Design of *in vitro* Synthetic Gene Circuits

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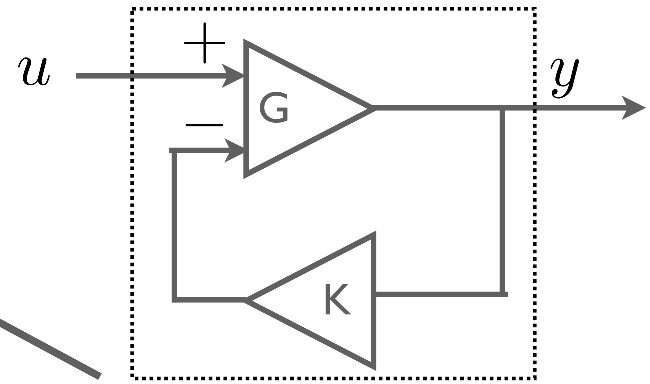
Richard M. Murray

15 June 2010

IWBDA

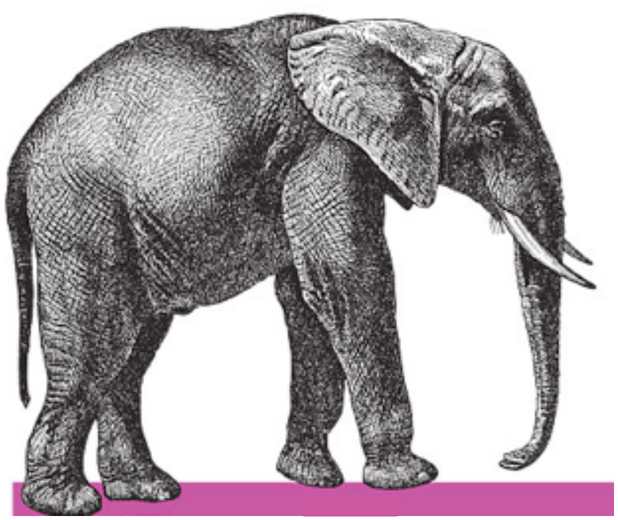
Programming large scale circuits



$$y = G(u - Ky)$$

$$y = u\frac{G}{1 + KG}$$

$$G \rightarrow \infty \Rightarrow y = \frac{1}{K}u$$



?

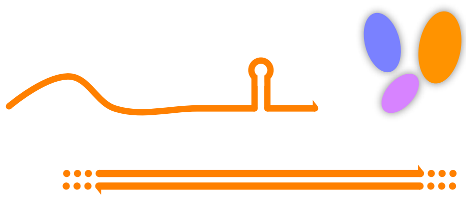
Bottom-up approach:
In vitro molecular programming

OBJECTIVES:

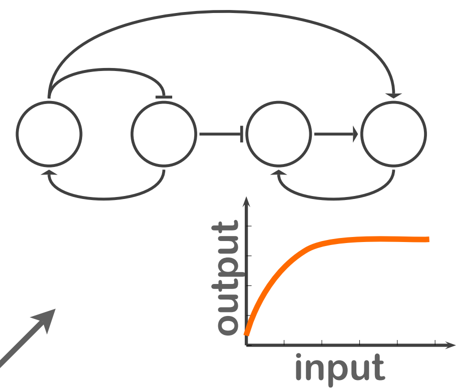
Design and synthesis of biologically plausible feedback loops

Understand and implement design principles

Use few components: DNA and enzymes (off the shelf)



DESIGN



MODELING

$$M_1 + M_2 \underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}} M_3$$

$$M_3 + M_4 \underset{k_2^-}{\overset{k_2^+}{\rightleftharpoons}} M_5$$

...

$$\frac{dc}{dt} = \nu \mathcal{E} R(c)$$

In this talk

In vitro genetic circuits: our tool kit

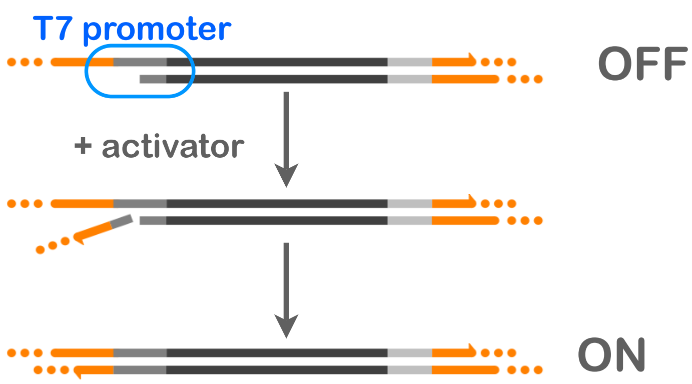
Programming a reaction network for rate regulation

Interconnecting modules

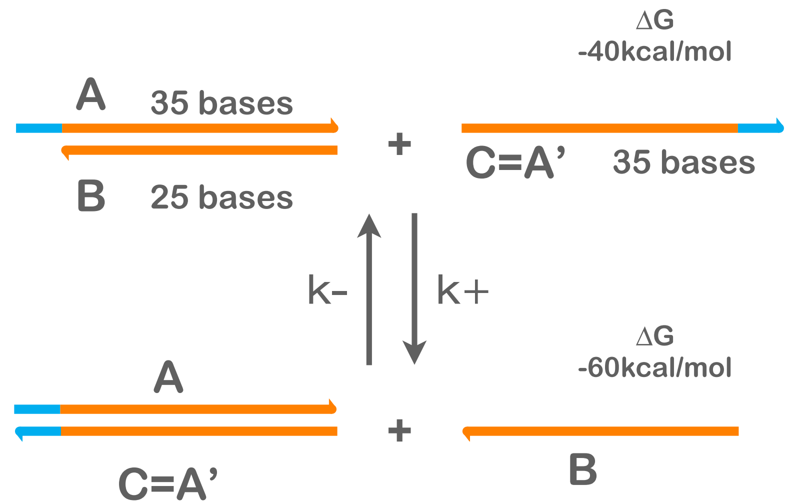
Insulating devices

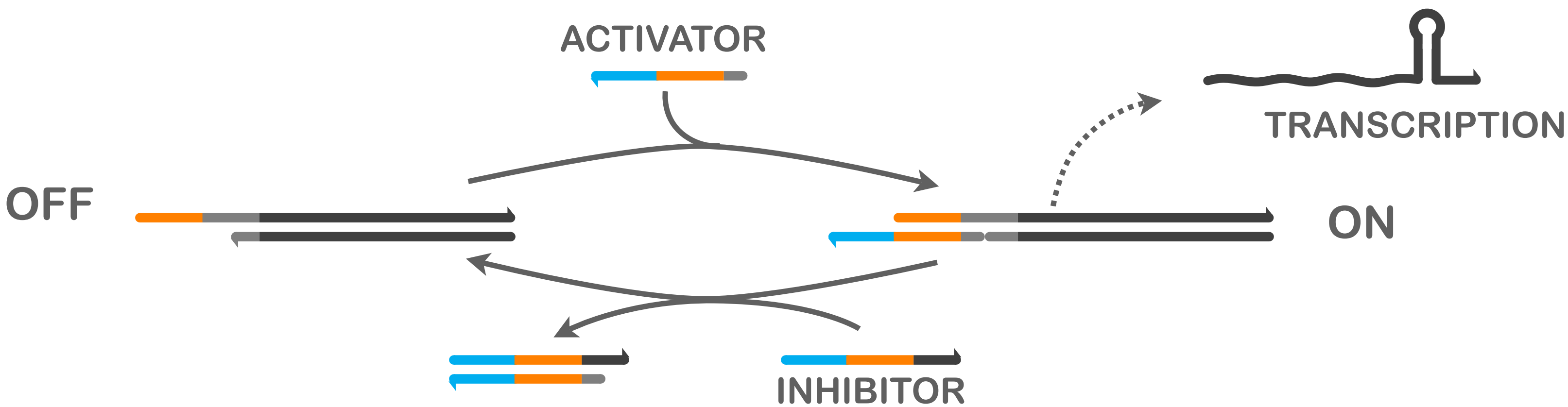
In vitro genetic circuits: the key ideas
Kim and Winfree Nature MSB06

