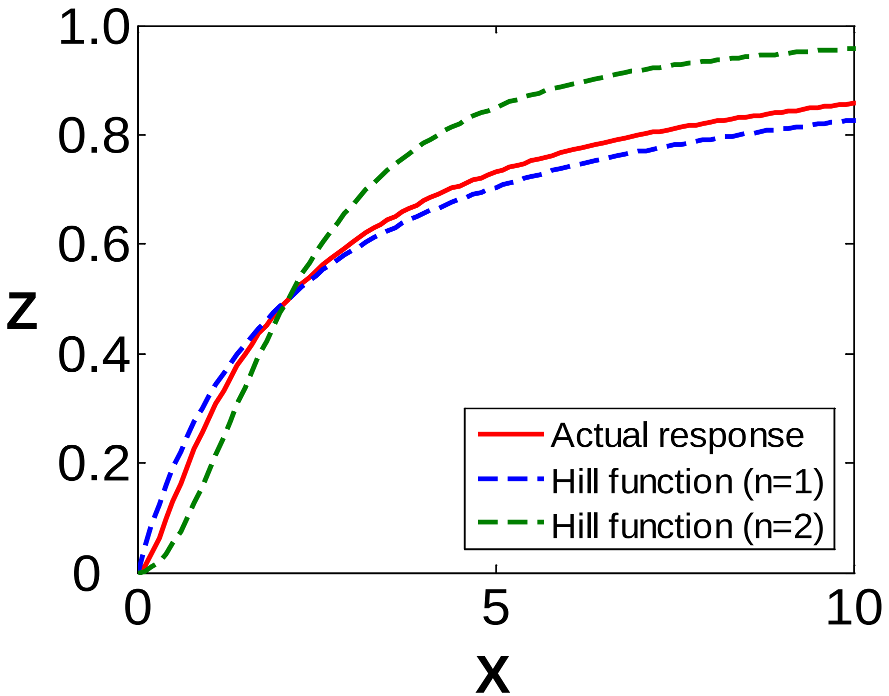




Assume at steady-state

$$[Y] = \frac{Y_{\max}[X]}{K_1 + [X]}$$

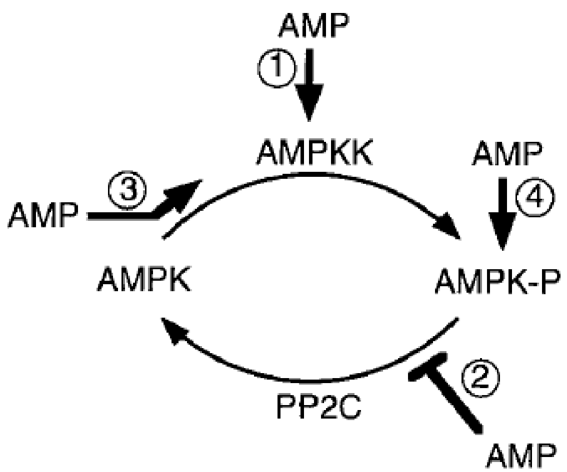
$$[Z] = \frac{Z_{\max}[X]}{K_2 + [X]} \frac{[Y]}{K_3 + [Y]}$$

$$[Z] = \frac{K_c[X]^2}{K_a^2 + [X]^2 + K_b[X]}$$

For multi-step signaling to generate ultrasensitivity, the converging paths have to act on processes that are **synergistic** rather than additive. The former denotes multiplication in mathematical term.

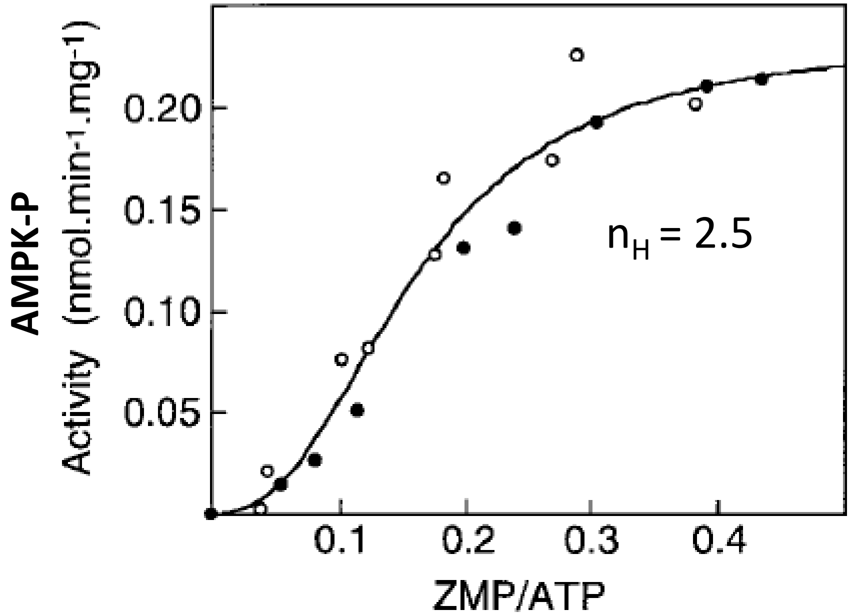
(where $K_a = \sqrt{\frac{K_1 K_2 K_3}{K_3 + Y_{\max}}}$ $\quad K_b = \frac{K_1 + K_2}{Y_{\max}} + \frac{K_2}{K_3}$ $\quad K_c = \frac{Z_{\max} Y_{\max}}{K_3 + Y_{\max}}$ *)*

(3) An example of multi-step signaling ultrasensitivity



Scheme 1 The AMPK cascade is activated by AMP via four mechanisms

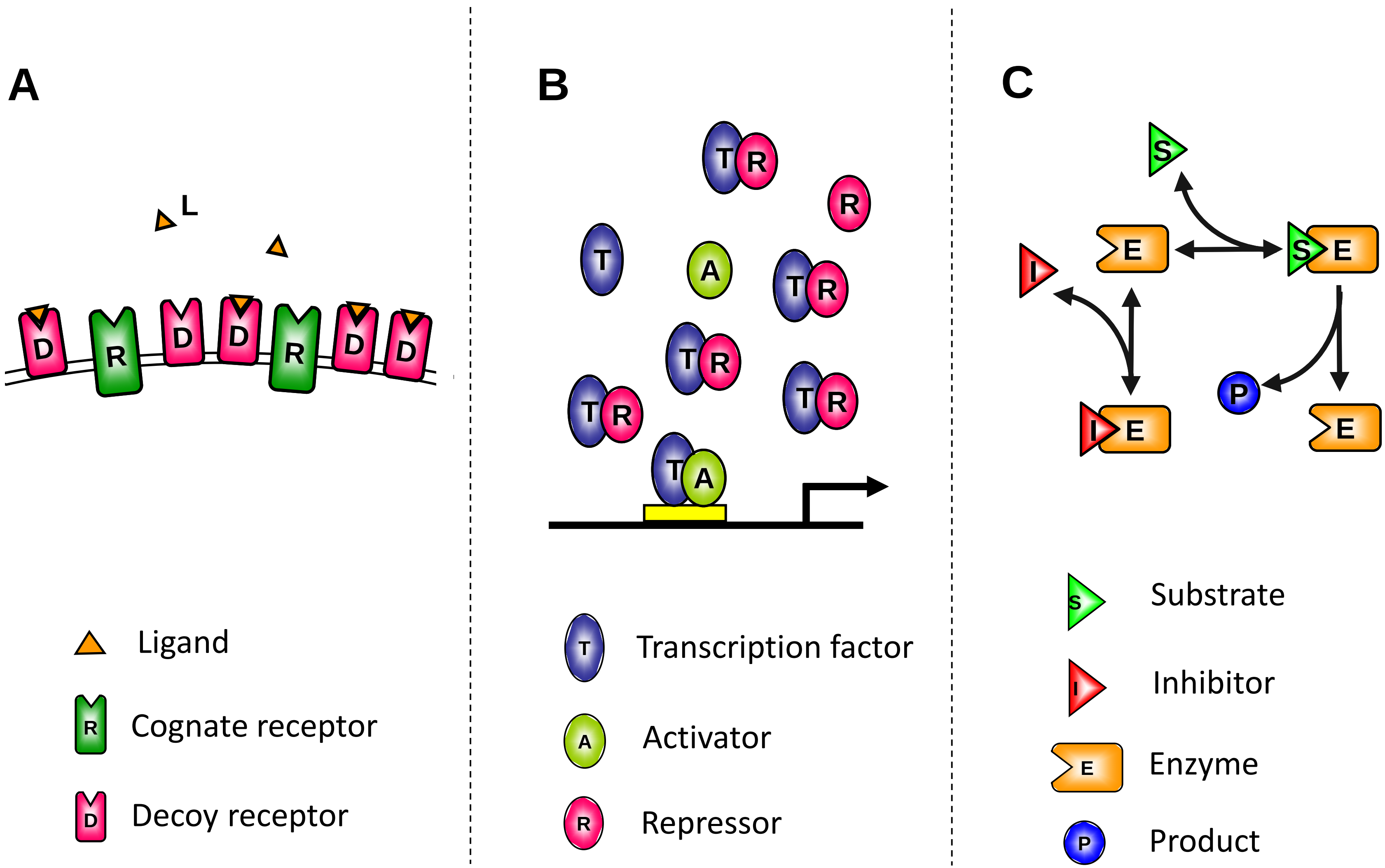
(1) Allosteric activation of AMPKK; (2) binding of AMP to AMPK, rendering it a poorer substrate for protein phosphatases; (3) binding of AMP to AMPK, making it a better substrate for the upstream kinase, AMPKK; and (4) allosteric activation of AMPK.



