Design of Bio-molecular Feedback Systems

Domitilla Del Vecchio
Electrical Engineering and Computer Science
University of Michigan, Ann Arbor

ASCC, August 2009

Outline

- **Part 1:** Overview, history, and enabling technology
- **Part 2:** Simple modules fabricated: oscillators, toggles, and inverters
- **Part 3:** The challenge of composing modules together
- **Part 4:** Fabrication *in vivo* and *in vitro*

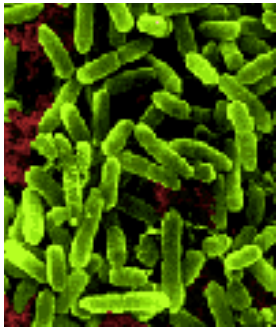
Part 1

Overview, history, and enabling technology

Why to Design Bio-molecular Feedback Systems?

MEDICAL APPLICATIONS
(e.g. targeted drug delivery)

ALTERNATIVE ENERGY
(e.g. bio-fuels)
Making bacteria that…
- Produce hydrogen or ethanol
- Transform waste into energy



COMPUTING APPLICATIONS
(e.g. molecular computing)

BIO-SENSING
(e.g. detecting pathogens or toxins)

Synthetic Biology: A Historical Perspective

Electronic Engineering

Electrical Engineering

Vacuum Tube era

Transistor era

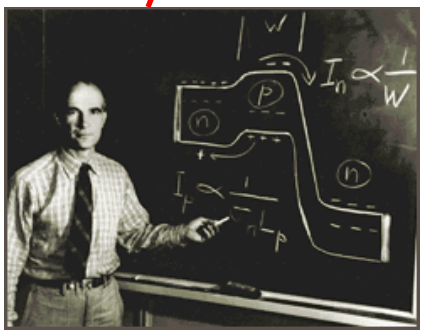
To Electronic computers

Ampere, Coulomb, Faraday, Gauss, Henry, Kirchhoff Maxwell Ohm

1904

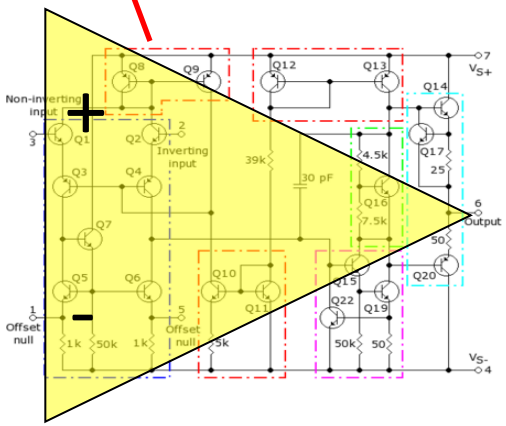
Fleming invented the diode (a two-terminal device)

1948



William Shockley explains how the bipolar junction transistor works (BJT) December 1947, Bell Laboratories (Nobel Prize in Physics in 1956)

1964



Operational Amplifier (OPAMP)
1964 Wildar at Fairchild Semiconductor

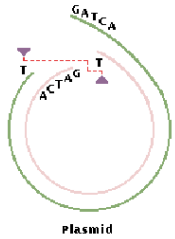
Synthetic Biology: A Historical Perspective

Birth of Genetic Engineering

recombinant DNA

Birth of Synthetic Biology?

1961 1968 1978 1980s 1983 2000



W. Arber discovers restriction enzymes (Nobel Prize winner)

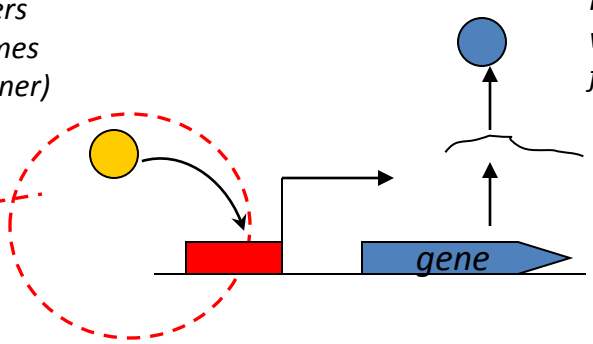
Insulin became first recombinant DNA drug

First reporter gene was isolated: green fluorescent protein (GFP)

K. Mullis: Polymerase Chain Reaction (PCR) (exponential amplification of DNA)

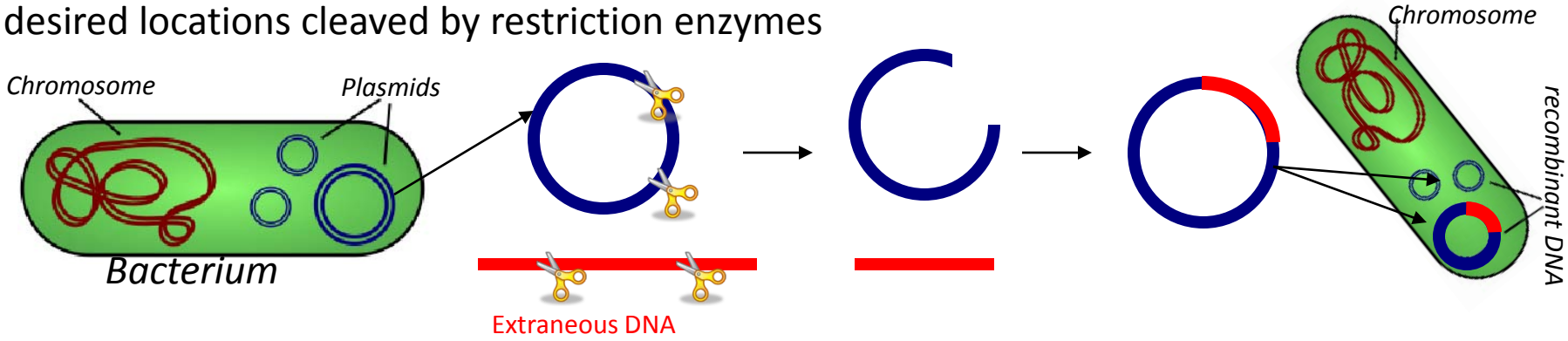
Early ``working'' synthetic circuits in E coli: Gardner et al. toggle switch, Elowitz and Leibler repressilator

Jacob and Monod introduce for the first time the concept of operon regulation

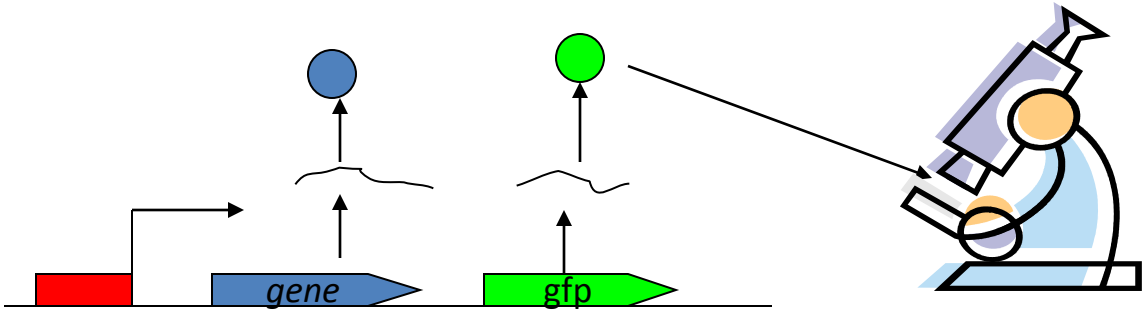


Key Enabling Technology

Recombinant DNA technology: allows to cut and paste pieces of DNA at desired locations cleaved by restriction enzymes



Fluorescent Proteins: allow through fluorescence microscopy to measure the concentration of a protein and thus the level of expression of the corresponding gene



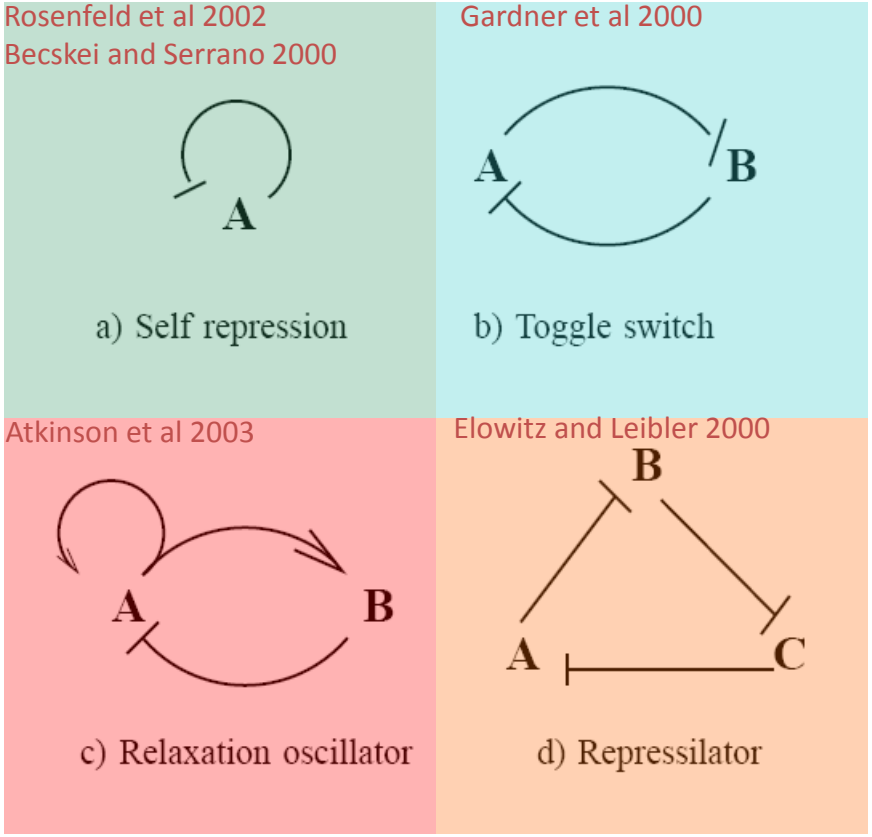
Early modules fabricated *in vivo*

Monostable modules

Bistable modules

Relaxation oscillators

Loop oscillators

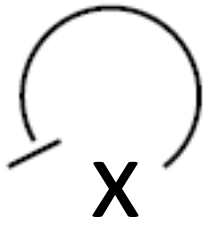


Part 2

Example modules

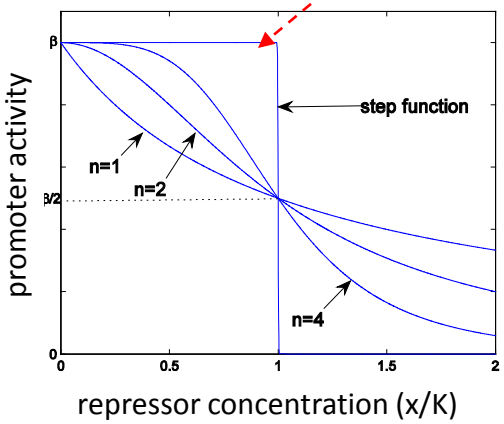
- Auto-repressed gene
- Toggle
- Loop oscillator
- Activator-repressor oscillator

A self repressed gene:Dynamics

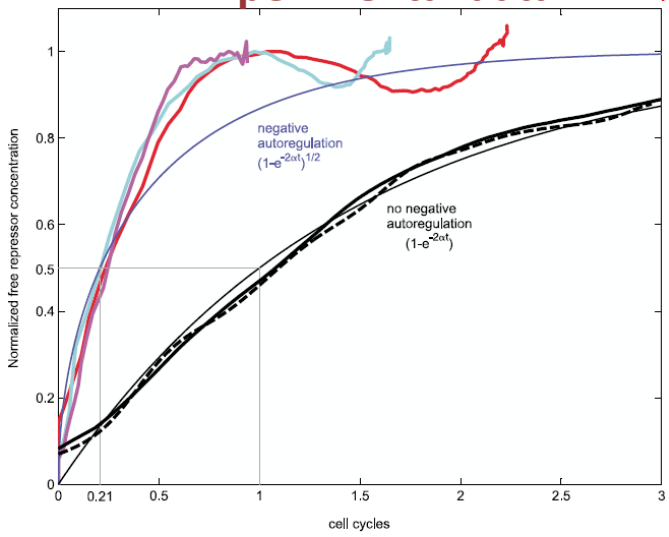


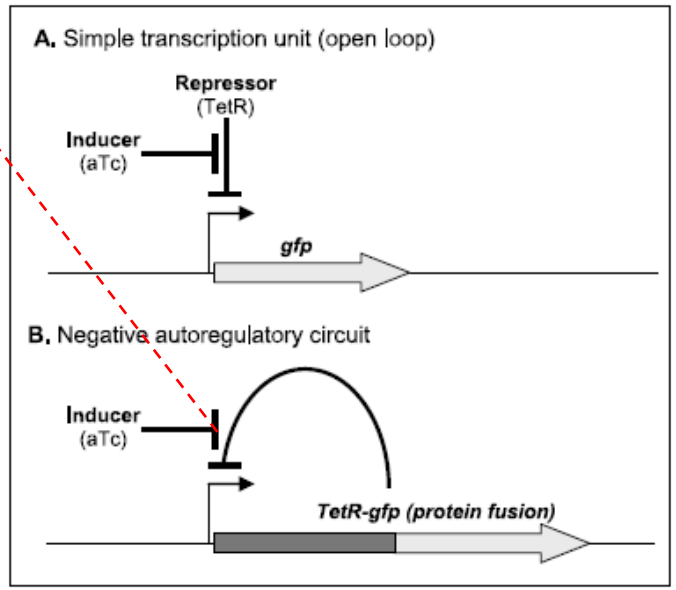
negative feedback

$$\frac{dx}{dt} = \frac{\beta}{1+(x/k)^n} - \alpha x$$



Experimental data





For n=1:

Without negative feedback

$$\frac{x_1(t)}{x_1^{st}} = 1 - e^{-\alpha t}$$

With negative feedback

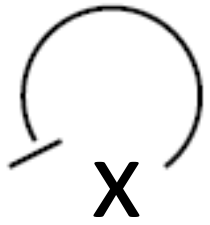
$$\frac{x_2(t)}{x_2^{st}} = \sqrt{1 - e^{-2\alpha t}}, \qquad \beta_2/\alpha \gg k$$

$$x_1^{st} = \beta_1/\alpha$$

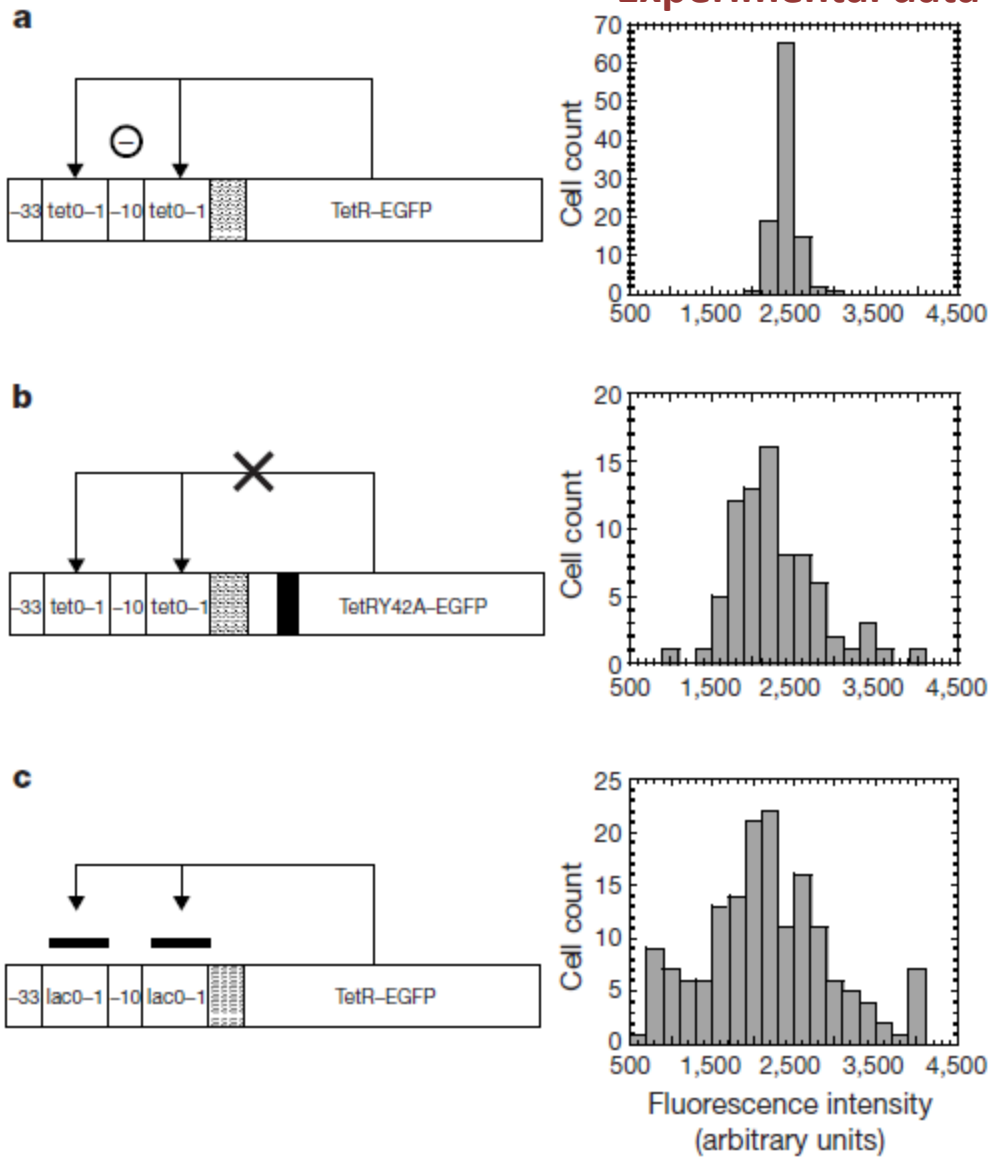
$$x_2^{st} = \sqrt{k\beta_2/\alpha}$$

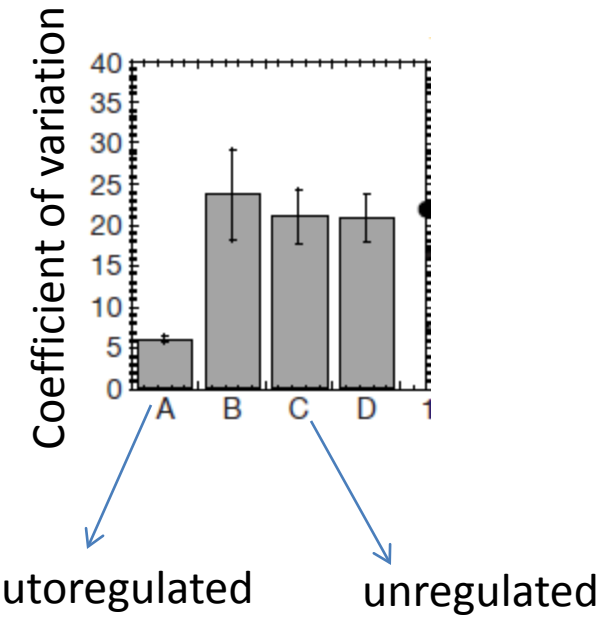
Negative feedback speeds up the response time (Rosenfeld et al 2002)

A self repressed gene: robustness



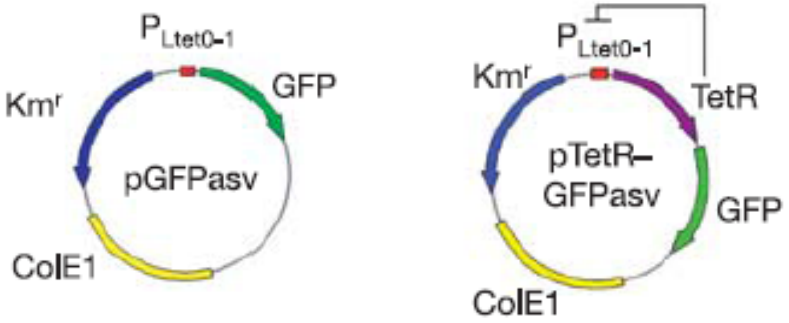
Experimental data



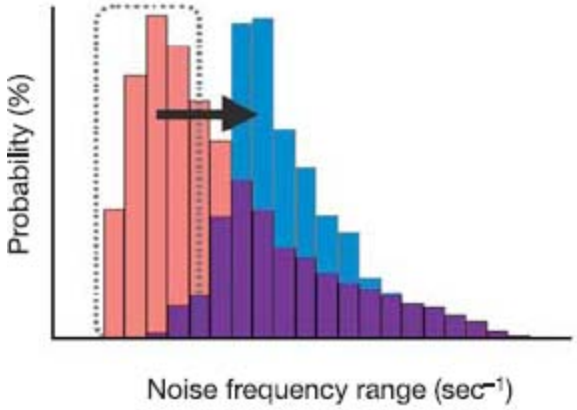


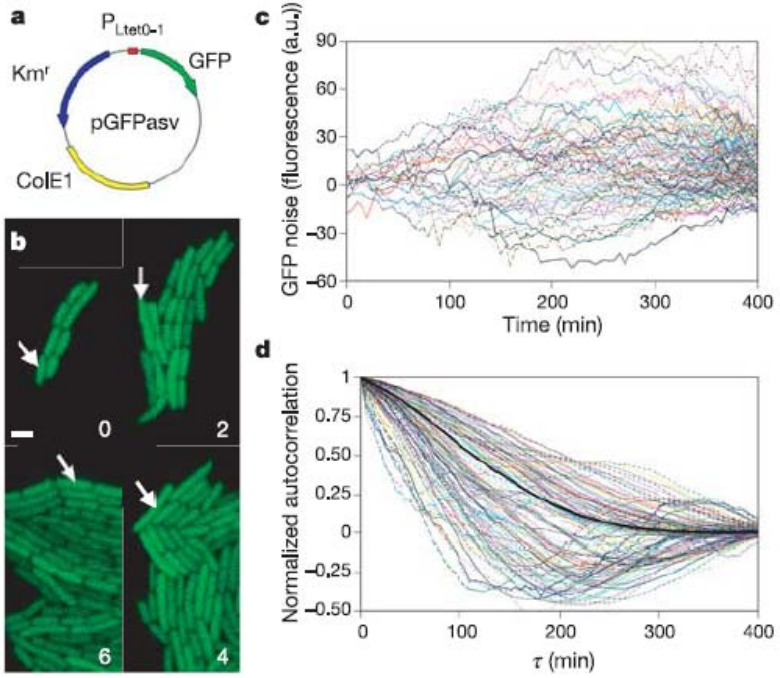
Becskei and Serrano, Nature 2000:
Negative autoregulation decreases noise