Simplification of feedback loops: Transcription is switched on and off <u>without transcription factors</u>



Switching: by toehold mediated branch migration - Yurke 03

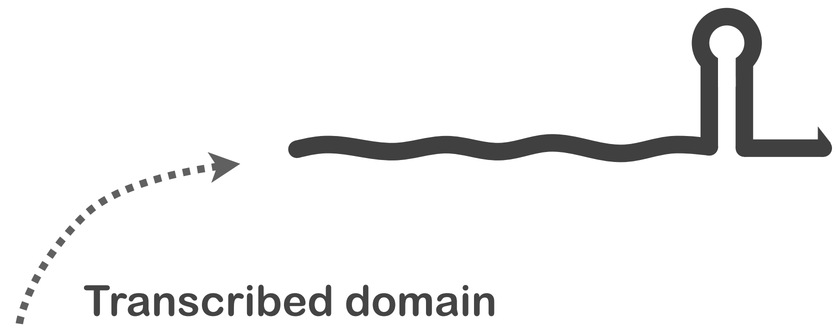


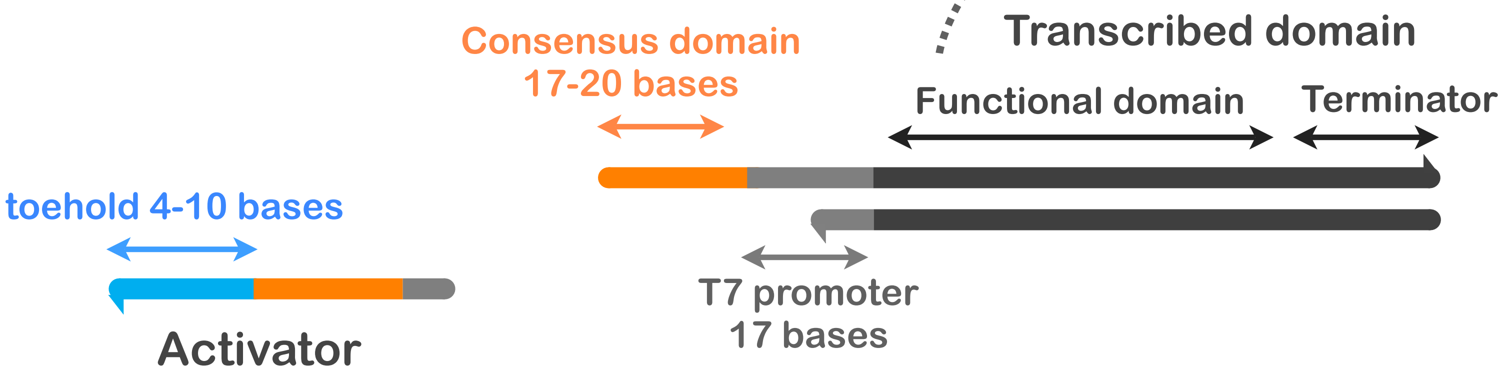


Design and specification of parts

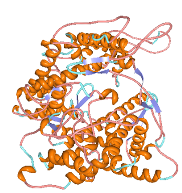
SOFTWARE:

- Maximize (-ΔG) of desired interactions
- Minimize (-ΔG) of unwanted interactions

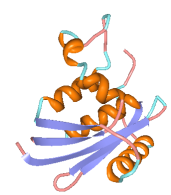




Enzymes off the shelf:



