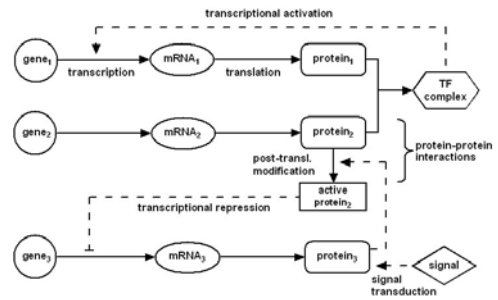


## Understanding the dynamics and function of cellular networks

### 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
- What is the relationship between the topological features of intracellular interaction networks and the dynamic behavior of cells?

## Signaling, gene regulation and protein interactions are intertwined



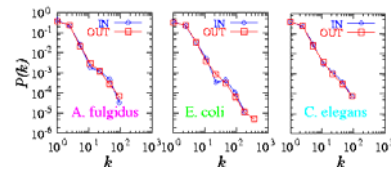
## Mapping of cellular interaction networks

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

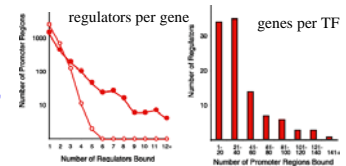
$$P_{in}(k) \approx k^{-2.2}$$

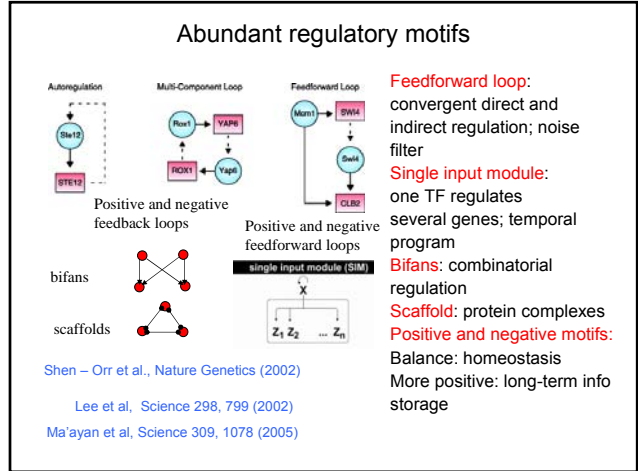
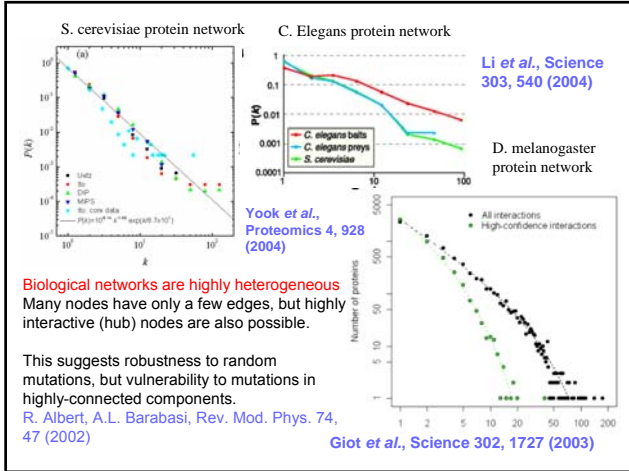
$$P_{out}(k) \approx k^{-2.2}$$

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

*S. cerevisiae*  
transcriptional network

Lee et al., Science 298, 799 (2002)





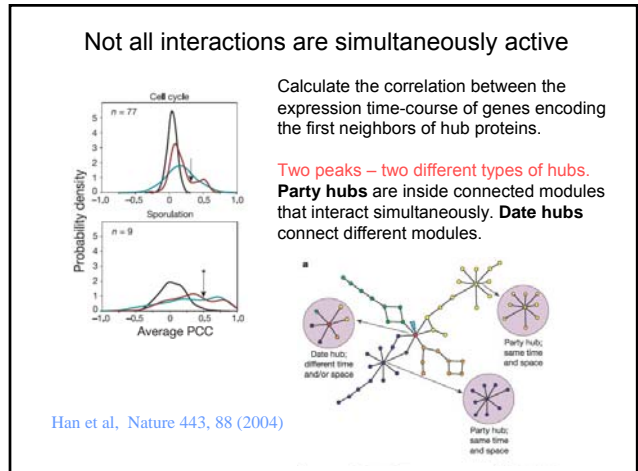
### Importance of a dynamical understanding

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

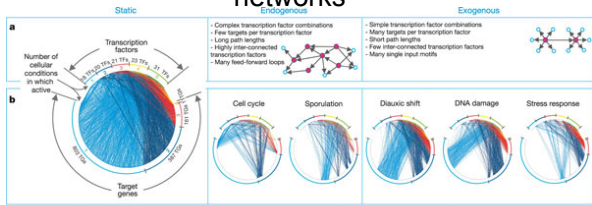
Han et al. 2004 – dynamical modularity of protein interaction networks

Luscombe et al. 2004 – endogenous and exogenous transcriptional subnetworks (see next two slides for a recap)

Q: How can we incorporate the information on the presence/absence or abundance of the molecules represented as nodes?