Self repressed gene: Frequency analysis

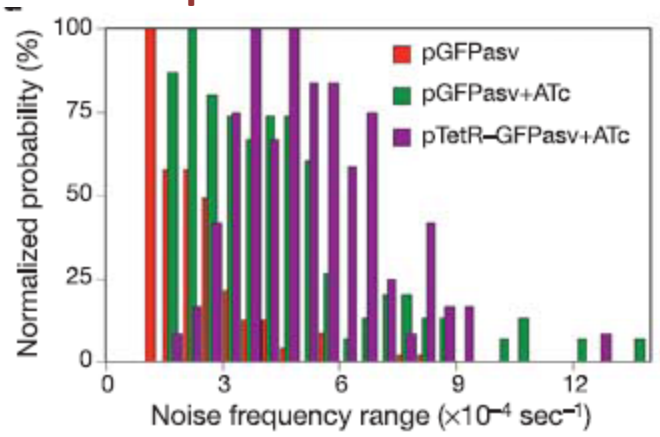


Simulation data (SSA)



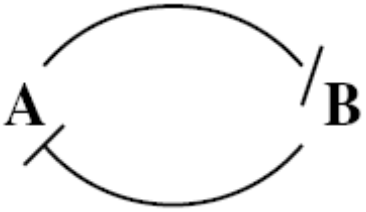


Experimental data

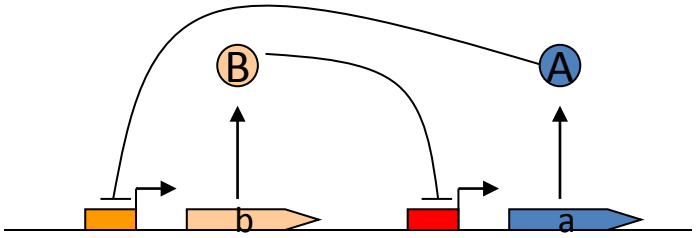


Austin et al. Nature 2006: Negative autoregulation shifts frequency content to high frequency

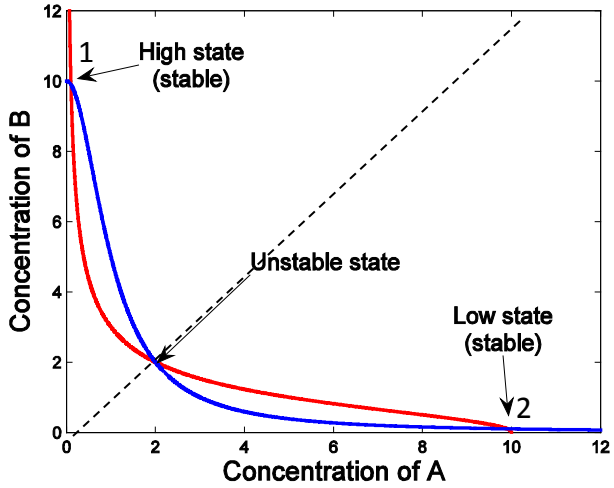
Toggle switch

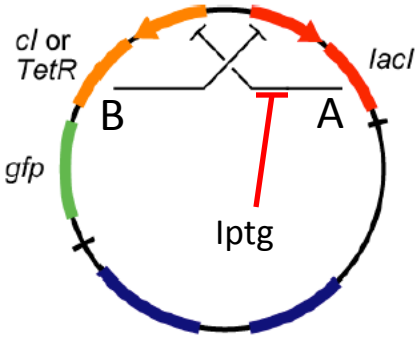


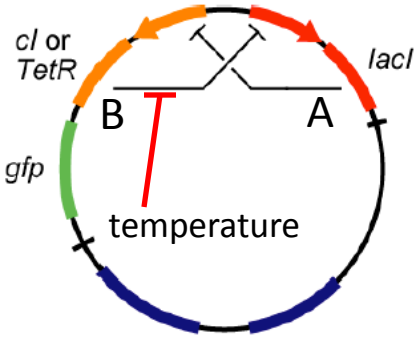
$$\frac{dA}{dt} = \frac{\beta}{1+(B/K_1)^n} - \alpha_1 A$$
$$\frac{dB}{dt} = \frac{\gamma}{1+(A/K_2)^n} - \alpha_2 B$$

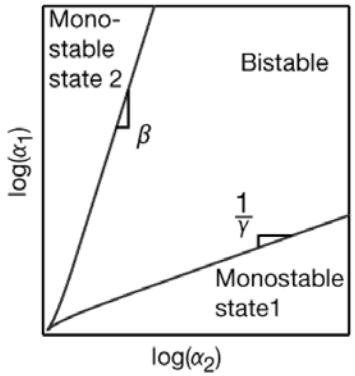


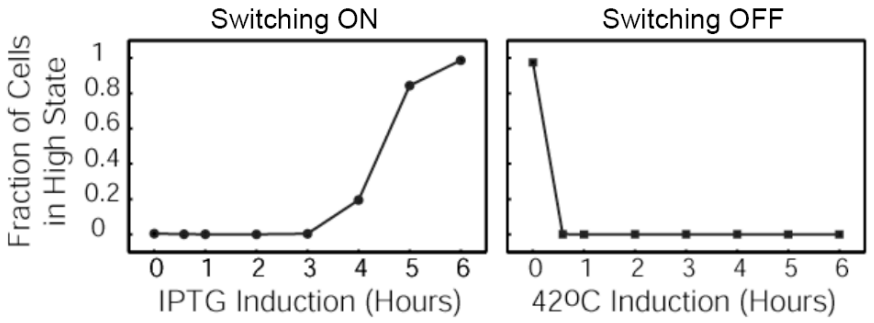
Symmetric design



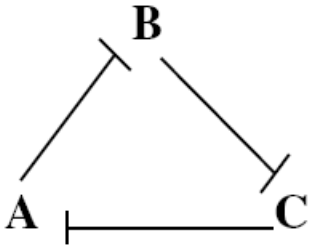


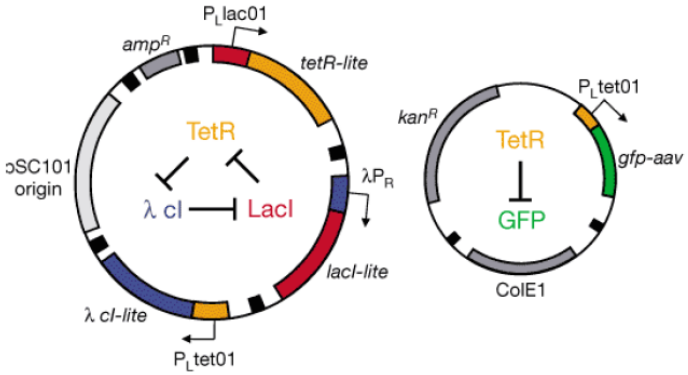






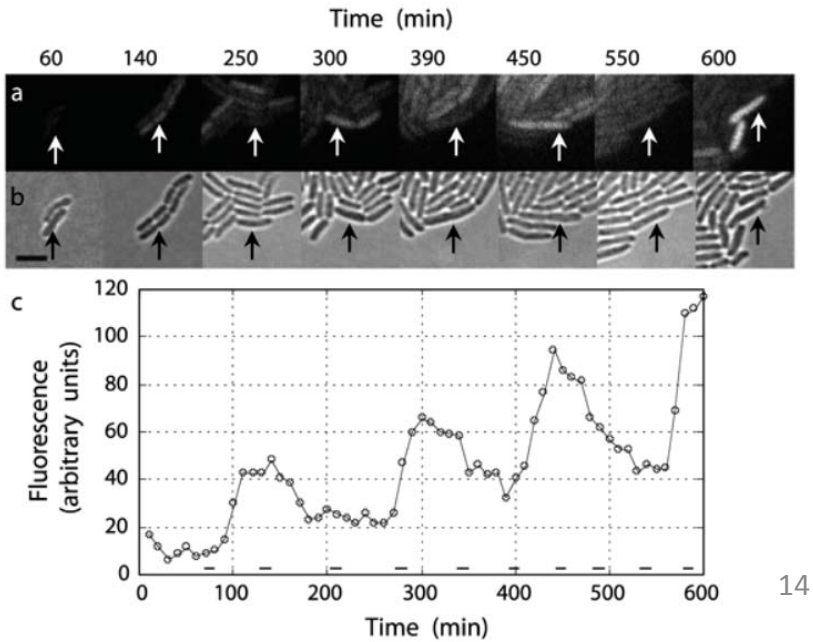
Loop oscillators: The repressilator





$$\frac{dr_A}{dt} = f(C) - \delta_r r_A$$
$$\frac{dA}{dt} = r_A - \delta A$$
$$\frac{dr_B}{dt} = f(A) - \delta_r r_B$$
$$\frac{dB}{dt} = r_B - \delta B$$
$$\frac{dr_C}{dt} = f(B) - \delta_r r_C$$
$$\frac{dC}{dt} = r_C - \delta C$$

$$f(p) = \frac{\alpha^2}{1+p^n}$$



Elowitz and Leibler, Nature 2000

Loop oscillators: Design criteria

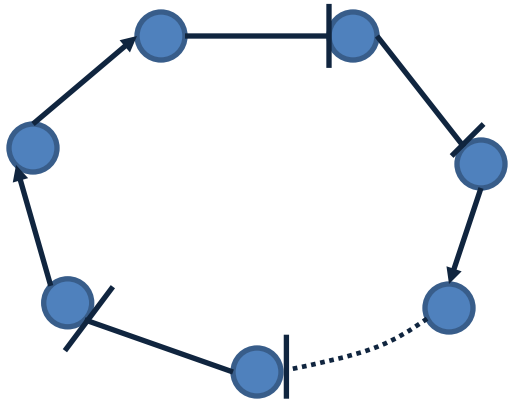
mRNA ← $\frac{dr_1}{dt} = f_1(p_n) - \alpha_1 r_1$

protein ← $\frac{dp_1}{dt} = r_1 - \gamma_1 p_1$

$\frac{dr_i}{dt} = f_i(p_{i-1}) - \alpha_i r_i$

$\frac{dp_i}{dt} = r_i - \gamma_i p_i$

$i = 2, 3, \ldots, n$



$f_i(p_{i-1}) = \frac{\beta_i p_{i-1}^n}{1 + p_{i-1}^n}$

Monotonically increasing

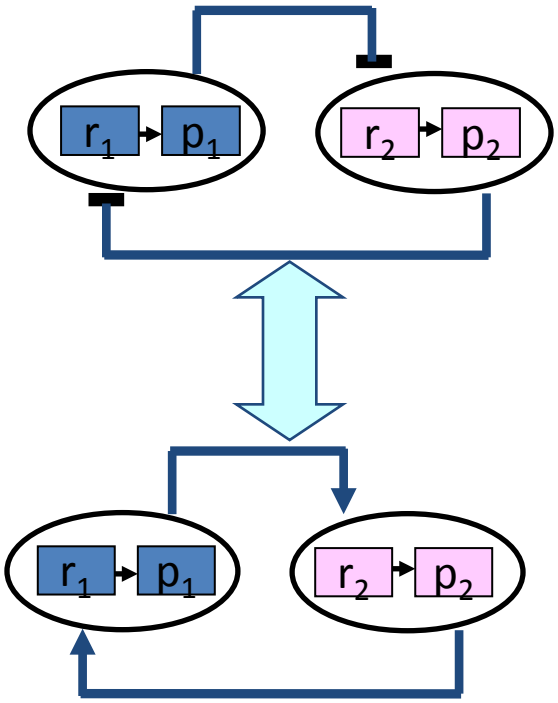
$f_i(p_{i-1}) = \frac{\beta_i}{1 + p_{i-1}^n}$

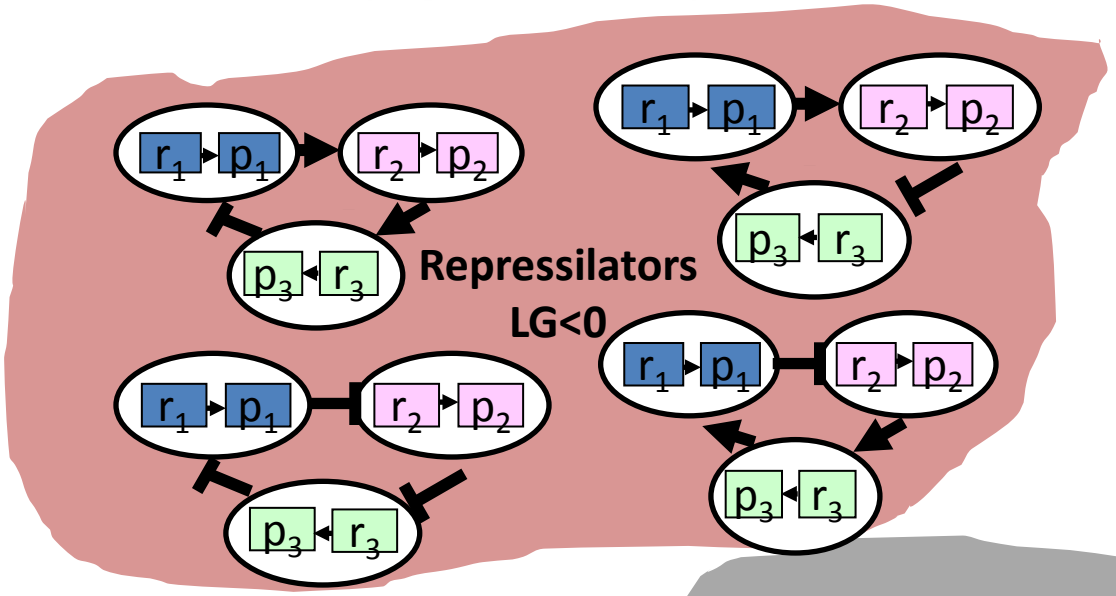
Monotonically decreasing

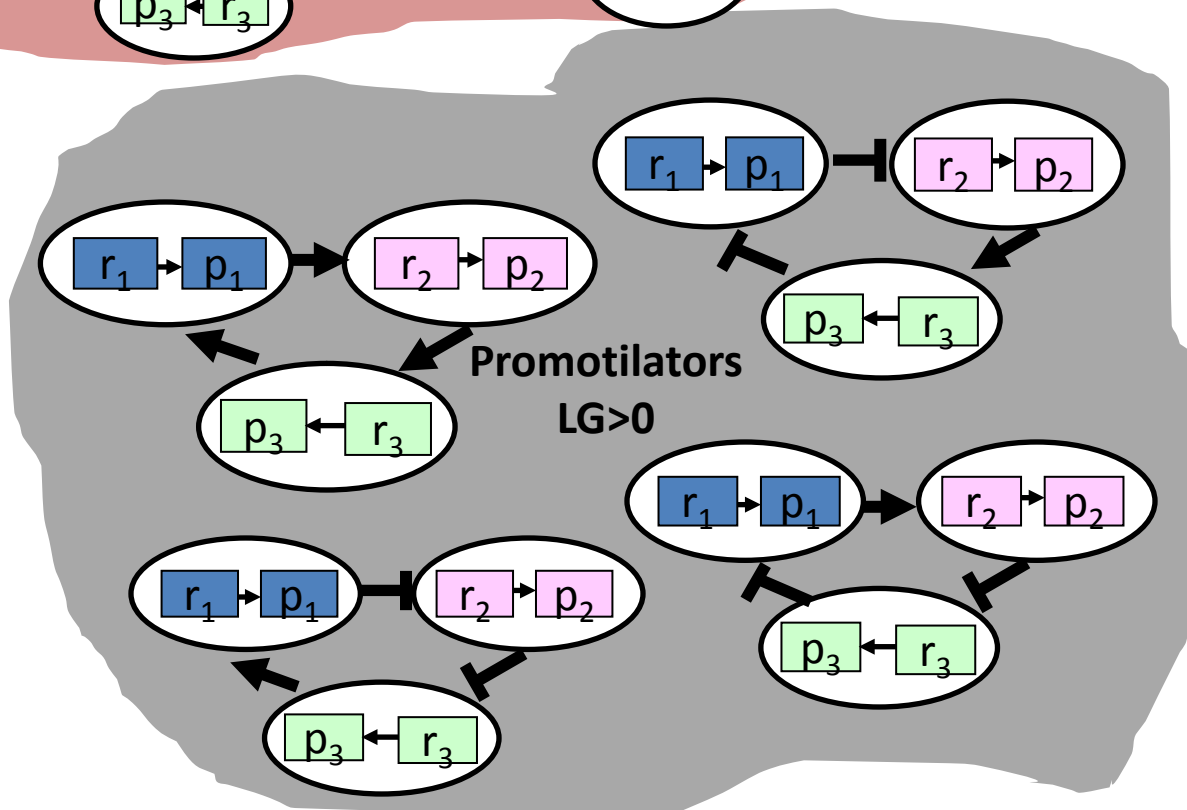
Loop gain

$$LG = \prod_{i=1}^{n} sign\left(\frac{\partial f_i}{\partial p_{i-1}}\right)$$

Circuits with same loop-gain are equivalent







Proof: Change of coordinates

$$r_1' = \frac{1}{\alpha_1} - r_1 \qquad r_2' = r_2$$

$$p_1' = \frac{1}{\alpha_1 \gamma_1} - p_1 \qquad p_2' = p_2$$

Dynamical behavior of repressilators and promotilators

Invariant set and attractor

$$B(1,1,1) = [0,\frac{1}{\alpha_1}]\times[0,\frac{1}{\alpha_1\gamma_1}]\times[0,\frac{1}{\alpha_2}]\times[0,\frac{1}{\alpha_2\gamma_2}].....\times[0,\frac{1}{\alpha_n}]\times[0,\frac{1}{\alpha_n\gamma_n}]$$

is a positively invariant set and it contains a compact attractor for the dynamics.

Omega limit set

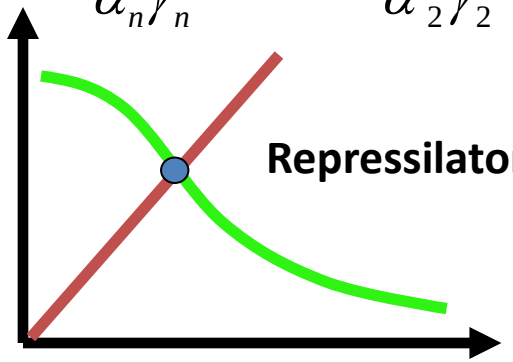
By Mallet-Paret and Smith Thorem (1990):

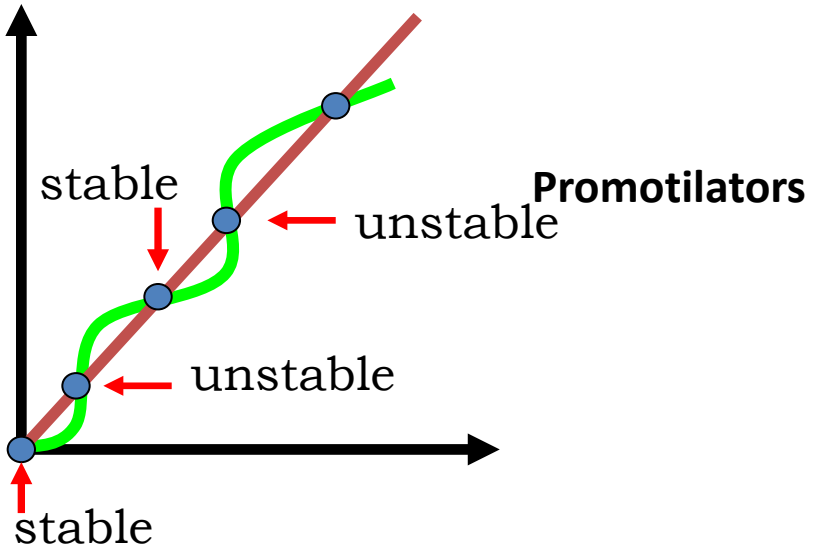
- Omega limit set is a single equilibrium, a single non-constant periodic orbit, or a structure consisting of a set of equilibria connected through homoclinic or heteroclinic orbits.
- Chaos can be ruled out.