T7 RNA polymerase



RNase H

Programming a simple biochemical network

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

$$T_1 \overset{k_1}{\rightharpoonup} R_1$$

$$T_2 \overset{k_2}{\rightharpoonup} R_2$$

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

<u>Objective</u>: steady flow of P

<u>Constraints</u>: avoid bottlenecks and waste of resources

<u>IDEAS:</u>

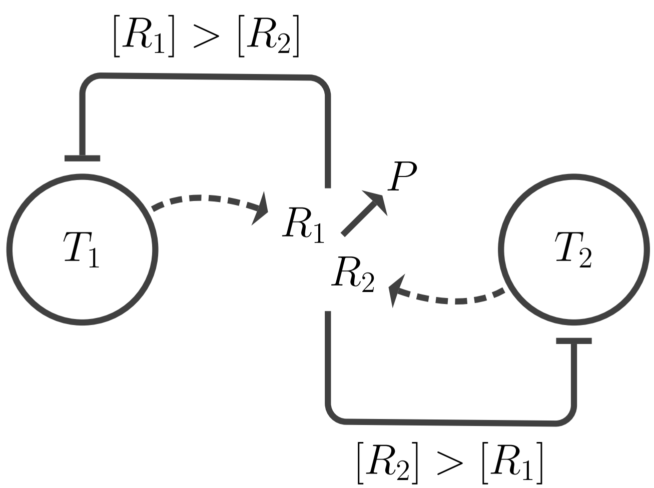
Negative feedback

Design network to decrease excess species

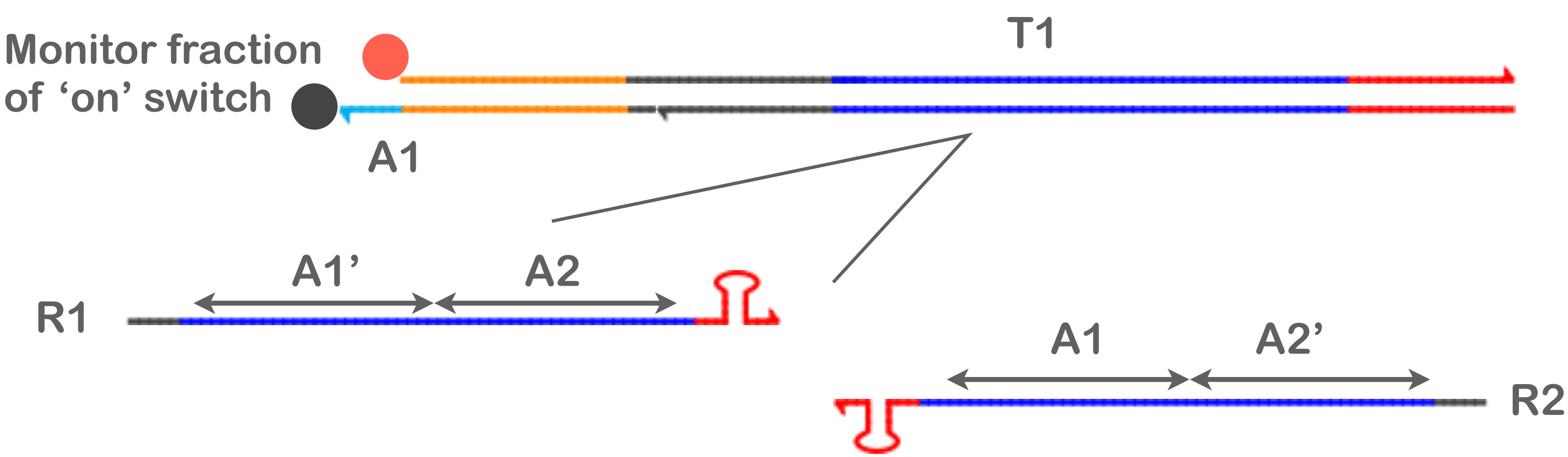
Positive feedback

Design network to stimulate production of less abundant species

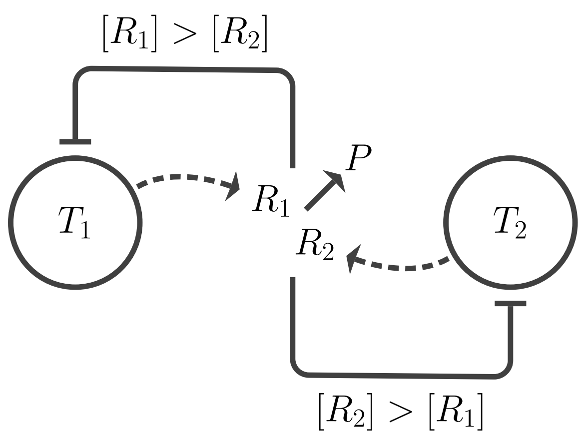
Self repression based flow regulation



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



Modeling the dynamics



Mass action kinetics

Activation

$$T_i + A_i \overset{k_{T_iA_i}}{\rightarrow} T_iA_i$$

Inhibition

$$R_i + A_i \overset{k_{R_iA_i}}{\rightarrow} R_iA_i$$

$$R_i + T_iA_i \overset{k_{R_iT_iA_i}}{\rightarrow} R_iA_i + T_i$$

Output

$$R_i + R_j \overset{k_{R_iR_j}}{\rightarrow} R_iR_j$$

Unwanted interactions

$$R_j + T_i \overset{k_{R_jT_i}}{\rightarrow} R_jT_i$$

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

Michaelis-Menten kinetics

$$\mathbf{R_p} + T_iA_i \underset{k^-_{ONii}}{\overset{k^+_{ONii}}{\rightleftarrows}} \mathbf{R_p} \cdot T_iA_i \overset{k_{catONii}}{\rightarrow} \mathbf{R_p} + T_iA_i + R_i$$

$$\mathbf{R_p} + T_i \underset{k^-_{OFFii}}{\overset{k^+_{OFFii}}{\rightleftarrows}} \mathbf{R_p} \cdot T_i \overset{k_{catOFFii}}{\rightarrow} \mathbf{R_p} + T_i + R_i$$

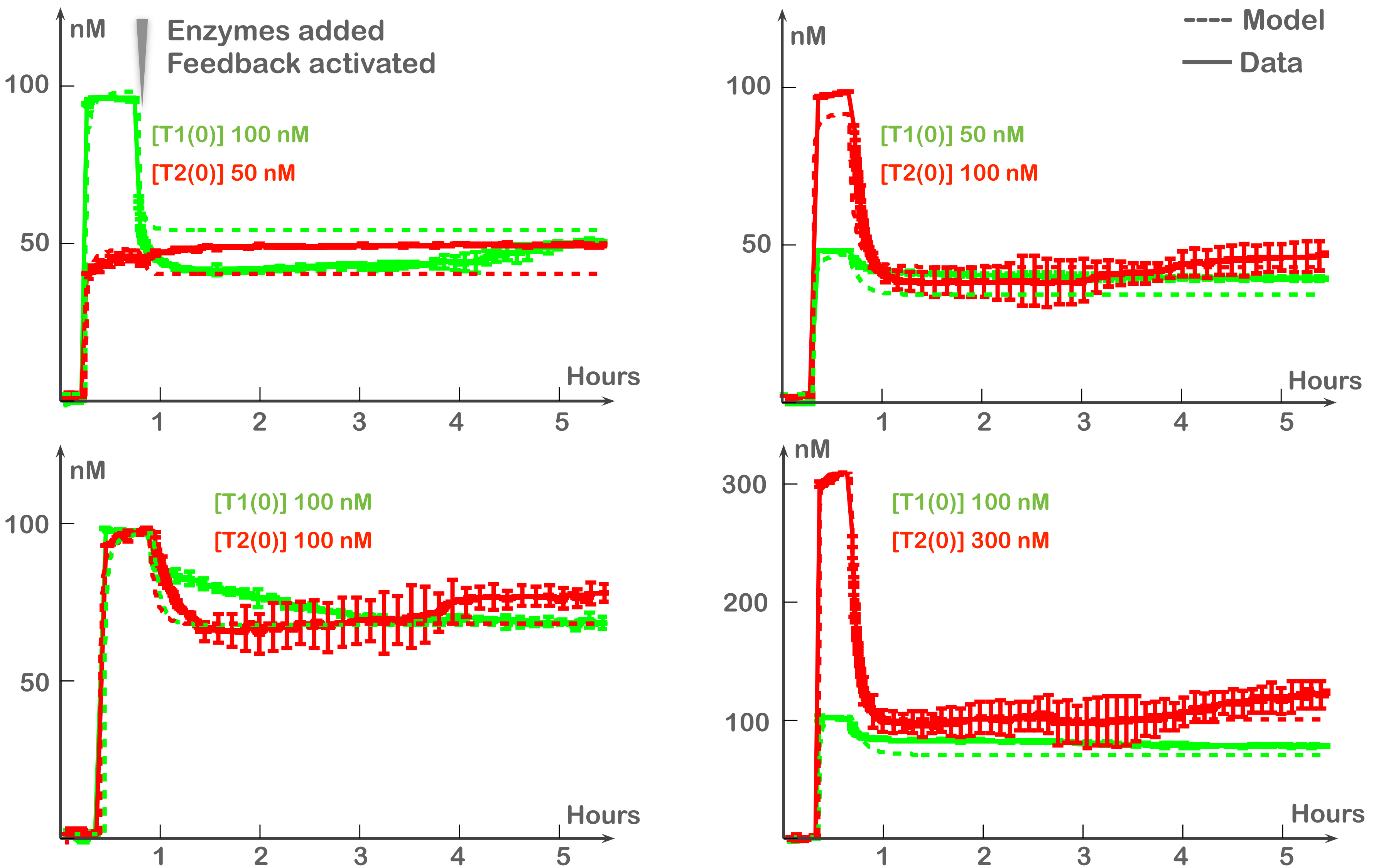
$$\mathbf{R_h} + R_iA_i \underset{k^-_{Hii}}{\overset{k^+_{Hii}}{\rightleftarrows}} \mathbf{R_h} \cdot R_iA_i \overset{k_{catHii}}{\rightarrow} \mathbf{R_h} + A_i$$

$$\mathbf{R_h} + R_jT_i \underset{k^-_{Hji}}{\overset{k^+_{Hji}}{\rightleftarrows}} \mathbf{R_h} \cdot R_jT_i \overset{k_{catHji}}{\rightarrow} \mathbf{R_h} + T_i$$

