Design of in vitro Synthetic Gene Circuits

Elisa Franco
Richard M. Murray

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IWBDA
Programming large scale circuits

\[ y = G(u - Ky) \]
\[ y = u \frac{G}{1 + KG} \]

\[ G \to \infty \Rightarrow y = \frac{1}{K} u \]
Bottom-up approach: In vitro molecular programming

OBJECTIVES:
Design and synthesis of biologically plausible feedback loops
Understand and implement design principles
Use few components: DNA and enzymes (off the shelf)

\[
M_1 + M_2 \xrightleftharpoons[k_1^-]{k_1^+} M_3 \\
M_3 + M_4 \xrightleftharpoons[k_2^-]{k_2^+} M_5 \\
\]

\[
\frac{dc}{dt} = \nu \mathcal{E} R(c)
\]
In vitro genetic circuits: our tool kit

Programming a reaction network for rate regulation

Interconnecting modules

Insulating devices
In vitro genetic circuits: the key ideas
Kim and Winfree Nature MSB06

Simplification of feedback loops:
Transcription is switched on and off without transcription factors

Switching: by toehold mediated branch migration - Yurke 03

T7 promoter
+ activator

OFF
ON

ACTIVATOR

INHIBITOR

TRANSCRIPTION

OFF
ON

A 35 bases
B 25 bases
C=A’ 35 bases

k-
k+

ΔG -40 kcal/mol

ΔG -60 kcal/mol
Design and specification of parts

SOFTWARE:

• Maximize (-\(\Delta G\)) of desired interactions
• Minimize (-\(\Delta G\)) of unwanted interactions

Enzymes off the shelf:

- T7 RNA polymerase
- RNase H

Activator

Transcribed domain

Functional domain

Terminator

Consensus domain 17-20 bases

toehold 4-10 bases
Programming a simple biochemical network

Produce reactants R1, R2
R1, R2 >> output P

$$T_1 \overset{k_1}{\rightarrow} R_1$$
$$T_2 \overset{k_2}{\rightarrow} R_2$$

$$R_1 + R_2 \overset{k^+}{\underset{k^-}{\rightarrow}} P$$

Objective: steady flow of P

Constraints: avoid bottlenecks and waste of resources

IDEAS:

Negative feedback
Design network to decrease excess species

Positive feedback
Design network to stimulate production of less abundant species
Self repression based flow regulation

- Transcriptional circuits implementation
- Reactants: transcripts
- Product: RNA complex
- Circuit design:

Monitor fraction of ‘on’ switch

R1

A1

A1'

A2

T1

R2

Monitor fraction of ‘on’ switch
Modeling the dynamics

Mass action kinetics

**Activation**
\[ T_i + A_i \xrightleftharpoons[k_{T_iA_i}]{k_{T_iA_i}} T_i A_i \]

**Inhibition**
\[ R_i + A_i \xrightleftharpoons[k_{R_iA_i}]{k_{R_iA_i}} R_i A_i \]
\[ R_i + T_i A_i \xrightleftharpoons[k_{R_iT_iA_i}]{k_{R_iT_iA_i}} R_i A_i + T_i \]

**Output**
\[ R_i + R_j \xrightarrow[k_{R_iR_j}]{k_{R_iR_j}} R_i R_j \]

**Unwanted interactions**
\[ R_j + T_i \xrightarrow[k_{R_jT_i}]{k_{R_jT_i}} R_j T_i \]

Enzymatic reactions:
- RNA polymerase - production of transcripts
- RNase H - degradation of DNA/RNA hybrids

**Michaelis-Menten kinetics**

\[ R_p + T_i A_i \xrightleftharpoons[k_{ON_{ii}}]{k_{ON_{ii}}} R_p \cdot T_i A_i \xrightarrow[k_{catON_{ii}}]{k_{catON_{ii}}} R_p + T_i A_i + R_i \]

\[ R_p + T_i \xrightleftharpoons[k_{OFF_{ii}}]{k_{OFF_{ii}}} R_p \cdot T_i \xrightarrow[k_{catOFF_{ii}}]{k_{catOFF_{ii}}} R_p + T_i + R_i \]

\[ R_h + R_i A_i \xrightarrow[k_{H_{ii}}]{k_{H_{ii}}} R_h \cdot R_i A_i \xrightarrow[k_{catH_{ii}}]{k_{catH_{ii}}} R_h + A_i \]

\[ R_h + R_j T_i \xrightarrow[k_{H_{jj}}]{k_{H_{jj}}} R_h \cdot R_j T_i \xrightarrow[k_{catH_{jj}}]{k_{catH_{jj}}} R_h + T_i \]

\[ R_p + R_j T_i \xrightarrow[k_{OFF_{jj}}]{k_{OFF_{jj}}} R_p \cdot R_j T_i \xrightarrow[k_{catOFF_{jj}}]{k_{catOFF_{jj}}} R_p + R_j T_i + R_i \]