Equilibria

Intersection of diagonal and

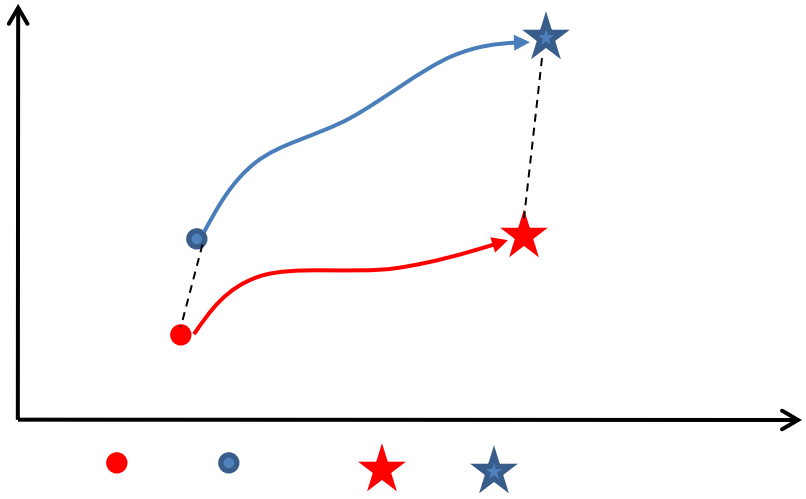
$$g(u) = \frac{1}{\alpha_n\gamma_n} f_n \circ .... \circ \frac{1}{\alpha_2\gamma_2} f_2 \circ \frac{1}{\alpha_1\gamma_1} f_1$$





Promotilators

Promotilators: Belong to the class of monotone dynamical systems (see Sontag 2005):

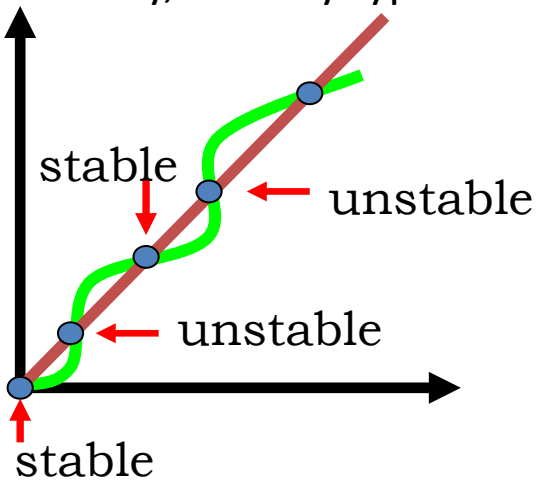


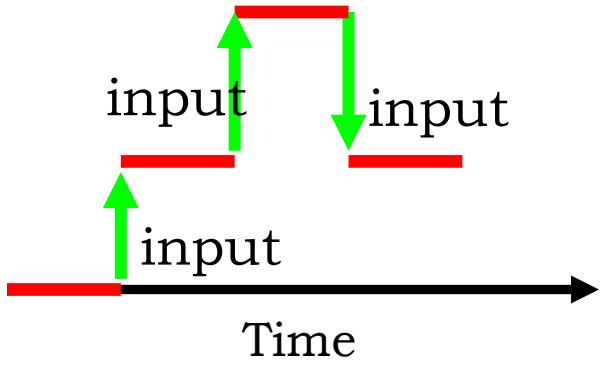
$$x(0) \leq y(0) \;\Rightarrow\; \varphi(t, x(0)) \leq \varphi(t, y(0))$$

Theorem (M. Hirsh, 1988)

Let *x(t)* be the flow of an irreducible monotone system with the property that all forward orbits have compact closures. If the set of steady-states of the system is discrete, then the set of points x_0 for which $x(t, x_0)$ does not converge to a steady-state has Lebesgue measure zero.

Alternate stability, stability type derived by looking at 1D plot (Sontag, 2005)

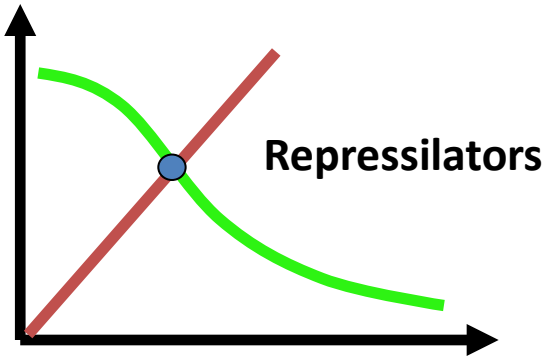




Repressilators

- Repressilators have only one equilibrium.
- Instability of equilibrium Is sufficient for existence of periodic solution:

Theorem (Hastings et al. 1977)



Let $\dot{x}_1 = f_1(x_n, x_1)$

$\dot{x}_j = f_j(x_{j-1}, x_j)$

Let $\frac{\partial f_j}{\partial x_j} < 0, \frac{\partial f_j}{\partial x_{j-1}} > 0, \frac{\partial f_1}{\partial x_n} < 0, f_i(0,0) \geq 0, \frac{\partial f_1}{\partial x_1}$ be bounded from above

Let $x^* = (x_1^*, x_2^*, \ldots, x_n^*)$ be the steady steady-state such that $f_1(x_n, x_1) < 0$

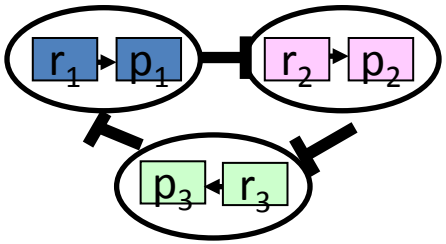
when $x_n > x_n^*, x_1 > x_1^*$ and $f_1(x_n, x_1) > 0$ when $x_n < x_n^*, x_1 < x_1^*$

If Jacobian has eigenvalues with positive real part, then system has a nonconstant periodic orbit

Repressilators design

- Can design oscillator by studying eigenvalues.
- Study robustness to parametric uncertainty
- Study tradeoffs

Symmetric design



Symmetric design

$$\frac{dr_1}{dt} = f_1(p_n) - \delta r_1 \qquad f_i(p) = \frac{\alpha^2}{1+p^n}$$

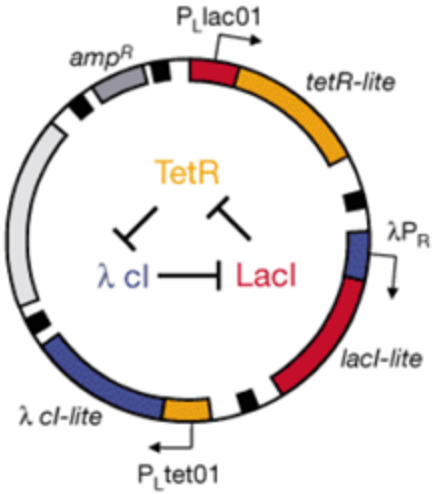
$$\frac{dp_1}{dt} = r_1 - \delta p_1$$

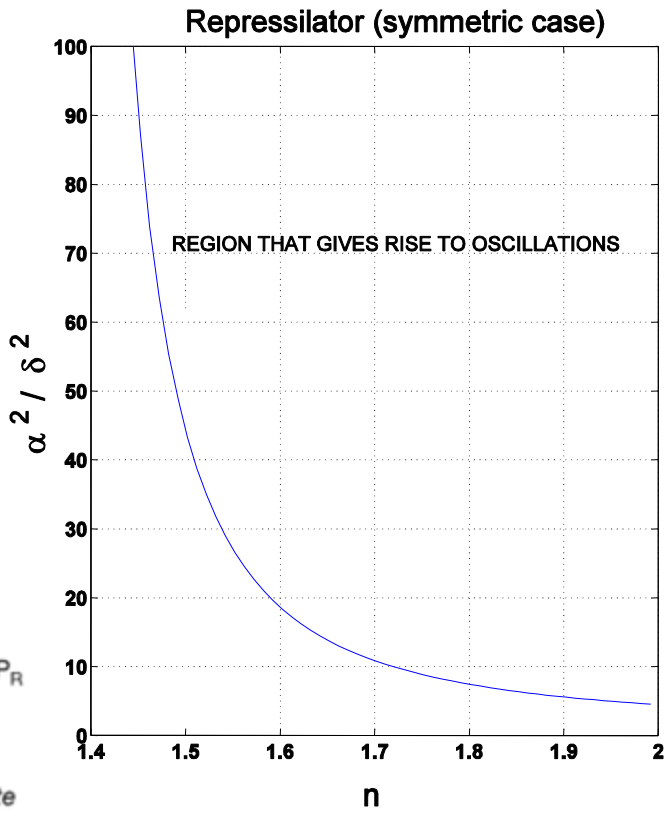
$$\frac{dr_i}{dt} = f_i(p_{i-1}) - \delta r_i$$

$$\frac{dp_i}{dt} = r_i - \delta p_i$$

Criterion employed in
Elowitz & Liebler Nature 2000

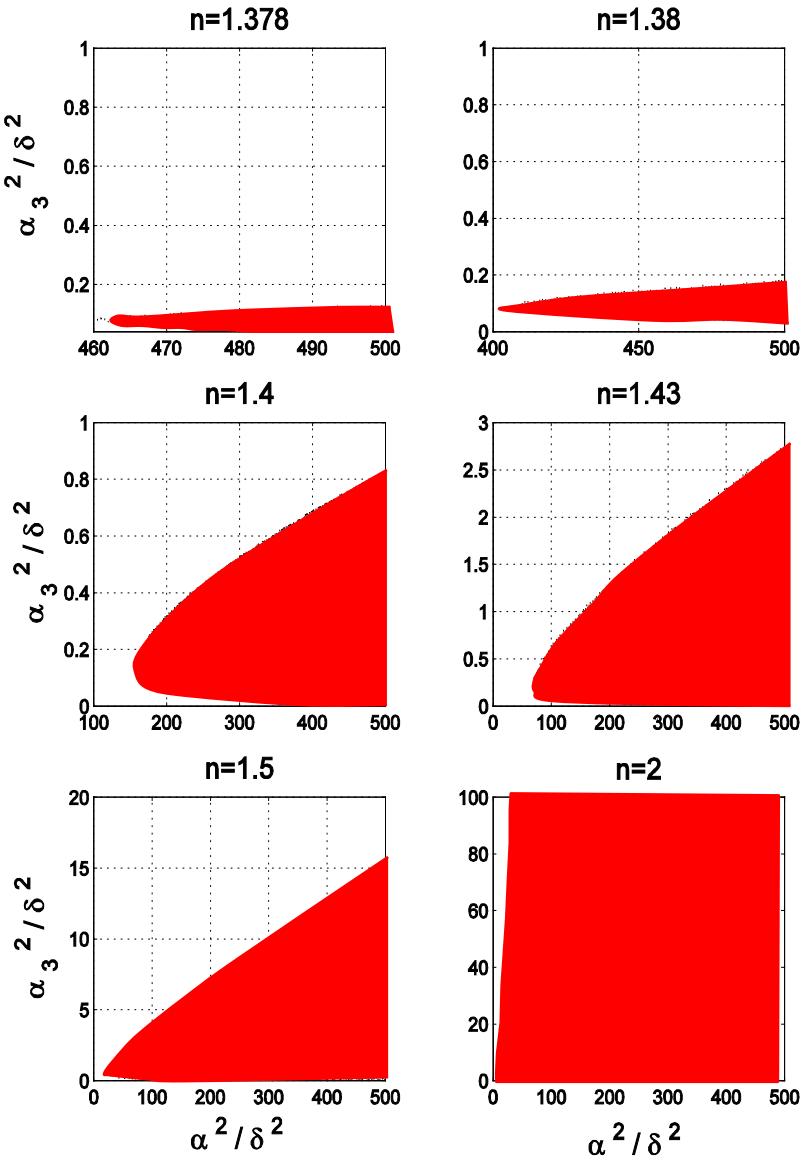
LacI, tetR, and lambdaCI all bind cooperatively to their target promoters: n~2



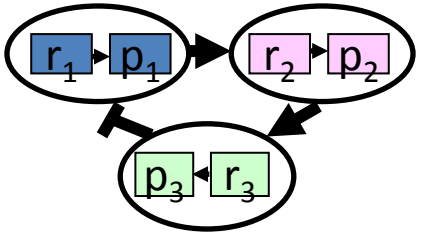


El Samad, Del Vecchio and Khammash, 2004

Repressilators design



Asymmetric design



$$\frac{dr_1}{dt} = f_1(p_n) - \delta r_1$$

$$\frac{dp_1}{dt} = r_1 - \delta p_1$$

$$\frac{dr_i}{dt} = f_i(p_{i-1}) - \delta r_i$$

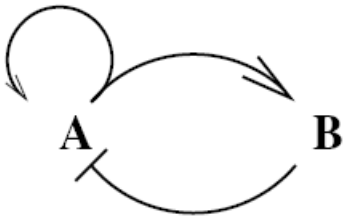
$$\frac{dp_i}{dt} = r_i - \delta p_i$$

$$f_1(p) = \frac{\alpha_3^{\,2}}{1+p^n}$$

$$f_{2,3}(p) = \frac{\alpha^2 p^n}{1+p^n}$$

Again: high cooperativity is a key for obtaining oscillations

Relaxation oscillators: Atkinson et al. clock

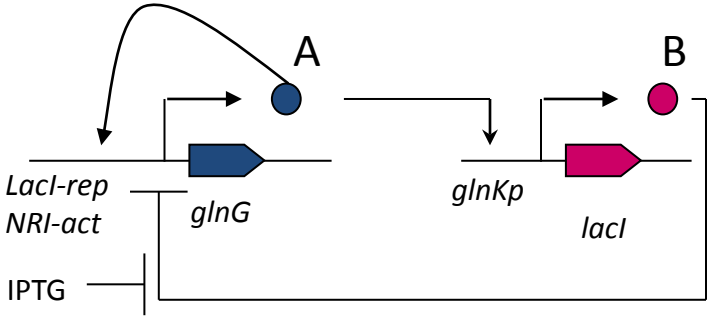


$$\frac{dr_A}{dt} = -\delta_1 r_A + F_1(A,B)$$
$$\frac{dA}{dt} = -\delta_A A + k_1 r_A$$
$$\frac{dr_B}{dt} = -\delta_2 r_B + F_2(A)$$
$$\frac{dB}{dt} = -\delta_B B + k_2 r_B,$$

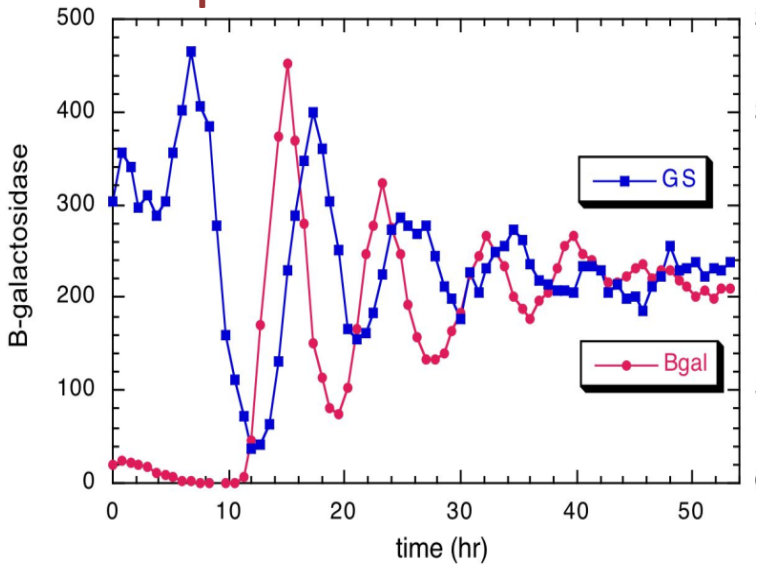
$$F_1(A,B) = \frac{K_1 A^n + K_{A0}}{1 + \gamma_1 A^n + \gamma_2 B^m}$$

$$F_2(A) = \frac{K_2 A^n + K_{B0}}{1 + \gamma_3 A^n}$$

Combinatorial promoter:
Promoter with two inputs



Experimental data



(Courtesy of Ninfa Lab at Umich)

(Cell population measurements)

2D Analysis and design

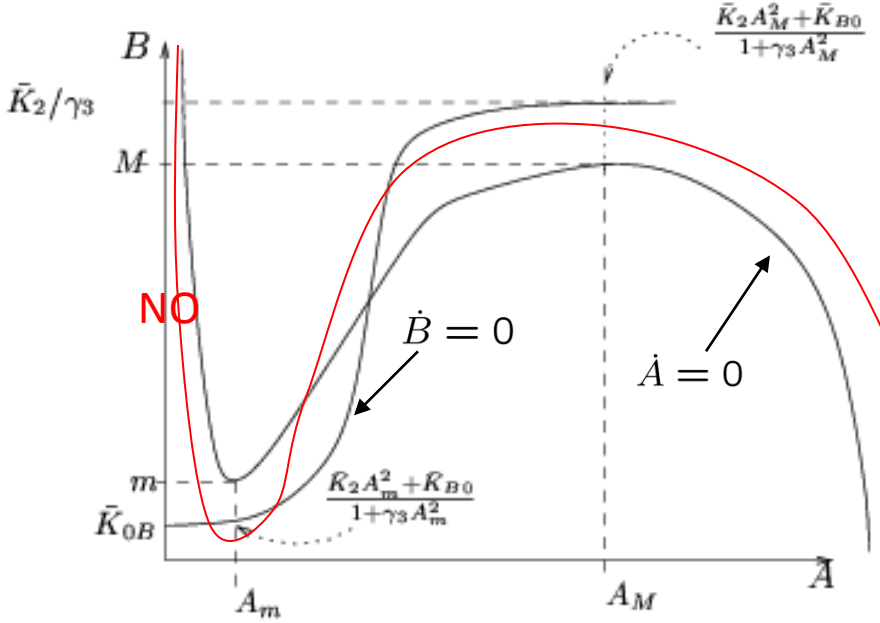
mRNA dynamics is 10 times faster than protein dynamics→ QSS approximation

$$\frac{dA}{dt} = -\delta_A A + f_1(A,B) \qquad f_1(A,B) = \frac{k_1}{\delta_1} F_1(A,B)$$

$$\frac{dB}{dt} = -\delta_B B + f_2(A), \qquad f_2(A) = \frac{k_2}{\delta_2} F_2(A)$$

The dynamics are bounded because the transcription functions are bounded.

