Repressilator

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Complex Genetic Networks

Synthetic Biology

- Top-down approach
  - Minimal system. Components vital for life
  - Living organism

- Bottom-up approach
  - Non-living components
  - Living bottom-up-only system
  - Synthetic biology

Increasingly complex

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The Central Dogma of Biology

DNA

RNA

Translation

Protein

NUCLEUS

CYTOPLASM

Integrated Gene Circuits: The Toggle Switch

Gene A

Protein A

Gene B

Protein B
The Repressilator System

Gene A

Protein A

Gene B

Protein B

Gene C

Protein C
The Repressilator System

Assumptions

- Stable temperature
  - Temperature affects binding and decay rates
- Closed system
  - No protein or mRNA leaving/entering the system
The Repressilator System: General Form

Rate of Change of the mRNA Concentration

\[
\frac{da}{dt} = -k_{dm}a + \frac{\alpha}{1 + C^n}
\]

Rate of Change of Protein Concentration

\[
\frac{dA}{dt} = \beta a - k_{dp}A
\]

- **a**: [mRNA a]
- **A**: [protein A]
- **C**: [protein C]
  - represses TX of gene A

Following parameters same for all species:
- **k_{dm}**: mRNA deg.
- **\alpha**: TX rate
- **n**: Hill’s coefficient
- **k_{dp}**: protein deg.
- **\beta**: TL rate
The Repressilator System: For All 3 Genes

Rate of Change of the mRNA Concentration

\[
\frac{da}{dt} = -k_{dm}a + \frac{\alpha}{1 + C^n} \\
\frac{db}{dt} = -k_{dm}b + \frac{\alpha}{1 + A^n} \\
\frac{dc}{dt} = -k_{dm}c + \frac{\alpha}{1 + B^n}
\]

Rate of Change of Protein Concentration

\[
\frac{dA}{dt} = \beta a - k_{dp}A \\
\frac{dB}{dt} = \beta b - k_{dp}B \\
\frac{dC}{dt} = \beta c - k_{dp}C
\]
The Repressilator System: Initial Conditions

Initial mRNA Concentrations

\[
a(t = 0) = 1 \\
b(t = 0) = 0 \\
c(t = 0) = 0
\]

Initial Protein Concentrations

\[
A(t = 0) = 0 \\
B(t = 0) = 0 \\
C(t = 0) = 0
\]

Parameter Values

\[
\alpha = 100 \\
n = 2 \\
k_{dm} = 1 \\
\beta = 1 \\
k_{dp} = 1
\]
Numerical Method - Explicit Runge-Kutta (RK4)

\[ y_{n+1} = y_n + \phi h \]

\[ k_1 = hf (x_n, y_n) \]
\[ k_2 = hf \left( x_n + \frac{1}{2} h, y_n + \frac{1}{2} k_1 h \right) \]
\[ k_3 = hf \left( x_n + \frac{1}{2} h, y_n + \frac{1}{2} k_2 h \right) \]
\[ k_4 = hf (x_n + h, y_n + k_3 h) \]

\[ y_{n+1} = y_n + \frac{h}{6} (k_1 + 2k_2 + 2k_3 + k_4) \]
Repressilator - Starting times
Repressilator - Steady State
Modulating Regulated Transcription Rate ($\alpha$)

Rate of Change of the mRNA Concentration

\[
\frac{da}{dt} = -k_{deg}a + \frac{\alpha}{1 + C^n}
\]

\[
\frac{db}{dt} = -k_{deg}b + \frac{\alpha}{1 + A^n}
\]

\[
\frac{dc}{dt} = -k_{deg}c + \frac{\alpha}{1 + B^n}
\]
Modulating Translation ($\beta$)

Rate of Change of Protein Concentration

\[
\frac{dA}{dt} = \beta a - k_{dP} A \\
\frac{dB}{dt} = \beta b - k_{dP} B \\
\frac{dC}{dt} = \beta c - k_{dP} C
\]
Modulating Cooperativity of Repressor and DNA

Rate of Change of the mRNA Concentration

\[
\frac{da}{dt} = -k_{deg}a + \frac{\alpha}{1 + C^n}
\]

\[
\frac{db}{dt} = -k_{deg}b + \frac{\alpha}{1 + A^n}
\]

\[
\frac{dc}{dt} = -k_{deg}c + \frac{\alpha}{1 + B^n}
\]
The Repressilator System with a Drug Inducer

**New Parameter**
- D = drug
  - Can diffuse in and out of the cell
  - Activates gene A

**Assumptions**
- Spike the media (extracellular fluid) with D
  - [D] outside the cell >> [D] inside the cell
  - Diffusion out of cell is negligible

D = drug
- Can diffuse in and out of the cell
- Activates gene A
The Repressilator System with a Drug Inducer

Rate of Change of the mRNA Concentration

\[
\frac{da}{dt} = -k_{dm}a + \frac{\alpha}{1 + C^n} + kD
\]

\[
\frac{db}{dt} = -k_{dm}b + \frac{\alpha}{1 + A^n}
\]

\[
\frac{dc}{dt} = -k_{dm}c + \frac{\alpha}{1 + B^n}
\]
The Repressilator System & Synchronization