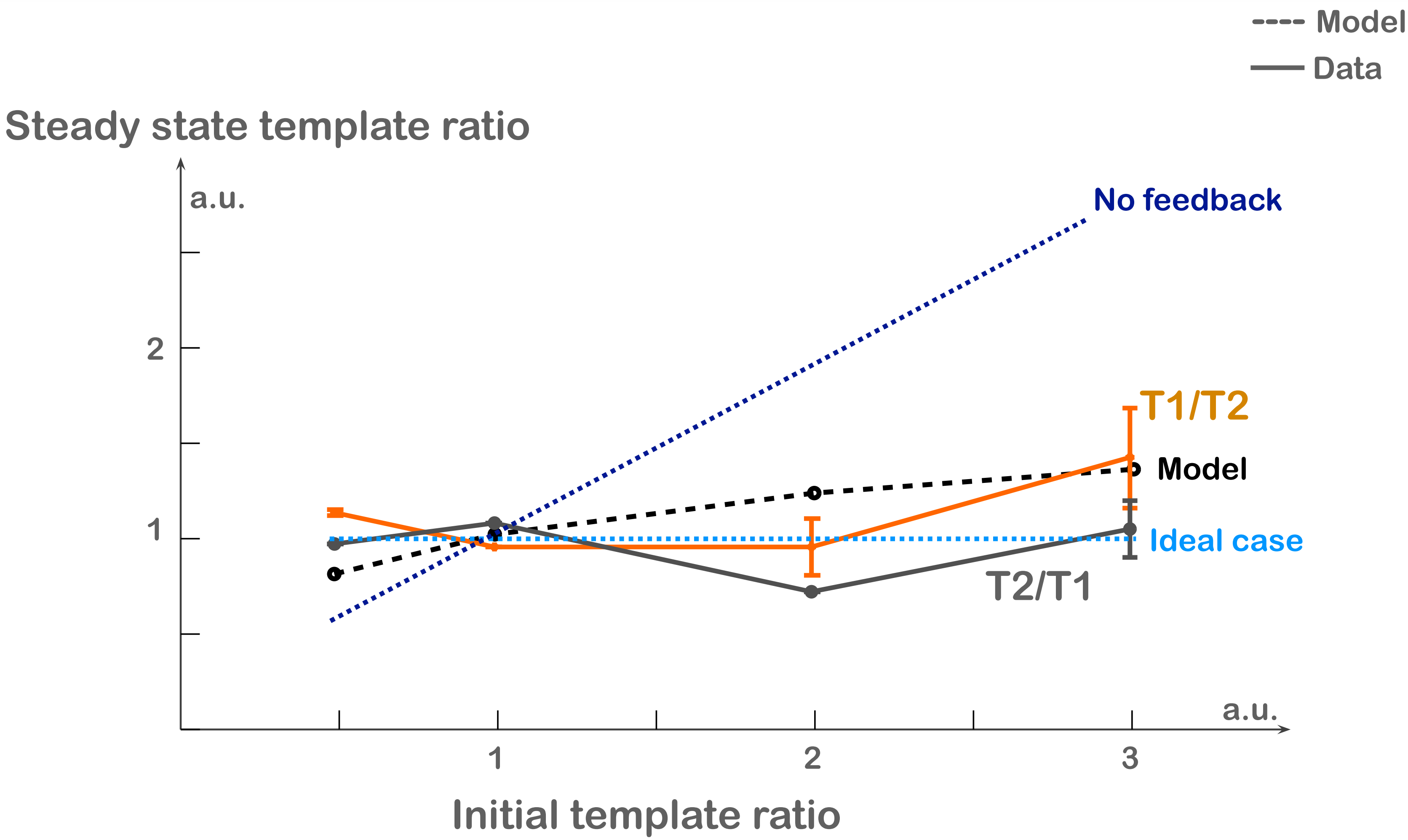
$$\mathbf{R_p} + R_jT_i \underset{k^-_{OFFji}}{\overset{k^+_{OFFji}}{\rightleftarrows}} \mathbf{R_p} \cdot R_jT_i \overset{k_{catOFFji}}{\rightarrow} \mathbf{R_p} + R_jT_i + R_i$$

Faster

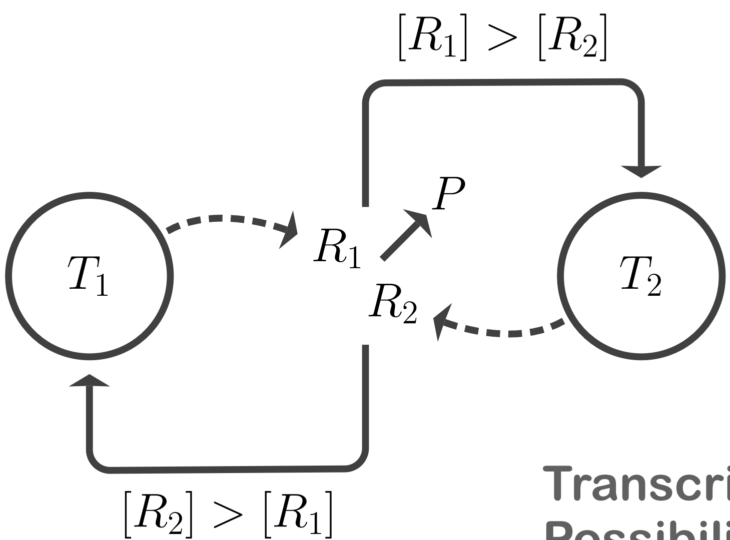
Experimental results



Ratio plot


---- Model
Data
Steady state template ratio
a.u.
No feedback
2
T1/T2
Model
1
Ideal case
T2/T1
a.u.
1
2
3
Initial template ratio