Faster
Experimental results

Enzymes added
Feedback activated

[T1(0)] 100 nM
[T2(0)] 50 nM

[T1(0)] 100 nM
[T2(0)] 100 nM

[T1(0)] 50 nM
[T2(0)] 100 nM

[T1(0)] 100 nM
[T2(0)] 300 nM
Ratio plot

Steady state template ratio

![Graph showing steady state template ratio with axes labeled 'Initial template ratio' and 'a.u.' and different lines representing 'Model', 'No feedback', 'T1/T2', 'T2/T1', 'Ideal case', and 'Data'.]
Cross activation circuit design

Reactants: transcripts

Product: RNA complex

Preliminary data
Current design is asymmetric

Steady state ‘on’ template ratio

\[ \frac{[R_2]}{[R_1]} > \frac{[R_2]}{[R_1]} \]

No domain sequestration (ideal)

\[ \frac{T_2}{T_1} \]

\[ \frac{T_1}{T_2} \]
In this talk

In vitro genetic circuits: our tool kit

Programming a reaction network for rate regulation

Interconnecting modules

Insulating devices
Interconnections can introduce unwanted dynamics

Interconnections introduce parasitic signals

RETROACTIVITY TO INPUTS AND OUTPUTS

\[ \dot{x} = f(x, u)s \]
\[ y = Y(x, u)s \]
\[ r = R(x, u, s) \]

Del Vecchio et al. Nature MSB 2008
Existing theoretical results suggest how to design an insulating device

Del Vecchio, Ninfa and Sontag, MSB08; Del Vecchio, Jayanthi, ACC08

Structural assumptions

1) \[ \Sigma : \begin{align*}
& \dot{x} = f(x, u, s) \\
& y = Y(x, u, s) \\
& r = R(x, u, s) \\
& x = (x_1, \ldots, x_n) \in D \subseteq \mathbb{R}^n_+
\end{align*} \]

2) \[ \Omega : \begin{align*}
& \dot{u} = f_0(t, u) \quad \text{prior to the interconnection}
\end{align*} \]

3) \[ \Sigma : \begin{align*}
& \dot{x} = \begin{pmatrix}
Gf_1(x, u) \\
Gf_2(x) \\
\vdots \\
Gf_{n-1}(x) \\
Gf_n(x)
\end{pmatrix}
\end{align*} \]

4) \[ \Lambda : \dot{\nu} = \begin{pmatrix}
g_1(\nu, y) \\
g_2(\nu) \\
\vdots \\
g_p(\nu)
\end{pmatrix} \]

5) Parasitic signals are additive

\[ \begin{align*}
\dot{u} &= f_0(t, u) + r(x, u) \\
\dot{x}_n &= \dot{y} = Gf_n(x) + s(\nu, y)
\end{align*} \]

6) Conservation laws

\[ \begin{align*}
r(x, u) &= -Gf_1(x, u) \\
s(\nu, y) &= -g_1(\nu, y)
\end{align*} \]
Existing theoretical results suggest how to design an insulating device

Stability assumption:

\[ \frac{\dot{x}}{F(x,u,s)} = f(x,u,s) \]
\[ y = Y(x,u,s) \]
\[ r = R(x,u,s) \]

Define:

\[ F : \mathbb{R}_+ \times \mathcal{D} \rightarrow \mathbb{R}^n \]
\[ F(a,x) = (f_1(x,a-x_1), f_2(x), \ldots, f_n(x)) \]

\[ a \in \mathbb{R}_+ \]
\[ x \in \mathcal{D} \]

The Jacobian:

\[ DF_x(a,x) \]

has all eigenvalues with negative real part in all its domain
The insulation property is achieved if the device is sufficiently fast.

\[
\begin{aligned}
\Omega & \xrightarrow{u} \Sigma \xrightarrow{y} \Lambda \\
\begin{array}{c}
\Omega \\
\Sigma \\
\Lambda
\end{array} & \begin{array}{c}
\Sigma \\
\nu
\end{array} & \sum : & \begin{pmatrix}
\dot{x} = f(x, u, s) \\
y = Y(x, u, s) \\
r = R(x, u, s)
\end{pmatrix}
\end{aligned}
\]

Claim 1
There exist G* sufficiently large such that for any G>\text{G*}:

\[
\| x^{\text{ref}}(t) - x(t) \| = \mathcal{O}(1/G)
\]

Where \( x^{\text{ref}}(t) \) is the state of the device when the load is absent, i.e. \( s(\nu, y) = 0 \)

Claim 2
There exist G’ sufficiently large such that for any G>\text{G’}:

\[
u(t) = \bar{u}(t) + \mathcal{O}(1/G)\quad \text{where} \quad \frac{d\bar{u}}{dt} = f_0(t, u) \left( \frac{1}{1 + \partial \gamma_1(\bar{u})/\partial \bar{u}} \right)
\]

Low output retroactivity

All proofs are based on timescale separation
Can we make an insulator in an in vitro setting?