There is one equilibrium point only if the nullclines intersect in one point only



$$\bar{K}_{A0} \ll \frac{\bar{K}_1^3}{27\gamma_1^2}$$

$$\bar{K}_{A0} \ll \frac{\bar{K}_1}{\gamma_1}$$

$$\frac{\bar{K}_1}{2\sqrt{\gamma_1\gamma_2}} \ll \frac{\bar{K}_2}{\gamma_3}$$

$$\sqrt{\frac{\bar{K}_{A0}}{A_m\gamma_2}} \geq \bar{K}_{B0}$$

The equilibrium is unstable and not a saddle if

$$\delta_A \gg \delta_B$$
$$n \geq 2,\ m \geq 1$$

Oscillator design requirements

By Poincarè-Bendixson theorem, since the system has one unstable equilibrium that is not a saddle and its trajectories are bounded in an invariant set, the omega-limit set is a periodic orbit

$$\frac{\bar{K}_1}{2\sqrt{\gamma_1\gamma_2}} \ll \frac{\bar{K}_2}{\gamma_3}$$

$$\sqrt{\frac{\bar{K}_{A0}}{A_m\gamma_2}} \geq \bar{K}_{B0}$$

→ Promoter of the repressor must be tight (not leaky)

Promoter of the activator must be leaky

The promoter controlling the repressor B must be very strong Compared to that controlling the activator

$$\delta_A \gg \delta_B$$
$$n \geq 2,\ m \geq 1$$

→ Half life of the activator much smaller than the one of repressor (safe choice): can add degradation tags to the activator

Cooperativity of the activator at least 2

4D analysis and design

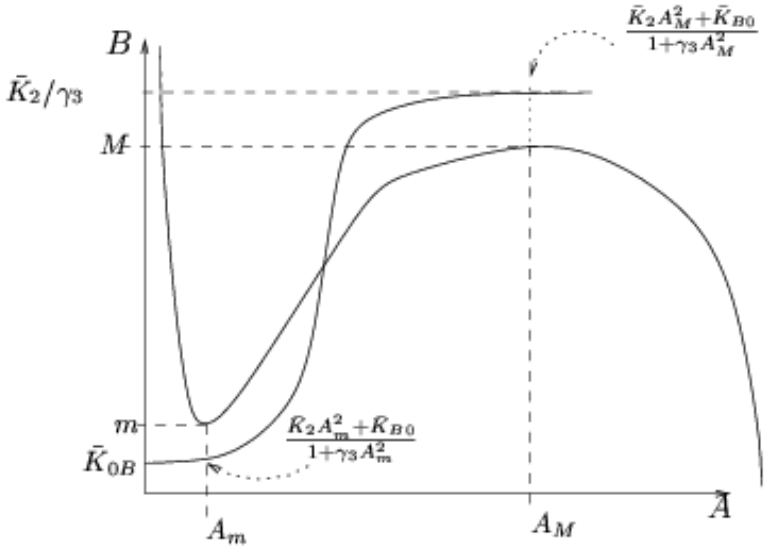
$$\frac{dr_A}{dt} = -\frac{\delta_1}{\epsilon} r_A + F_1(A,B)$$
$$\frac{dA}{dt} = \nu\left(-\delta_A A + \frac{k_1}{\epsilon} r_A\right)$$
$$\frac{dr_B}{dt} = -\frac{\delta_2}{\epsilon} r_B + F_2(A)$$
$$\frac{dB}{dt} = -\delta_B B + \frac{k_2}{\epsilon} r_B,$$

Study the behavior as these two parameters are varied

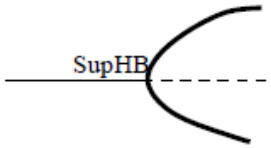
$$F_1(A,B) = \frac{K_1 A^n + K_{A0}}{1 + \gamma_1 A^n + \gamma_2 B^m}$$

$$F_2(A) = \frac{K_2 A^n + K_{B0}}{1 + \gamma_3 A^n}.$$

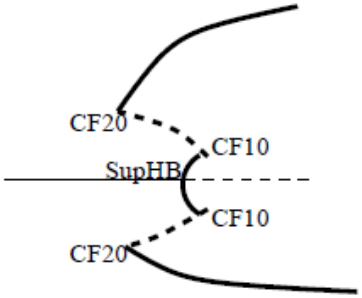
Choose K_i, k_i, δ_i, δ_A, and δ_B such that there is only one equilibrium point



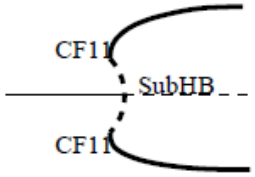
Perform bifurcation analysis



$1/\epsilon \in (100, 1000]$



$1/\epsilon \in [20, 100]$

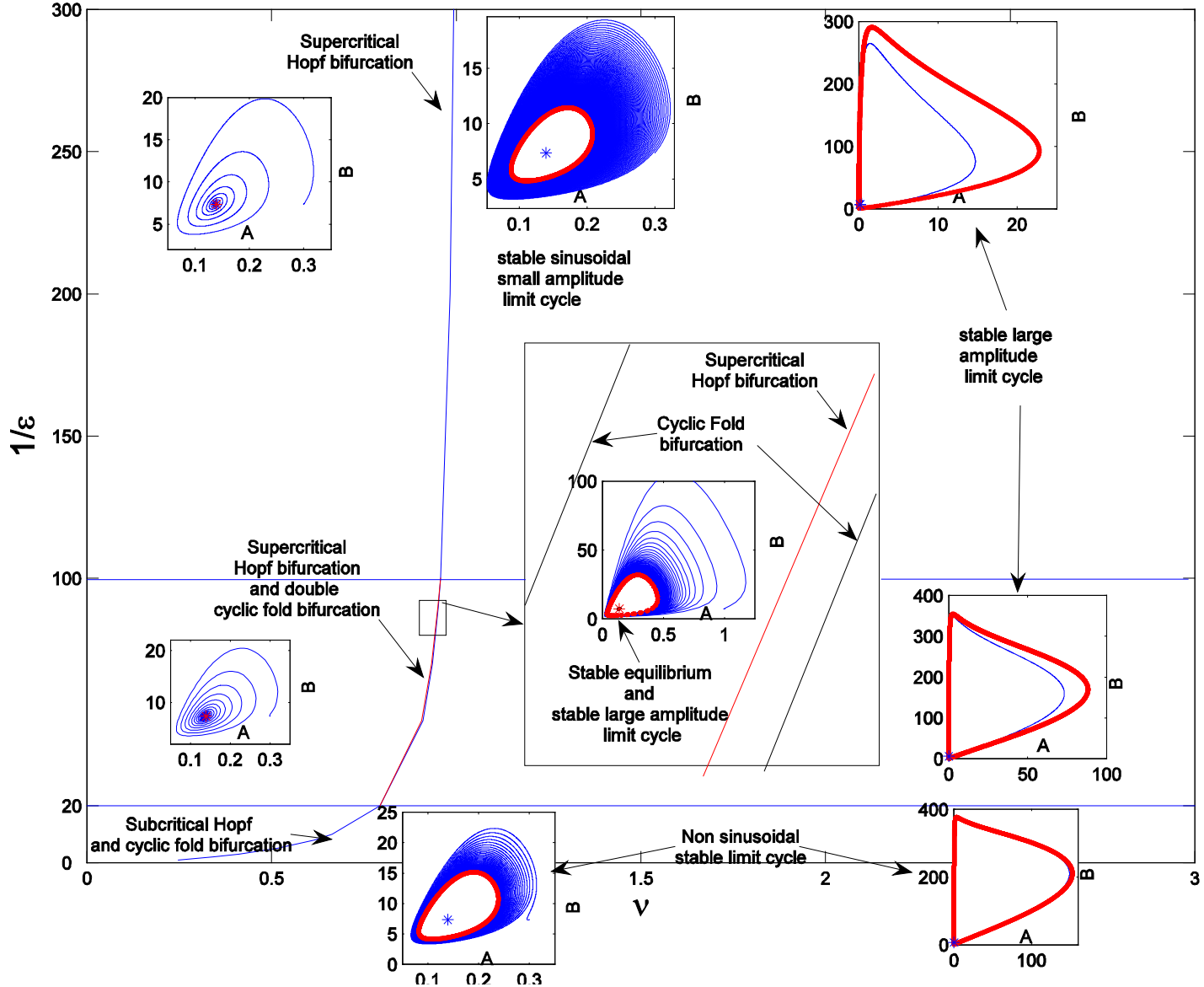


$1/\epsilon \in [1, 20)$

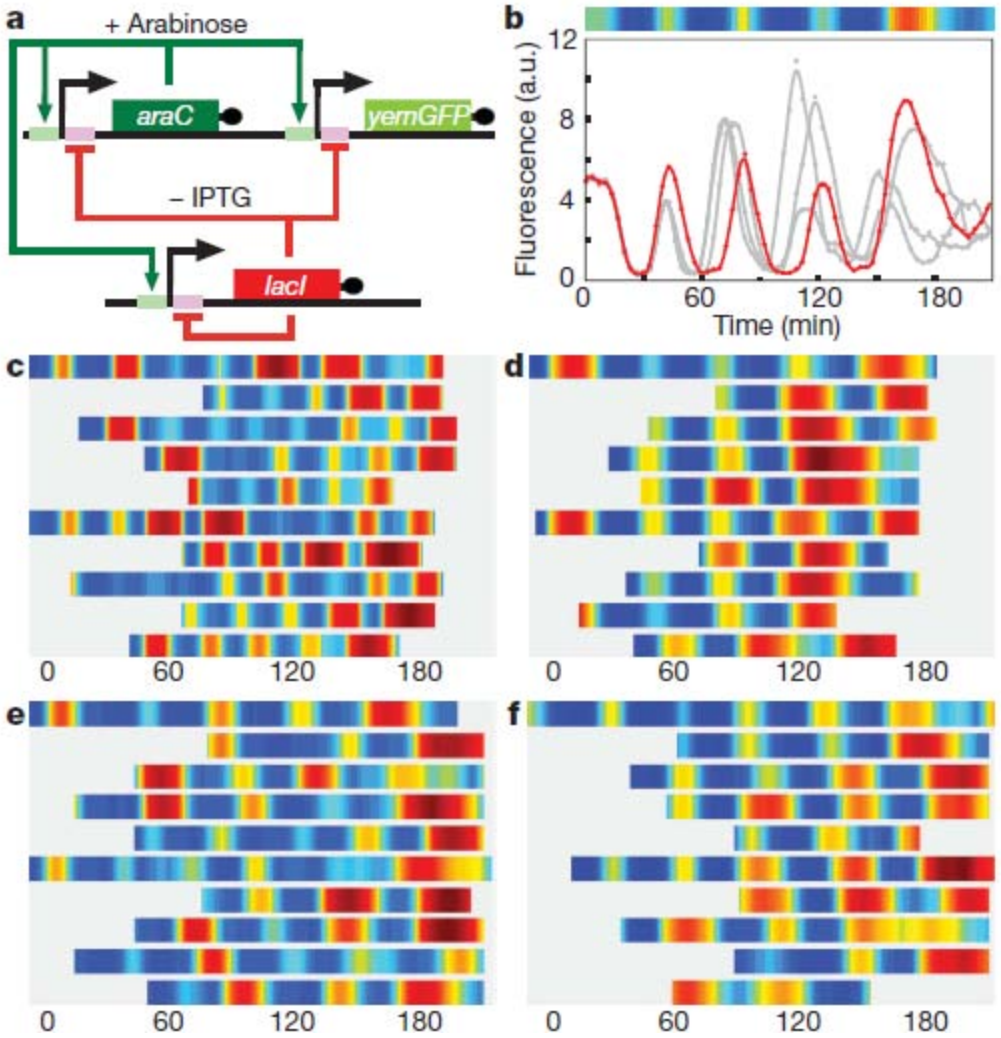
Bifurcation Analysis

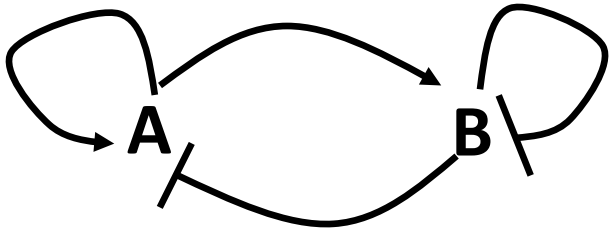
$$\begin{aligned}\dot{r}_A &= -\delta_1/\epsilon\, r_A + F_1(A,B)\\ \dot{A} &= \nu(-\delta_A A + k_1/\epsilon\, r_A)\\ \dot{r}_B &= -\delta_2/\epsilon\, r_B + F_2(A)\\ \dot{B} &= -\delta_B B + k_2/\epsilon\, r_B,\end{aligned}$$

Hopf bifurcation and saddle node bifurcation (cyclic fold) of the periodic orbit as ν and ε change



A robust Oscillator





Source of robustness: delay in the Negative feedback loops deriving From processes involved in the Formation of protein

Positive feedback loop: confers Robustness and tunability (the period can be tuned by changing the amounts Of inducers

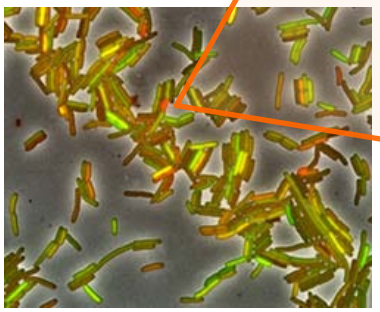
Stricker et al. Nature 2008

Part 3

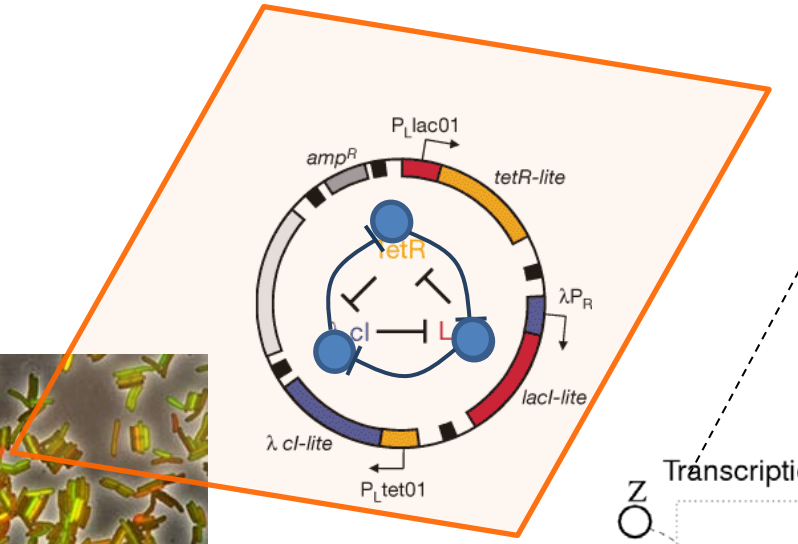
The challenge of composing modules together

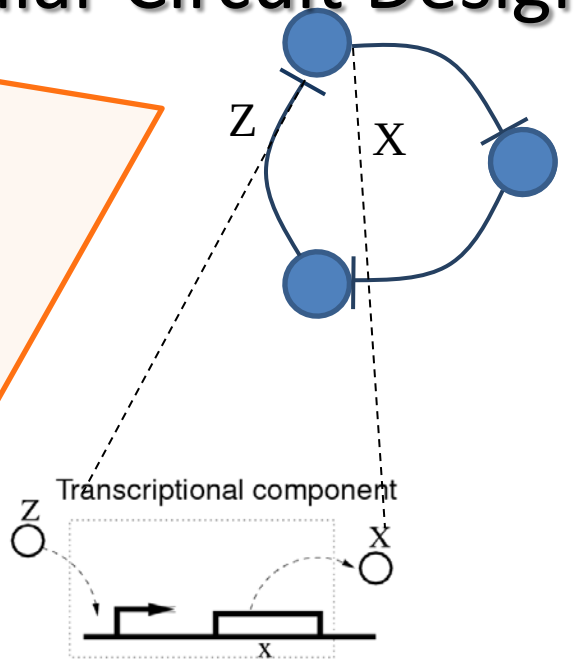
- **Retroactivity phenomenon and its modeling**
- **Insulation devices**
- **Implementation examples**

Synthetic Biology: Enabling Technology for Bio-molecular Circuit Design



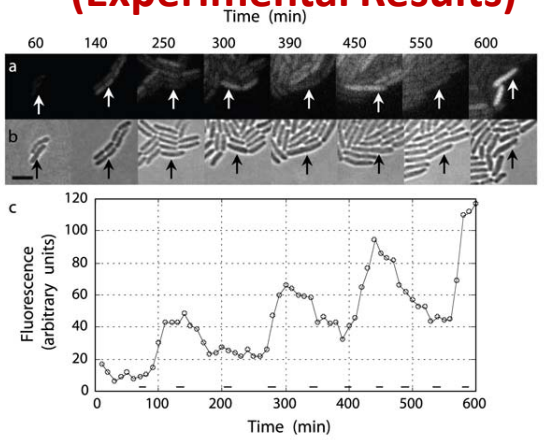
Courtesy of Elowitz Lab



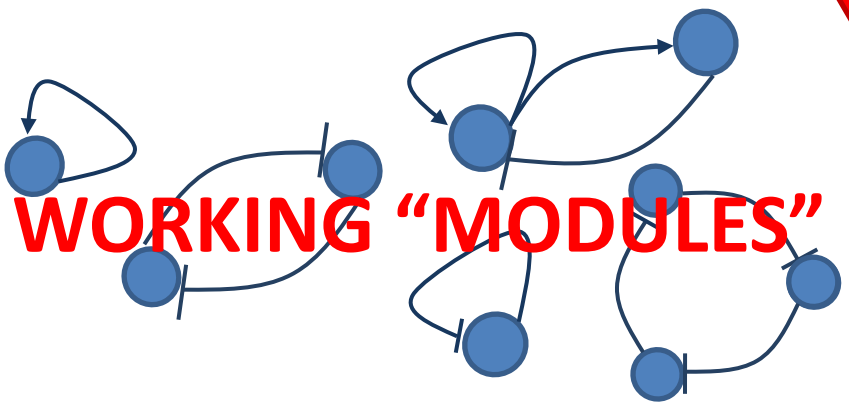


Repressilator

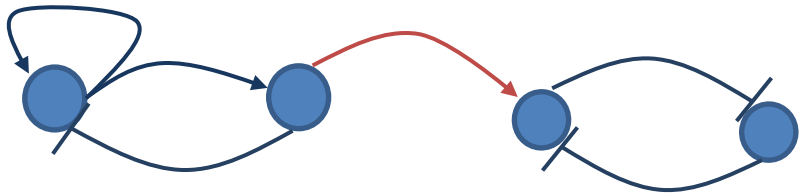
(Experimental Results)



(Elowitz and Leibler, *Nature* 2000)

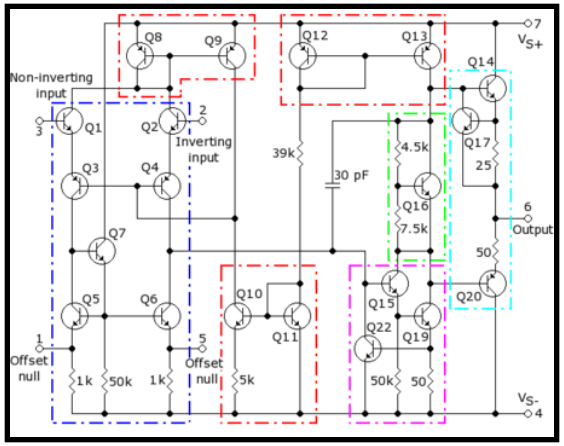


WORKING "MODULES"



NOT WORKING INTERCONNECTIONS !

Modularity: A Fundamental Property

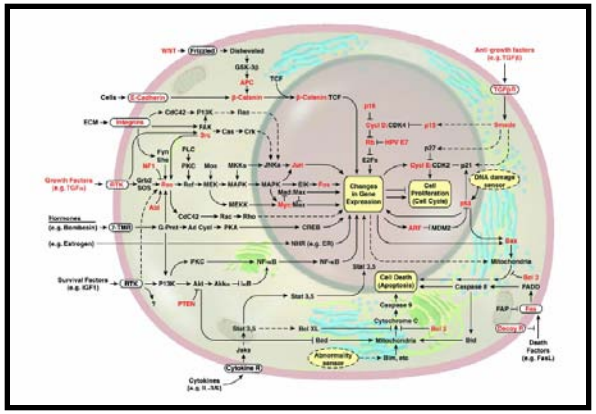


Internal circuitry of an OPAMP:
It is composed of well defined modules

Modularity guarantees that the input/output behavior of a component (a module) does not change upon interconnection.

Electronics and Control Systems Engineering rely on modularity to predict the behavior of a complex network by the behavior of the composing subsystems.

Result: Computers, Videos, cell phones…



The Emergent integrated circuit of the cell
[Hanahan & Weinberg (2000)]

Functional modules seem to recur also in biological networks (e.g. Alon (2007)). But…

But can they be interconnected and still maintain their behavior unchanged?

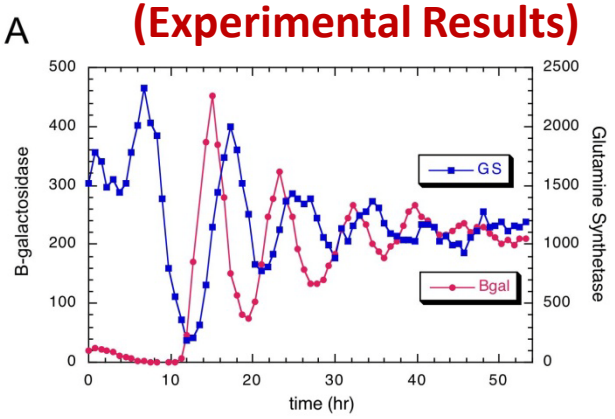
If not, what mechanism can be used to interconnect modules without altering their behavior?

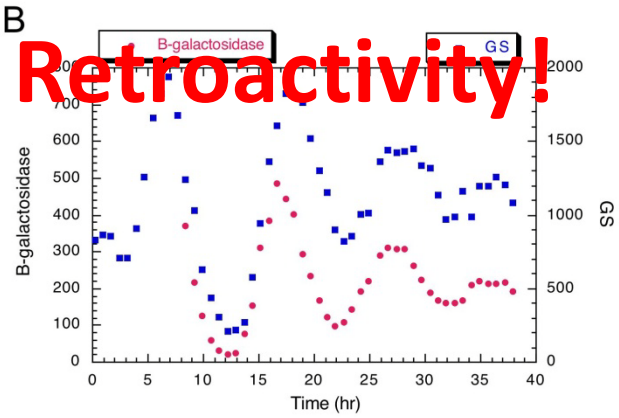
Does nature already employ such mechanisms?

Modularity is **not** a Natural Property of Bio-molecular Circuits

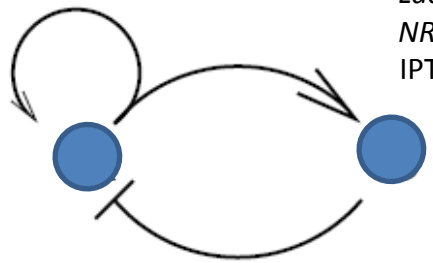
Activator/Repressor Clock

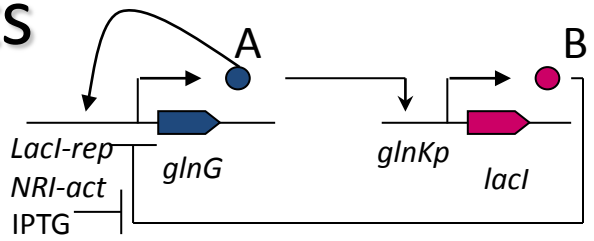
(Experimental Results)





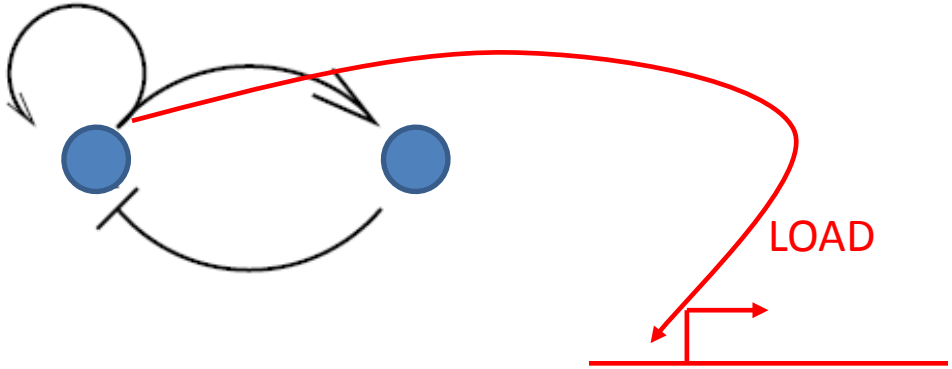
Courtesy of Ninfa Lab at Umich





(Atkinson et al, *Cell* 2003)

Retroactivity!