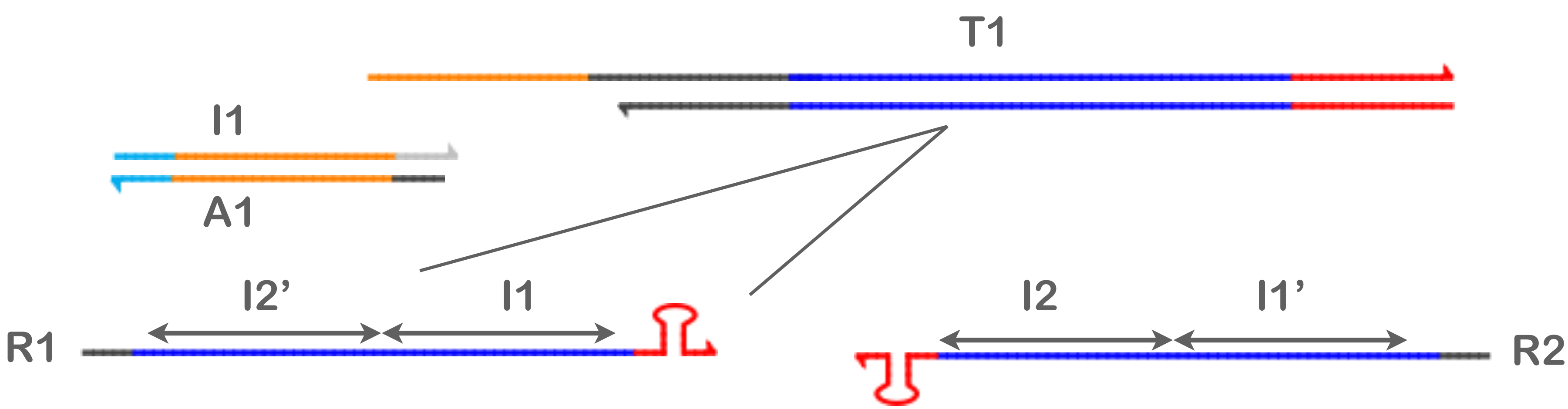
Cross activation circuit design



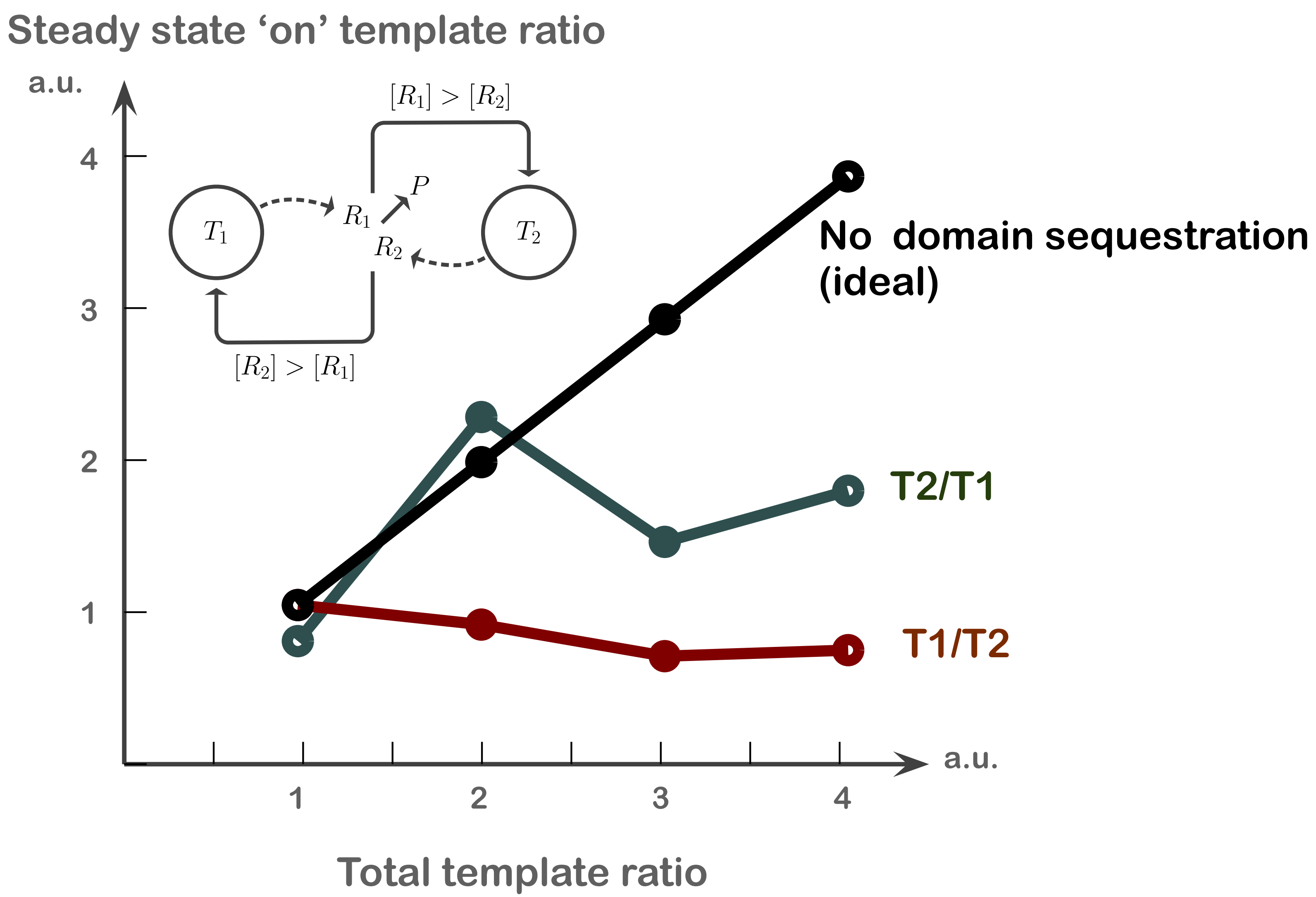
Reactants: transcripts

Product: RNA complex

Transcripts: 'releaser' strands for the activators. Possibility of unwanted self inhibition.



Preliminary data
Current design is asymmetric



In this talk

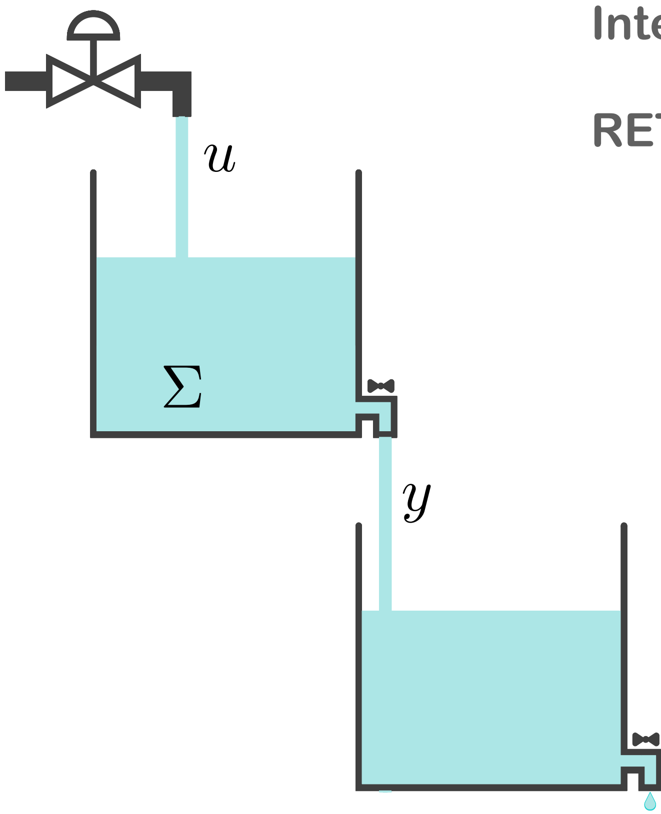
In vitro genetic circuits: our tool kit

Programming a reaction network for rate regulation

Interconnecting modules

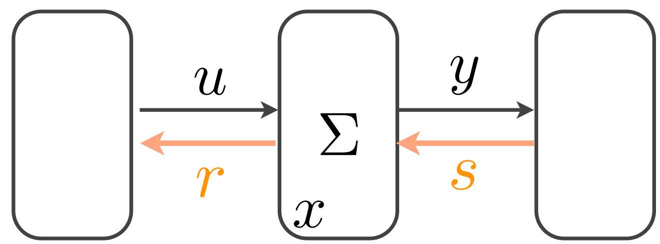
Insulating devices

Interconnections can introduce unwanted dynamics



Interconnections introduce parasitic signals

RETROACTIVITY TO INPUTS AND OUTPUTS

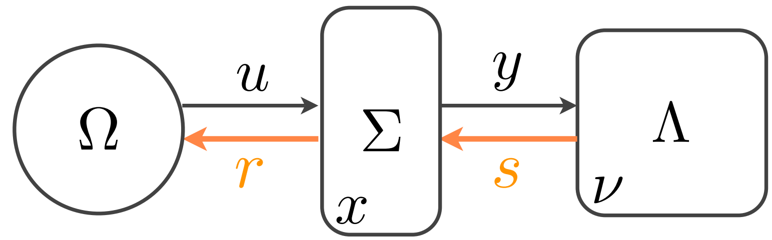


$$\dot{x} = f(x, u, s)$$
$$y = Y(x, u, s)$$
$$r = R(x, u, s)$$

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

$$\Sigma: \quad \begin{aligned} \dot{x} &= f(x,u,s) \\ y &= Y(x,u,s) \\ r &= R(x,u,s) \\ x &= (x_1, ..., x_n) \in \mathcal{D} \subseteq \mathbb{R}^n_+ \end{aligned}$$

