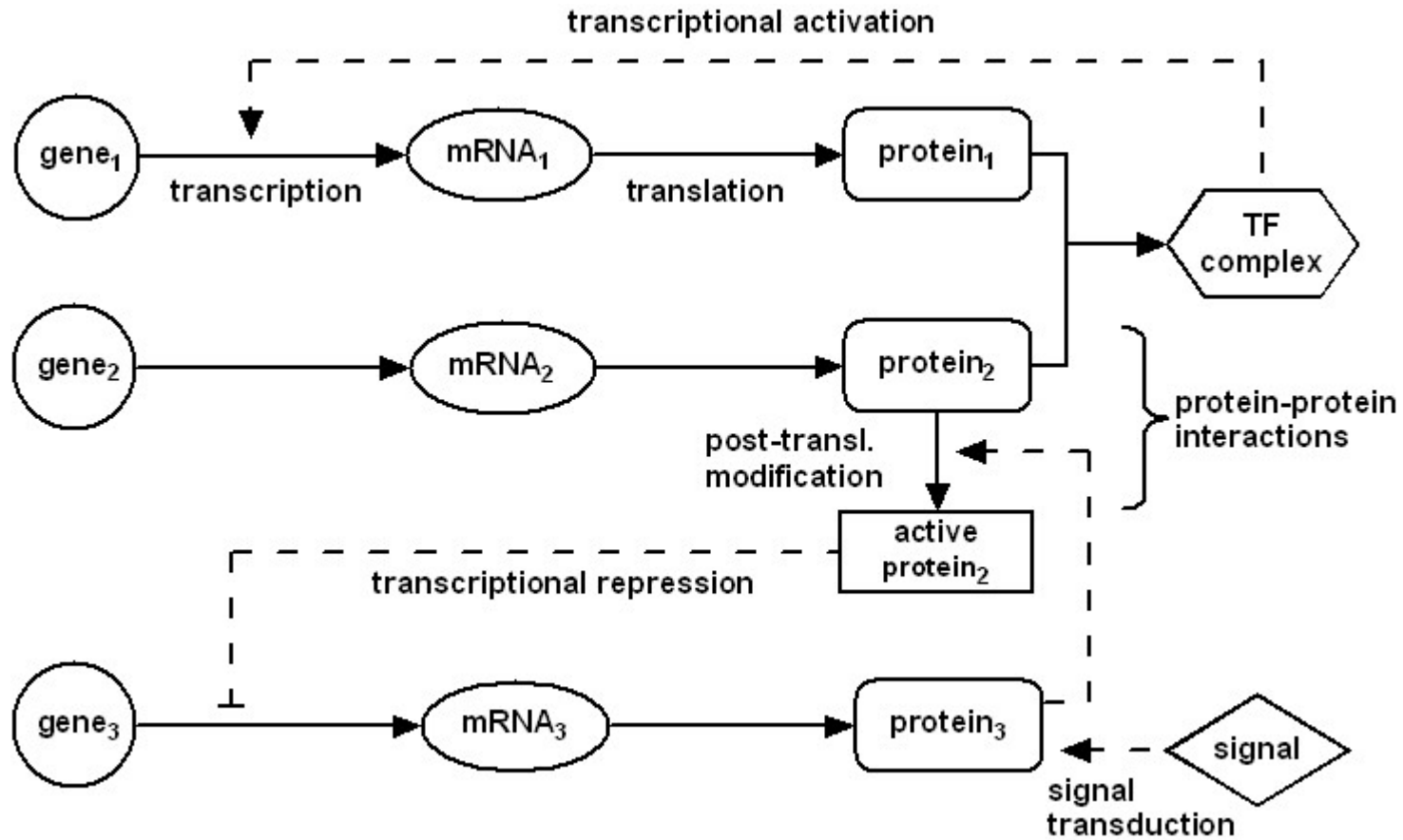


Understanding the dynamics and function of cellular networks

Cells are complex systems

- functionally diverse elements
- diverse interactions that form networks
 - signal transduction-, gene regulatory-, metabolic-
- have a function that needs to be performed
 - sense and respond to the environment
 - maintain homeostasis
 - replicate
- need certain dynamical features
 - sensitive to some changes, insensitive/adaptable to others
 - robust to unwanted perturbations
 - evolvable, shaped by evolution
- Which network topological features can ensure reliable and robust dynamics?

Signaling, gene regulation and protein interactions are intertwined



Mapping of cellular interaction networks

Experimental advances allow the construction of genome-wide cellular interaction networks