Minimize $s, r$
• Input U drives a switch
• RNA output binds to RNA load
Reactions for a transcriptional insulator

**Input stage**
\[
\emptyset p_U(t) U(t) \xrightarrow{d_U(t)} \emptyset \\
D_I + U \xrightarrow{k_{IU}^+} \bar{D}_I \bar{U} \\
\bar{D}_A \bar{D}_I + U \xrightarrow{k_{AIU}} \bar{D}_I \bar{U} + D_A
\]

**Core device**
\[
\begin{align*}
D_A + D_T & \xrightarrow{k_{AT}} \bar{D}_A \bar{D}_T, \\
\bar{D}_A \bar{D}_T + D_I & \xrightarrow{k_{AIT}} \bar{D}_T + \bar{D}_A D_I, \\
D_A + D_I & \xrightarrow{k_{AI}} \bar{D}_A D_I.
\end{align*}
\]
\[
p_{RY}(t) = \alpha \, h \left( \frac{[\bar{D}_A \bar{D}_T]}{K_{MP}} \right)
\]
\[
d_{DD_{RY}}(t) = \gamma \, h \left( \frac{[\bar{D}_D R_Y]}{K_{MH}} \right)
\]

**Output stage**
\[
\emptyset p_L(t) \xrightarrow{p_L(t)} R_L(t), \\
R_Y + R_L \xrightarrow{k_{YL}} \bar{R}_Y \bar{R}_L
\]
Structural assumptions: dynamic gain can be tuned

\[ \dot{U} = \pm p_U(t) - d_U(t) - k_{IU}^+ D_I U + k_{IU}^- \dot{D}_I U - k_{AIU} D_A D_I U \]

\[ \dot{D}_I U = +k_{IU}^+ D_I U - k_{IU}^- D_I U + k_{AIU} D_A D_I U \]

\[ \dot{D}_A D_I = +k_{AI} D_A D_I - k_{AIU} D_A D_I U \]

\[ \dot{D}_A D_T = +k_{AT} D_A D_T - k_{AIT} D_I D_A D_T \]

\[ \dot{D}_D = -k_{DY} D_D R_Y + \gamma h \left( \frac{D_D R_Y}{K_{MH}} \right) \]

\[ \dot{R}_Y = +\alpha h \left( \frac{D_A D_T}{K_{MP}} \right) - k_{DY} D_D R_Y - k_{YL} R_Y R_L \]

\[ \dot{R}_L = +p_L(t) - k_{YL} R_Y R_L \]

- Fast toehold kinetics
- High enzyme concentrations/activities

\[ \dot{x} = \begin{pmatrix} Gf_1(x, u) \\ Gf_2(x) \\ \vdots \\ Gf_{n-1}(x) \\ Gf_n(x) \end{pmatrix} \]
Structural assumptions: additive retroactivity and conservation laws

\[
\dot{U} = \pm_{U}(t) - d_{U}(t) - k_{IU}^+ D I U + k_{IU}^- \hat{D}_I U - k_{AIU} D_A D_I U
\]

\[
\hat{D}_I U = +k_{IU}^+ D_I U - k_{IU}^- \hat{D}_I U + k_{AIU} D_A D_I U
\]

\[
\hat{D}_A D_I = +k_{AI} D_A D_I - k_{AIU} D_A D_I U
\]

\[
\hat{D}_A D_T = +k_{AT} D_A D_T - k_{AIT} D_I D_A D_T
\]

\[
\dot{D}_D = -k_{DY} D_D R_Y + \gamma h \left( \frac{\hat{D}_D R_Y}{K_{MH}} \right)
\]

\[
\dot{R}_Y = +\alpha h \left( \frac{\hat{D}_A D_T}{K_{MP}} \right) - k_{DY} D_D R_Y - k_{Y_L} R_Y R_L
\]

\[
\dot{R}_L = +p_L(t) - k_{Y_L} R_Y R_L
\]

\[
\dot{u} = f_0(t, u) + r(x, u)
\]

\[
\dot{x} = \begin{pmatrix}
Gf_1(x, u) \\
Gf_2(x, u) \\
Gf_3(x) \\
Gf_4(x) \\
Gf_5(x) + Gs(\nu, y)
\end{pmatrix}
\]

\[
\dot{\nu} = f_\nu(t) + Gs(\nu, y).
\]
Structural assumptions: stability

Jacobian of the dynamics of the core device:

$$DF_x(a, x) = \begin{bmatrix} P & \emptyset \\ L & Q \end{bmatrix}$$

The eigenvalues have negative real part at any equilibrium point.

All structural assumptions are verified.

• Claim 1 holds: Low output retroactivity
• Claim 2 holds: Low input retroactivity

Note: Analytical mapping I/O not available, device may work in non linear regime
Simulation results

Low enzyme amounts, bottleneck

High enzyme amounts, time scale separation works again!
Programming synthetic biomolecular systems: embedding engineering principles in the hardware of life

Designing and building biosynthetic systems is today
• Easier
• Faster
• Cheaper

• In vitro bio-computational networks

Example: flow control systems

• Scaling up networks, modularity

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