1) u, $y = x_n$ positive scalars

2) $\Omega: \quad \dot{u} = f_0(t,u)$ prior to the interconnection

3) $$\Sigma: \quad \dot{x} = \begin{pmatrix} Gf_1(x,u) \\ Gf_2(x) \\ \vdots \\ Gf_{n-1}(x) \\ Gf_n(x) \end{pmatrix}$$

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

5) Parasitic signals are additive

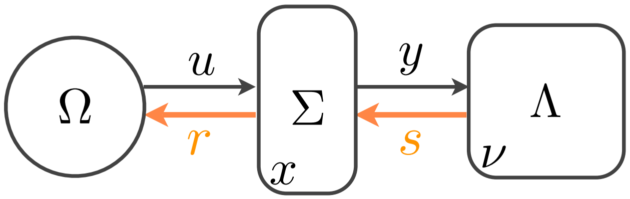
$$\dot{u} = f_0(t,u) + r(x,u)$$
$$\dot{x}_n = \dot{y} = Gf_n(x) + s(\nu, y)$$

6) Conservation laws

$$r(x,u) = -Gf_1(x,u)$$
$$s(\nu, y) = -g_1(\nu, y)$$

Existing theoretical results suggest how to design an insulating device

Stability assumption:



$$\Sigma : \begin{aligned} \dot{x} &= f(x,u,s) \\ y &= Y(x,u,s) \\ r &= R(x,u,s) \end{aligned}$$

$$\dot{x} = \begin{pmatrix} Gf_1(x,u) \\ Gf_2(x) \\ \vdots \\ Gf_{n-1}(x) \\ Gf_n(x) \end{pmatrix}$$

Define:

$$F : \mathbb{R}_+ \times \mathcal{D} \rightarrow \mathbb{R}^n$$

$$F(a,x) = (f_1(x, a - x_1), f_2(x), ..., f_n(x))$$

$$a \in \mathbb{R}_+$$

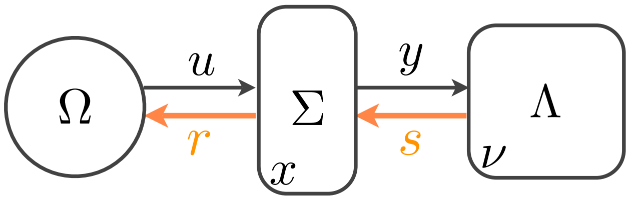
$$x \in \mathcal{D}$$

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



$$\Sigma: \begin{array}{l} \dot{x} = f(x,u,s) \\ y = Y(x,u,s) \\ r = R(x,u,s) \end{array}$$

$$\dot{x} = \begin{pmatrix} Gf_1(x,u) \\ Gf_2(x) \\ \vdots \\ Gf_{n-1}(x) \\ Gf_n(x) \end{pmatrix}$$

Claim 1

There exist G* sufficiently large such that for any G>G*:

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

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

Claim 2

There exist G' sufficiently large such that for any G>G':

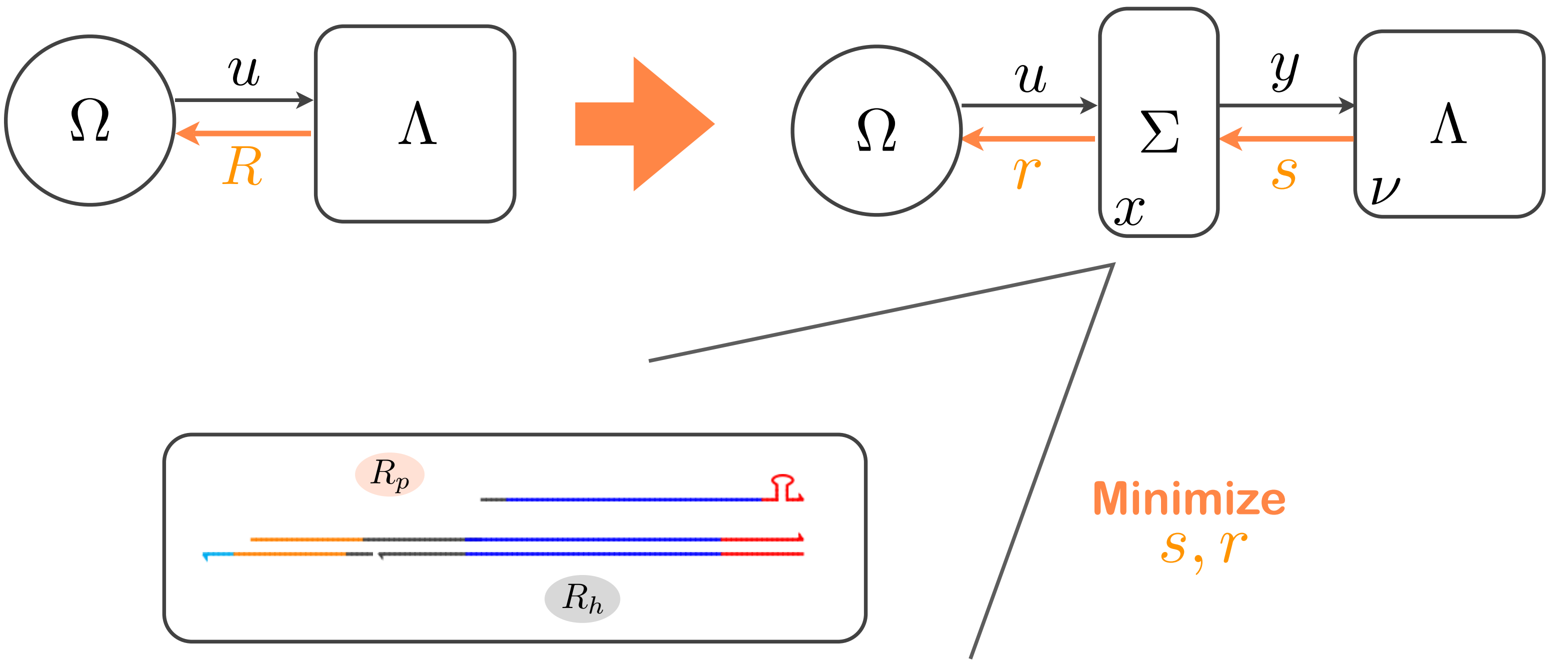
$$u(t) = \bar{u}(t) + \mathcal{O}(1/G)$$

where $\frac{d\bar{u}}{dt} = f_0(t,u)\left(\frac{1}{(1 + \partial\gamma_1(\bar{u})/\partial\bar{u})}\right)$