- **Protein networks:**

Uetz et al. 2000, Ito et al., 2001 – *S. cerevisiae*,

Giot et al. 2003 – *Drosophila melanogaster* , Li et al. 2004 – *C. elegans*

Human interactome

- **Metabolic networks:**

KEGG, WIT, Ecocyc

- **Transcriptional regulatory networks**

Shen-Orr et al. 2002 – *E. coli*,

Guelzim et al 2002, Lee et al. 2002 - *S. cerevisiae*,

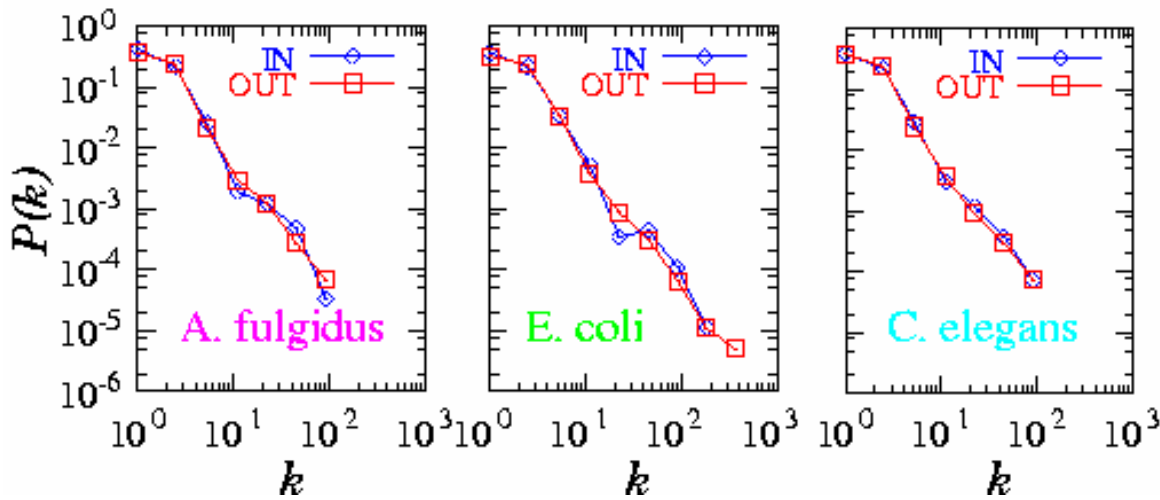
Davidson *et al.* 2002 – sea urchin

- **Signal transduction networks**

Ma'ayan et al. 2005 – mammalian hippocampal neuron

Graph analysis uncovered common architectural features of cellular networks: **Connected, short path length, heterogeneous (scale-free), conserved interaction motifs**

node degree: number of edges (indicating regulation by/of multiple components)
 degree distribution: fraction of nodes with a given degree



Metabolites

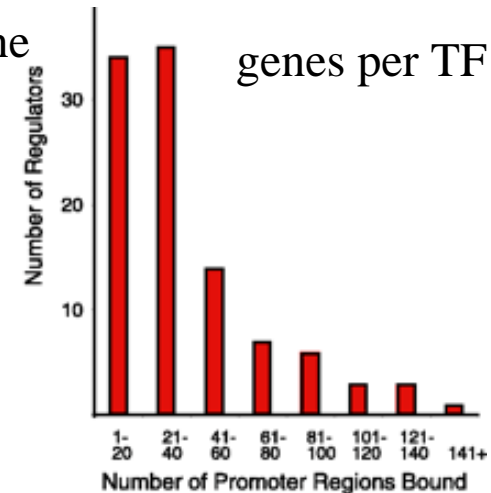
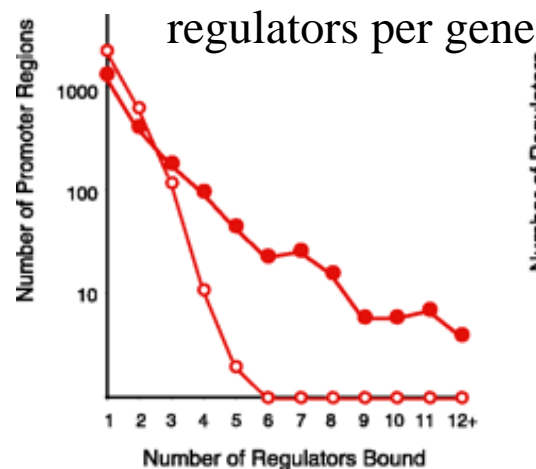
$$P_{in}(k) \approx k^{-2.2}$$

$$P_{out}(k) \approx k^{-2.2}$$

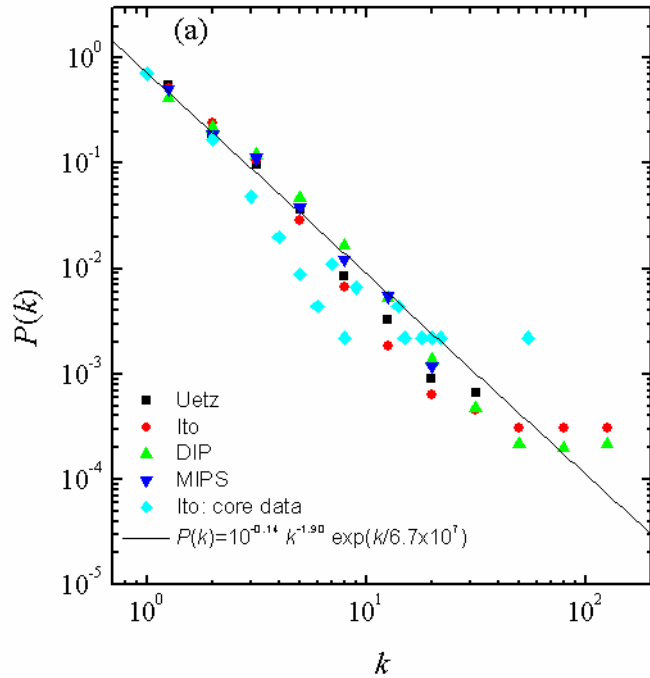
H. Jeong et al., Nature 407, 651 (2000)