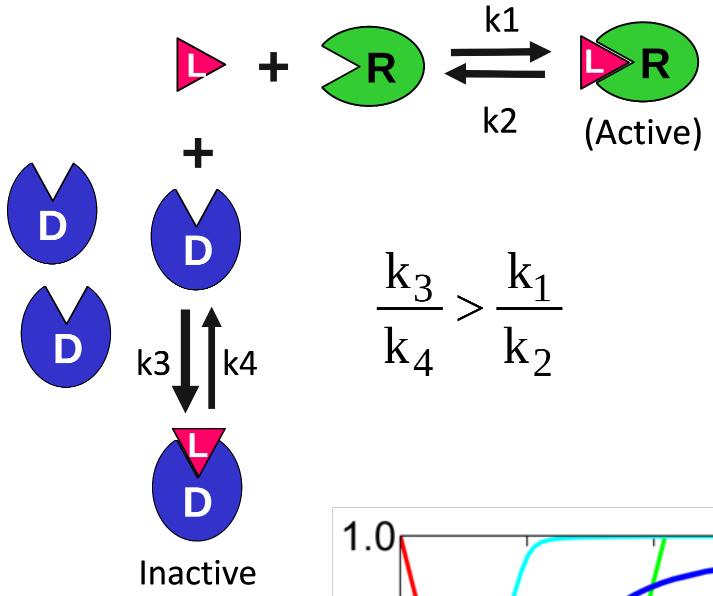
ZMP: an AMP mimic

Hardie et al, Biochem J 1999

(4) Molecular titration

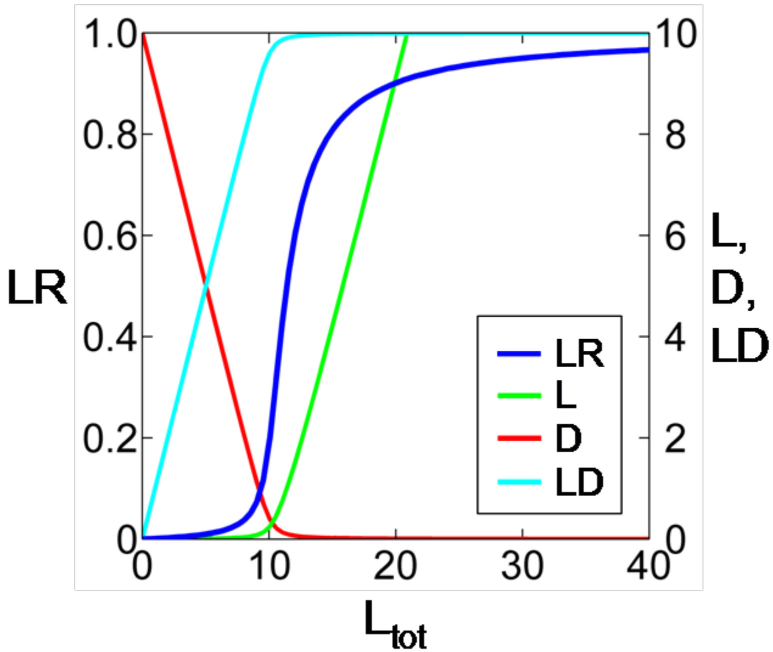


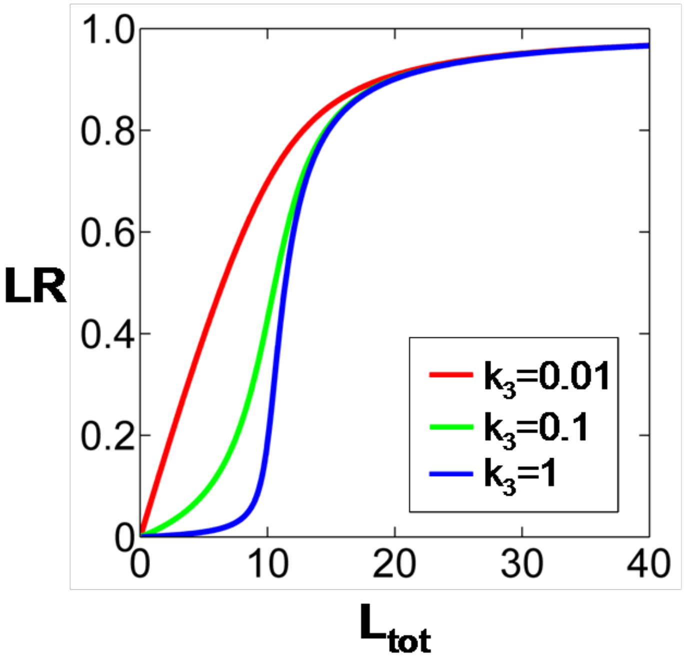
(4) Molecular titration



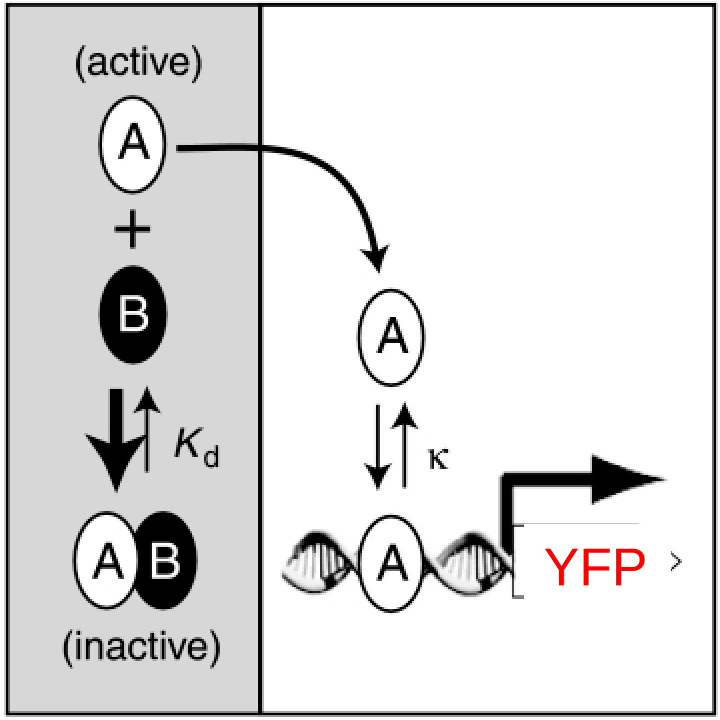
$$\frac{k_3}{k_4} > \frac{k_1}{k_2}$$

- Ultrasensitivity occurs when binding affinity between L and D (stoichiometric inhibitor) is much greater than that between L and R. The higher the former, the steeper the response is.
- At low concentrations, L is mostly soaked up by D. When total L increases to a level where all Ds are nearly used up, any further addition of L into the system will be all available for R. This is the point at which the abrupt change occurs.
- Note the input is the total amount of L not free L.