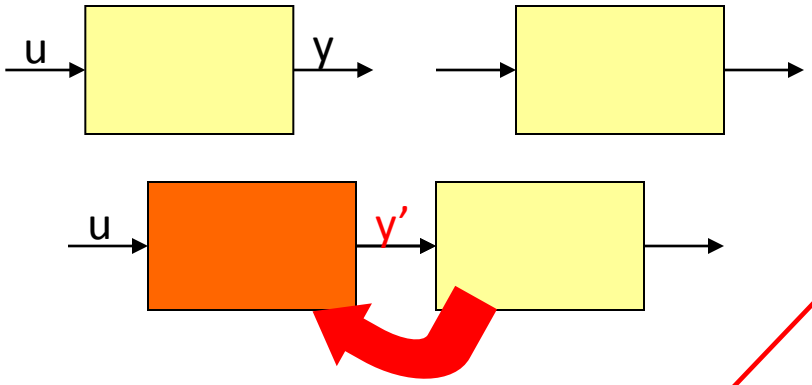
How do we model these effects? How do we prevent them?

Outline

- A modeling framework for systems with retroactivity
- Retroactivity in transcriptional networks
- A lesson from OPAMPs: Insulation devices
- Design of a bio-molecular insulation device based on protease-feedback
- Fast time-scales as a key mechanism for insulation: phosphorylation
- Compromise between retroactivity attenuation and noise

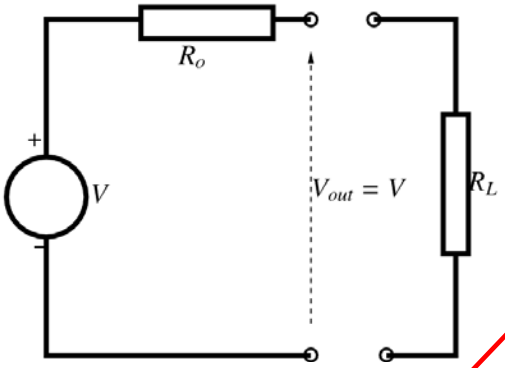
A systems theory with retroactivity

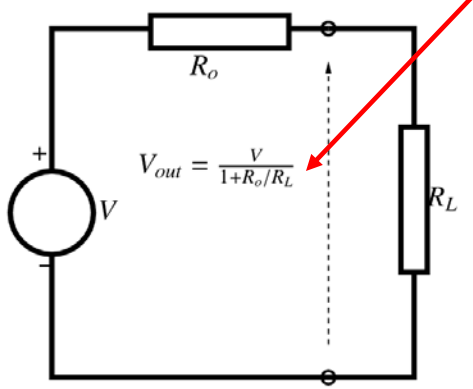
Basic Idea:

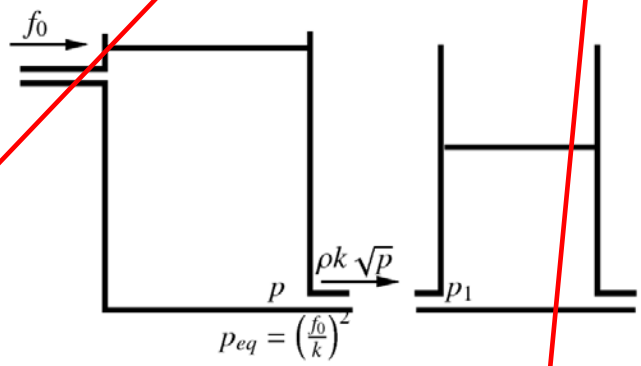


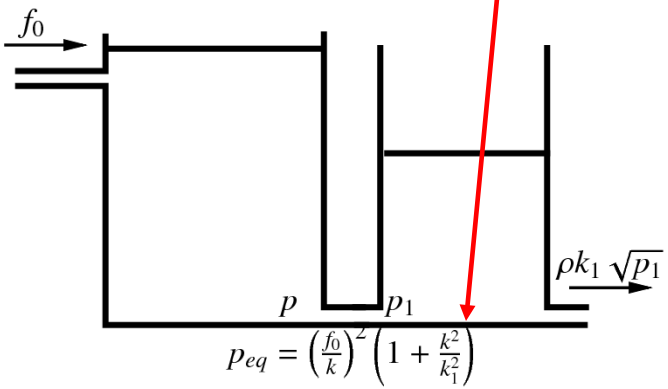
The interconnection changes the behavior of the upstream system

Familiar Examples:

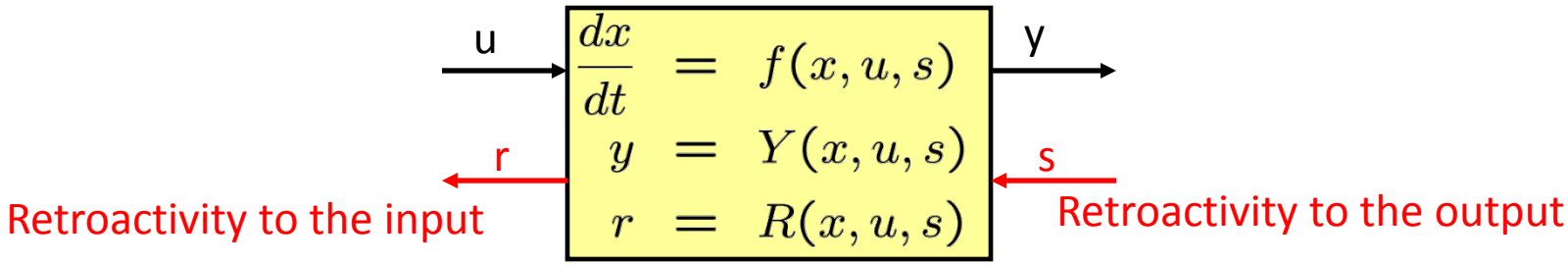






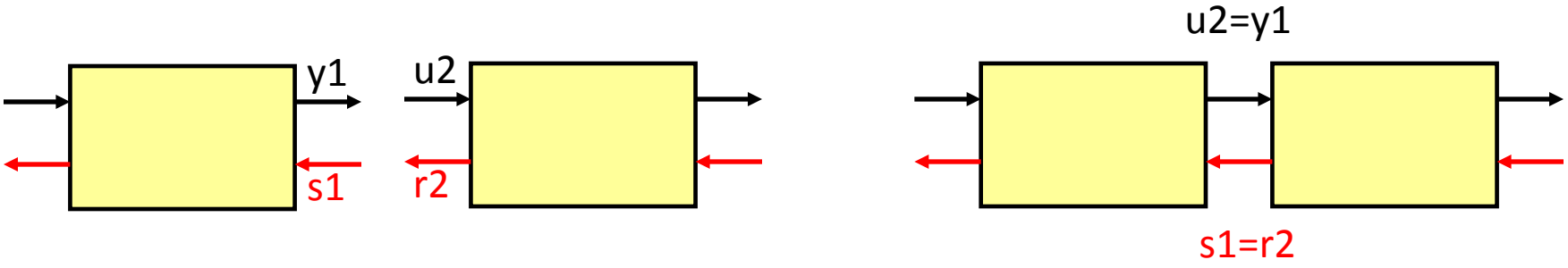


A systems theory with retroactivity



Def: The I/O model of the **isolated system** is obtained when s=0 and when r is not an additional output

The interconnection of two systems is possible only when the internal state variable sets are disjoint:



Outline

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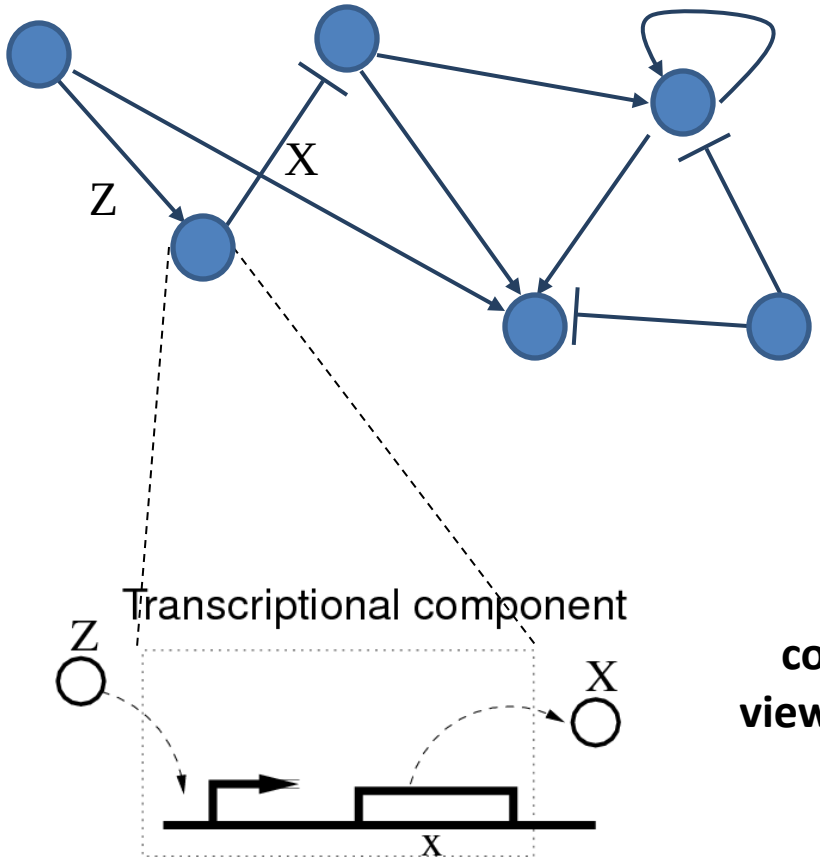
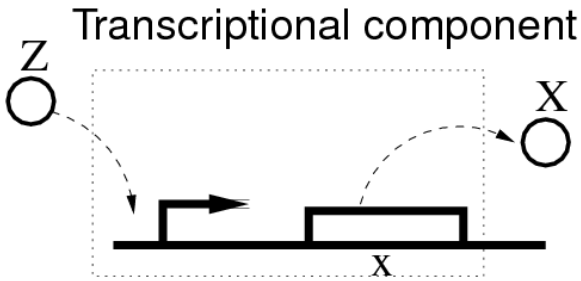
Gene regulatory circuitry: A network of transcriptional modules



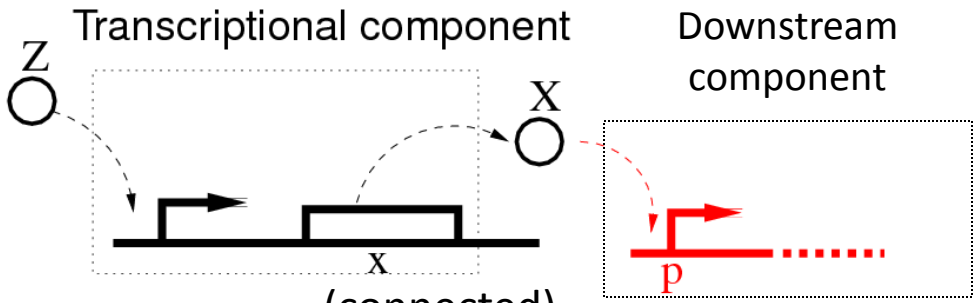
A transcriptional component is typically viewed as an input/output module

But, is its input/output response unchanged upon interconnection?

Retroactivity in transcriptional networks has dramatic effects on the dynamics



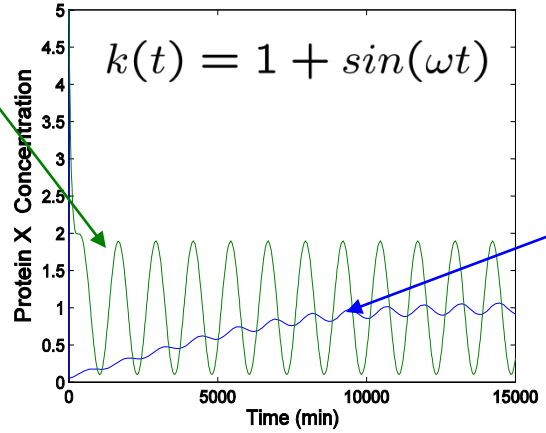
(isolated)



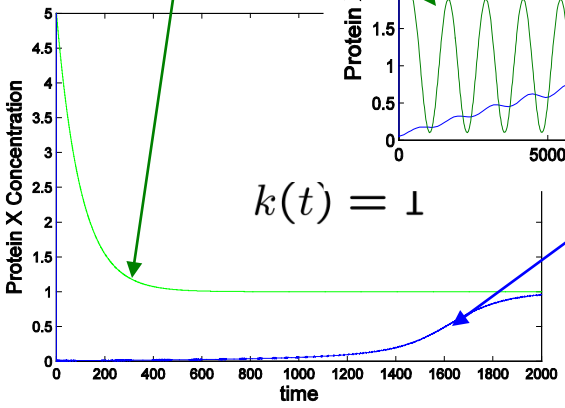
(connected)

$$\frac{dX}{dt} = k(t) - \delta X$$

$$k(t) = 1 + sin(\omega t)$$



$$k(t) = \perp$$



$$X + p \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} C,$$

$$p + C = p_{TOT}$$

$$\frac{dX}{dt} = k(t) - \delta X + \overbrace{k_{off}C - k_{on}(p_{TOT} - C)X}^{s}$$

$$\frac{dC}{dt} = -k_{off}C + k_{on}(p_{TOT} - C)X,$$

Measure of the retroactivity

We seek to quantify the difference in the dynamics of the state X between the connected and isolated system

Isolated system (1D)

$$\frac{dX}{dt} = k(t) - \delta X$$

Connected system (2D)

$$\begin{aligned}\frac{dX}{dt} &= k(t) - \delta X + \overbrace{k_{off}C - k_{on}(p_{TOT} - C)X}^{s} \\ \frac{dC}{dt} &= -k_{off}C + k_{on}(p_{TOT} - C)X,\end{aligned}$$

To compare the X dynamics we seek a 1D approximation for the connected system:

$$\frac{d\bar{X}}{dt} = k(t) - \delta\bar{X} + \bar{s}$$

Measure of retroactivity will be given by $\bar{s}$

Calculation of $\bar{s}$

We exploit the time-scale separation between the output X dynamics and the dynamics of the input stage of the downstream component

$$\frac{dX}{dt} = k(t) - \delta X + k_{off}C - k_{on}(p_{TOT} - C)X$$
$$\frac{dC}{dt} = -k_{off}C + k_{on}(p_{TOT} - C)X,$$

$k_{off} \gg \delta$ and $k_{on} = k_{off}/k_d$

$$\epsilon = \delta/k_{off} \qquad y = X + C$$

$$\frac{dy}{dt} = k(t) - \delta(y - C)$$
$$\epsilon\frac{dC}{dt} = -\delta C + \frac{\delta}{k_d}(p_{TOT} - C)(y - C),$$

$\epsilon = 0 \Rightarrow C = \gamma(y)$ (slow manifold)

$$\frac{d\bar{X}}{dt} = k(t) - \delta\bar{X} - (k(t) - \delta\bar{X})\frac{d\gamma(\bar{y})}{d\bar{y}}$$

$\bar{s}$

$$\bar{s} = 0 \Leftrightarrow \frac{d\gamma(\bar{y})}{d\bar{y}} = 0 \longrightarrow$$ we take $\frac{d\gamma(\bar{y})}{d\bar{y}}$ as a measure of retroactivity

Meaning and value of $\frac{d\gamma(\bar{y})}{d\bar{y}}$

Isolated system dynamics: $\frac{dX}{dt} = f_I(X,t),\ f_I(X,t) = k(t) - \delta X$

Approximate connected system dynamics:

$$\frac{dX}{dt} = f_C(X,t),\ f_C(X,t) = k(t) - \delta X - (k(t) - \delta X)\frac{d\gamma(y)}{dy}$$

$$\frac{d\gamma(y)}{dy} = \frac{|f_I(X,t) - f_C(X,t)|}{|f_I(X,t)|}$$

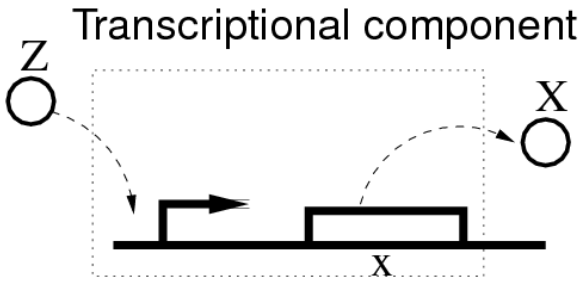
percentage difference between the isolated system dynamics and the approximate connected system dynamics

$$\frac{d\gamma(y)}{dy} = \frac{1}{1 + \frac{(1+X/k_d)^2}{p_{TOT}/k_d}} =: \mathcal{R}(X)$$

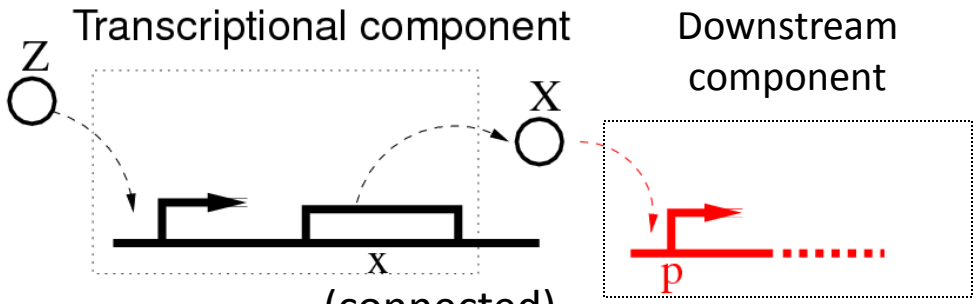
The value of the retroactivity measure for the interconnection through transcriptional regulation

Del Vecchio, Ninfa, and Sontag, Nature/EMBO-MSB 2008

Effect of R(X) on the dynamics



(isolated)



(connected)

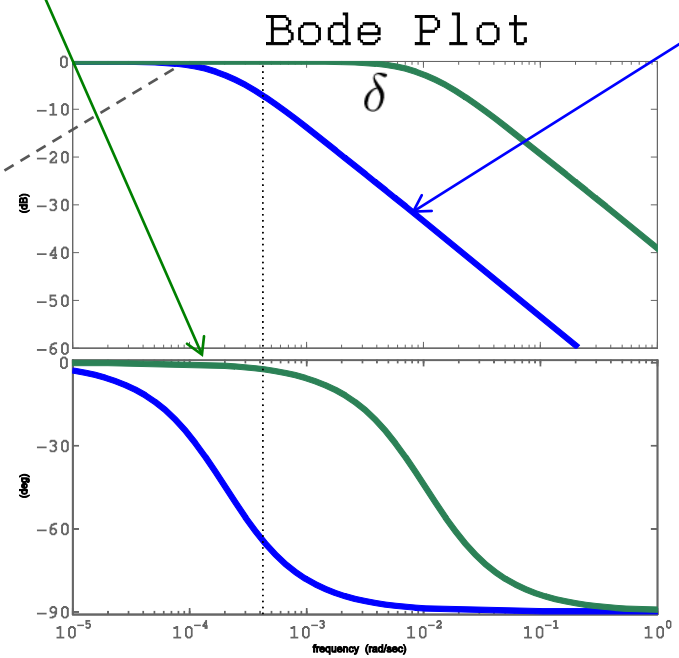
$$\frac{dX}{dt} = k(t) - \delta X$$

$$\frac{dX}{dt} = (k(t) - \delta X)\,(1 - \mathcal{R}(X))$$

$$\frac{\delta}{1 + R_l}$$

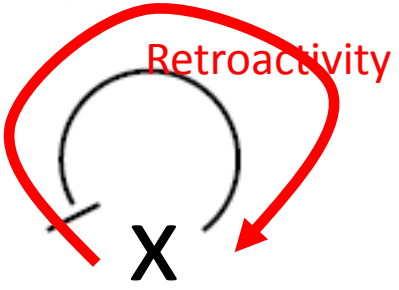
$$R_l = \frac{k_d\, p_{TOT}}{(\bar{k}/\delta + k_d)^2}$$

$$k(t) = \bar{k} + A_0 \sin(\omega t)$$

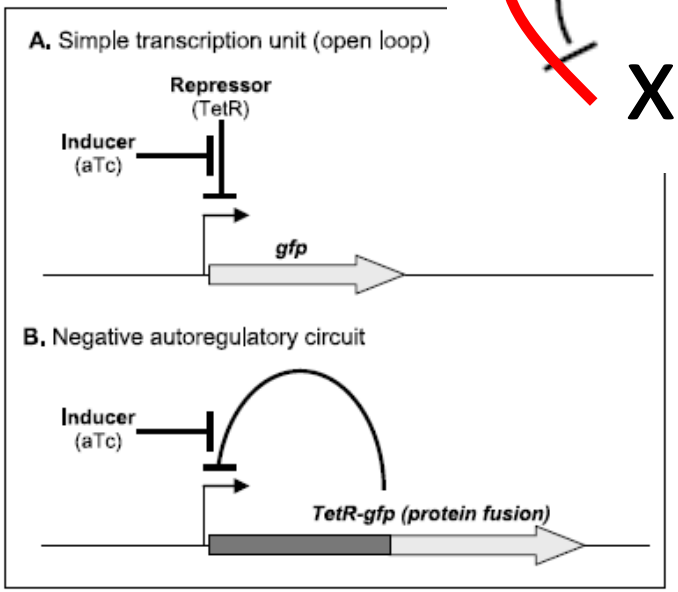


Retroactivity shifts the poles of the transfer function of the linearized system toward low frequency

Does negative feedback always speeds up the response time?



$$\begin{aligned}\frac{dm_X}{dt} &= u(t) - \delta_1 m_X \\ \frac{dX}{dt} &= k m_X - \delta_2 X,\end{aligned}$$



$$\begin{aligned}\frac{dm_X}{dt} &= k_1(p_{TOT} - C) - \delta_1 m_X \\ \frac{dX}{dt} &= k m_X - \delta_2 X - k_{on}(p_{TOT} - C)X + k_{off} C, \\ \frac{dC}{dt} &= k_{on}(p_{TOT} - C)X - k_{off} C.\end{aligned}$$

Open loop system linearization matrix

$$F = \begin{bmatrix} -\delta_1 & 0 \\ k & -\delta_2 \end{bmatrix} \qquad \bar{F} = \begin{bmatrix} -\delta_1 & -k_1 \frac{\bar{p}}{k_D + X} \\ k & -\delta_2 \end{bmatrix} \Delta$$

$$\Delta = \mathrm{diag}\left(1, \frac{1}{1 + \bar{p}/(k_D + X)}\right)$$

$$\frac{k k_1}{\delta_2(\delta_1 - \delta_2)} < 1 \quad \Rightarrow \quad \lambda_{MAX}(F) < \lambda_{MAX}(\bar{F})$$

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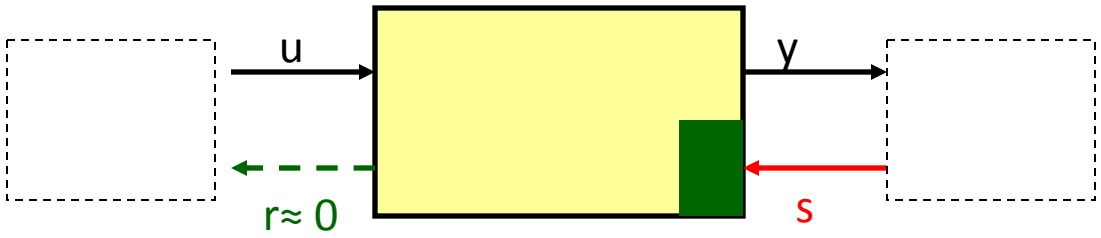
- Compromise between retroactivity attenuation and noise

Dealing with retroactivity: Insulation devices

In general, we cannot design the downstream system (the load) such that it has low retroactivity. But, we can design an insulation system to be placed between the upstream and downstream systems.