S. cerevisiae
 transcriptional network

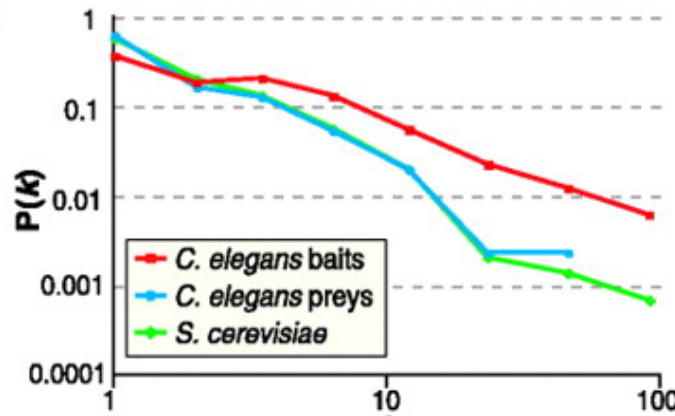
Lee et al, Science 298, 799 (2002)



S. cerevisiae protein network



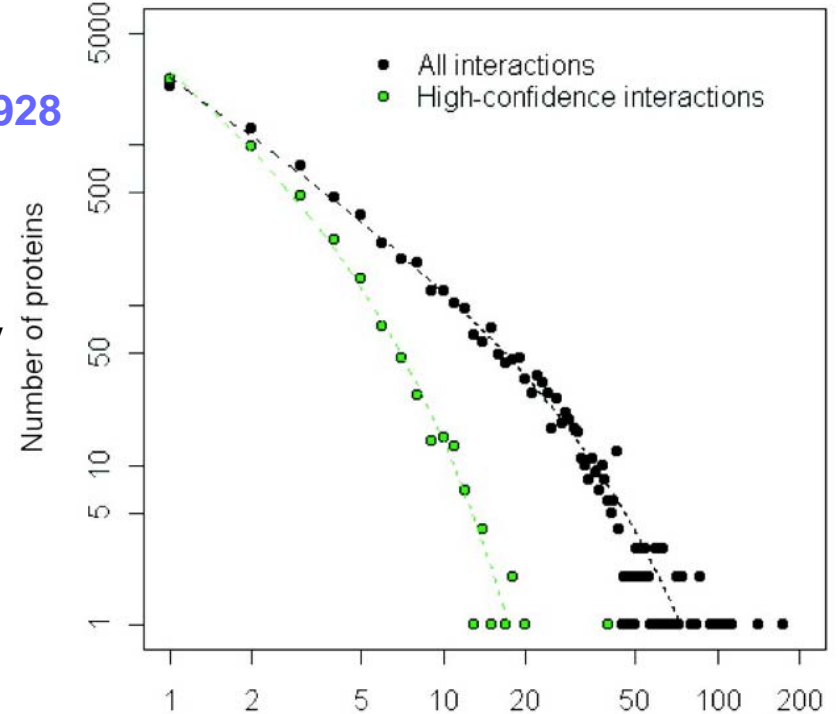
C. Elegans protein network



Li et al., Science 303, 540 (2004)

Yook et al., Proteomics 4, 928 (2004)

D. melanogaster protein network



Giot et al., Science 302, 1727 (2003)

Biological networks are highly heterogeneous

Many nodes have only a few edges, but highly interactive (hub) nodes are also possible.

This suggests robustness to random mutations, but vulnerability to mutations in highly-connected components.

R. Albert, A.L. Barabasi, Rev. Mod. Phys. 74, 47 (2002)

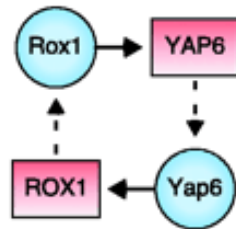
Abundant regulatory motifs

Autoregulation



Positive and negative feedback loops

Multi-Component Loop

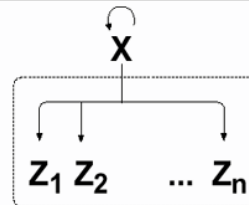


Positive and negative feedforward loops

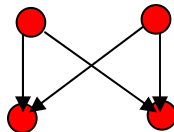
Feedforward Loop



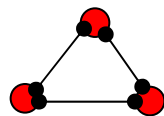
single input module (SIM)



bifans



scaffolds



Feedforward loop:

convergent direct and indirect regulation; noise filter

Single input module:

one TF regulates several genes; temporal program

Bifans: combinatorial regulation

Scaffold: protein complexes

Positive and negative motifs:

Balance: homeostasis

More positive: long-term info storage

Shen – Orr et al., Nature Genetics (2002)

Lee et al, Science 298, 799 (2002)

Ma'ayan et al, Science 309, 1078 (2005)

Importance of a dynamical understanding

Only subsets of the genome-wide interaction networks are active in a given external condition

[Han et al. 2004](#) – dynamical modularity of protein interaction networks
[Luscombe et al. 2004](#) – endogeneous and exogeneous transcriptional subnetworks

Network topology needs to be complemented by a description of network dynamics – states of the nodes and changes in the state

First step - **pseudo-dynamics**: propagation of reactions in chemical(interaction) space, starting from a source (signal)

Complete dynamical description is only feasible on smaller networks (modules):

Signal transduction in bacterial chemotaxis, NF-kB signaling module, the yeast cell cycle, Drosophila embryonic segmentation

Access dynamics through modeling

First step: define the system; collect known states or behavior

Input: components; states of components

Hypotheses: interactions; kinetics (rates, parameters).

Validation: capture known behavior.