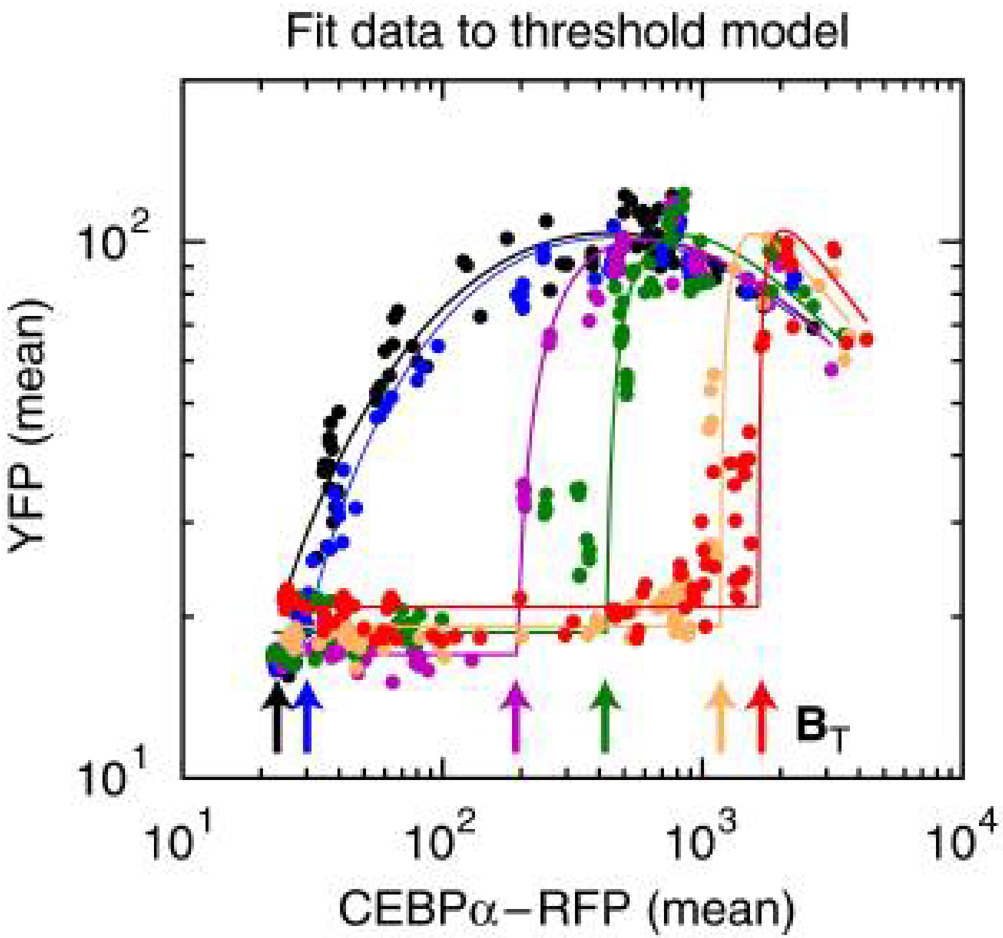




(4) An example of molecular titration ultrasensitivity

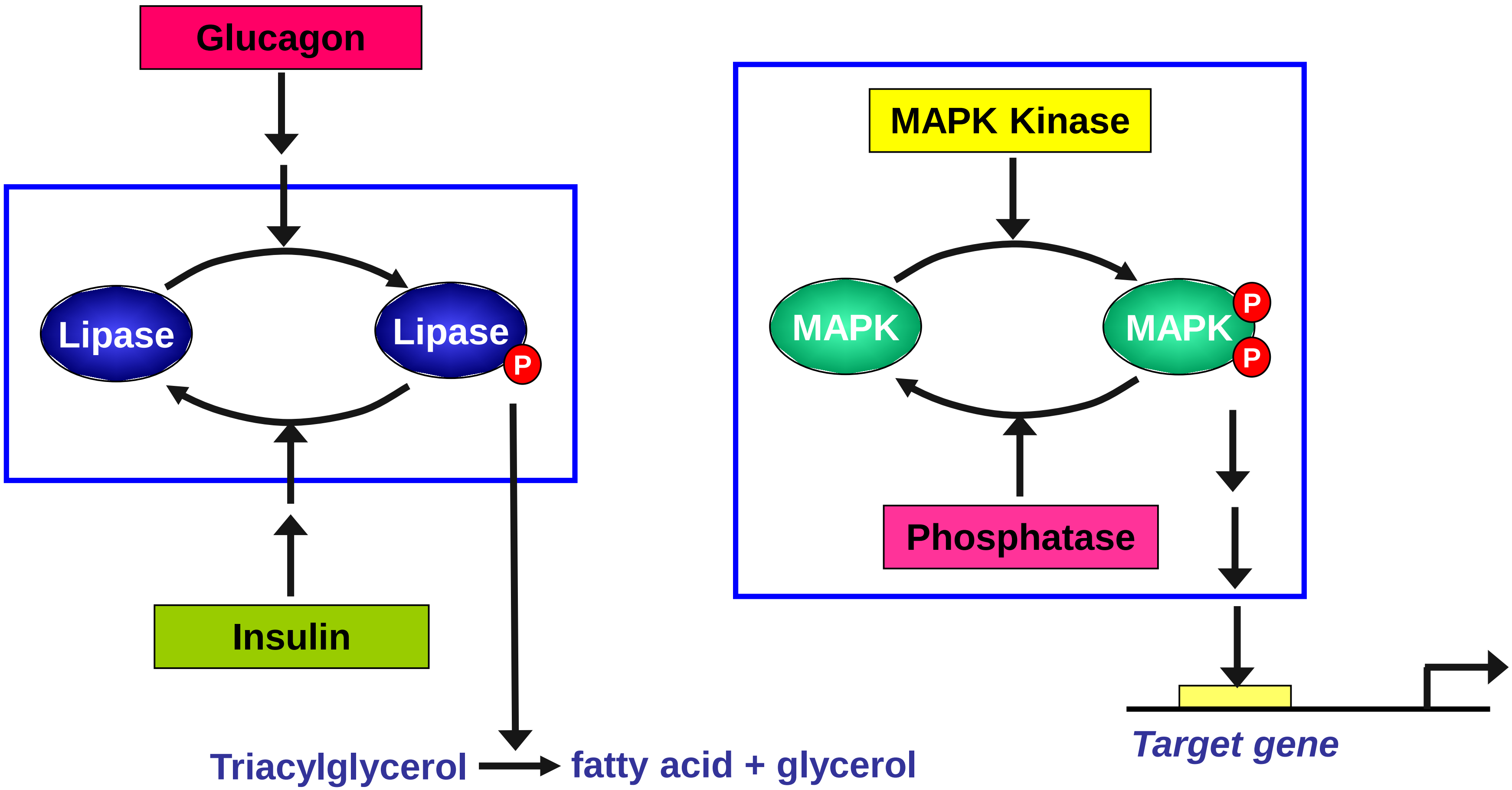


A: CEBPα-RFP

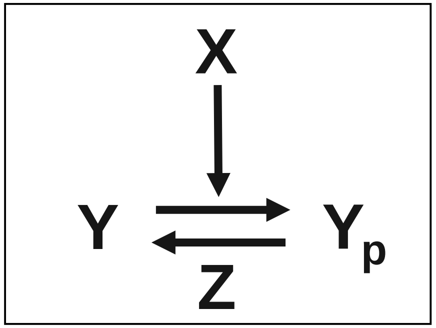


Buchler and Cross, Mol Sys Biol 2009

(5) Covalent modification cycle

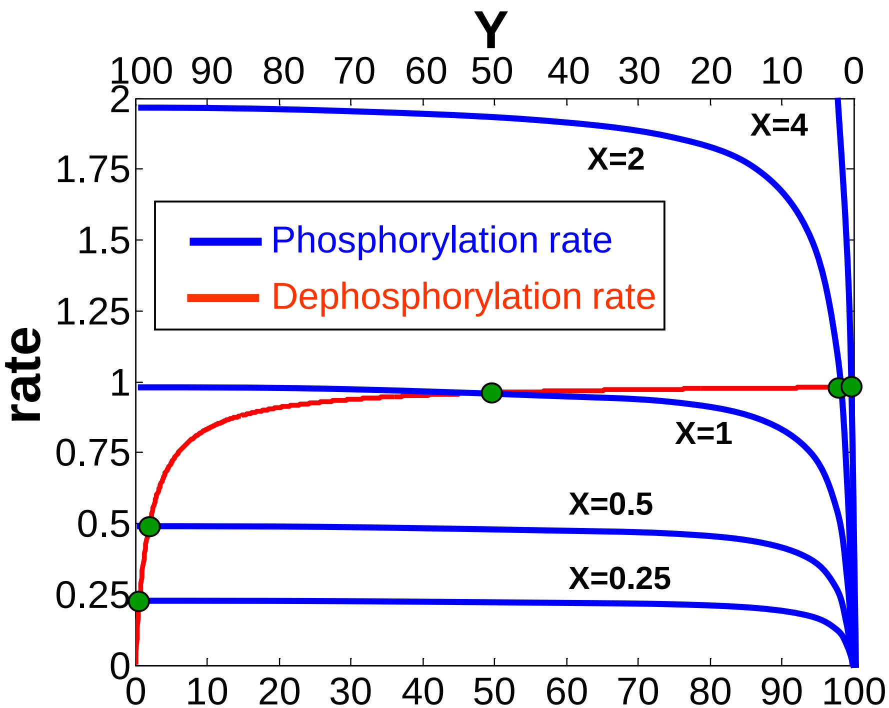


(5) Covalent modification cycle (zero-order ultrasensitivity)

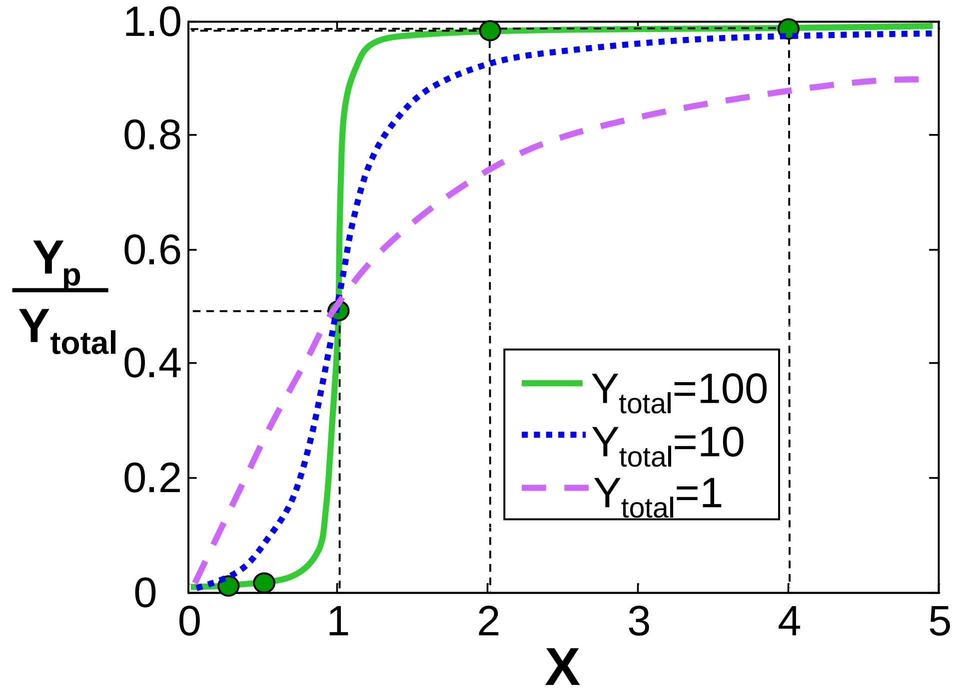


$$\frac{d[Y_p]}{dt} = \overbrace{\frac{k_1[X][Y]}{K_{m(x)}+[Y]}}^{\text{Phosphorylation rate}} - \overbrace{\frac{k_2[Z][Y_p]}{K_{m(z)}+[Y_p]}}^{\text{Dephosphorylation rate}}$$

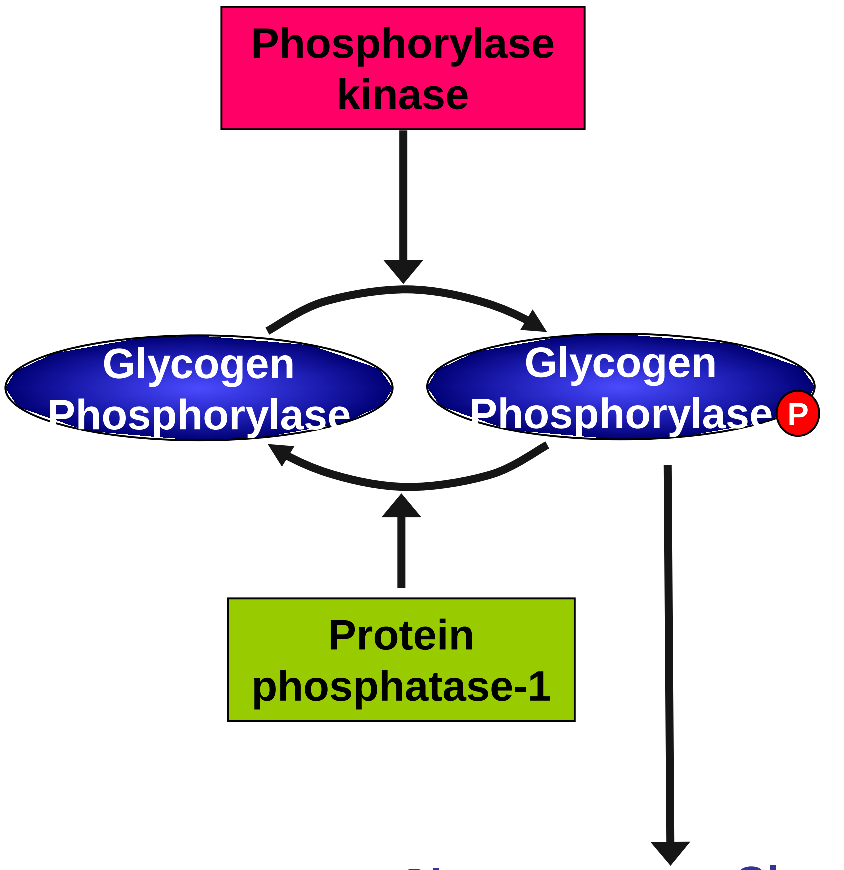
$$(Y_{total} = [Y]+[Y_p]) \quad (k_1 = k_2 = K_{m(x)} = K_{m(z)} = [Z] = 1)$$



When Y_{total}=100>>$K_{m(x)}$, $K_{m(z)}$ Y_p



(5) An example of zero-order ultrasensitivity



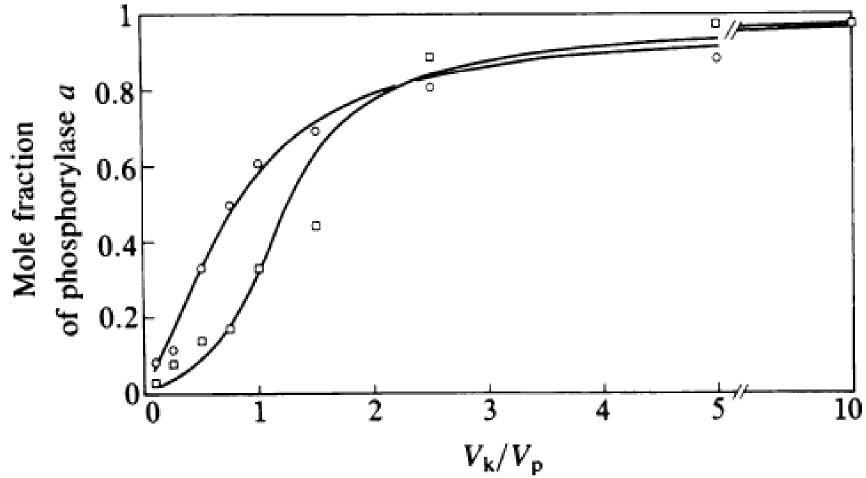
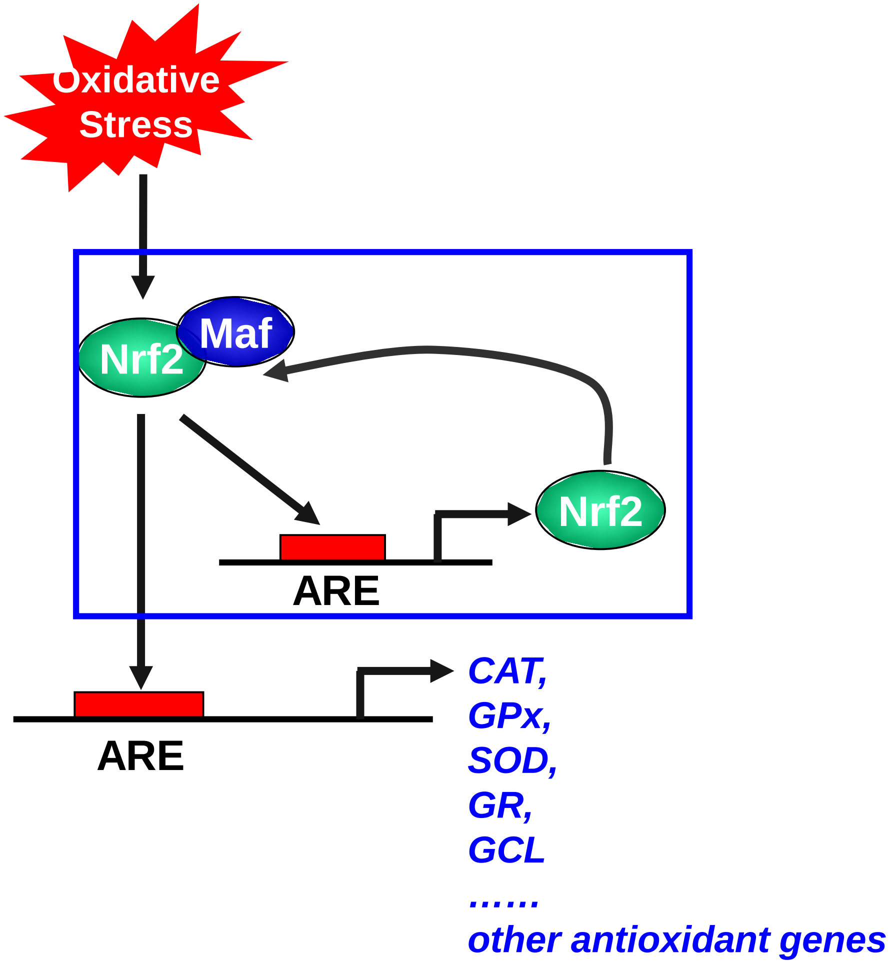


