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
\[ P_{in}(k) \approx k^{-2.2} \]
\[ P_{out}(k) \approx k^{-2.2} \]


S. cerevisiae transcriptional network

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.

Abundant regulatory motifs

**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)


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 – endogeneus and exogeneus 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

A \rightarrow \text{Product}

Rate of reaction = rate of disappearance of A = \( r_A = \frac{\text{d}[A]}{\text{dt}} = \)

# of moles of A reacting (“disappearing”) per unit time per unit volume

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

Reaction rate law is an \textit{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 \textit{experimentally}

Measuring [A] as a function of time and calculating slope \((\text{d}[A]/\text{dt})\) at various time points.
Basics of Chemical Kinetics - 2

\[ A + B \rightarrow \text{Product} \]

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

**Temperature dependence**

**Concentration dependence**

**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)

Other factors impacting rate constant:
- Catalyst
- Pressure
- Ionic strength (pH)
- Solvent
Basics of Chemical Kinetics - 3

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

\[
A + 2B \rightarrow C \quad r_A = -k \cdot [A] \cdot [B]^2
\]

➢ 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\textsuperscript{st} or 2\textsuperscript{nd} order

(Probability of three molecules colliding very low)

➢ Reversible reactions:

\[ A + 2B \leftrightarrow C \]

\[ A + 2B \rightarrow C \quad A + 2B \leftarrow C \]

Forward Reaction  Backward Reaction
Basics of Chemical Kinetics - 4

**Reaction Stoichiometry + Law of Conservation of Mass**

\[ aA + bB \rightarrow cC + dD \]

\[
\begin{align*}
\frac{\text{d}[A]}{\text{dt}} &= -a \cdot v \\
\frac{\text{d}[B]}{\text{dt}} &= -b \cdot v \\
\frac{\text{d}[C]}{\text{dt}} &= c \cdot v \\
\frac{\text{d}[D]}{\text{dt}} &= d \cdot v
\end{align*}
\]

(Irrespective of whether reaction is elementary or not)

Specify rate law

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

Specify initial conditions

\[ [A]_{(t=0)} = [A]_0 \]
\[ [B]_{(t=0)} = [B]_0 \]
\[ [C]_{(t=0)} = [C]_0 \]
\[ [D]_{(t=0)} = [D]_0 \]
Ex. 1 \[ A + B \rightarrow C \]

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

Assume the reaction is elementary. Determine the rate of change of \([A], [B], [C]\)
Ex. 1

\[ A + B \rightarrow C \]

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

Example:

\[ A + B \xrightleftharpoons[k_1, k_{-1}]{k_1} C \]

For simplicity, we’ll leave off the brackets from \([A], ..\)

\[
\frac{dA}{dt} = \frac{dB}{dt} = -k_1AB + k_{-1}C
\]

\[
\frac{dC}{dt} = k_1AB - k_{-1}C
\]

Mass conservation: \( A + C = A_0 \) \hspace{1cm} B + C = B_0

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

\[ A + B \xrightarrow{k_1} C \xleftarrow{k_{-1}} \]

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

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

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

$$E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P$$

Ex. Draw two possible network representations of this process.
Michaelis-Menten kinetics

\[ E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P \]

\[
\frac{dS}{dt} = -k_1 ES + k_{-1} ES \quad \frac{dP}{dt} = k_2 ES
\]

\[
\frac{dES}{dt} = k_1 ES - k_{-1} ES - k_2 ES
\]

Mass conservation: \( E_T = E + ES \)

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

\[
\frac{dES}{dt} = 0, \quad ES = ES \frac{k_1}{k_{-1} + k_2}
\]
Michaelis-Menten kinetics (cont.)

\[ E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + P \]

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

\[ \frac{dP}{dt} = k_2 ES \]

\[ ES = ES \frac{k_1}{k_{-1} + k_2} \]

\[ E_T = E + ES \]

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

\[ \frac{dP}{dt} = k_2 E_T \frac{S}{K_M + S} \]
Michaelis-Menten kinetics (cont.)

\[
E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P
\]

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

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 \( \frac{k_2}{K_M} \) ?
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 \(10^9 \text{s}^{-1} \text{mol}^{-1}\).
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

Kinetics of protein synthesis and degradation

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

Protein degradation: protein $\rightarrow$

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

Steady state: $R_{ss} = \frac{k_1 S}{k_2}$
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

$$\frac{dR_p}{dt} = k_1 SR - k_2 R_p$$

Steady state:

$$R_{p,ss} = R_T \frac{S}{k_2/k_1 + S}$$

production

degradation

$$R_T = R + R_p$$
Phosphotransfer with Michaelis-Menten kinetics

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

\[ k_1 S R \rightarrow k_1 S \frac{R}{K_{M1} + R} \quad k_2 R_P \rightarrow k_2 \frac{R_P}{K_{M2} + R_P} \]

\[ \frac{dR_P}{dt} = k_1 S \frac{R_T - R_P}{K_{M1} + R_T - R_P} - \frac{k_2 R_P}{K_{M2} + R_P} \]
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: \[R_{pss} = R_T G \left( k_1 S, k_2, \frac{K_{M1}}{R_T}, \frac{K_{M2}}{R_T} \right)\]

G - Goldbeter-Koshland function
The signal acts on R both directly, and through an intermediary.

Negative feed-forward

\[
\frac{dR}{dt} = k_1 S - k_2 X \quad R
\]

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

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

$$R_{ss}^1 = \frac{k_1}{k_2} \quad \frac{dR}{dt} = k_1S - k_2X \quad R$$

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

$$R_{ss}^2 = \frac{k_1}{k_2} = R_{ss}^1 \quad \text{adaptation}$$
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?
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 \( E_P \)

\[
E_{Pss} = E_T G\left( k_3 R, k_4, \frac{K_{M3}}{E_T}, \frac{K_{M4}}{E_T}\right)
\]
Positive feedback (cont.)

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

For \( S < S_{\text{crit}} \) there are three possible steady-state \( R \) values.

Two of these solutions are stable - bistability

At \( S = S_{\text{crit}} \) the response increases abruptly and irreversibly – one-way switch
Negative feedback

R inhibits the enzyme catalyzing its synthesis.

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

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

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