Explore: study cases that are not accessible experimentally
change parameters, change assumptions

Tyson 1991 – cell cycle

Barkai & Leibler 1997, Spiro et al. 1997 – chemotaxis

Bhalla & Iyengar 1999 - EGF pathway

Kholodenko 2000 – MAPK signaling module

G. von Dassow et al. 2000 – segment polarity gene network

Hoffman et al. 2002 - NF-kB signaling

Types of models

1. **Continuous** - similar to chemical kinetics
 - differential equations
 2. **Discrete** - assume a small set of qualitative states
 - e.g. active or inactive; basal, intermediate, high
 - the changes in state are given by discrete (logical) rules
-
1. **Deterministic** - no randomness is involved in the development of future states of the system
 2. **Stochastic** - non-deterministic in that the next state of is not fully determined by the previous state.
 - can take into account the fluctuations in mRNA/protein numbers and external noise

Basics of Chemical Kinetics - 1



- Rate of reaction = rate of disappearance of A = $r_A = -d[A]/dt =$
of moles of A reacting (“disappearing”) per unit time per unit volume

[A] = concentration of A = (# moles/volume) ; 1 mole = 6.023×10^{23} molecules

- Reaction rate law is an algebraic equation involving concentrations
(not a differential equation)

$$r_A = -k [A]$$

$$r_A = -k [A]^2$$

$$r_A = -k_1 [A]/(1+k_2[A])$$

- For a given reaction, the rate law is determined **experimentally**
- Measure [A] as a function of time and calculate slope ($d[A]/dt$) at various time points.

Basics of Chemical Kinetics - 2



➤ In general : $r_A = -k(T) \cdot f([A],[B],\dots)$

**Temperature
dependence**

Rate Constant

**Concentration
dependence**

Other factors impacting
rate constant

- Catalyst
- Pressure
- Ionic strength (pH)
- Solvent

(Not really “constant”, just
independent of concentration)

➤ Reaction Order: $r_A = -k \cdot [A]^\alpha \cdot [B]^\beta$

The reaction is of order α with respect to A and of order β with respect to B

➤ Reaction order can be fractional $r_A = -k \cdot [A]^1 \cdot [B]^{0.5}$

➤ Not every reaction has an order! $r_A = -k_1 \cdot [A] / (1 + k_2 \cdot [B])$

(Temperature and concentration dependence not separable)

Basics of Chemical Kinetics - 3

- **Elementary Reaction:** Reaction order of each species is identical with the stoichiometric coefficient of that species



- Elementary reactions hypothesized to happen exactly how they are written!

(One molecule of A colliding with 2 molecules of B to produce C)

- Elementary reactions are typically 1st or 2nd order

(Probability of three molecules colliding very low)

- Reversible reactions:



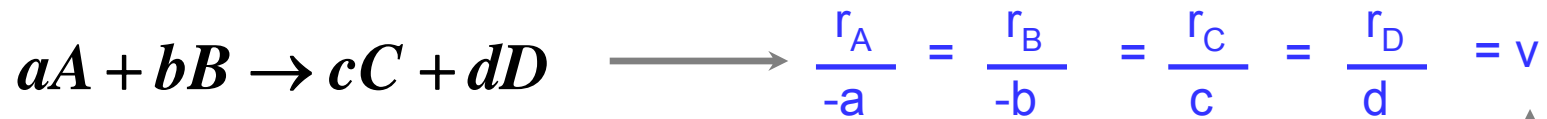
Forward Reaction



Backward Reaction

Basics of Chemical Kinetics - 4

➤ Reaction Stoichiometry + Law of Conservation of Mass



(Irrespective of whether reaction is elementary or not)

Reaction flux

Specify rate law

$$d[A] / dt = -a \cdot v$$

$$v = -k \cdot [A]^a \cdot [B]^b$$

$$d[B] / dt = -b \cdot v$$

$$v = -k \cdot [A] \cdot [B]$$

$$d[C] / dt = c \cdot v$$

Specify initial conditions

$$d[D] / dt = d \cdot v$$

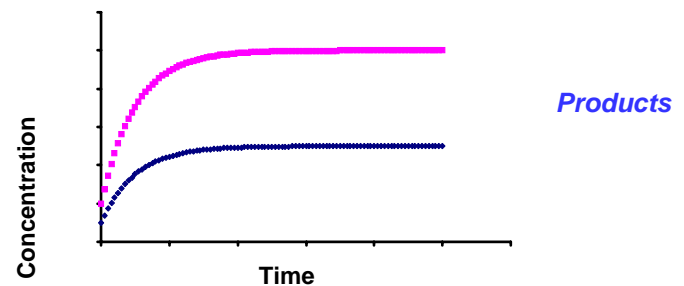
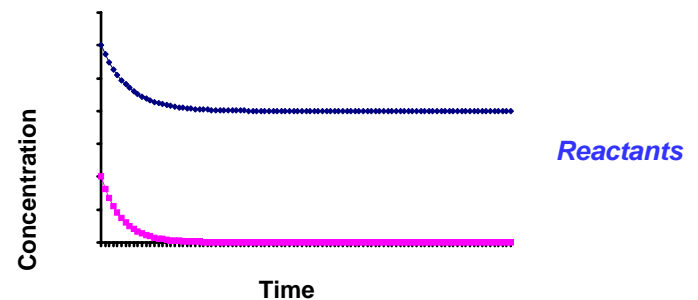
$$[A]_{(t=0)} = [A]_0$$

$$[B]_{(t=0)} = [B]_0$$

$$[C]_{(t=0)} = [C]_0$$

$$[D]_{(t=0)} = [D]_0$$

Concentration Time Course





Determine the relation between the reaction rates and the reaction flux.