## Condition-dependent transcription sub-networks



### Endogenous

- Complex TF combination
- Few targets per TF
- Long path length
- Inter connected TF
- Many FFL

### Exogenous

- Simple TF combination
- Many targets per TF
- Short path length
- Few Inter connected TF
- Single input motifs

72	63
676	362
262	566
1.6	1.6
3.22	3.22
2.2	2.2
0.09	0.08
65.7%	58.1%
228 (57.3%)	78 (50.2%)
141 (17.0%)	80 (50.7%)
829	386

Luscombe et al, Nature 431, 308 (2004)

## Toward network dynamics

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

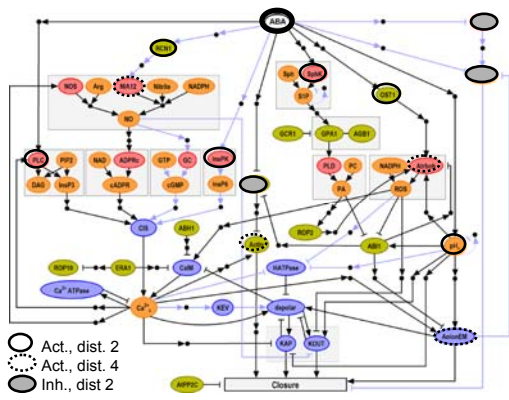
First step - **pseudo-dynamics**: propagation of reactions in chemical (interaction) space, starting from a source (signal)

This can only be done in directed networks. In effect we use topological analysis as a proxy for dynamic information on signal propagation.

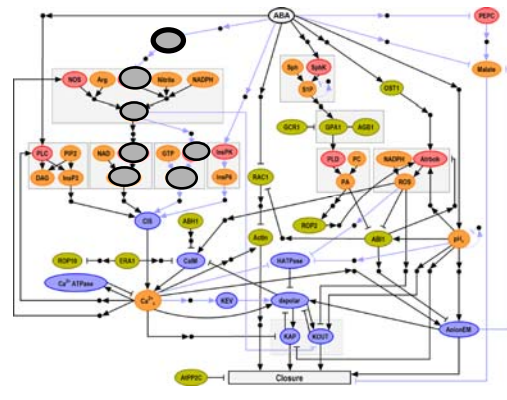
Q: What topological properties should be studied and what dynamic properties do they reflect?

Complete dynamical description is only feasible on smaller networks (modules): Signal transduction in bacterial chemotaxis, NF- $\kappa$ B signaling module, the yeast cell cycle, Drosophila embryonic segmentation

## Pseudodynamic signal propagation



## Pseudodynamic effects of knockouts



## Forward and reverse dynamic modeling

### Dynamic modeling of interaction network:

**Input:** components; interactions; states of components

**Hypotheses:** interactions; kinetics (rates, parameters)

**Output:** behavior of components in time

**Validation:** capture known behavior

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

### Reverse problem: Network inference from dynamic information:

**Input:** components; states of components (in time)

**Hypotheses:** regulatory framework

**Output:** proposed regulatory network

**Validation:** capture known interactions

We will study network inference later in the course.

## Types of dynamic 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 =  $6.023 \times 10^{23}$  molecules

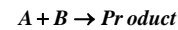
➤ Reaction rate law is an **algebraic equation** involving concentrations  
(not a differential equation)

$$r_A = -k[A] \quad r_A = -k[A]^2 \quad r_A = -k_1[A]/(1+k_2[A])$$

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

Other factors impacting rate constant

**Temperature dependence**

**Concentration dependence**

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

**Rate Constant**

(Not really "constant", just independent of concentration)

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

*The reaction is of order  $\alpha$  with respect to A and of order  $\beta$  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



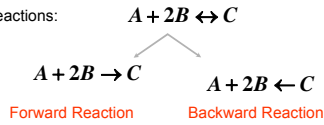
➤ Elementary reactions hypothesized to happen exactly how they are written!