Cell 1:
- Gene A
- Protein A
- Gene B
- Protein B
- Gene C
- Protein C

Cell 2:
- Gene A
- Protein A
- Gene B
- Protein B
- Gene C
- Protein C

Cell 3:
- Gene A
- Protein A
- Gene B
- Protein B
- Gene C
- Protein C
The Repressilator: 3 Cell System (i=1,2,3)

mRNA concentrations: a, b, c

\[
\frac{da_i}{dt} = -k_{dm}a_i + \frac{\alpha}{1 + c_i^n} + kD
\]

\[
\frac{db_i}{dt} = -k_{dm}b_i + \frac{\alpha}{1 + a_i^n}
\]

\[
\frac{dc_i}{dt} = -k_{dm}c_i + \frac{\alpha}{1 + b_i^n}
\]

Protein concentrations: A, B, C & Drug concentration

\[
\frac{dA_i}{dt} = \beta a_i - k_{dp}A_i
\]

\[
\frac{dB_i}{dt} = \beta b_i - k_{dp}B_i
\]

\[
\frac{dC_i}{dt} = \beta c_i - k_{dp}C_i
\]

\[
\frac{dD}{dt} = -k_{dd}D
\]
The Repressilator: 3 Cell System Initial Conditions

<table>
<thead>
<tr>
<th>Cell 1</th>
<th>Cell 2</th>
<th>Cell 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_1(t = 0) = 1$</td>
<td>$a_2(t = 0) = 0$</td>
<td>$a_3(t = 0) = 0$</td>
</tr>
<tr>
<td>$b_1(t = 0) = 0$</td>
<td>$b_2(t = 0) = 1$</td>
<td>$b_3(t = 0) = 0$</td>
</tr>
<tr>
<td>$c_1(t = 0) = 0$</td>
<td>$c_2(t = 0) = 0$</td>
<td>$c_3(t = 0) = 1$</td>
</tr>
</tbody>
</table>

$A(t=0)=B(t=0)=C(t=0)=0$ for all cells
Repressilator with Sensing - 3 cell synchronization
Repressilator with Sensing - 3 cell synchronization

![Graph showing protein concentration over time for three cells labeled 1, 2, and 3, with analyte markers for Protein A and Drug D.](image-url)
The Repressilator System: Adding Complexity

A single cell

Gene A → Protein A
Gene B → Protein B
Gene C → Protein C
Gene D

D → D
Quorum Sensing: Adding Even More Complexity
Extra Slides
The Effect of Basal Transcription Rate ($\alpha_0$)

Rate of Change of the mRNA Concentration

$$\frac{da}{dt} = -k_{deg} a + \frac{\alpha}{1 + C^n} + \alpha_0$$

$$\frac{db}{dt} = -k_{deg} b + \frac{\alpha}{1 + A^n} + \alpha_0$$

$$\frac{dc}{dt} = -k_{deg} c + \frac{\alpha}{1 + B^n} + \alpha_0$$
The Effect of Protein Degradation

Rate of Protein Degradation

Species
- A
- B
- C
Coupled RK4

\[
\begin{align*}
\frac{dy_1}{dt} &= f_1(t, y_1, y_2), \\
\frac{dy_2}{dt} &= f_2(t, y_1, y_2),
\end{align*}
\]

\[
\begin{align*}
k_{1,1} &= f_1(t_k, y_{1,k}, y_{2,k}), \\
k_{2,1} &= f_2(t_k, y_{1,k}, y_{2,k}), \\
k_{1,2} &= f_1(t_k + 0.5h, y_{1,k} + 0.5k_{1,1}h, y_{2,k} + 0.5k_{2,1}h), \\
k_{2,2} &= f_2(t_k + 0.5h, y_{1,k} + 0.5k_{1,1}h, y_{2,k} + 0.5k_{2,1}h), \\
k_{1,3} &= f_1(t_k + 0.5h, y_{1,k} + 0.5k_{1,2}h, y_{2,k} + 0.5k_{2,2}h), \\
k_{2,3} &= f_2(t_k + 0.5h, y_{1,k} + 0.5k_{1,2}h, y_{2,k} + 0.5k_{2,2}h), \\
k_{1,4} &= f_1(t_k + h, y_{1,k} + k_{1,3}h, y_{2,k} + k_{2,3}h), \\
k_{2,4} &= f_2(t_k + h, y_{1,k} + k_{1,3}h, y_{2,k} + k_{2,3}h),
\end{align*}
\]

\[
\begin{align*}
y_{1,k+1} &= y_{1,k} + \frac{h}{6} (k_{1,1} + 2k_{1,2} + 2k_{1,3} + k_{1,4}), \\
y_{2,k+1} &= y_{2,k} + \frac{h}{6} (k_{2,1} + 2k_{2,2} + 2k_{2,3} + k_{2,4}).
\end{align*}
\]