Assume the reaction is elementary. Determine the rate of change of [A], [B], [C]



Determine the relation between the reaction rates and the reaction flux.

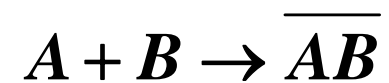
Assume the reaction is elementary. Determine the rate of change of [A], [B], [C]

$$\frac{d[A]}{dt} = \frac{d[B]}{dt} = -k[A][B] \quad \frac{d[C]}{dt} = k[A][B]$$

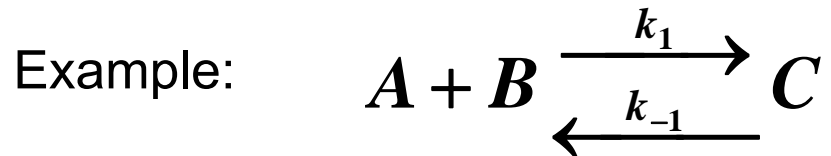
Ex. 2

Write the condition(s) of mass conservation.

Hint: think of the reaction as a complex formation



Reversible reactions



For simplicity, we'll leave off the brackets from $[A]$, ..

$$\frac{dA}{dt} = \frac{dB}{dt} = -k_1 AB + k_{-1} C$$

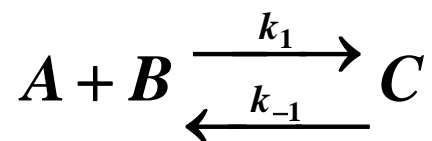
$$\frac{dC}{dt} = k_1 AB - k_{-1} C$$

Mass conservation: $A + C = A_0$ $B + C = B_0$

Units: $k_1 - (\text{mol}/\text{volume}/\text{time})^{-1}$, $k_{-1} - (\text{time})^{-1}$

Steady states

If the rates of the forward and backward reactions are equal, the system is able to reach a steady state where the concentrations do not change in time



$$\frac{dA}{dt} = \frac{dB}{dt} = \frac{dC}{dt} = 0 \quad \text{if} \quad k_1 AB - k_{-1} C = 0$$

$$C_{ss} = \frac{k_1}{k_{-1}} A_{ss} B_{ss} = \frac{k_1}{k_{-1}} (A_0 - C_{ss})(B_0 - C_{ss})$$

Solve for C_{ss}

Enzyme-catalyzed reactions

Most reactions in biological systems would not take place at perceptible rates in the absence of **enzymes**.

Enzymes are specialized proteins that bind specific reactants, get them close together, and by this, accelerate the reaction up to a million times.

In this context, the reactants are called **substrates**.

In enzyme-catalyzed reactions the rate of product synthesis depends **nonlinearly** on the concentration of the substrate.

Michaelis-Menten model of enzymatic reactions

Leonor Michaelis, Maud Menten (1913)

1. A specific enzyme-substrate complex is a necessary intermediate in catalysis
2. The product does not revert to the original substrates



Ex. Draw two possible network representations of this process.

Michaelis-Menten kinetics



$$\frac{dS}{dt} = -k_1 E S + k_{-1} \overline{ES} \qquad \frac{dP}{dt} = k_2 \overline{ES}$$

$$\frac{d\overline{ES}}{dt} = k_1 E S - k_{-1} \overline{ES} - k_2 \overline{ES}$$

Mass conservation: $E_T = E + \overline{ES}$

Assumption: the enzyme-substrate complex is in quasi-steady-state

$$\frac{d\overline{ES}}{dt} = 0, \quad \overline{ES} = ES \frac{k_1}{k_{-1} + k_2}$$

Michaelis-Menten kinetics (cont.)



Goal: express the rate of product synthesis as a function of substrate concentration

$$\frac{dP}{dt} = k_2 \overline{ES}$$

$$\left. \begin{aligned} \overline{ES} &= ES \frac{k_1}{k_{-1} + k_2} \\ E_T &= E + \overline{ES} \\ K_M &= \frac{k_{-1} + k_2}{k_1} \end{aligned} \right\} \frac{dP}{dt} = k_2 E_T \frac{S}{K_M + S}$$

Michaelis-Menten kinetics (cont.)



$$\frac{dP}{dt} = k_2 E_T \frac{S}{K_M + S} \quad K_M = \frac{k_{-1} + k_2}{k_1}$$

Ex. 1

Draw the dependence of the rate of product synthesis on the substrate concentration. Characterize three limits/points on the curve.

Ex. 2

What is the upper limit for k_2/K_M ?

Enzyme-catalyzed reactions

$$\frac{dP}{dt} = k_2 E_T \frac{S}{K_M + S}$$

K_M is equal to the substrate concentration at which the reaction rate is half its maximal value.

Limit 1 $\frac{dP}{dt} \approx k_2 E_T$

$k_2 E_T$ is the number of substrate molecules converted in a unit time when the enzyme is fully saturated with substrate.