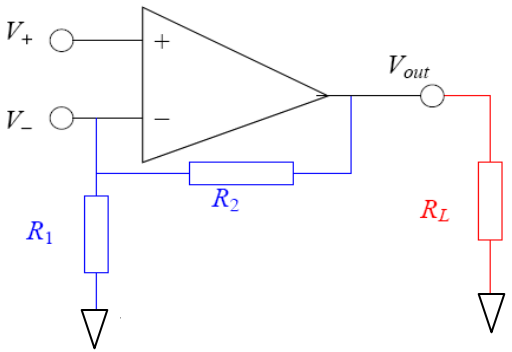
1. The retroactivity to the input is approx zero: r≈0

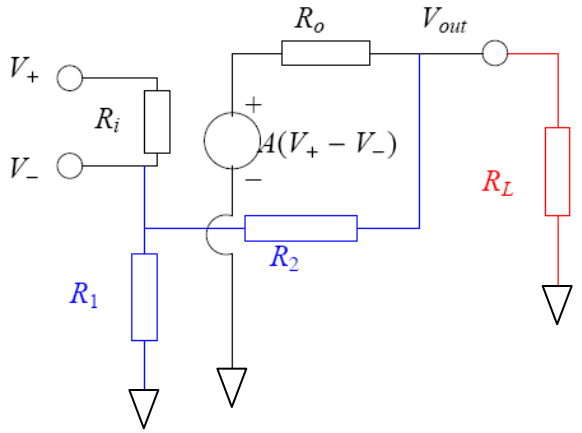
2. The retroactivity to the output s is attenuated

3. The output is proportional to the input: y=c u

Reaching small retroactivity to the input r

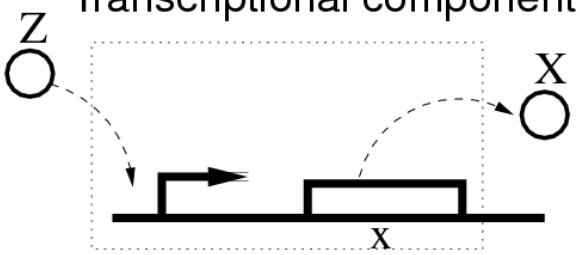
Non-inverting amplifier:





$R_i = \infty$ because the input stage of an OPAMP absorbs almost zero current

Transcriptional component



Choose the biochemical parameters of the input stage to allow a small value of $\mathcal{R}(Z)$

For example:

$$\mathcal{R}(Z) = \frac{1}{1 + \frac{(1+Z/k_d)^2}{p_{TOT}/k_d}}$$

Dealing with retroactivity: Insulation devices

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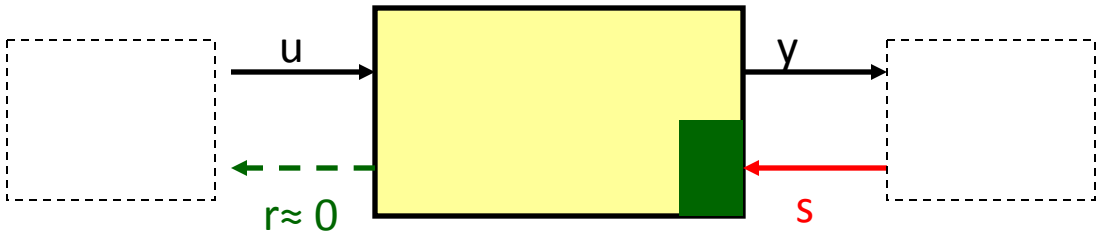


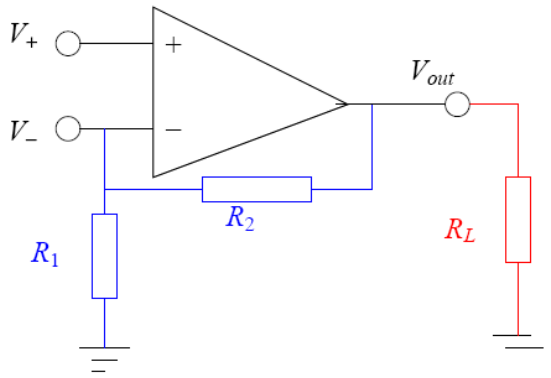
1. The retroactivity to the input is approx zero: r≈0

2. The retroactivity to the output s is attenuated

3. The output is proportional to the input: y=c u

Attenuation of the retroactivity to the output "s": Large feedback and large amplification

Non-inverting amplifier:



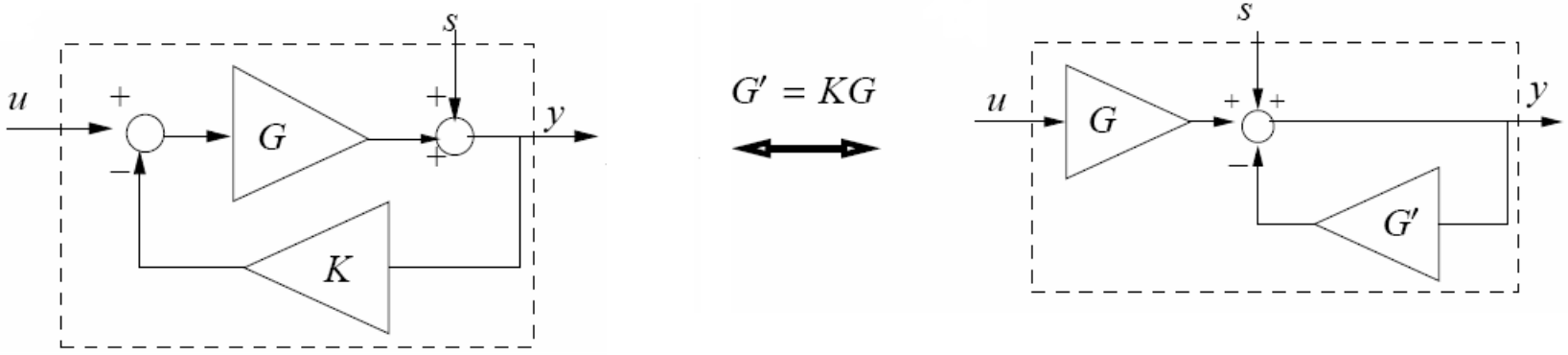
$$V_{out} = G(V_+ - V_-)$$

For G large enough:

$$V_{out} = \frac{V_+}{K},\ K = \frac{R_1}{R_1 + R_2}$$

Conceptually:

$$y = G(u - Ky) + s \ \Rightarrow\ y = u\frac{G}{1+KG} + \frac{s}{1+KG}$$



Attenuation of the retroactivity to the output "s" in the transcriptional component

Connected system approximated dynamics

$$\frac{d\bar{X}}{dt} = (k(t) - \delta\bar{X})(1 - \frac{d\gamma(\bar{y})}{d\bar{y}})$$

Isolated system

$$\frac{dX}{dt} = k(t) - \delta X$$

Apply large input amplification G and large output feedback G'

$$\frac{d\bar{X}}{dt} = (Gk(t) - G'\bar{X} - \delta\bar{X})(1 - \frac{d\gamma(\bar{y})}{d\bar{y}})$$

$$\frac{dX}{dt} = Gk(t) - G'X - \delta X$$

Lemma. Consider the system

$$\frac{dX}{dt} = G(t)(u(t) - KX)$$

in which $G(t) \geq G_0 > 0$ and $|u'(t)| \leq V$ uniformly in t. Then,

$$|X(t) - \frac{u(t)}{K}| \leq \exp(-tG_0K)|X(0) - \frac{u(0)}{K}| + \frac{V}{G_0K^2}.$$

Let $G' = GK$, then as G grows the signals $\bar{X}(t)$ and $X(t)$ become close to each other

How do we realize a large input amplification and a large negative feedback?

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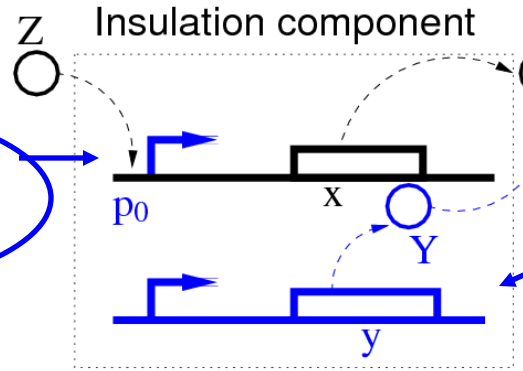
A protease-based design for a biological insulation device



Large feedback through a protease Y for X, constitutively produced by a strong promoter

Large amplification through a strong non-leaky promoter

Protease Y reaction

$$X + Y \underset{\eta_2}{\overset{\eta_1}{\rightleftharpoons}} W \xrightarrow{\beta} Y,$$

$$\begin{aligned}
\frac{dZ}{dt} &= k(t) - \delta Z + k_- Z_{bound} - k_+ Z(p_{0,TOT} - Z_{bound}) \\
\frac{dZ_{bound}}{dt} &= k_+ Z(p_{0,TOT} - Z_{bound}) - k_- Z_{bound} \\
\frac{dm_X}{dt} &= G Z_{bound} - \delta_1 m_X \\
\frac{dX}{dt} &= \nu m_X - \eta_1 Y X + \eta_2 W - \delta_2 X + k_{on} C - k_{off} X(p_{TOT} - C) \\
\frac{dW}{dt} &= \eta_1 X Y - \eta_2 W - \beta W \\
\frac{dY}{dt} &= -\eta_1 Y X + \beta W + \alpha G - \gamma Y + \eta_2 W \\
\frac{dC}{dt} &= -k_{on} C + k_{off} X(p_{TOT} - C),
\end{aligned}$$

r

s

Full ODE Model

Simplified analysis: why should it attenuate "s"?

$$\begin{aligned}
\frac{dZ}{dt} &= k(t) - \delta Z + k_- Z_{bound} - k_+ Z(p_{0,TOT} - Z_{bound}) \\
\frac{dZ_{bound}}{dt} &= k_+ Z(p_{0,TOT} - Z_{bound}) - k_- Z_{bound} \\
\frac{dm_X}{dt} &= G Z_{bound} - \delta_1 m_X
\end{aligned}$$

For a suitable signal $v(t)$ we have $m_X = Gv(t)$

$$\begin{aligned}
\frac{dX}{dt} &= \nu m_X - \eta_1 Y X + \eta_2 W - \delta_2 X + k_{on} C - k_{off} X(p_{TOT} - C) \\
\frac{dW}{dt} &= \eta_1 XY - \eta_2 W - \beta W \\
\frac{dY}{dt} &= -\eta_1 YX + \beta W + \alpha G - \gamma Y + \eta_2 W \\
\frac{dC}{dt} &= -k_{on} C + k_{off} X(p_{TOT} - C),
\end{aligned}$$

Simplify to a one-step reaction model

$X + Y \xrightarrow{\beta} Y$

and assuming Y at its equilibrium $\alpha G/\gamma$

$$\begin{aligned}
\frac{dX}{dt} &= \nu G v(t) - \beta(\alpha G/\gamma) X - \delta_2 X + k_{on} C - k_{off} X(p_{TOT} - C) \\
\frac{dC}{dt} &= -k_{on} C + k_{off} X(p_{TOT} - C),
\end{aligned}$$

Applying singular perturbation, we obtain the dynamics after a fast transient as

$$\frac{d\bar{X}}{dt} = (\nu G v(t) - (G' + \delta_2)\bar{X})(1 - \frac{d\gamma(\bar{y})}{d\bar{y}}),$$

with $G' = KG$ and $K = \beta\alpha/\gamma$

As G and G' grow, $\bar{X}(t)$ becomes closer to $X(t)$ generated by the isolated system

The input stage of the insulation device can be designed so as to have small "r"

$$\frac{dZ}{dt} = k(t) - \delta Z + k_- Z_p - k_+ Z(p_{0,TOT} - Z_p) \longrightarrow \text{r}$$

$$\frac{dZ_p}{dt} = k_+ Z(p_{0,TOT} - Z_p) - k_- Z_p$$

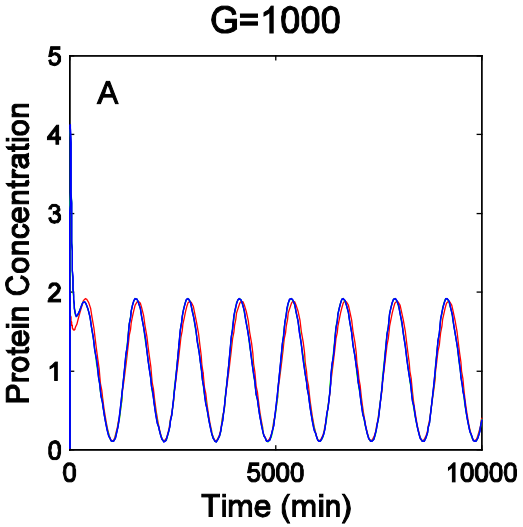
Applying singular perturbation we obtain the quasi steady state dynamics as

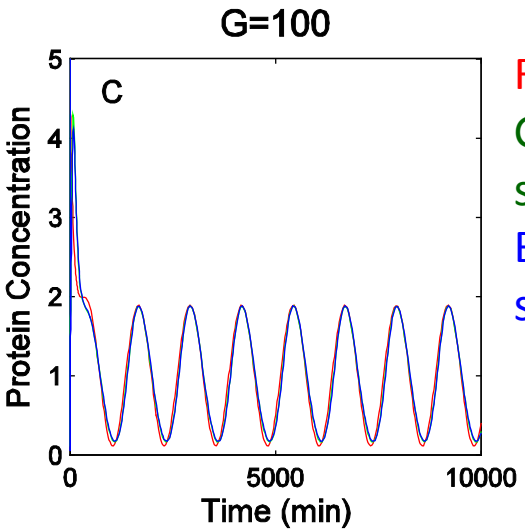
$$\frac{d\bar{Z}}{dt} = (k(t) - \delta\bar{Z})(1 - \mathcal{R}(\bar{Z})) \qquad \mathcal{R}(\bar{Z}) = \frac{1}{1 + \frac{(1+\bar{Z}/\bar{k}_d)^2}{p_{0,TOT}/\bar{k}_d}} \quad \bar{k}_d = k_-/k_+$$

choose $p_{0,TOT}/\bar{k}_d \ll 1$ to make $\mathcal{R}(\bar{Z})$ smaller

If $\bar{Z} \ll \bar{k}_d$, then the $\bar{Z}$ to X relationship is about linear

Simulation results for the full system

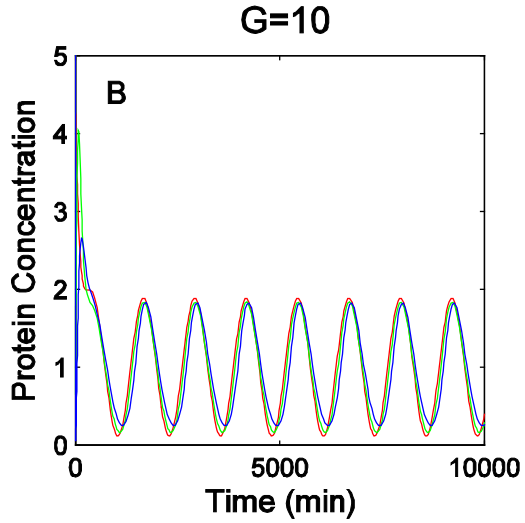


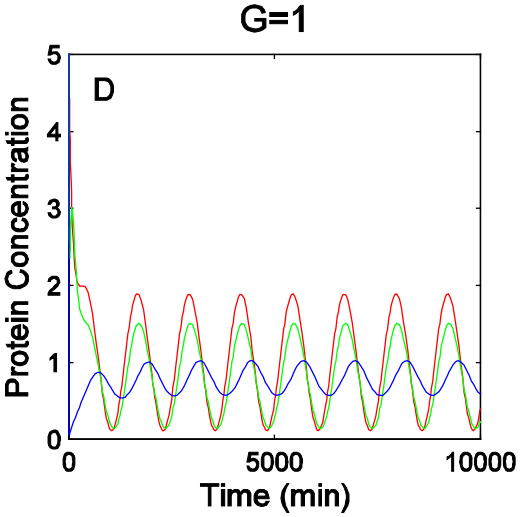


Red = Z

Green = X for the isolated system

Blue= X for the connected system





protein half life $= 0.01 min^{-1}$

clock period = 12 times protein half life

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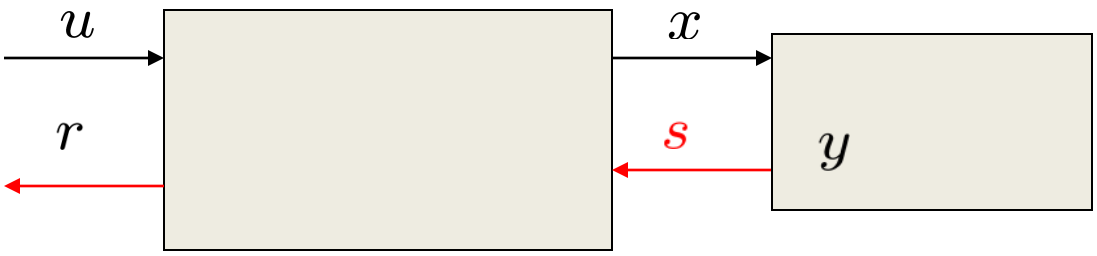
Fast time scales: A key mechanism for insulation

Basic Idea:



$$\frac{du}{dt} = f_0(t,u) + r(u,x)$$

$$\frac{dx}{dt} = Gf_1(u,x) + Gs(x,y)$$

$$\frac{dy}{dt} = -Gs(x,y)$$

Large

Interconnection
through binding/unbinding
(possibly large)

Claim: if G is large enough, signal x at the QSS is not affected by y.

