Natural and synthetic gene regulatory networks

Friday, November 13, 2015

SBU: CHE/PHY558, Physical & Quantitative Biology
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Networks (Graphs)

- A *network (graph)* is a system of interconnected objects

- Components of a network:
  - *Nodes (vertices)*
  - *Links (edges)*, which can be:
    - Directed
    - Undirected
    - Signed (+/-)
    - Unsigned
Examples of networks

Internet

Protein-protein interaction network

Social network

Neuronal network
Gene-regulatory networks consist of regulatory interactions between genes.

- Some proteins called transcription factors can bind to promoter regions.
- Some transcription factors (activators) can activate the synthesis of other proteins.
- Others (repressors) can inhibit it.
Real gene regulatory networks are large and complex
The lac operon of the bacterium *Escherichia coli* consists of 3 genes: *lacZ*, *lacY*, *lacA*.

Without lactose, the repressor LacI binds DNA and prevents transcription of these genes.
Example: lac operon ON

When lactose (inducer) appears, it binds the repressor LacI, forcing it to change conformation.

LacI can no longer bind DNA, thus allowing polymerase to transcribe *lacZ*, *lacY*, *lacA*.
Model of gene repression

We focus on: repressor (R), promoter bound (B), unbound (D), mRNA (M), protein (P).

Promoter:

\[
\begin{align*}
D + R & \xrightleftharpoons[k_{OFF}]{k_{ON}} B \\
\frac{dD}{dt} &= -k_{ON} DR + k_{OFF} B \\
D + B &= \text{const} = 1
\end{align*}
\]

mRNA:

\[
\begin{align*}
D & \xrightarrow{k_M} M + D \\
\frac{dM}{dt} &= k_M D - g_M M
\end{align*}
\]

Protein:

\[
\begin{align*}
M & \xrightarrow{k_P} P + M \\
\frac{dP}{dt} &= k_P M - g_P P
\end{align*}
\]
Gene expression versus repressor level

Promoter: \( k_{ON} (1 - B)R = k_{OFF} B \)

\[ D = 1 - B = \frac{K}{K + R} \]

\[ K = \frac{k_{OFF}}{k_{ON}} \]

mRNA:

\[ M = \frac{k_M}{g_M} B \]

Protein:

\[ P = \frac{k_P}{g_P} M \]

\[ P = \frac{k_P}{g_P} \frac{k_M}{g_M} B \]

\[ P = \frac{k_P}{g_P} \frac{k_M}{g_M} \frac{K}{K + R} \]
Model of gene activation

We focus on: activator (A), promoter bound (B), unbound (D), mRNA (M), protein (P).

Promoter:

\[ D + A \xrightleftharpoons[k_{OFF}]{k_{ON}} B \]

\[ \frac{dD}{dt} = -k_{ON}DA + k_{OFF}B \]

\[ D + B = const = 1 \]

mRNA:

\[ B \xrightarrow{k_M} M + B \]

\[ \frac{dM}{dt} = k_M B - g_M M \]

Protein:

\[ M \xrightarrow{k_P} P + M \]

\[ \frac{dP}{dt} = k_P M - g_P P \]
Gene expression versus activator level

Promoter: \( k_{ON} (1 - B) A = k_{OFF} B \)  \[ B = \frac{A}{K + A} \]
\[ K = \frac{k_{OFF}}{k_{ON}} \]

mRNA: \( M = \frac{k_M}{g_M} B \)

Protein: \( P = \frac{k_p}{g_P} M \)
\( P = \frac{k_p k_M}{g_p g_M} B \)
\( P = \frac{k_p k_M}{g_p g_M} \frac{A}{K + A} \)
What if more repressors/activators?