FIG. 2. Effect of varying the kinase/phosphatase activity ratio on the mole fraction of phosphorylase in the active (a) form at 20 μM (○) and 70 μM (□) phosphorylase. The points shown are averages, based on the results of assays performed on triplicate steady-state incubations on each of 2 (20 μM) or 3 (70 μM) separate days. The overall standard deviation for the fitted curve at 20 μM was 0.088 and 0.056 at 70 μM. The ratio V_k/V_p was varied by holding the phosphatase constant and varying the amount of kinase.

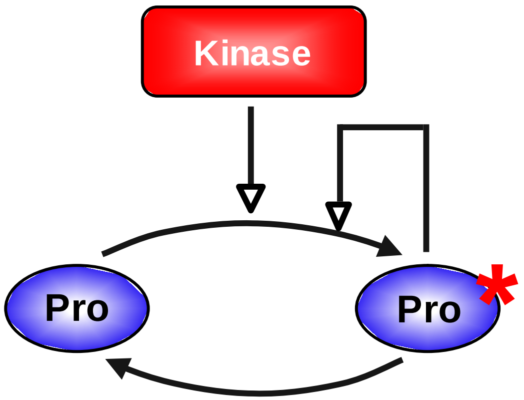
Meinke et al, PNAS 1986

(6) Positive feedback

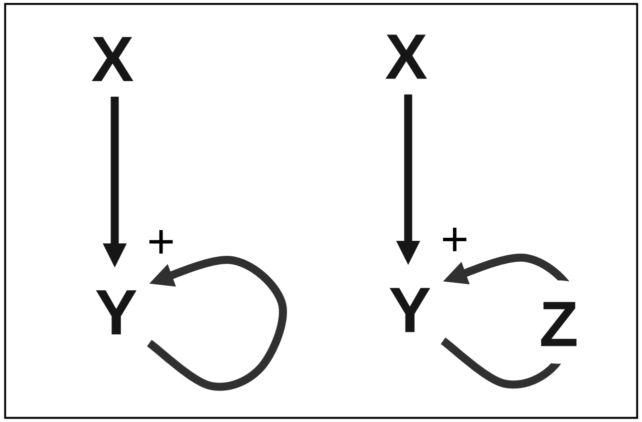
Gene auto-regulation

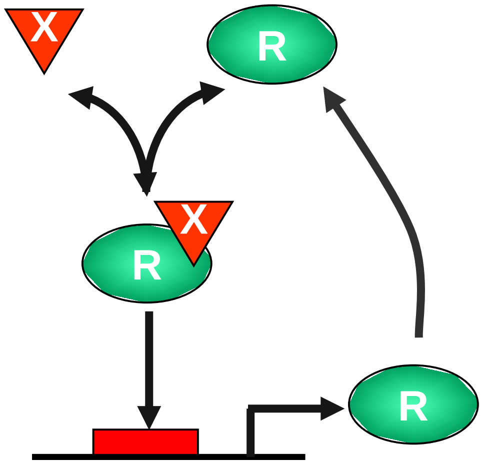


Auto-catalysis

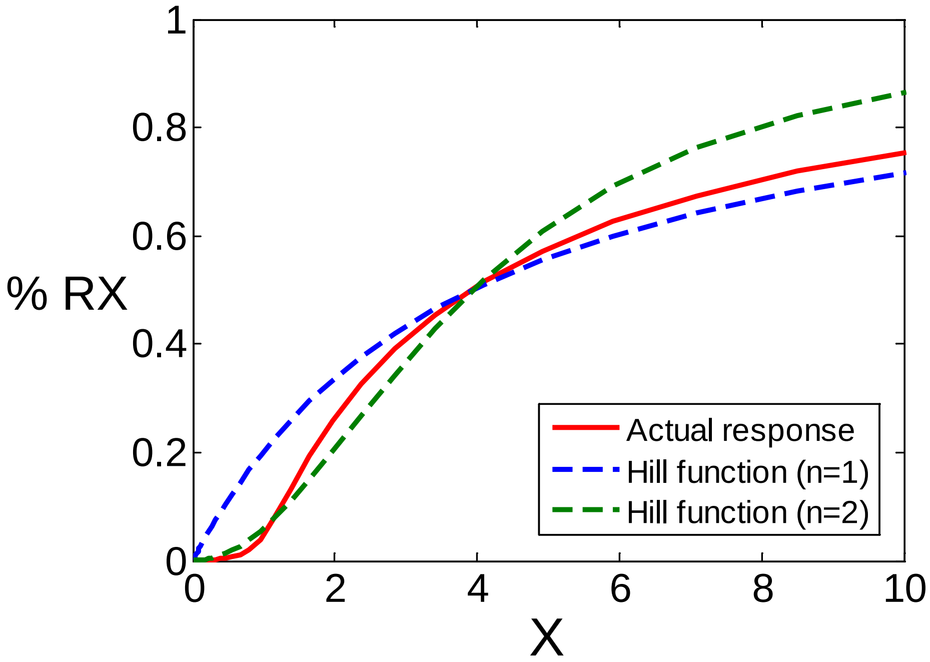


(6) Positive feedback



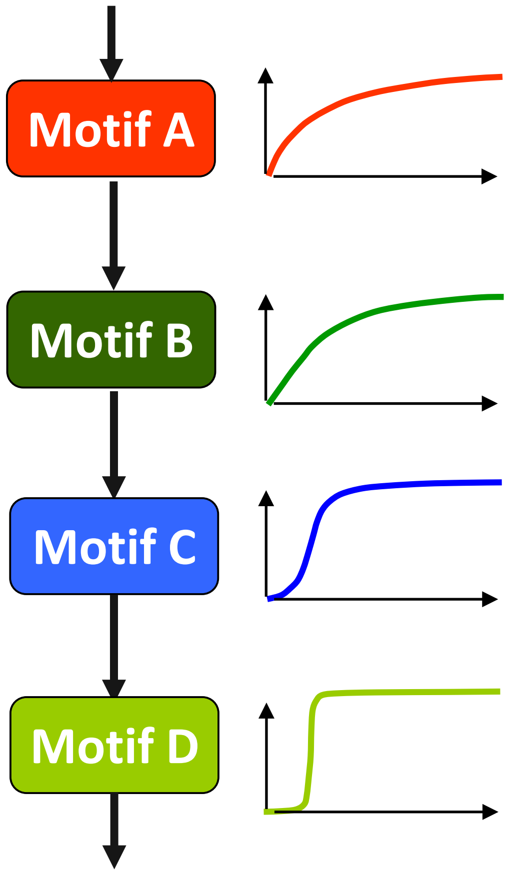


Ultrasensitivity arises even when every activation step in the feedback loop is linear.

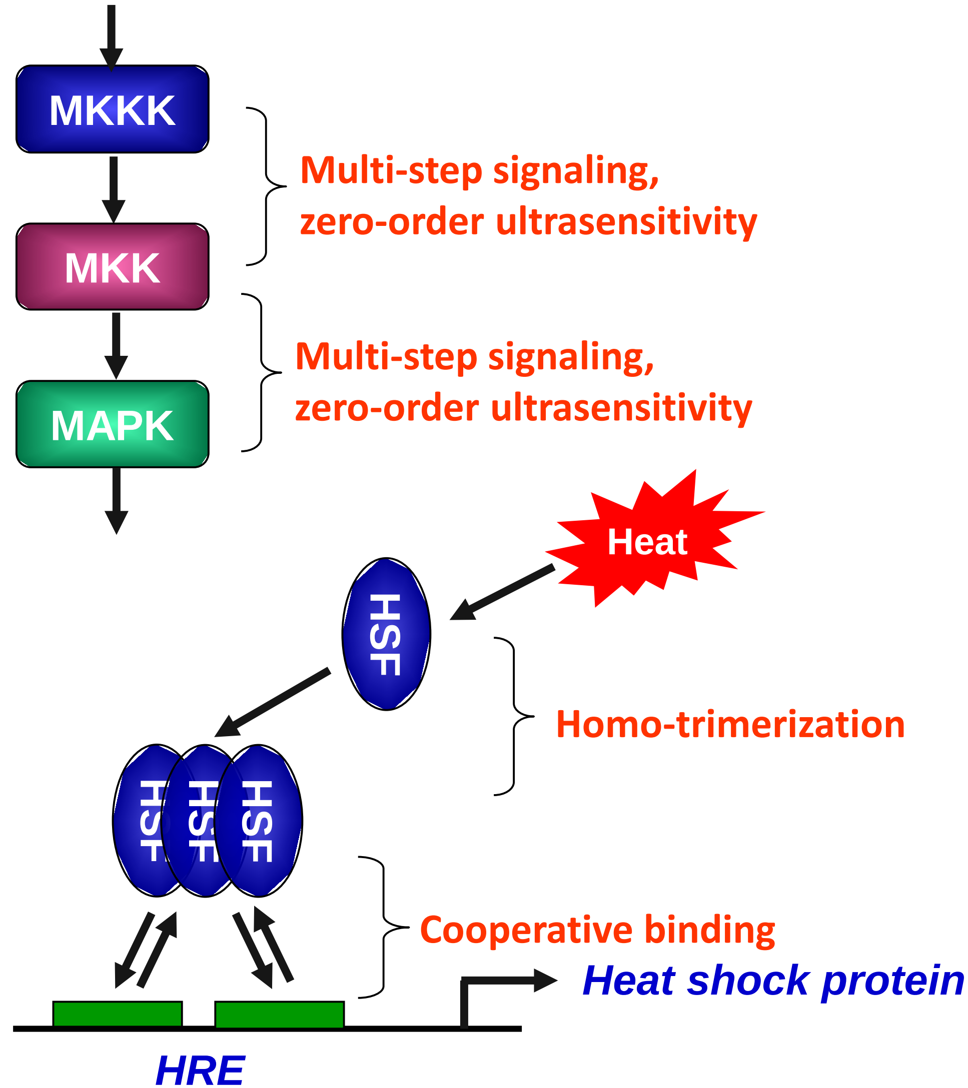


The ultrasensitive response can not be satisfyingly fitted with Hill function of any Hill coefficient.