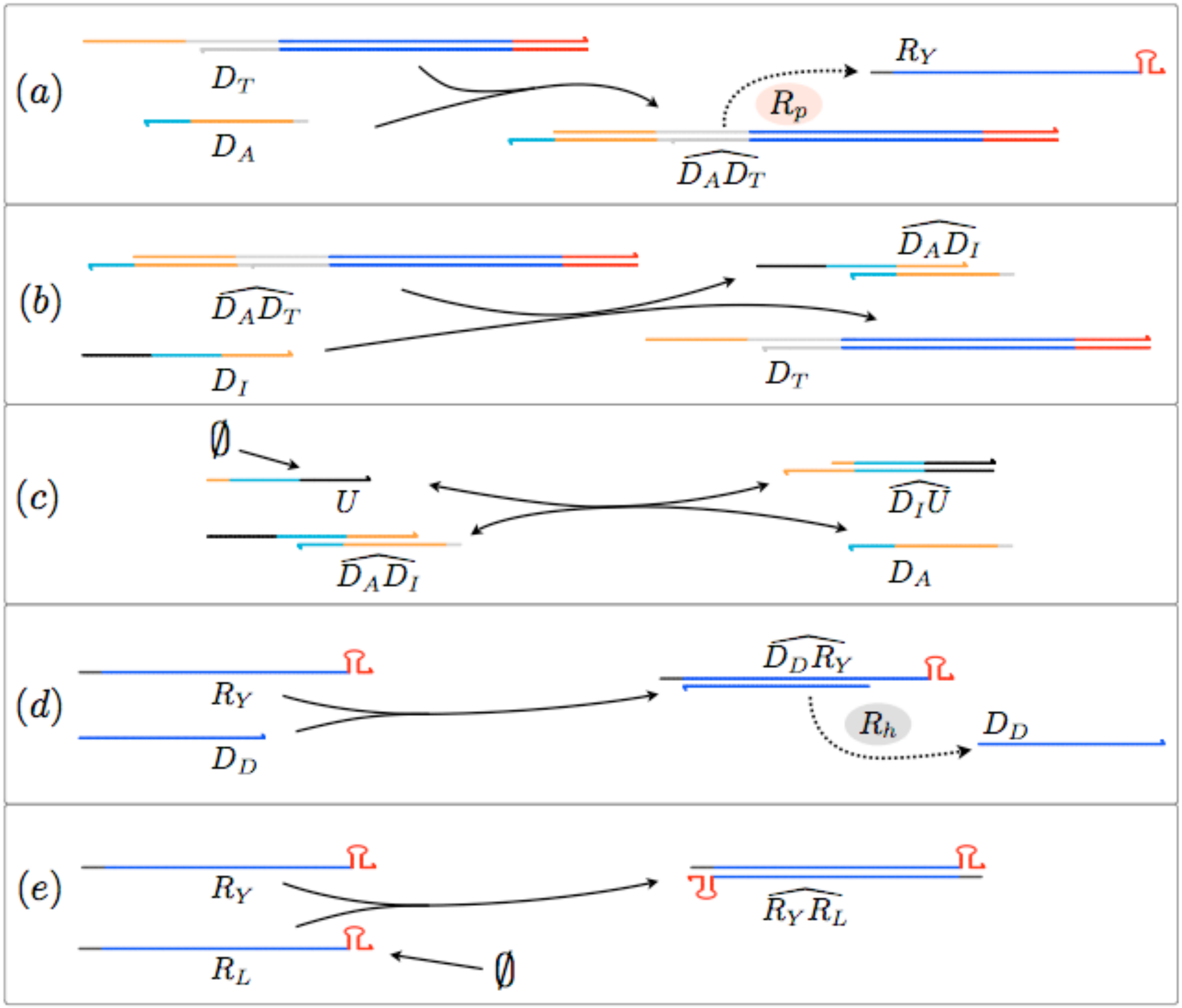
<u>Low output retroactivity</u>

All proofs are based on timescale separation

Can we make an insulator in an in vitro setting?



- Input U drives a switch
- RNA output binds to RNA load



Elisa Franco, Caltech

Reactions for a transcriptional insulator

Input stage

$$\emptyset \xrightharpoonup{p_U(t)} U(t) \xrightharpoonup{d_U(t)} \emptyset$$

$$D_I + U \underset{k_{IU}^-}{\overset{k_{IU}^+}{\rightleftharpoons}} \widehat{D_I U}$$

$$\widehat{D_A D_I} + U \xrightharpoonup{k_{AIU}} \widehat{D_I U} + D_A$$

Core device

$$D_A + D_T \xrightharpoonup{k_{AT}} \widehat{D_A D_T},$$

$$\widehat{D_A D_T} + D_I \xrightharpoonup{k_{AIT}} D_T + \widehat{D_A D_I},$$

$$D_A + D_I \xrightharpoonup{k_{AI}} \widehat{D_A D_I}.$$

$$p_{R_Y}(t) = \alpha\, h\left(\frac{[\widehat{D_A D_T}]}{K_{MP}}\right)$$

$$d_{D_D R_Y}(t) = \gamma\, h\left(\frac{[\widehat{D_D R_Y}]}{K_{MH}}\right)$$

Output stage

$$\emptyset \xrightharpoonup{p_L(t)} R_L(t),$$

$$R_Y + R_L \xrightharpoonup{k_{YL}} \widehat{R_Y R_L}$$

Structural assumptions: dynamic gain can be tuned

Input

$$\dot{U} = +p_U(t) - d_U(t) - k^+_{IU} D_I U + k^-_{IU} \widehat{D_I U} - k_{AIU} \widehat{D_A D_I} U$$

Core device

$$
\begin{aligned}
\dot{\widehat{D_I U}} &= +k^+_{IU} D_I U - k^-_{IU} \widehat{D_I U} + k_{AIU} \widehat{D_A D_I} U \\
\dot{\widehat{D_A D_I}} &= +k_{AI} D_A D_I - k_{AIU} \widehat{D_A D_I} U \\
\dot{\widehat{D_A D_T}} &= +k_{AT} D_A D_T - k_{AIT} D_I \widehat{D_A D_T} \\
\dot{D}_D &= -k_{DY} D_D R_Y + \gamma\, h \left(\frac{\widehat{D_D R_Y}}{K_{MH}} \right) \\
\dot{R}_Y &= +\alpha\, h \left(\frac{\widehat{D_A D_T}}{K_{MP}} \right) - k_{DY} D_D R_Y - k_{YL} R_Y R_L
\end{aligned}
$$

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

Output

- Fast toehold kinetics
- High enzyme concentrations/ activities

$$\dot{x} = \begin{pmatrix} G f_1(x, u) \\ G f_2(x) \\ \vdots \\ G f_{n-1}(x) \\ G f_n(x) \end{pmatrix}$$

Structural assumptions: additive retroactivity and conservation laws

Input

$$\dot{U} = +p_U(t) - d_U(t) - k^+_{IU} D_I U + k^-_{IU} \widehat{D_I U} - k_{AIU} \widehat{D_A D_I} U$$