We can use binding polynomials:

\[ Q = D \frac{1}{1 + K_1A + K_2A^2 + ... + K_nA^n} \]

Empty promoter:

\[ D = \frac{1}{1 + K_1A + K_2A^2 + ... + K_nA^n} \]

Promoter bound by 1 activator:

\[ B_1 = \frac{K_1A}{1 + K_1A + K_2A^2 + ... + K_nA^n} \]

And so on.
Complete cooperativity on promoter

Promoter: \( k_{ON} (1 - B) A^n = k_{OFF} B \)

\[ B = \frac{A^n}{K^n + A^n} \]
\[ K = \sqrt{n \frac{k_{OFF}}{k_{ON}}} \]

mRNA:
\[ M = \frac{k_M}{g_M} B \]

Protein:
\[ P = \frac{k_P}{g_P} M \]
\[ P = \frac{k_P}{g_P} \frac{k_M}{g_M} B \]
\[ P = \frac{k_P}{g_P} \frac{k_M}{g_M} \frac{A^n}{K^n + A^n} \]
Dynamical systems: Steady states (equilibria)

Equilibrium: when synthesis = degradation

Equation: \[
\frac{dP}{dt} = k - \gamma P = 0
\]

Equilibrium: when time derivative = 0

Equilibrium: when synthesis = degradation

Equilibrium: Protein level = \( P_{eq} = \frac{k}{\gamma} \)
Types of equilibria (steady states)

1. Stable
   \[ \frac{d^2 \Phi}{dx^2} < 0 \]
   Return if perturbed

2. Neutral
   \[ \frac{d^2 \Phi}{dx^2} = 0 \]
   Equilibrium everywhere

3. Unstable
   \[ \frac{d^2 \Phi}{dx^2} > 0 \]
   Depart if perturbed
Synthetic Biology:

**Building biological systems for predefined purposes**

making oscillators, switches, gates, biofuels, medicine

**Electronic components:**
(transistors, resistors, capacitors, etc.)

- Standard
- Well-characterized
- Reliable
- Low noise
- No replication

**Biological components:**
(genes, promoters, RNA-s, etc.)

- Diverse
- Uncharacterized
- Mutate & evolve
- Noisy
- Replication
Toggle switch: design

Gene 1 = lacI
Promoter 1

TetR

Gene 2 = tetR
Promoter 2

ATc

IPTG

Gene 2 ON

IPTG

Gene 1 ON

Toggle switch: dynamics

Equations (ODE system)

Protein 1 = $u$; Protein 2 = $v$

\[
\begin{align*}
\frac{du}{dt} &= \frac{\alpha_1}{1 + v^\beta} - u \\
\frac{dv}{dt} &= \frac{\alpha_2}{1 + u^\gamma} - v
\end{align*}
\]

The Repressilator: A synthetic oscillator

Equations (ODE system)

Proteins = \( p_i \); mRNA-s = \( m_i \)

\[
\frac{dm_i}{dt} = -m_i + \frac{\alpha}{1 + p_j^n} + \alpha_0 \\
\frac{dp_i}{dt} = -\beta(p_i - m_i)
\]

\( i = lacI, \text{tetR}, cl \) \\
\( j = cl, lacI, \text{tetR} \)

Repressilator: dynamics

Can we build a gene expression dimmer?

Synthetic genetic switches have been built.

Can we build a genetic dimmer?
Starting with a regulatory cascade

\[
\frac{dx}{dt} = a - bxy
\]

Potential

Higher ATc

rate of change

synthesis  degradation

repressor, x

represents the rate of change where synthesis is shown in blue and degradation in red.

Potential graph shows the relationship between reppressor, x, and potential.
NR: dose-response

- Gating
- Mean
- Noise

Fluorescence (a.u.) vs ATc (ng/ml)

- NR
- NR low
- NR high

Normalized cell count vs ATc (ng/ml)

CV vs ATc (ng/ml)
NR: gene expression at intermediate induction
The “Linearizer”

\[
\frac{dx}{dt} \approx aF(x) - bxy
\]

\[
F(x) = \frac{K^n}{K^n + x^n}
\]

TetR represses its own promoter (in addition to the target gene)

- Negative Feedback
- Identical promoters
Comparison: NR and NF mean and CV

Precise gene expression tuner = dimmer

Nevozhay et al., PNAS 106:5123, 2009
NF: gene expression at intermediate induction