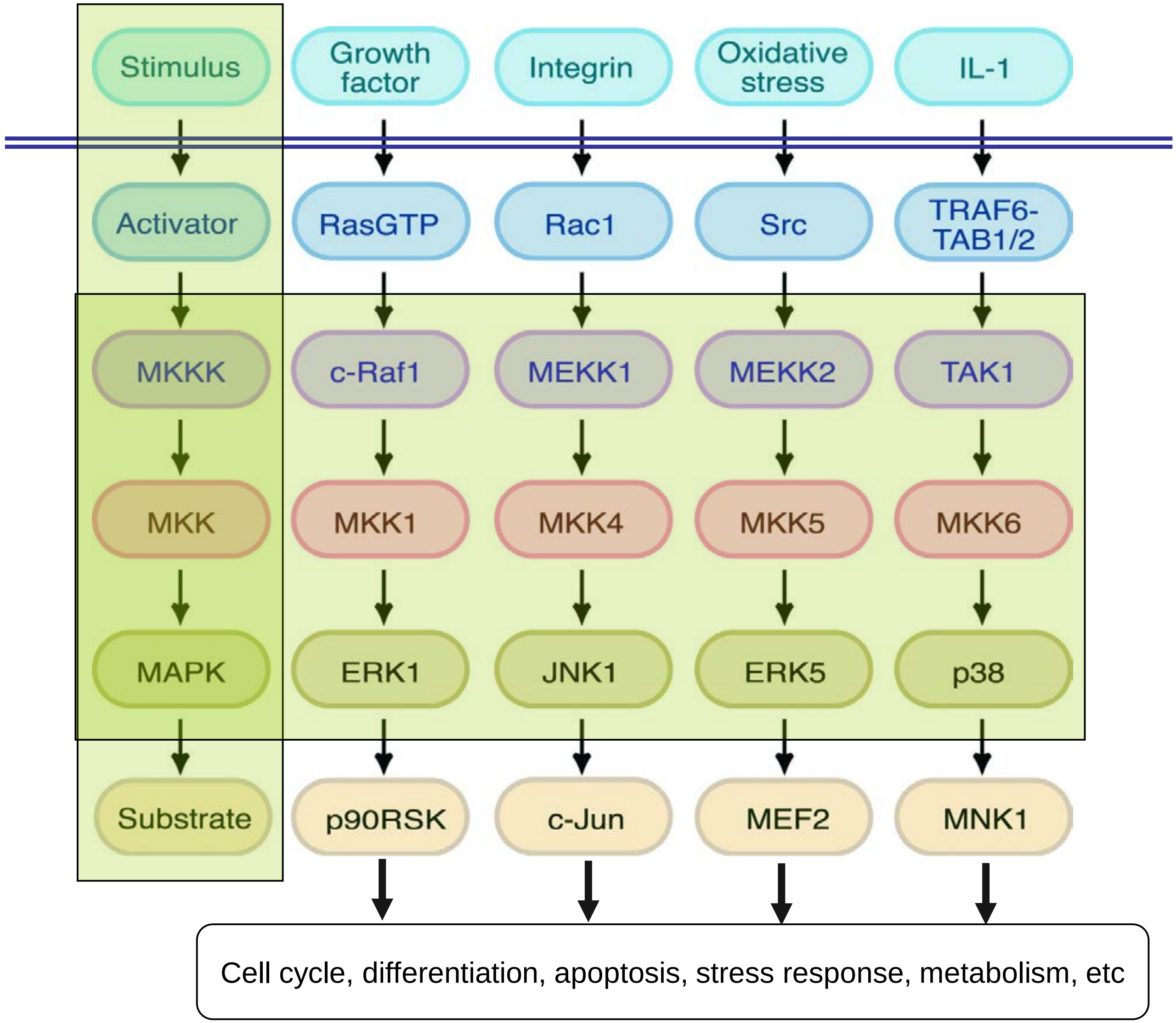
Combinations of Ultrasensitive Motifs



A number of slightly ultrasensitive motifs can be linked in sequence to give rise to an overall steeply sigmoid, or switch-like response.



MAPK cascade, motif, and function

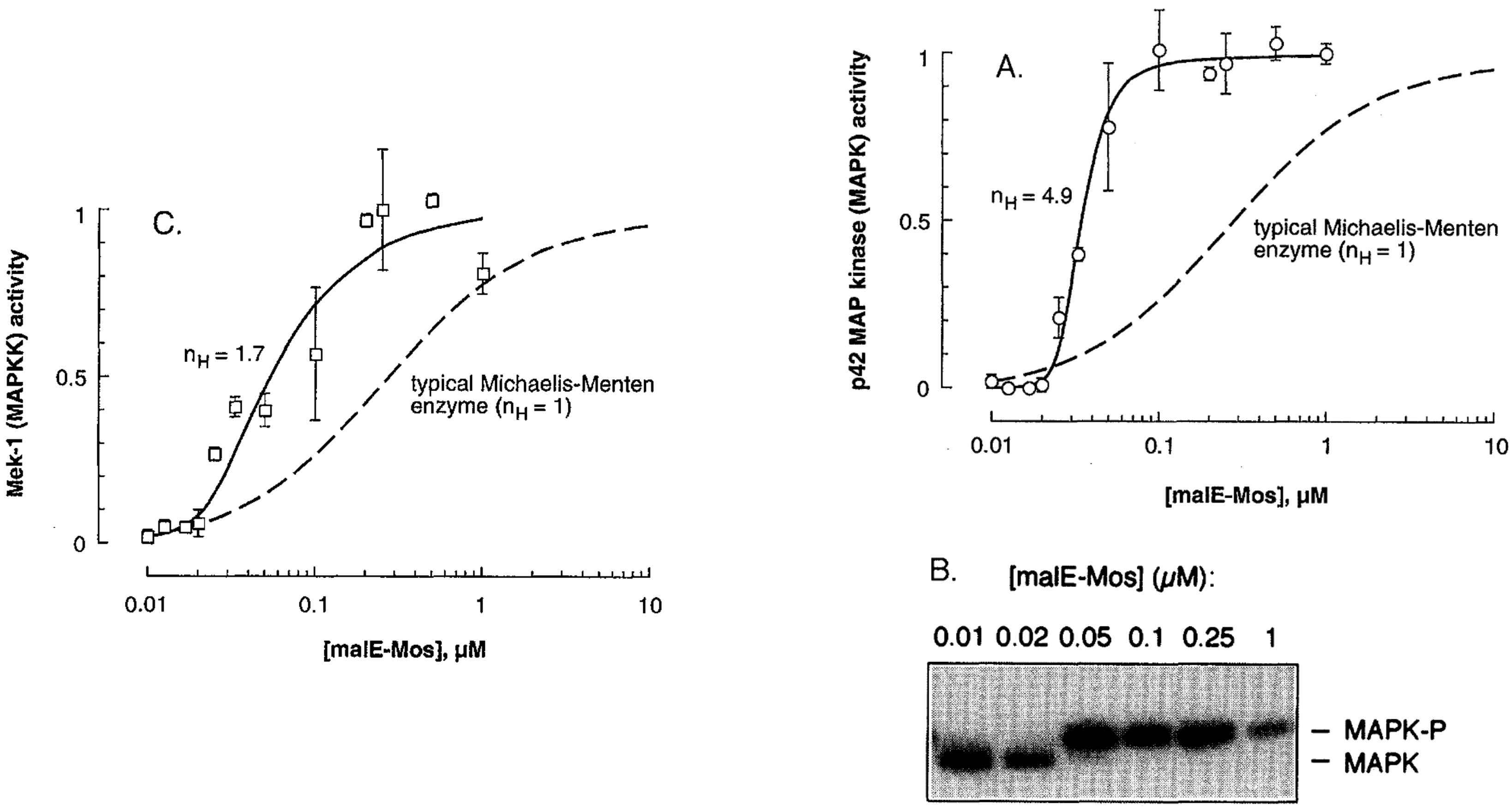


Adapted from Johnson and Lapadat, Science 2002

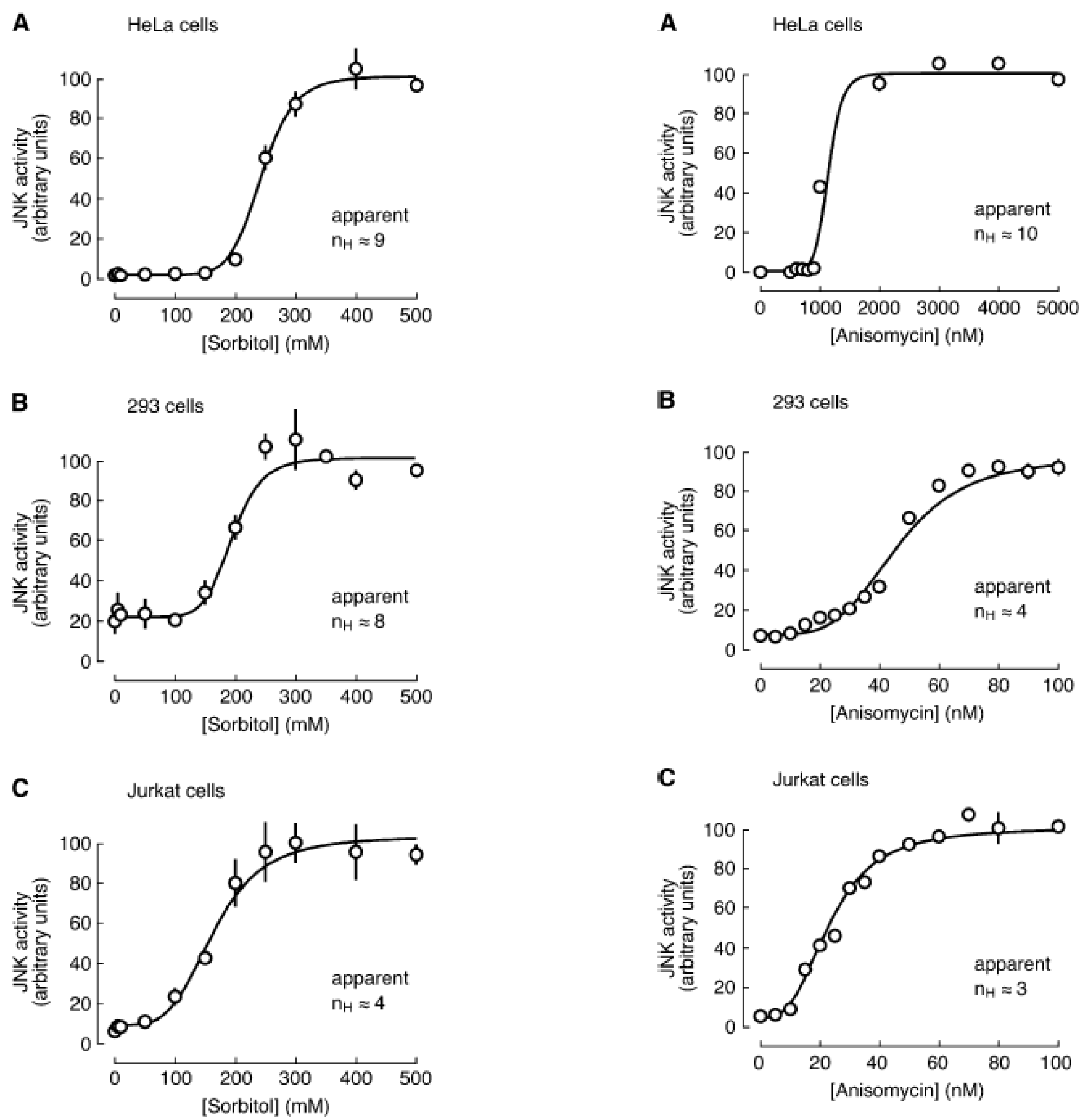
Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 10078–10083, September 1996
Biochemistry