Limit 2 $\frac{dP}{dt} \approx \frac{k_2}{K_M} E_T S$

The efficiency of an enzyme can be described by k_2 / K_M

The ultimate limit for enzyme efficiency is the diffusion-limited encounter of enzyme and substrate, or $10^9 s^{-1} mol^{-1}$

Chemical kinetics-like models of cellular processes

Assumption: cellular synthesis and degradation processes can be described as simple or enzyme-catalyzed reactions

Ex.: receptor - ligand binding

methylation reactions – catalyzed by methylating enzymes,

phosphorylation - catalyzed by kinases

dephosphorylation – spontaneous or catalyzed by phosphatases

protein synthesis –catalyzed by mRNA,

protein degradation – spontaneous or catalyzed

[J. Tyson, K. Chen, B. Novak, Curr. Opin. Cell Biology 15, 221 \(2003\)](#)

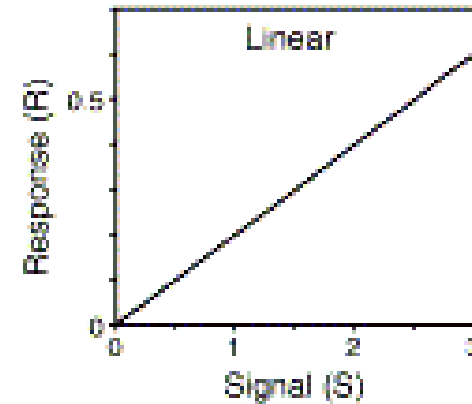
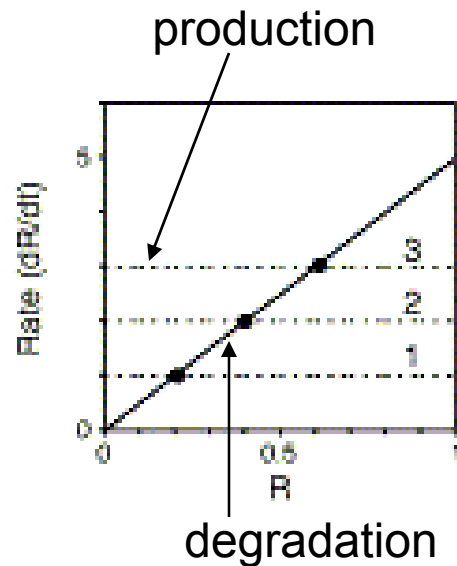
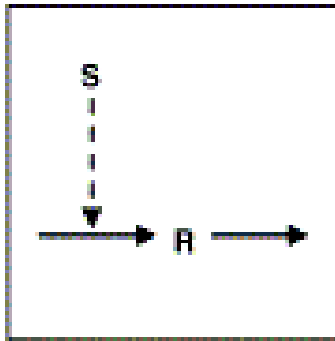
Kinetics of protein synthesis and degradation

Protein synthesis: mRNA \rightarrow protein (sufficient supply of amino-acids)

Protein degradation: protein \rightarrow

$$\frac{dR}{dt} = k_1 S - k_2 R \quad \text{Steady state:} \quad R_{ss} = \frac{k_1 S}{k_2}$$

(a)



Kinetics of phosphotransfer

Phosphorylation: protein \rightarrow phospho-protein

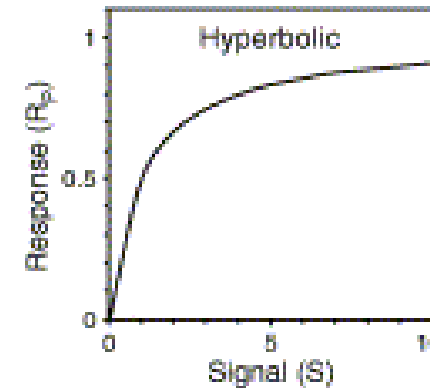
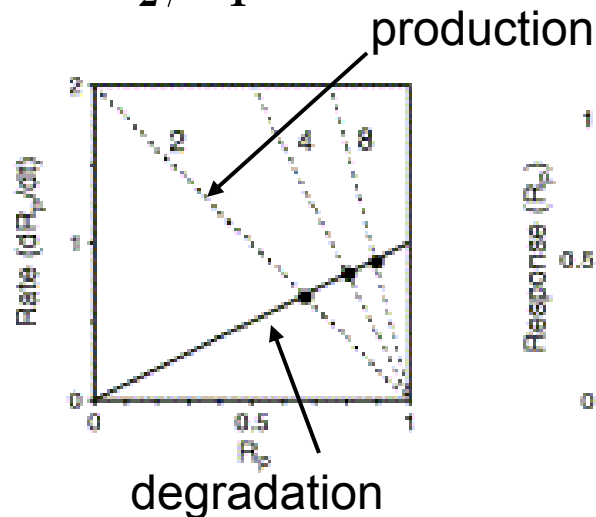
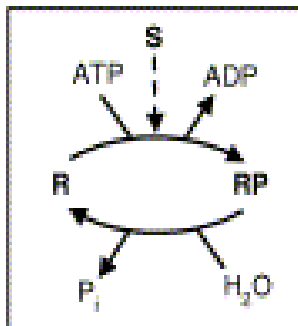
Dephosphorylation: phospho-protein \rightarrow protein

The first reaction is catalyzed by a kinase, **assume** first –order kinetics

$$\frac{dR_P}{dt} = k_1 S R - k_2 R_P \quad R_T = R + R_P$$

Steady state: $R_{P_{ss}} = R_T \frac{S}{k_2/k_1 + S}$

(b)

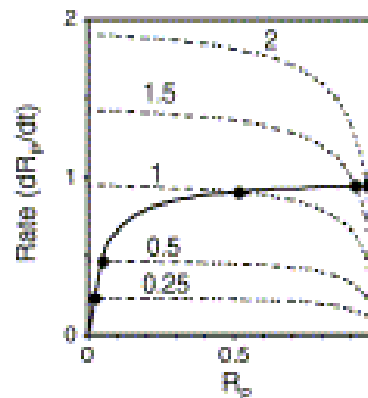
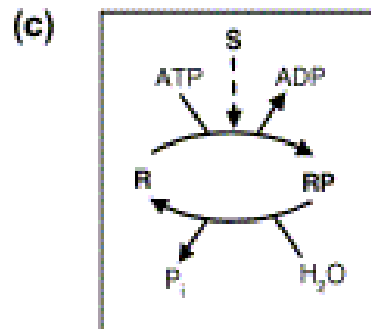


