Lecture 2: Modeling intracellular dynamics (introductory)

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Regulation
- Feedback loops
- Cooperativity
- Alostery

Relation of output to input

Input changes with time (e.g. concentration of TF increases)

Output changes with time

Regulatory dynamics

Example of an inherently dynamical system: Circadian oscillators
Reminder/exercize first lecture

\[ p = \frac{([A]/K_d)^n}{1 + ([A]/K_d)^n} \]

\[ \frac{dp}{dA} = \frac{n}{4K_d} \quad \text{(calculated at } A = K_d) \]

\[ \frac{dp}{dA} = \frac{n}{4} \quad \text{for } K_d = 1 \]

Note that dissociation constant is much smaller in the case myoglobin compared to hemoglobin

\[ \frac{dp}{dA} = \frac{n}{4K_d} \]

Plotting things on log scale eliminates \( K_d \) and “isolates” the effect of differences in cooperativity \( (n) \)
Change of concentration with time

\[
\frac{dc_i(t)}{dt} = f (c_1, c_2, ..., c_n; k_1, k_2, ..., k_m)
\]

\[c_1, c_2, ..., c_n\] Relevant concentrations

\[k_1, k_2, ..., k_m\] Rates of chemical reactions

What is the form of \(f\)?
Degradation of molecules

\[
\frac{dc(t)}{dt} = -kc(t)
\]

Dependence of concentration on time

\[
c(t) = c_0 \exp\left(-t/\tau\right)
\]

\[
\tau = 1/k
\]

Characteristic decay time

Figure 15.6 Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Reversible reaction

\[ A \Leftrightarrow B \]

\[ \frac{dc_A}{dt} = -k_+ c_A + k_- c_B \quad \frac{dc_A}{dt} = - \frac{dc_B}{dt} \]

Bimolecular reactions

\[ A + B \rightarrow AB \]

\[ \frac{dc_{AB}(t)}{dt} = k_{AB} c_A c_B \]
From dynamics to equilibrium

\[ L + R \rightleftharpoons LR \]

\[ \frac{d[LR]}{dt} = -k_{\text{off}}[LR] + k_{\text{on}}[L][R] \]

In the case of equilibrium:

\[ -k_{\text{off}}[LR]_{eq} + k_{\text{on}}[L]_{eq}[R]_{eq} = 0 \]

\[ K_d = \frac{[L]_{eq}[R]_{eq}}{[LR]_{eq}} = \frac{k_{\text{off}}}{k_{\text{on}}} \]

\[ \text{Reaction dissociation constant} \]
Approximation of unequilibrium system by equilibrium

$$A \xleftrightarrow{k_+}{k_-} B \rightarrow C$$

$$k_+, k_- \gg r$$

Unequilibrium process

However, A and B make equilibrium very fast

$$B(t) / A(t) = k_+ / k_-$$
Michaelis-Menten kinetics

\[ E + S \xrightarrow{k_+} ES \xrightarrow{k_-} E + P \]

**Assumption:** \( k_+, k_- \gg r \)

\[ \frac{d[P]}{dt} = r[ES] = V_{\text{max}} \frac{[S]/K_m}{1+[S]/K_m} \]

**Michaelis Menten kinetics**

\[ [S] \ll K_m \Rightarrow \frac{d[P]}{dt} = V_{\text{max}} \frac{[S]}{K_m} \]

**The mass action low**

*Speed of ATP hydrolysis by myosine*
Example 1: **CRISPR/Cas** – Advanced bacterial immune systems

**CRISPR transcript processing**

**CRISPR/Cas**

Advanced **bacterial immune system** which is based on expression of small RNA molecules

First theoretically predicted (bioinformatics + specific model) and subsequently experimentally confirmed
CRISPR/Cas system

- CRISPR/Cas = CRISPR  + Cas proteins
- CRISPR array: sequences which are repeated “R” are separated by variable sequences “S”
Mechanism of resistance to infection

CRISPR array is transcribed as a long transcripts (pre-crRNA), which are then processed to small RNA molecules (crRNA) by Cas proteins.