Ultrasensitivity in the mitogen-activated protein kinase cascade

CHI-YING F. HUANG AND JAMES E. FERRELL, JR.†

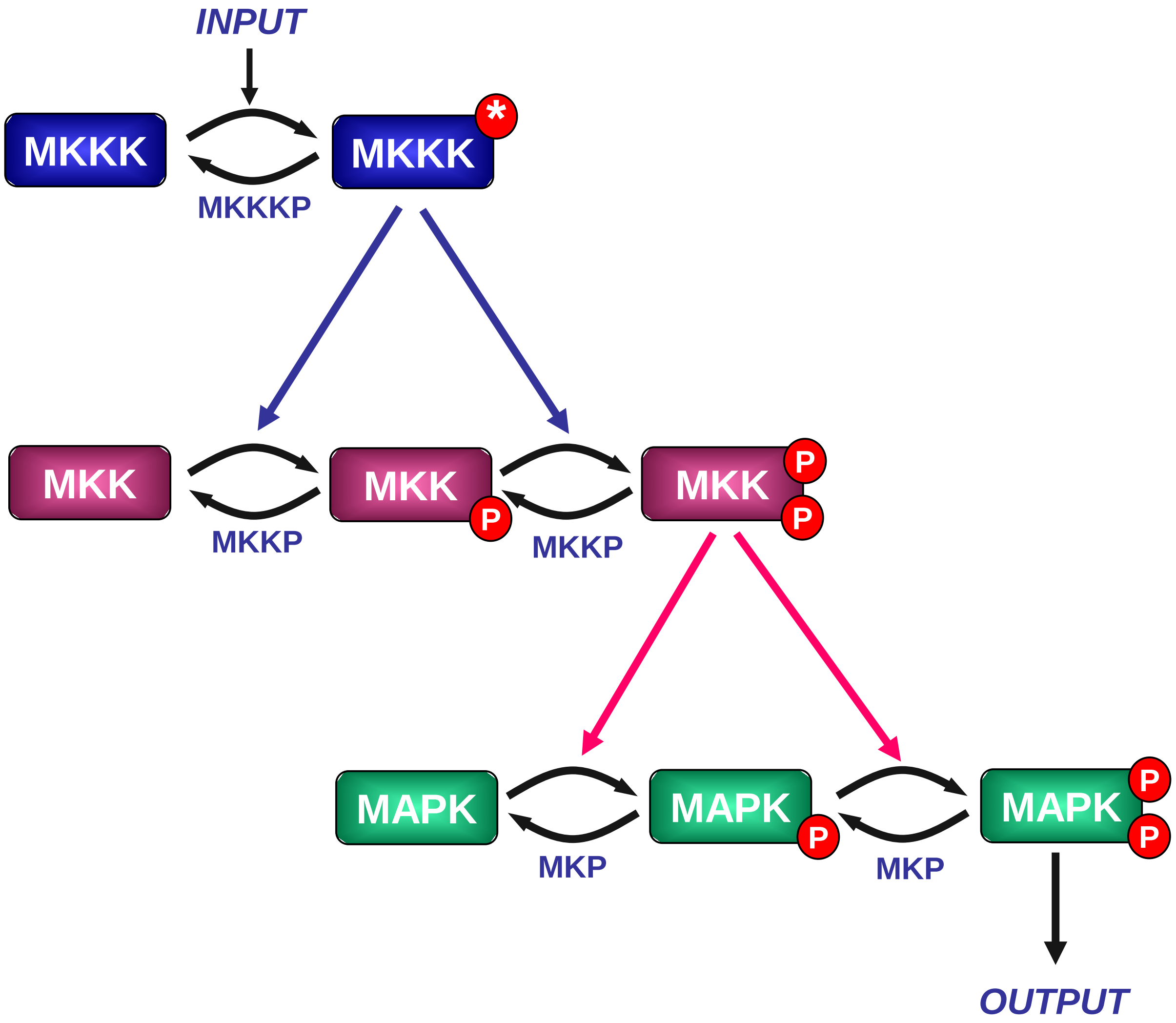


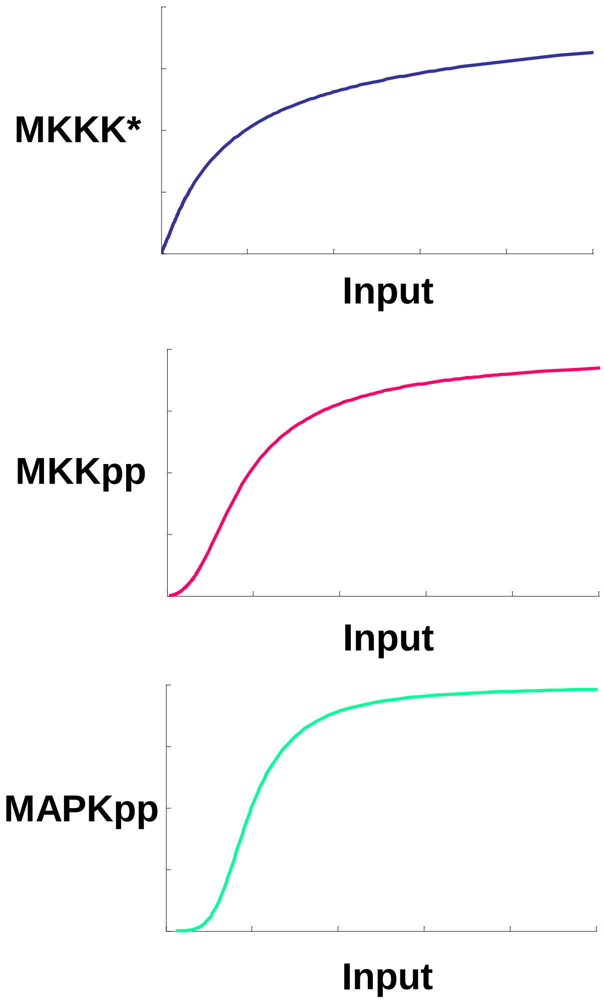
JNK ultrasensitivity in mammalian cells



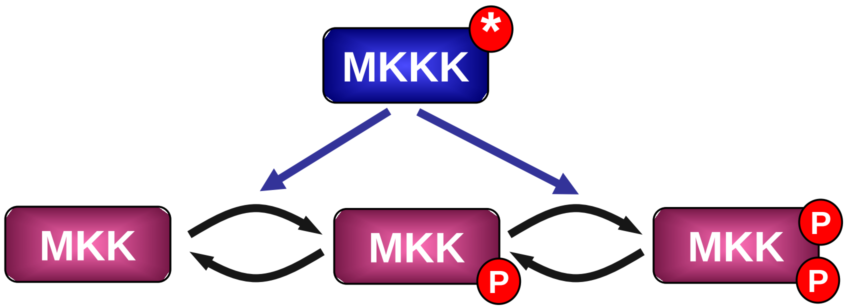
Bagowski et al, Current Biology 2003

MAPK cascade outputs increasing degree of ultrasensitivity



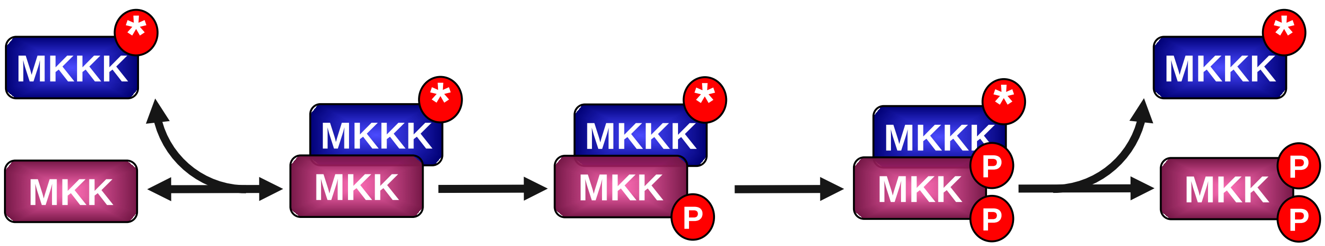


Origin of MAPK ultrasensitivity (I): multi-step signaling

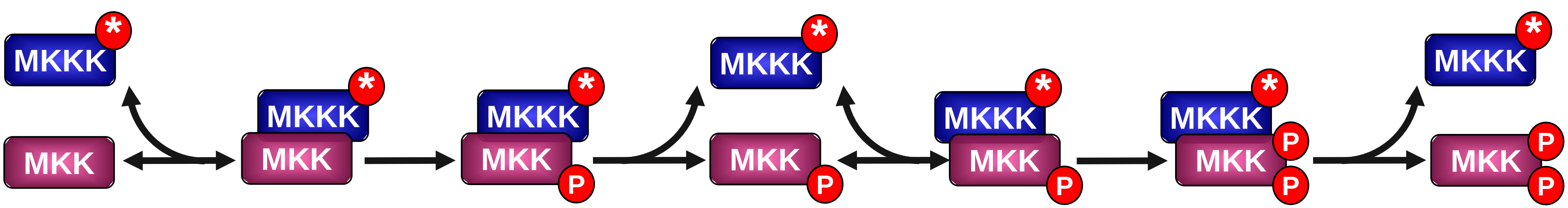


- Scenario 2 is what actually happens with dual-phosphorylation of MKK.
- Two separate collisions mean MKKK will appear (twice) as a non-linear term for the rate of dual-phosphorylation of MKK. This is a form of multi-step signaling, one of the sources for ultrasensitivity.
- Dual-phosphorylation of MAPK also proceeds similarly via two collisions.