Phosphotransfer with Michaelis-Menten kinetics

Assume that the phosphorylation and dephosphorylation reactions follow Michaelis-Menten kinetics



$$\frac{dR_P}{dt} = k_1 S \frac{R_T - R_P}{K_{M1} + R_T - R_P} - \frac{k_2 R_P}{K_{M2} + R_P}$$

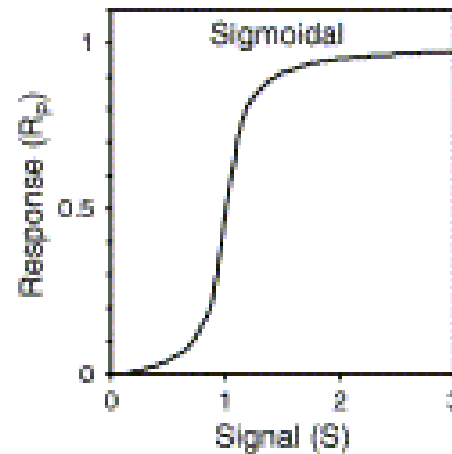


Phosphotransfer with Michaelis-Menten kinetics

$$\frac{dR_P}{dt} = k_1 S \frac{R_T - R_P}{K_{M1} + R_T - R_P} - \frac{k_2 R_P}{K_{M2} + R_P}$$

Steady state: $R_{P_{ss}} = R_T G\left(k_1 S, k_2, \frac{K_{M1}}{R_T}, \frac{K_{M2}}{R_T}\right)$

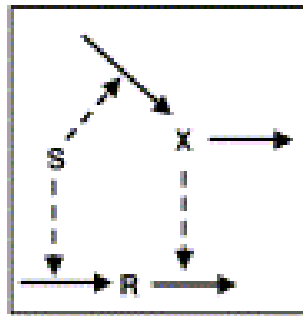
G - Goldbeter-Koshland function



Feed-forward loop

The signal acts on R both directly, and through an intermediary.

Negative feed-forward



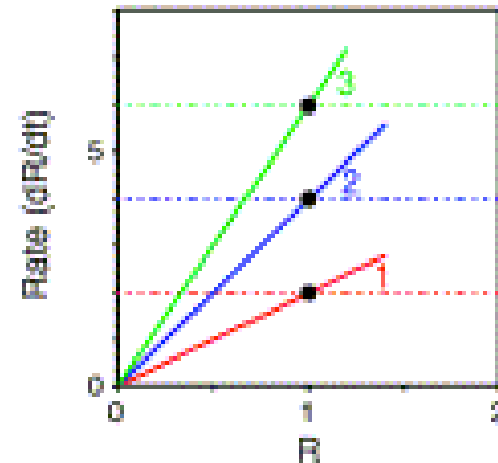
$$\frac{dR}{dt} = k_1 S - k_2 X R$$

$$\frac{dX}{dt} = k_3 S - k_4 X$$

Steady state for X:

$$X_{ss} = \frac{k_3 S}{k_4}$$

$$R_{ss} = \frac{k_1 k_4}{k_2 k_3}$$



Perfect adaptation

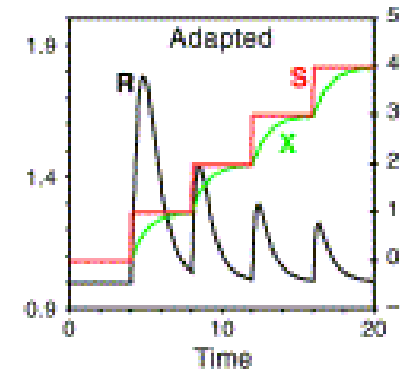
Assume that S has several step changes, assume $k_3 = k_4$

$$X_{SS}^1 = S^1 \quad \frac{dX}{dt} = k_3(S - X) \quad X_{SS}^2 = S^2$$

X increases until it reaches another steady state

$$R_{SS}^1 = \frac{k_1}{k_2} \quad \frac{dR}{dt} = k_1 S - k_2 X R$$

R increases, then starts to decrease, and finally settles into a new steady state



$$R_{SS}^2 = \frac{k_1}{k_2} = R_{SS}^1$$

adaptation

Ex: positive feed-forward loop

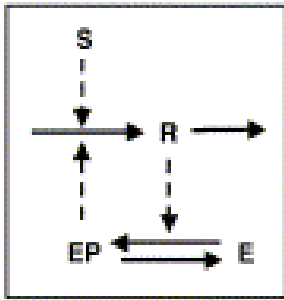
The synthesis of protein R is activated by two catalysts: S and X. The degradation of R is not catalyzed. S activates the synthesis of X, while X decays freely.

1. Draw the network diagram for this process.
2. Write down the equations for the rate of change of the concentrations of R and X.
3. Assume that X is in a steady state. How does the rate of synthesis and decay of R depend on the concentration of R and S?
4. Find the steady state concentration of R. How does this differ from the case when only S catalyzes R synthesis?
5. What is your expectation for the dynamical behavior of R if S goes through consecutive step changes?