Fast time scales: A key mechanism for insulation

Why would it work?

$$\frac{du}{dt} = f_0(t,u) + r(u,x)$$

$$\frac{dx}{dt} = Gf_1(u,x) + Gs(x,y)$$

$$\frac{dy}{dt} = -Gs(x,y)$$

$$\tilde{x} := x + y$$

$$\epsilon := 1/G$$

$$\frac{du}{dt} = f_0(t,u) + r(u,x)$$

$$\epsilon\frac{d\tilde{x}}{dt} = f_1(u,x)$$

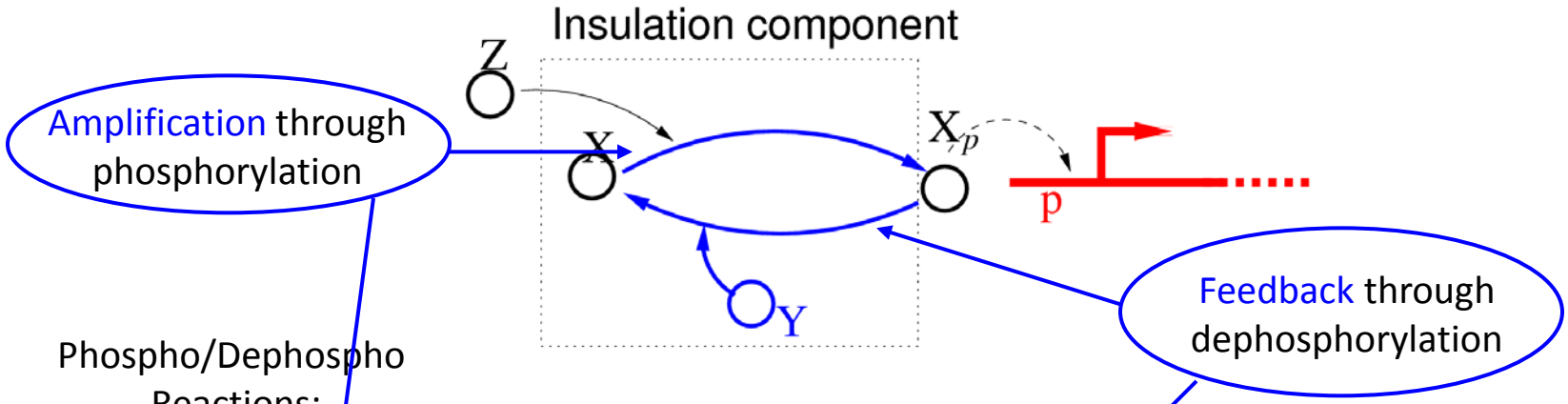
$$\epsilon\frac{dy}{dt} = -s(x,y)$$

If the slow manifold is locally exponentially stable then $x = \gamma(u)$ with $f_1(u,\gamma(u)) = 0$

x(t) does not depend on y on the slow manifold

Del Vecchio and Jayanthi, *CDC* 2008

A phosphorylation-based design for a bio-molecular insulation device



Phospho/Dephospho Reactions:

$$X + Z \underset{\beta_2}{\overset{\beta_1}{\rightleftharpoons}} C_1 \xrightarrow{k_1} X_p + Z, Y + X_p \underset{\alpha_2}{\overset{\alpha_1}{\rightleftharpoons}} C_2 \xrightarrow{k_2} X + Y,\ Y_{TOT} = Y + C_2,\ X_{TOT} = X + X_p + C_1 + C_2 + C$$

$$\begin{aligned}
\frac{dZ}{dt} &= k(t) - \delta Z\left[-\beta_1 Z X_{TOT}\left(1 - \frac{X_p}{X_{TOT}} - \frac{C_1}{X_{TOT}} - \frac{C_2}{X_{TOT}} - \boxed{\frac{C}{X_{TOT}}}\right) + (\beta_2 + k_1)C_1\right] \\
\frac{dC_1}{dt} &= -(\beta_2 + k_1)C_1 + \beta_1 Z X_{TOT}\left(1 - \frac{X_p}{X_{TOT}} - \frac{C_1}{X_{TOT}} - \frac{C_2}{X_{TOT}} - \boxed{\frac{C}{X_{TOT}}}\right) \\
\frac{dC_2}{dt} &= -(k_2 + \alpha_2)C_2 + \alpha_1 Y_{TOT} X_p\left(1 - \frac{C_2}{Y_{TOT}}\right) \\
\frac{dX_p}{dt} &= k_1 C_1 + \alpha_2 C_2 - \alpha_1 Y_{TOT} X_p\left(1 - \frac{C_2}{Y_{TOT}}\right) + \boxed{k_{off} C - k_{on} X_p (p_{TOT} - C)} \\
\frac{dC}{dt} &= -k_{off} C + k_{on} X_p (p_{TOT} - C)
\end{aligned}$$

r

s

Full ODE Model

Full system: The fast time-scale of the device is a key feature for attenuating "s"

Phosphorylation and dephosphorylation reactions are often much faster than protein production and decay:

$$\epsilon = \delta/k_{off},\ k_{on} = k_{off}/k_d,\ b_1 = \beta_1 X_{TOT}\epsilon/\delta,$$
$$a_1 = \alpha_1 Y_{TOT}\epsilon/\delta,\ b_2 = \beta_2\epsilon/\delta,\ a_2 = \alpha_2\epsilon/\delta,\ c_i = \epsilon k_i/\delta$$

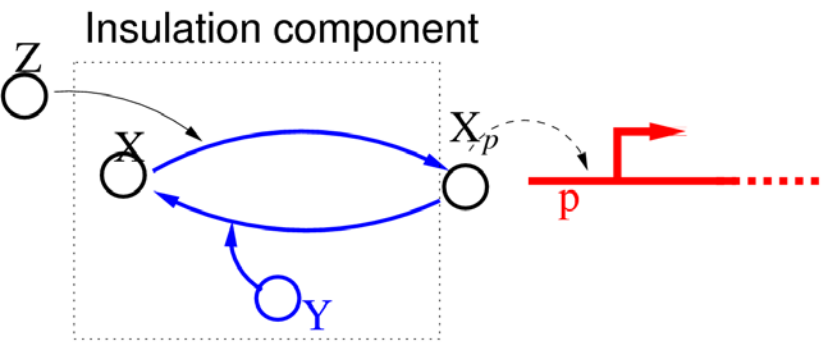
$$X_{TOT} \gg p_{TOT}$$

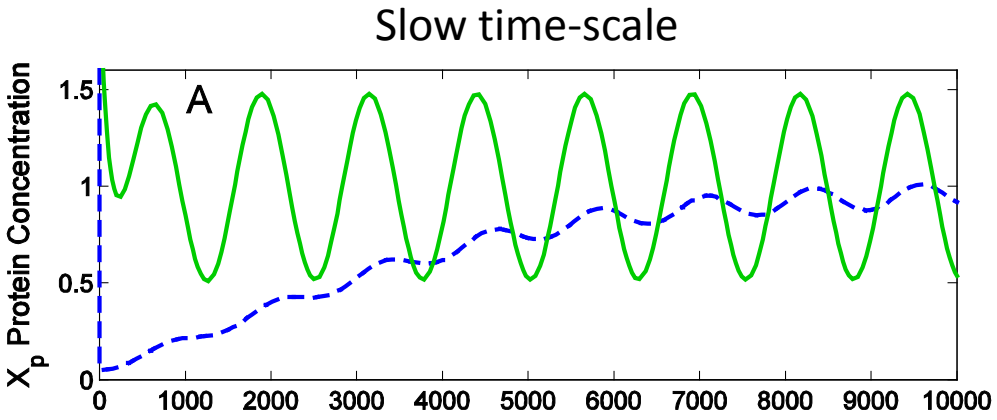
When one sets $\epsilon = 0$, this part becomes zero

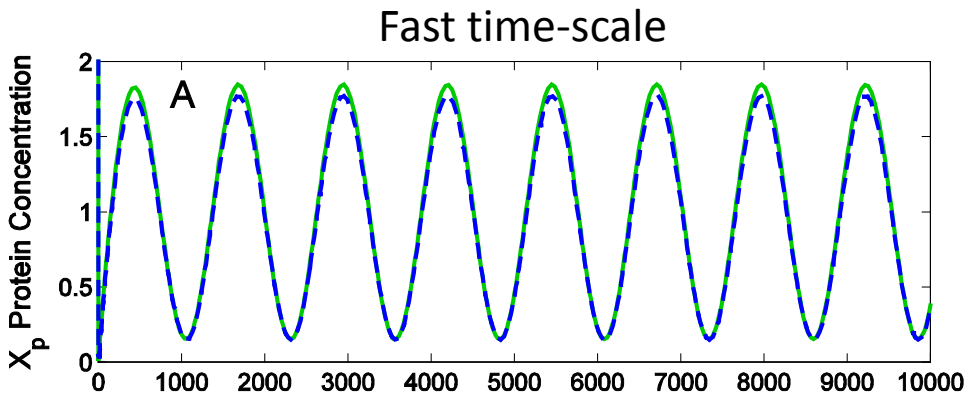
$$\begin{aligned}
\frac{dz}{dt} &= k(t) - \delta(z - C_1) \\
\epsilon\frac{dC_1}{dt} &= -\delta(b_2 + c_1)C_1 + \delta b_1(z - C_1)(1 - \frac{X_p}{X_{TOT}} - \frac{C_1}{X_{TOT}} - \frac{C_2}{X_{TOT}}) \\
\epsilon\frac{dC_2}{dt} &= -\delta(c_2 + a_2)C_2 + \delta a_1 X_p(1 - \frac{C_2}{Y_{TOT}}) \\
\epsilon\frac{dX_p}{dt} &= \delta c_1 C_1 + \delta a_2 C_2 - \delta a_1 X_p(1 - \frac{C_2}{Y_{TOT}}) + \boxed{\delta C - \delta/k_d(p_{TOT} - C)X_p} \\
\epsilon\frac{dC}{dt} &= -\delta C + \delta/k_d(p_{TOT} - C)X_p
\end{aligned}$$

The retroactivity s has no effect on the approximated dynamics of $X_p(t)$

Simulation results for the pho/depho insulation device







The fast time-scale of the phosphorylation cycle allows to reach insensitivity to very large loads (*p*=100)

Xp for the isolated system

Xp for the connected system

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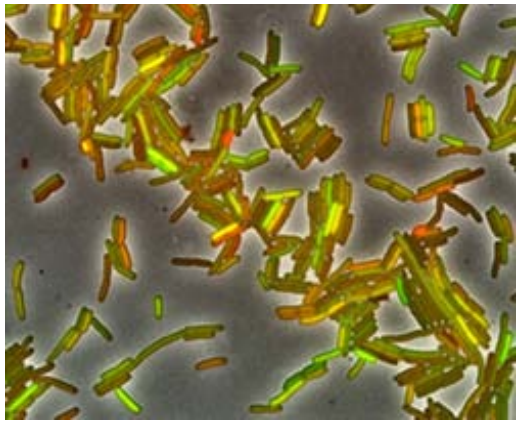
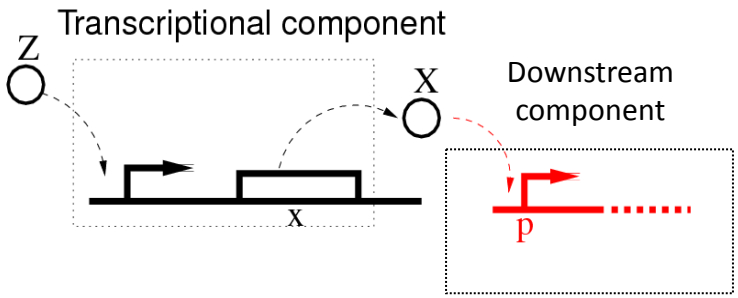
High gains improve signal-to-noise ratio…



Courtesy of Elowitz lab (Caltech)

Bio-molecular processes are intrinsically stochastic

How do high gains (required for retroactivity attenuation) impact noise?



$$\text{SNR} = \frac{A}{\sigma}$$

calculated by linearizing the system about its equilibrium corresponding to $\bar{k}$

calculated the Fokker-Planck Equation deriving from the Master Equation

$$\begin{aligned}\dot{X} &= Gk(t) - G\delta X + k_{off}C - k_{on}(p_{TOT} - C)X \\ \dot{C} &= -k_{off}C + k_{on}(p_{TOT} - C)X\end{aligned}$$

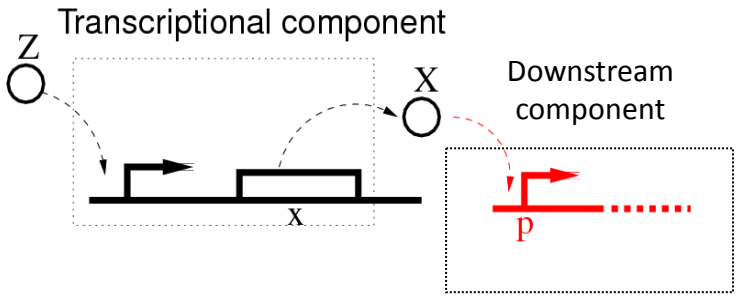
$$k(t) = \bar{k} + A_0 \sin(\omega t)$$

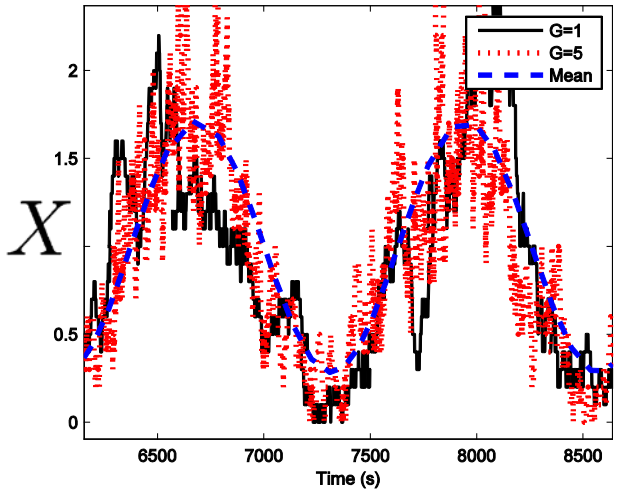
$$SNR_X(\omega) = \frac{\Omega}{k\delta} \frac{G^2}{G^2 + \frac{\omega^2}{\delta^2}(1+R_l)^2} A_0^2$$

$$SNR_C(\omega) = \frac{\Omega}{k\delta} \frac{G^2 R_l}{G^2 + \frac{\omega^2}{\delta^2}(1+R_l)^2} A_0^2$$

$$R_l = \frac{k_d\, p_{TOT}}{(\bar{k}/\delta + k_d)^2}$$

…but they also increase intrinsic noise at higher frequency





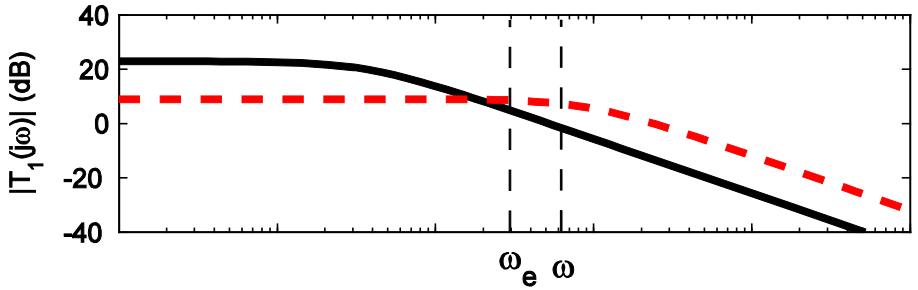
Use linearized Langevin approximation

$$\dot{X} = G\tilde{k}(t) - G\delta X - k_{on}(p_{TOT} - \bar{C})X + k_{on}\bar{X}C + k_{off}C + \sqrt{2G\bar{k}}N_1(t) - \sqrt{2k_{off}\bar{C}}N_2(t)$$

$$\dot{C} = k_{on}(p_{TOT} - \bar{C})X - k_{on}\bar{X}C - k_{off}C + \sqrt{2k_{off}\bar{C}}N_2(t)$$

$$T_1(s) = \frac{X(s)}{N_1(s)} = \frac{\sqrt{2G\bar{k}}}{s(1+R_l) + G\delta}$$

$$\omega_e = \frac{\delta\sqrt{G}}{(1+R_l)}$$



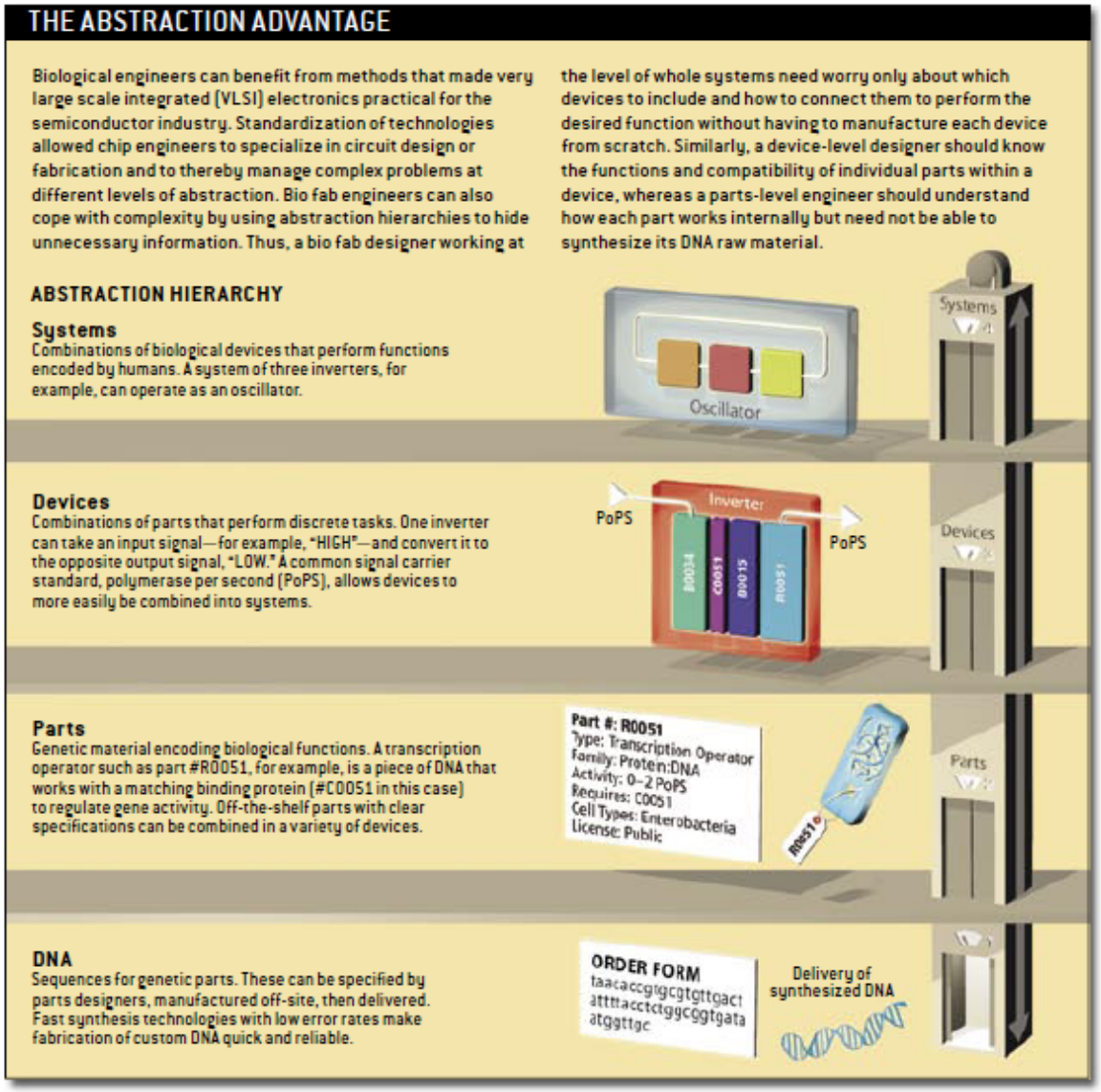
Jayanthi and Del Vecchio, CDC 2009

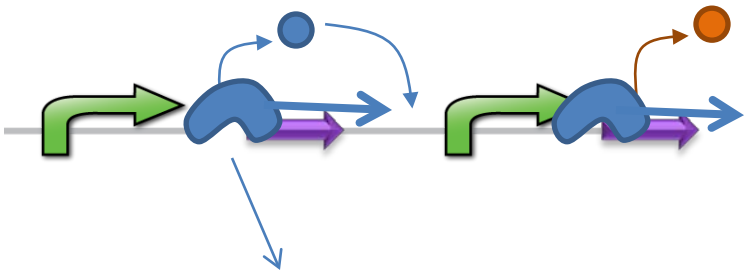
Part 4

Fabrication Technology

- **Biobricks: overview**
- ***In vitro* transcriptional circuits**

Parts, Devices, Systems



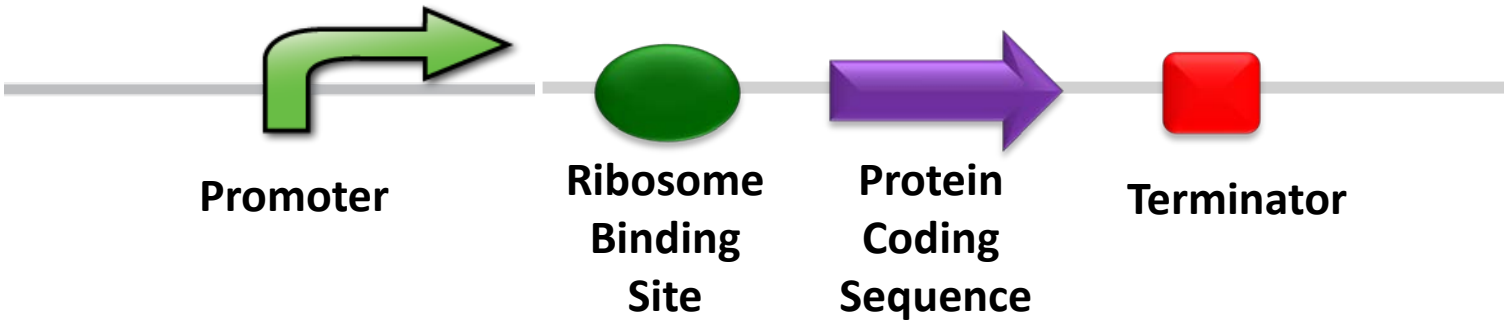


RnaPolymerase

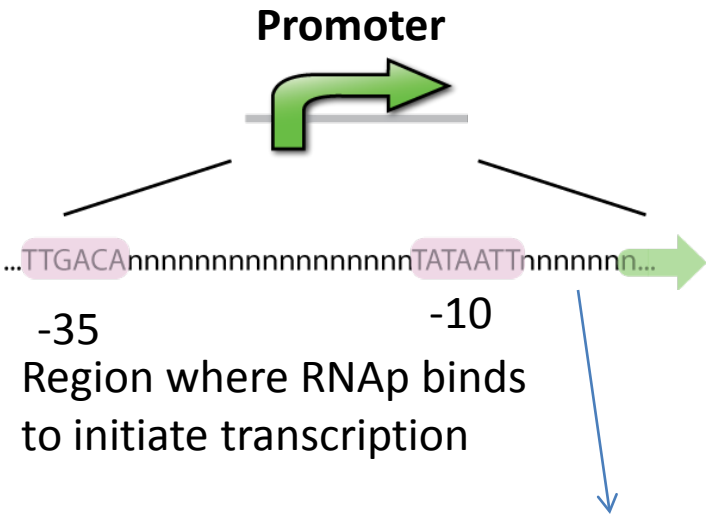
PoPS= Polymerase Per Second

David Baker,
George Church,
Jim Collins,
Drew Endy,
Joseph Jacobson,
Jay Keasling,
Paul Modrich,
Christina Smolke
and Ron Weiss
Scientific American
2006