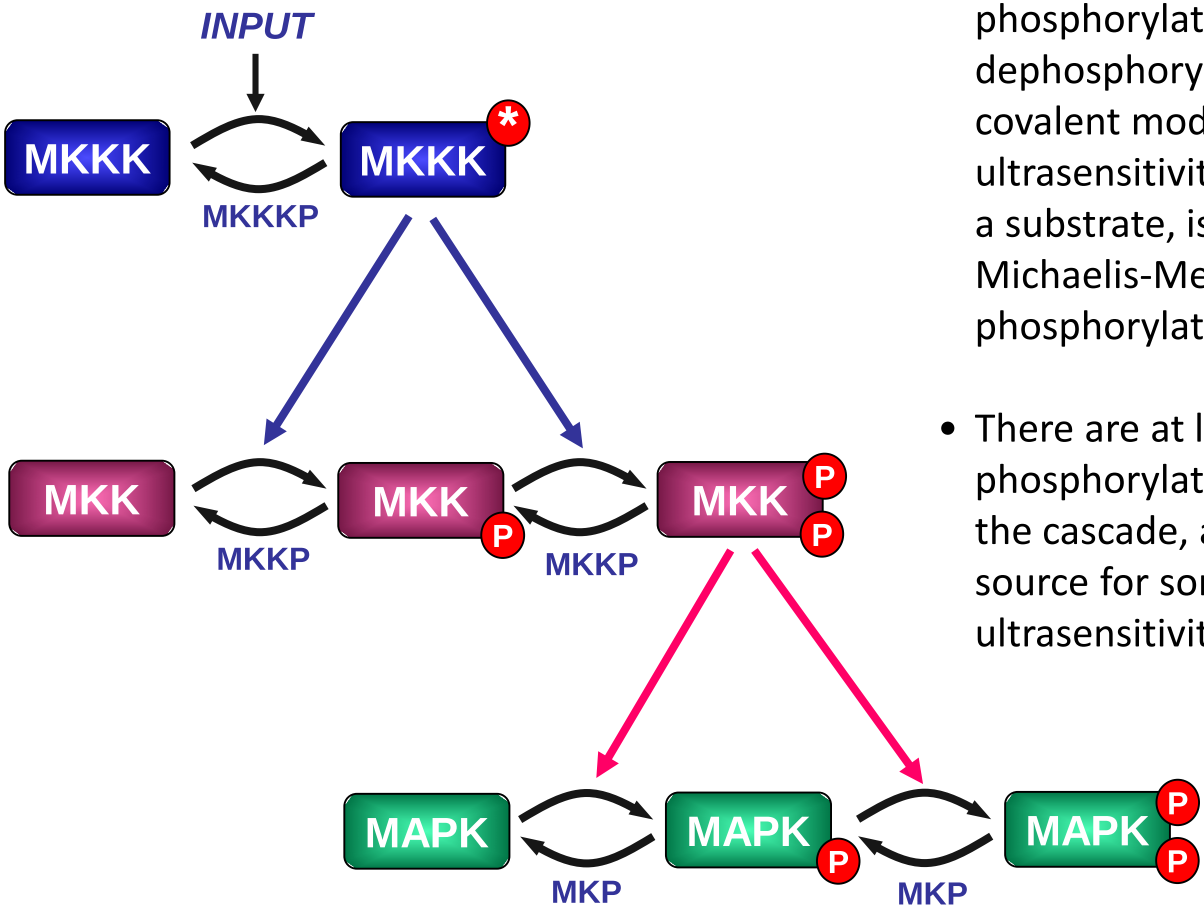
Scenario 1: one collision (processive)



Scenario 2: two collisions (nonprocessive, multi-step signaling → ultrasensitivity)



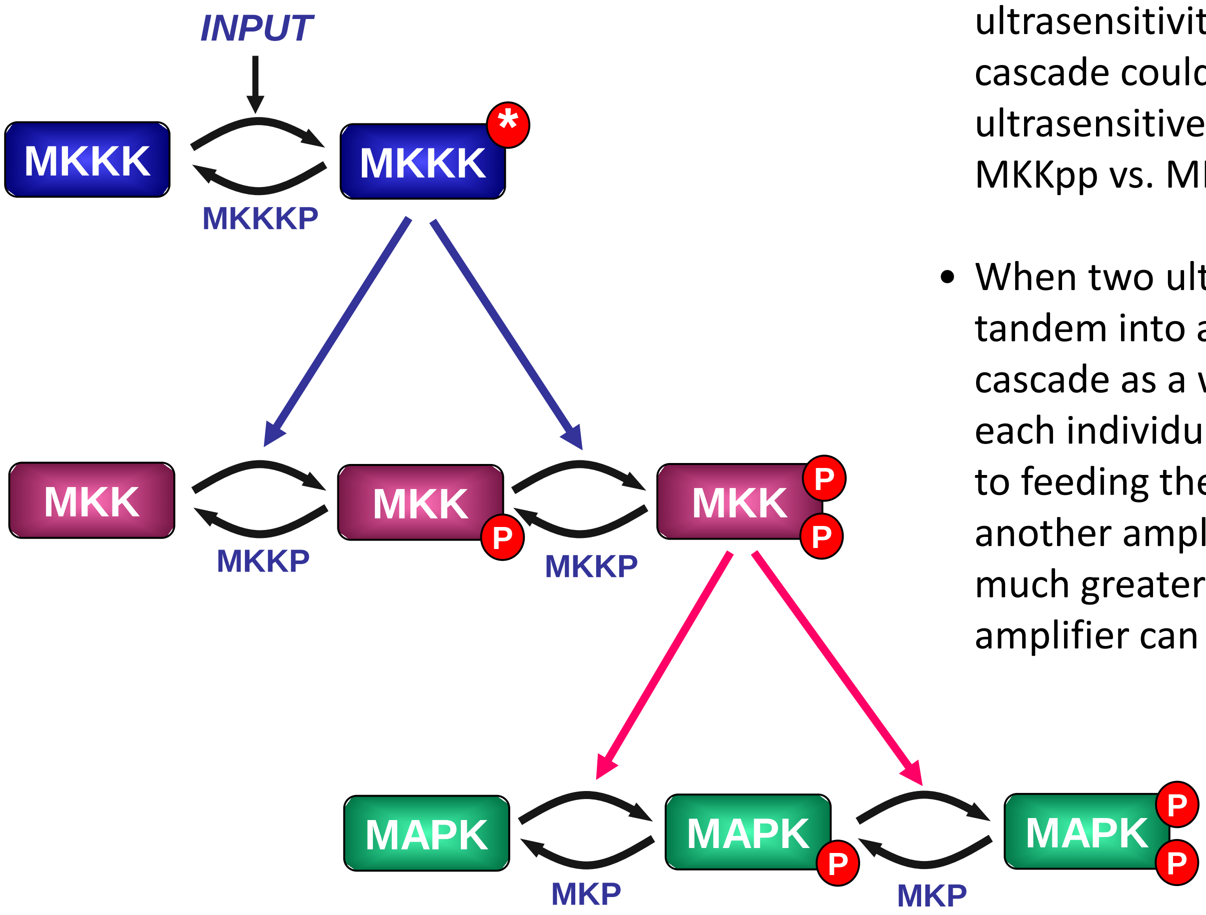
Origin of MAPK ultrasensitivity (II): zero-order ultrasensitivity



- In the MAPK cascade, each kinase is phosphorylated by its upstream kinase and dephosphorylated by a phosphatase. This covalent modification cycle may generate ultrasensitivity if the amount of the kinase, as a substrate, is comparable or greater than the Michaelis-Menten constants for its phosphorylation and dephosphorylation.

- There are at least four phosphorylation/dephosphorylation cycles in the cascade, and each could be a potential source for some degree of zero-order ultrasensitivity.

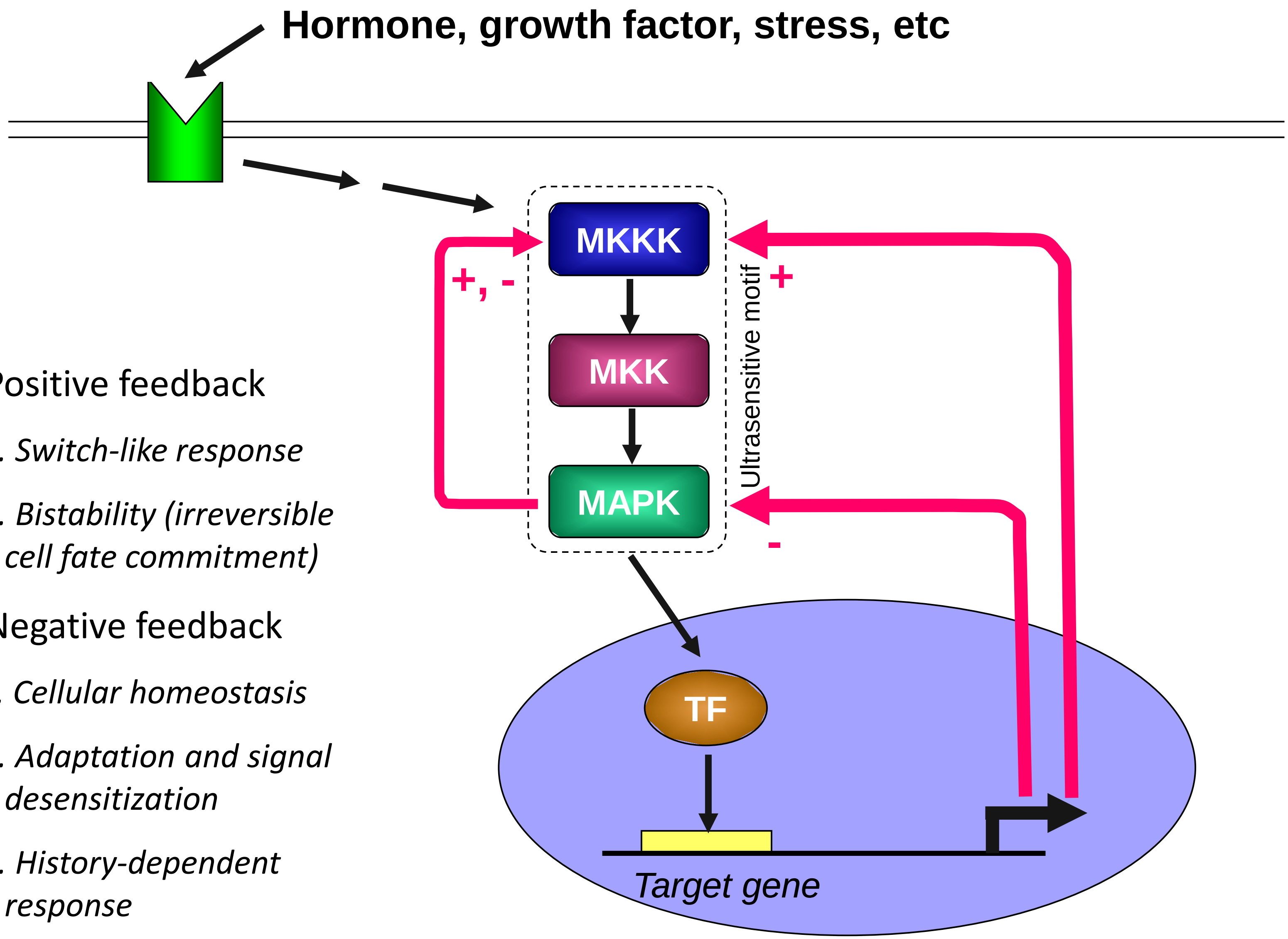
Origin of MAPK ultrasensitivity (III): layered arrangement



- With multi-step signaling and zero-order ultrasensitivity, each layer of the MAPK cascade could have some degree of ultrasensitive response of its own, e.g., MKKpp vs. MKKK*, and MAPKpp vs. MKKpp.

- When two ultrasensitive layers are linked in tandem into a cascade, it is possible that the cascade as a whole is more ultrasensitive than each individual layer alone. This is analogous to feeding the output of one amplifier into another amplifier, together they generate a much greater output than each individual amplifier can do.