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

➤ Elementary reactions are typically 1<sup>st</sup> or 2<sup>nd</sup> order

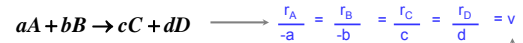
(Probability of three molecules colliding very low)

➤ Reversible reactions:



### Basics of Chemical Kinetics - 4

➤ Reaction Stoichiometry + Law of Conservation of Mass



(Irrespective of whether reaction is elementary or not)

Reaction flux  $\uparrow$

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

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

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

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

Specify rate law

$$v = -k \cdot [A]^a \cdot [B]^b \quad \text{or}$$

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

Specify initial conditions

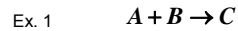
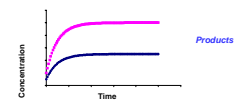
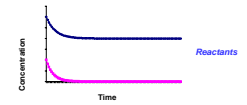
$$[A]_{(t=0)} = [A]_0$$

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

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

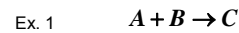
$$[D]_{(t=0)} = [D]_0$$

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]

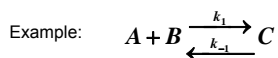
$$\frac{d[A]}{dt} = \frac{d[B]}{dt} = -k[A][B] \quad \frac{d[C]}{dt} = k[A][B]$$

Ex. 2

Write the condition(s) of mass conservation.

Hint: think of the reaction as a complex formation  $A + B \rightarrow \overline{AB}$

## Reversible reactions



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

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

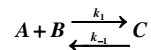
$$\frac{dC}{dt} = k_1 AB - k_{-1} C$$

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.

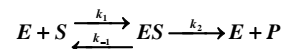
In this context, the reactants are called **substrates**.

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

## Michaelis-Menten model of enzymatic reactions

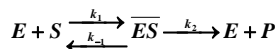
Leonor Michaelis, Maud Menten (1913)

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



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

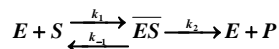
$$\frac{d\overline{ES}}{dt} = k_1 E S - k_{-1} \overline{ES} - k_2 \overline{ES} \quad \frac{dP}{dt} = k_2 \overline{ES}$$

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

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

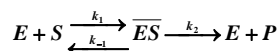
$$\overline{ES} = ES \frac{k_1}{k_{-1} + k_2}$$

$$E_T = E + \overline{ES}$$

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

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

### 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**  $S \gg K_M \Rightarrow \frac{dP}{dt} \approx k_2 E_T$

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

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

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  $10^9 \text{ s}^{-1} \text{ mol}^{-1}$

## 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)

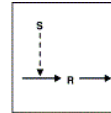
## Protein synthesis and degradation

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

Protein degradation: protein →

Notations in Tyson et al 2003: The source element (here the mRNA) is denoted S (for signal). One component (here the protein) is designated as the response.

Network diagram:



Solid edge: mass flow  
 Dashed edge: regulation

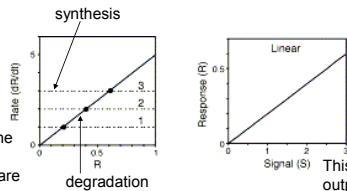
Q: Draw an alternative network, more in line with what we have seen before, where edges connect two nodes and signify regulation.

## Kinetics of protein synthesis and degradation

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

Protein degradation: protein →

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



The points where the synthesis and degradation terms are equal indicate the steady states.

This is the input-output characteristic of the system.

## Kinetics of phosphotransfer

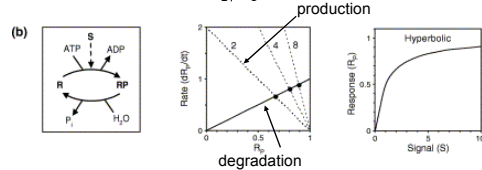
Phosphorylation: protein → phospho-protein

Dephosphorylation: phospho-protein → protein

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

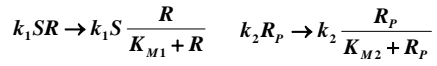
$$\frac{dR_p}{dt} = k_1 S R - k_2 R_p \quad R_r = R + R_p$$

$$\text{Steady state: } R_{P,ss} = R_r \frac{S}{k_2/k_1 + S}$$

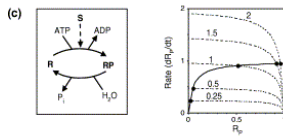


### Phosphotransfer with Michaelis-Menten kinetics

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



$$\frac{dR_p}{dt} = k_1S \frac{R_T - R_p}{K_{M1} + R_T - R_p} - \frac{k_2R_p}{K_{M2} + R_p}$$



### Phosphotransfer with Michaelis-Menten kinetics

$$\frac{dR_p}{dt} = k_1S \frac{R_T - R_p}{K_{M1} + R_T - R_p} - \frac{k_2R_p}{K_{M2} + R_p}$$

Steady state:  $R_{p,ss} = R_T G\left(k_1S, k_2, \frac{K_{M1}}{R_T}, \frac{K_{M2}}{R_T}\right)$

G - Goldbeter-Koshland function