Positive feedback

R is catalyzing the phosphorylation of E, and E_P feeds back to R

Assume Michaelis-Menten kinetics for the phosphotransfer.



$$\frac{dR}{dt} = k_0 E_P (R) + k_1 S - k_2 R$$

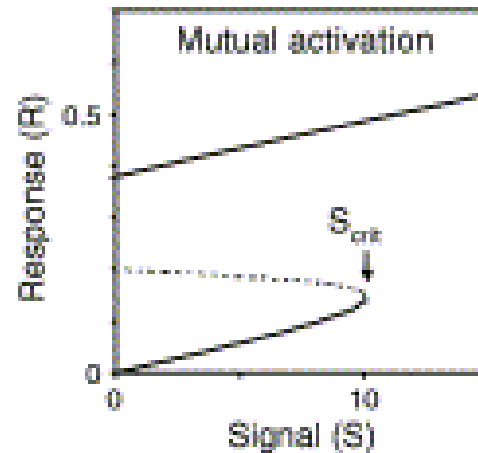
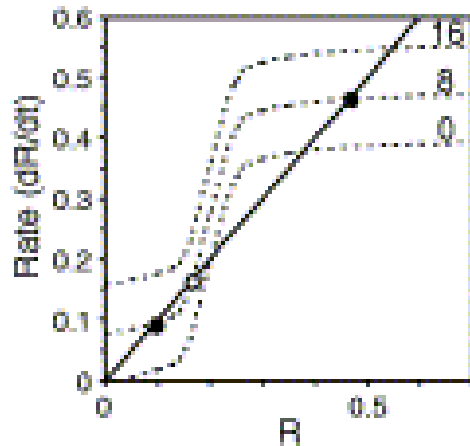
$$\frac{dE_P}{dt} = k_3 R \frac{E_T - E_P}{K_{M3} + E_T - E_P} - k_4 \frac{E_P}{K_{M4} + E_P}$$

Steady state for E_P

$$E_{P_{ss}} = E_T G \left(k_3 R, k_4, \frac{K_{M3}}{E_T}, \frac{K_{M4}}{E_T} \right)$$

Positive feedback (cont.)

$$\frac{dR}{dt} = k_0 E_T G(k_3 R, ..) + k_1 S - k_2 R$$



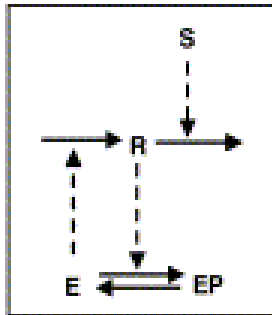
For $S < S_{crit}$ there are three possible steady-state R values.

Two of these solutions are stable - **bistability**

At $S = S_{crit}$ the response increases abruptly and irreversibly –
one-way switch

Negative feedback

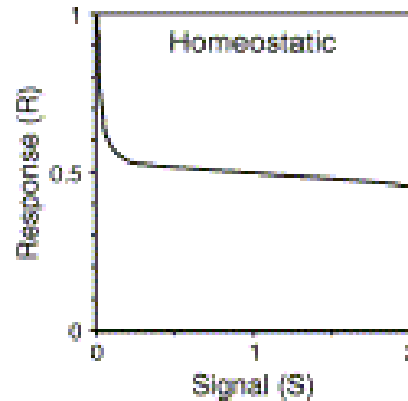
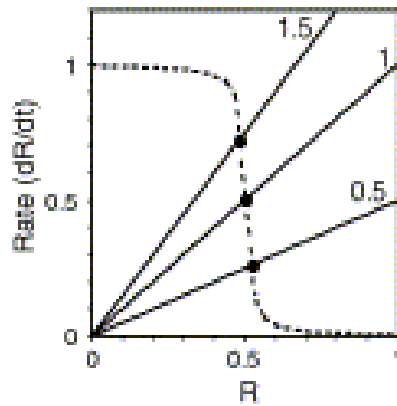
R inhibits the enzyme catalyzing its synthesis.

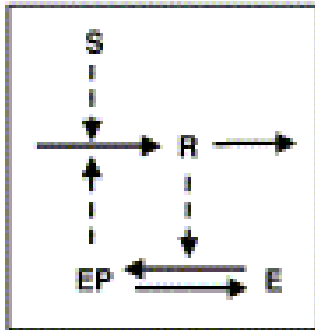


$$\frac{dR}{dt} = k_0 E(R) - k_1 S R$$

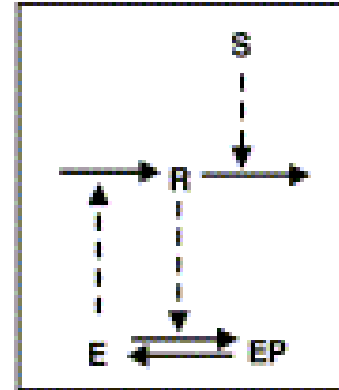
$$\frac{dE}{dt} = -k_3 R \frac{E}{K_{M3} + E} + k_4 \frac{E_T - E}{K_{M4} + E_T - E}$$

$$E_{SS} = E_T \left(1 - G \left(k_3 R, k_4, \frac{K_{M3}}{E_T}, \frac{K_{M4}}{E_T} \right) \right)$$





Positive feedback



Negative feedback

1. What other difference is between these two processes besides the nature of the feedback? Is it important for the end result?
2. The negative regulation in all these examples was taken into account as a catalysis of the degradation process. How would you represent negative regulation of the synthesis?