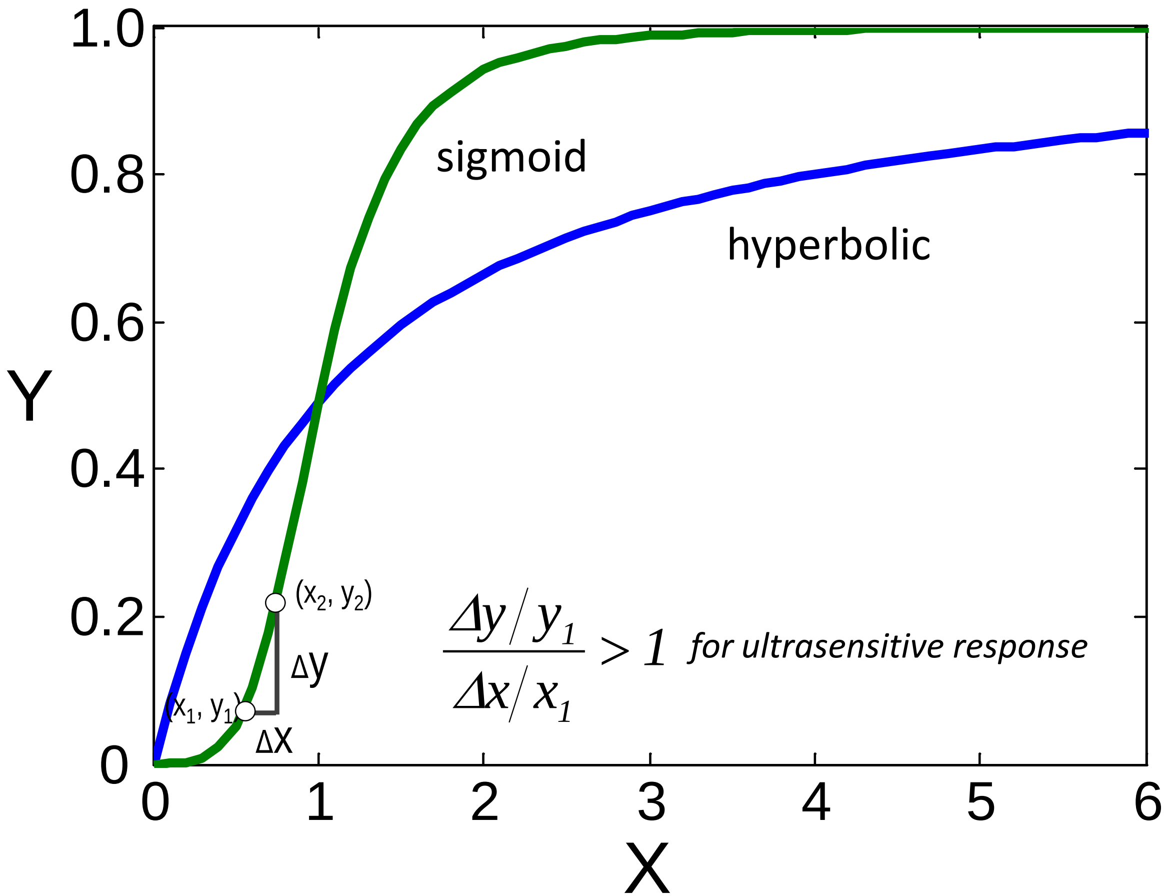
MAPK cascade is embedded in larger networks



- Positive feedback
  1. *Switch-like response*
  2. *Bistability (irreversible cell fate commitment)*
- Negative feedback
  1. *Cellular homeostasis*
  2. *Adaptation and signal desensitization*
  3. *History-dependent response*

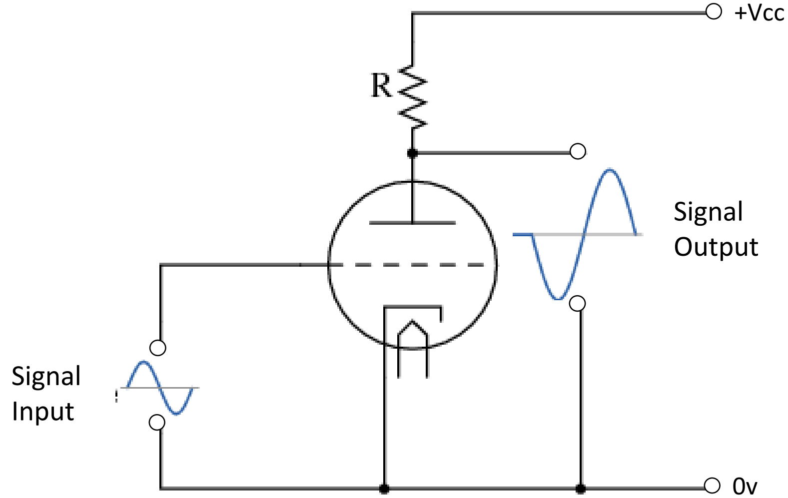
Ultrasensitive response motifs

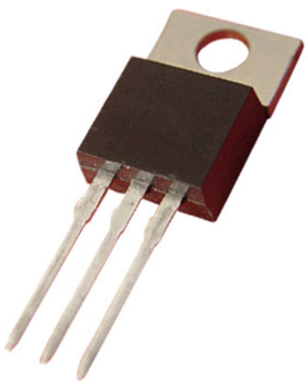
Ultrasensitivity refers to a (steady-state) stimulus-response that is significantly steeper than the hyperbolic, Michaelis-Menten form such that it appears globally as a sigmoid curve on a **<u>linear</u>** scale.

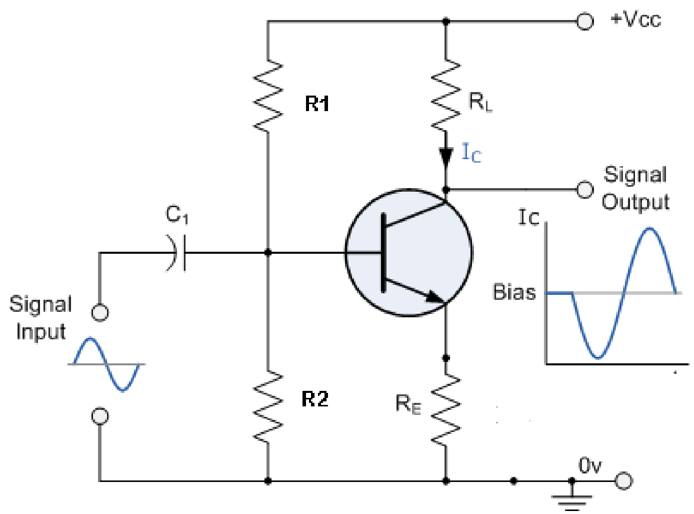


- Ultrasensitive response allows amplification of **<u>percentage</u>** change locally.
- The steepness of the globally sigmoid curve is usually approximated by Hill function.

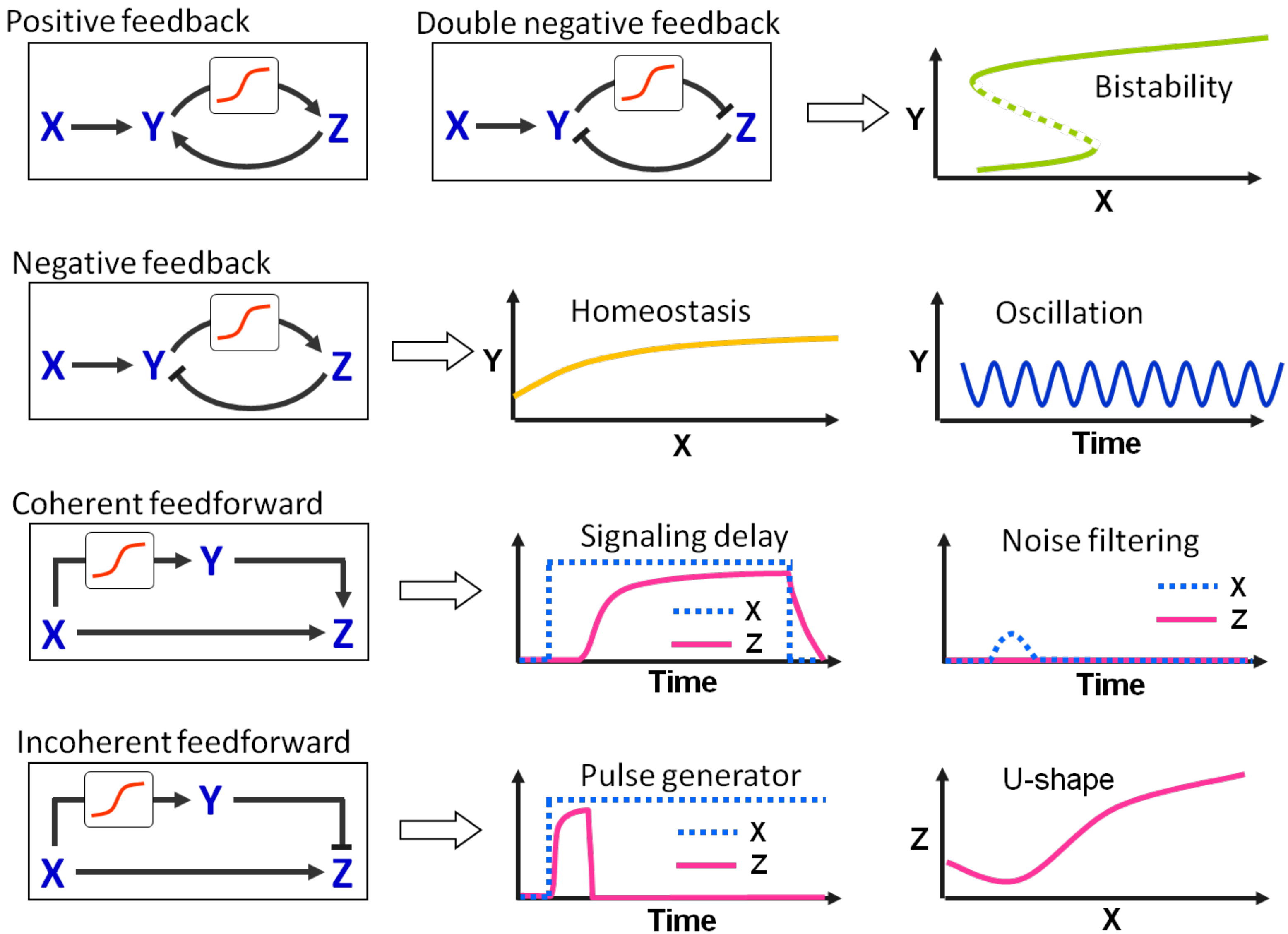
Invention of the vacuum tube triode and later the transistor – *both of which can amplify electrical signals* – heralded the age of modern electronics







Ultrasensitivity is required for complex dynamics



Summary

- Ultrasensitive motifs transfer signal in a sigmoid manner such that they amplify the percentage changes in the input signal.
- Motifs that may generate ultrasensitivity include positive cooperative binding, homo-multimerization, multi-step signaling, molecular titration, zero-order covalent modification cycle, and positive feedback.
- Ultrasensitive motifs can be linked in sequence to generate steeply sigmoid, or even switch-like response.
- The MAPK cascade transfers signal in an ultrasensitive manner.
- Ultrasensitive motifs are required to generate more complex behaviors, including bistability, robust homeostasis, oscillation, and others.