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

Core device

$$\begin{aligned}
\dot{\widehat{D_I U}} &= +k^+_{IU} D_I U - k^-_{IU} \widehat{D_I U} + k_{AIU} \widehat{D_A D_I} U \\
\dot{\widehat{D_A D_I}} &= +k_{AI} D_A D_I - k_{AIU} \widehat{D_A D_I} U \\
\dot{\widehat{D_A D_T}} &= +k_{AT} D_A D_T - k_{AIT} D_I \widehat{D_A D_T} \\
\dot{D}_D &= -k_{DY} D_D R_Y + \gamma\, h\left(\frac{\widehat{D_D R_Y}}{K_{MH}}\right) \\
\dot{R}_Y &= +\alpha\, h\left(\frac{\widehat{D_A D_T}}{K_{MP}}\right) - k_{DY} D_D R_Y - k_{YL} R_Y R_L
\end{aligned}$$

$$\dot{x} = \begin{pmatrix} G f_1(x,u) \\ G f_2(x,u) \\ G f_3(x) \\ G f_4(x) \\ G f_5(x) + G s(\nu, y) \end{pmatrix}$$

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

$$\dot{\nu} = f_\nu(t) + G s(\nu, y).$$

Output

Structural assumptions: stability

Jacobian of the dynamics of the core device:

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

Lower diagonal

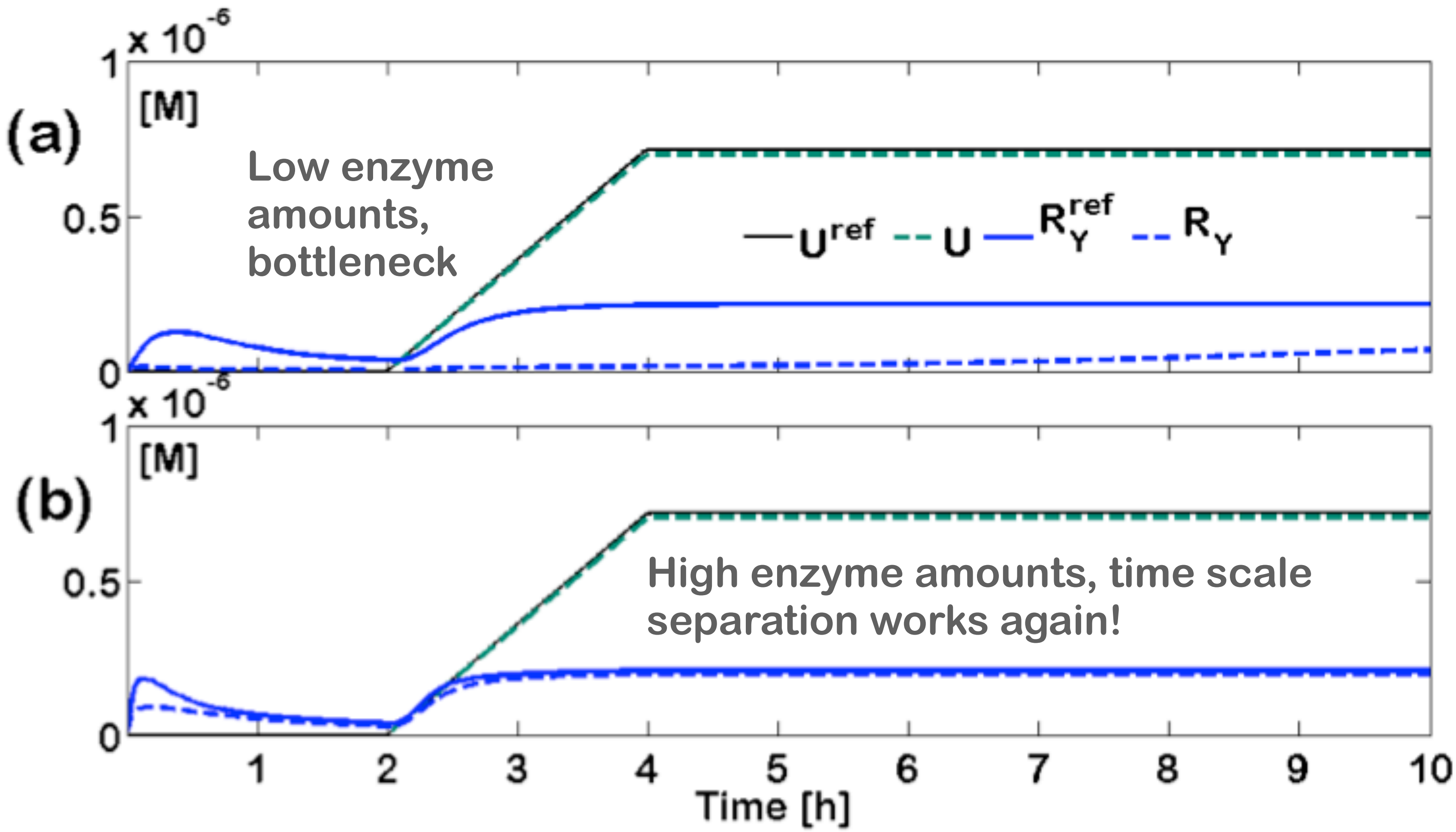
The eigenvalues have negative real part at any equilibrium point.

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

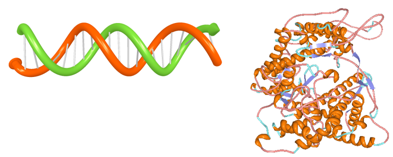
Simulation results



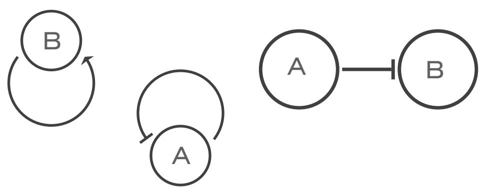
Programming synthetic biomolecular systems: embedding engineering principles in the hardware of life

Designing and building biosynthetic systems is today

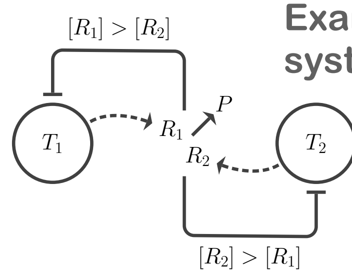
- Easier
- Faster
- Cheaper

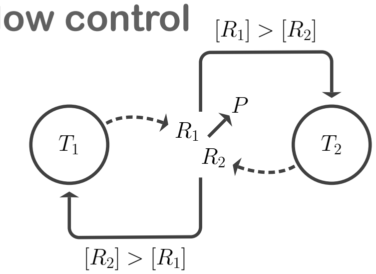


- In vitro bio-computational networks

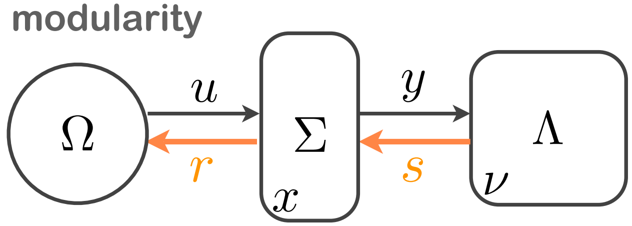


Example: flow control systems





- Scaling up networks, modularity



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