As soon as crRNA recognizes virus sequence, Cas proteins will be recruited to the target, and the virus DNA is destroyed.
CRISPR transcript processing

**Cas gene expression**

pre-crRNA decreases for factor less than 10

Much larger (more than two orders of magnitude) increase of crRNA.

crRNAs are very stable!

pre-crRNAs are very stable, which is a consequence of non-specific degradation.

Pougach et. al., Mol. Microbiol., 2010
Expression of cas genes leads to strong linear amplification of transcripts:

\[ \Delta[crRNA] = -\frac{\lambda_{pre-crRNA}}{\lambda_{crRNA}} \Delta[pre-crRNA] \]

Interestingly this strong amplifications crucially depends on non-specific processing of pre-crRNA transcripts.

Expression of *cas* genes

Experimental conditions

![Graphs showing expression of pre-crRNA and crRNA under different saturation conditions of crRNA amount.](image)

Saturation of crRNA amount

Joint increase of transcription and processing rates

Saturation in crRNA can be relieved if pre-crRNA production is also increased. This joint increase can lead to very large production of crRNA.
Repression by H-NS

Promoters for CRISPR and \textit{cas} genes are repressed by H-NS-a.

Joint increase of processing and pre-crRNA generation rate is probably directly relevant for functioning of the system in natural conditions.
Conclusion CRISPR/Cas

- System can generate very large quantities of crRNA very fast.
- This fast production is based on control of the system at the level of RNA processing, and on tuning the system parameters (e.g. on extreme transcript stability values).
- Unidentified nuclease which is responsible for fast pre-crRNA degradation is probably the most important control element of CRISPR/Cas.
Example 2: Oscillatory systems
Oscillatory systems

• Many cell processes are repeated in regular manner.
• Two important examples are cell cycle and circadian rhythms (track change between day and night).
• Biological oscillators are typically complex, but can often be reduced to feedback between activator and receptor.
Inertia/Oscillations

Mechanical oscillations are based on inertia. Inside cell (in cytoplasm) inertia plays a minor role, due to a large viscosity. Oscillations inside cell are based on biochemical reactions and feedback loops.
What is crucial is existence of a positive regulator (cyclin) and negative regulator (cyclin dependent kinase), and feedbacks between them.
Circadian oscillators

Make track of changes between day and night – an extreme example are photoreceptor cells in this spider.
Different biological processes – the same mechanism

Feedback loop between activator and repressor leads to oscillations.

One of the major goals of systems biology: Understand common design principles behind mechanistically otherwise different biological systems.
The Nobel Assembly at Karolinska Institutet has today decided to award the
2017 NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE

Jeffrey C. Hall
Michael Rosbash
Michael W. Young

“For their discoveries of molecular mechanisms controlling the circadian rhythm”
More on oscillators

Working session 2: Biological rhythms (genetic oscillators): delay and relaxation oscillator

Relaxation oscillator – combination of positive and negative feedback, basically the type of oscillator that we talked about today.

Delay oscillator – just negative feedback, but with the delay.
Literature:

**General:** The same as in the first lecture

**CRISPR transcript processing:**

**Biological oscillators (more on this on Working session 3 – Friday):**

**Bistable switches & bistability & bifurcations (Working session 2-today):**


Problem

The following kinematic scheme is given:

a) Write differential equations that determine amounts of pre-crRNA and crRNA transcripts. Assume the following:

- $u$ – pre-crRNA amount
- $p$ – crRNA amount
- $\lambda_u$ – pre-crRNA decay rate
- $\lambda_p$ – crRNA decay rate
- $\varphi$ – pre-crRNA transcription rate
- $k$ – pre-crRNA y crRNA transcription rate
b) Write equations that determine equilibrium pre-crRNA and crRNA amounts.

c) Assume that the system is induced so that Cas proteins are expressed so that the degradation rate of pre-crRNA to crRNA is increased from $k$ to $k'$, while all other parameters remain the same. Show that there is the following relationship between the change of pre-crRNA and crRNA.

$$\Delta[p] = -\frac{\lambda_u}{\lambda_p} \Delta[u]$$

d) Simulate the system dynamics, that is reproduce the crRNA dynamics shown in slide 15. The parameters are:

$$\varphi = 10 \text{ nM/min} \quad \lambda_u = 1 \text{ 1/min} \quad \lambda_p = 1/100 \text{ 1/min}$$