Library of standard Parts: Biobricks



Catalog of parts & devices



Region where RNAp binds to initiate transcription

Operator sites:
Regions where regulatory proteins bind

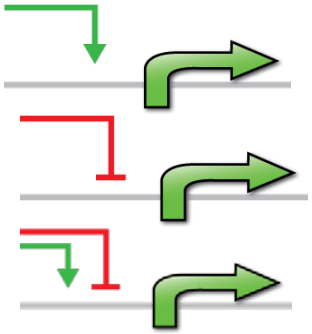
A promoter can be:

(1) Constitutive: no inputs, it is always ON

(2) Positively regulated

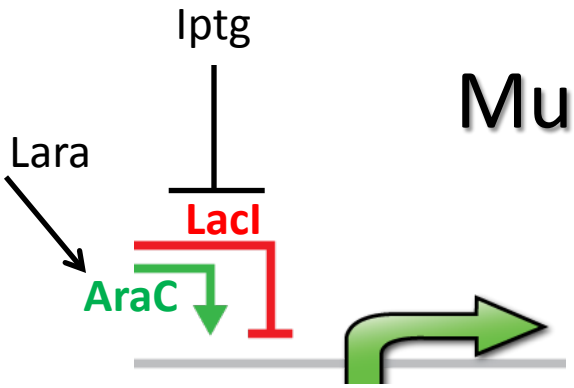
(3) Negatively regulated

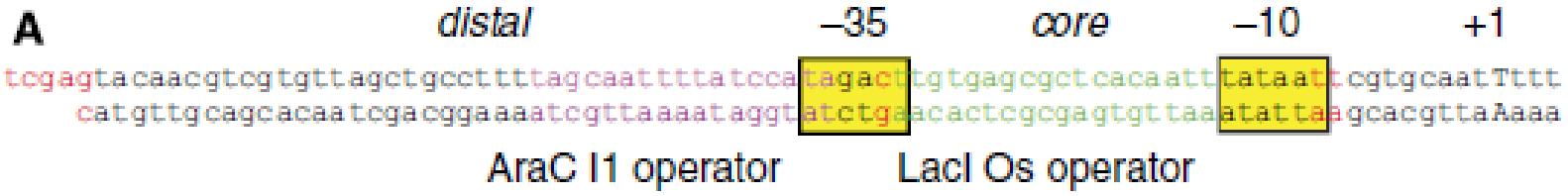
(4) Multi-regulated
Combinatorial promoters

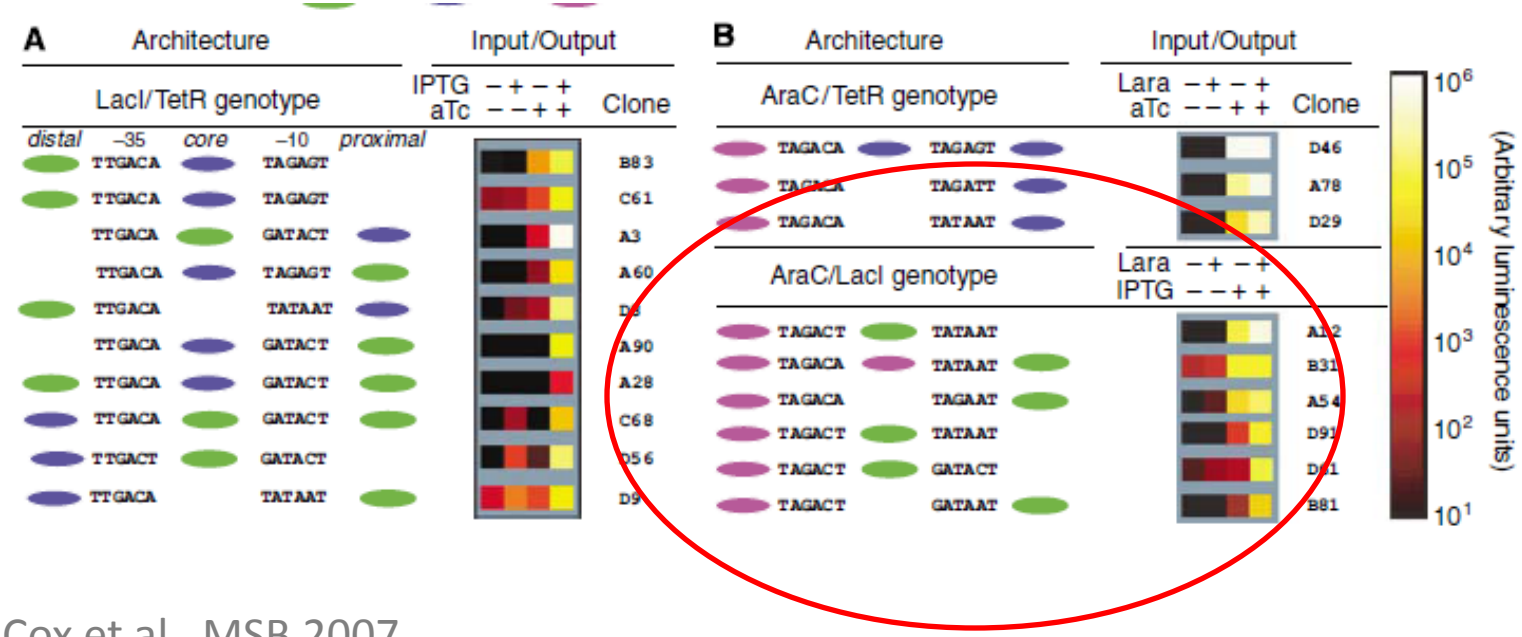


http://partsregistry.org/

Multi-regulated promoters

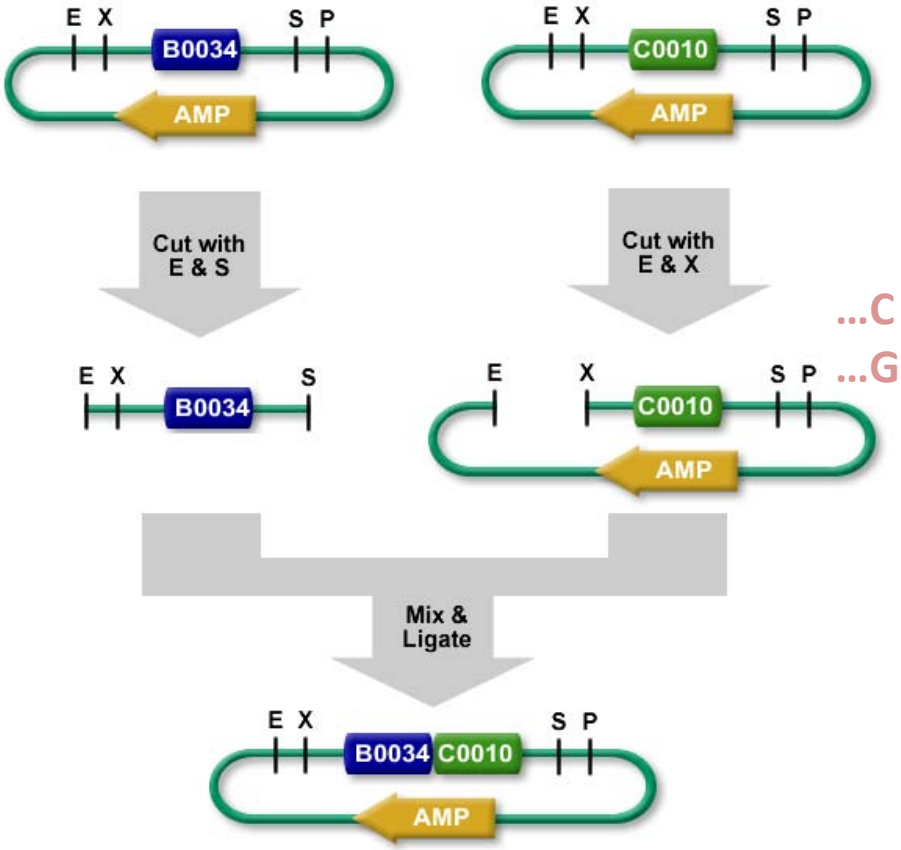






Cox et al. MSB 2007

Biobrick standard assembly



Restriction sites: E=EcoRI; X=XbaI
S=SpeI; P=PstI

Example: cutting with EcoRI

…C A G T C T A G A G C …
…G T C A G A T C T C G …

…T A A T C T A G A A A …
…A T T A G A T C T T T …

C T A G A G C …
T C G …

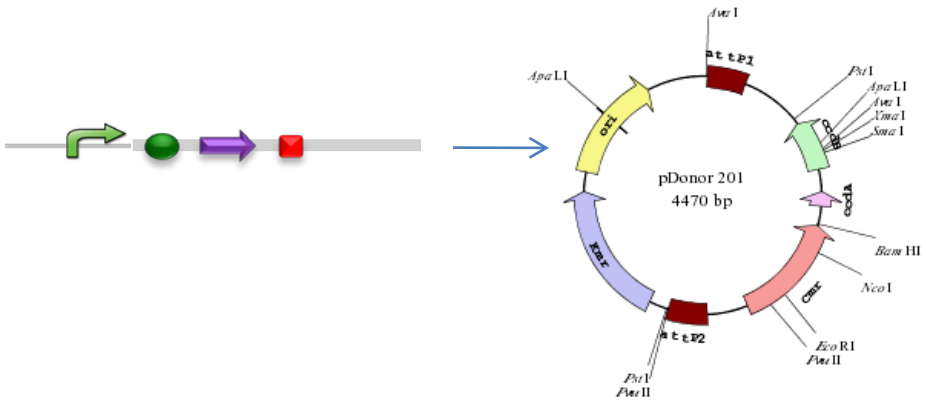
+

…T A A T
…A T T A G A T C T

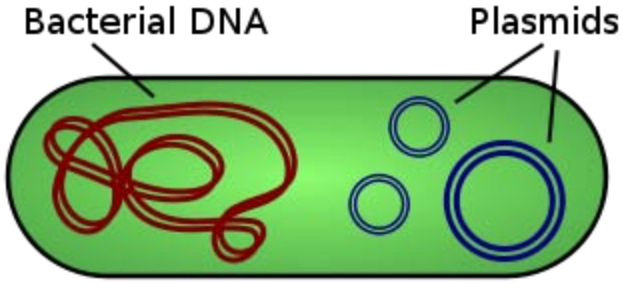
… T A A T C T A G A G C …
… A T T A G A T C T C G …

Any two biobricks can be combined in any order to form a new biobrick → Modular assembly

In vivo Implementation



Plasmid: circular portion of DNA separate from the chromosomal DNA, which is capable of replicating independently of the chromosomal DNA



Transformation: process of inserting the plasmid within in the cell. It occurs by rendering the cells *competent,* (external membrane becomes permeable).

Inducers: signaling molecules that bind to repressors and disable them. The net effect is to start transcription. They can be added to the cell population to provide input forcing to the circuit

Reporter Genes: express proteins that produce an easily observable phenotype, for example, green fluorescent proteins, which causes cells to fluoresce green under blue Light. They are inserted after a gene of interest to measure its production rate. They Provide an easily measurable output to the system.

Catalog of parts & devices

Example of a device: An inverter

aTc: inducer: external input

Constitutive promoter

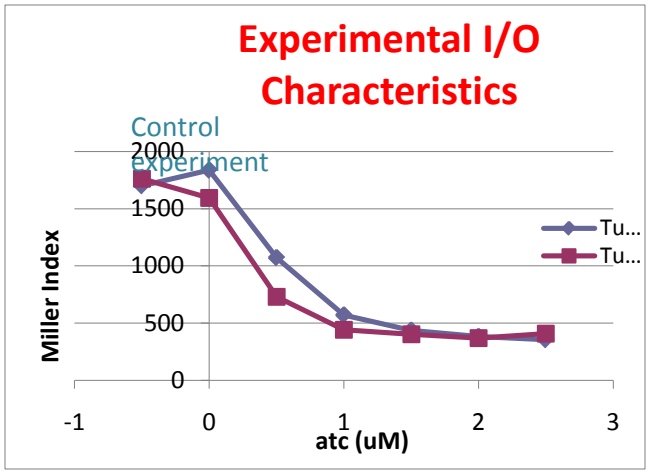
Negatively Regulated promoter

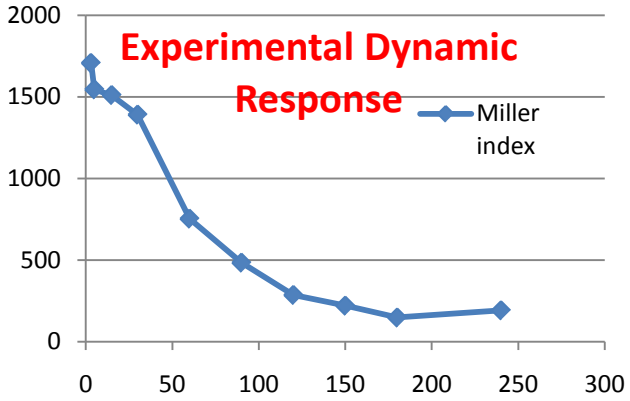
Negatively Regulated promoter

lacIQ | tetR | P(TetR) | lacI-LVA | P(Lac) | lacZ

Measurable Output

aTc → Expected I/O characteristics → LacZ



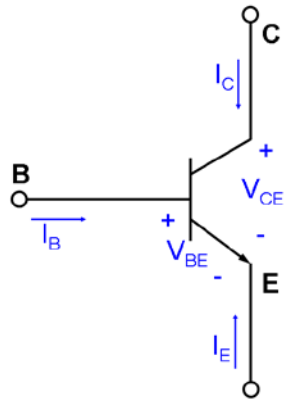


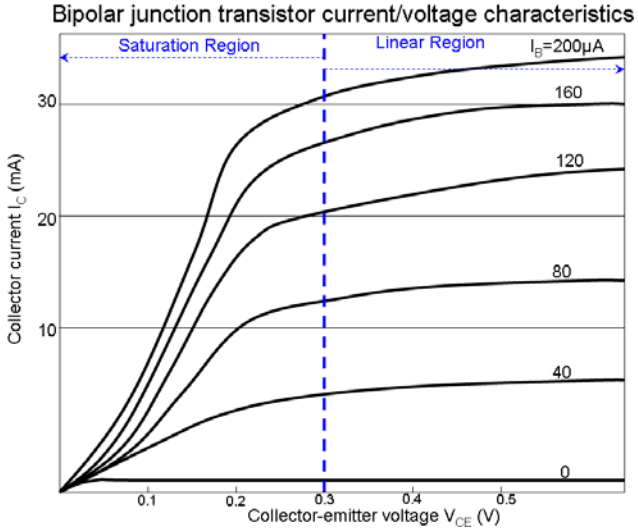
Expected dynamic response

$$LacZ(t) \approx K * aTc * (1 - e^{-\alpha t})$$

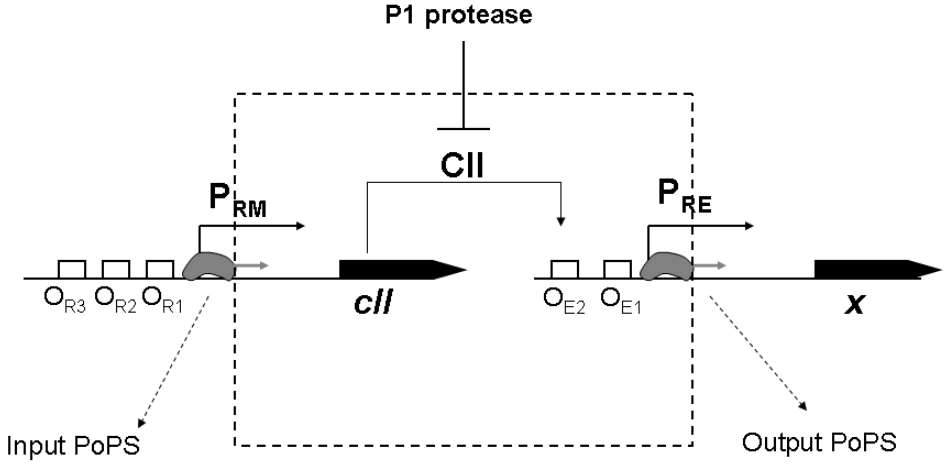
Example of a device: A three-terminal device

Idea: design a BJT-like device





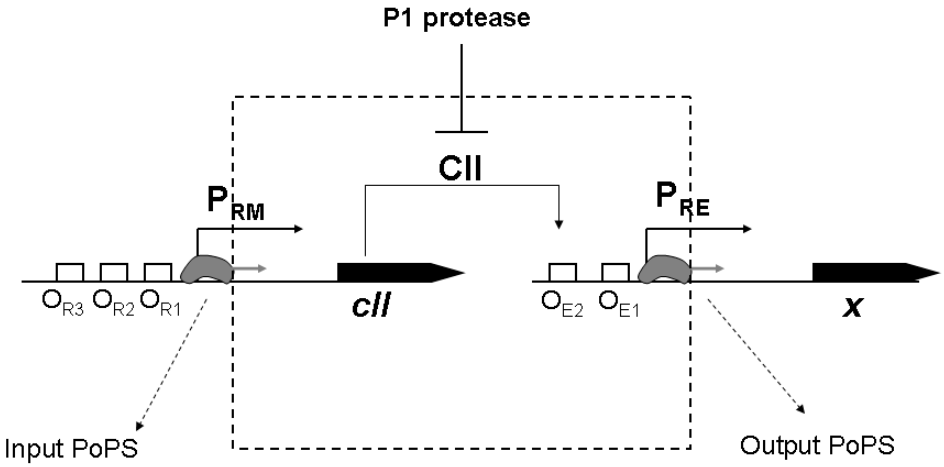
Bio-molecular implementation: using parts from lambda-switch and signal carrier: current/PoPS and voltage/protein concentration

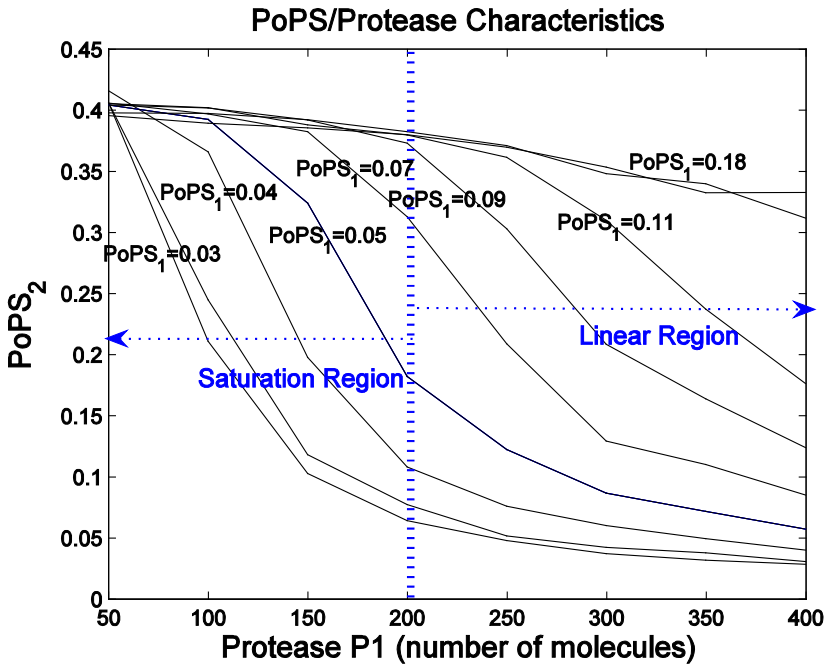


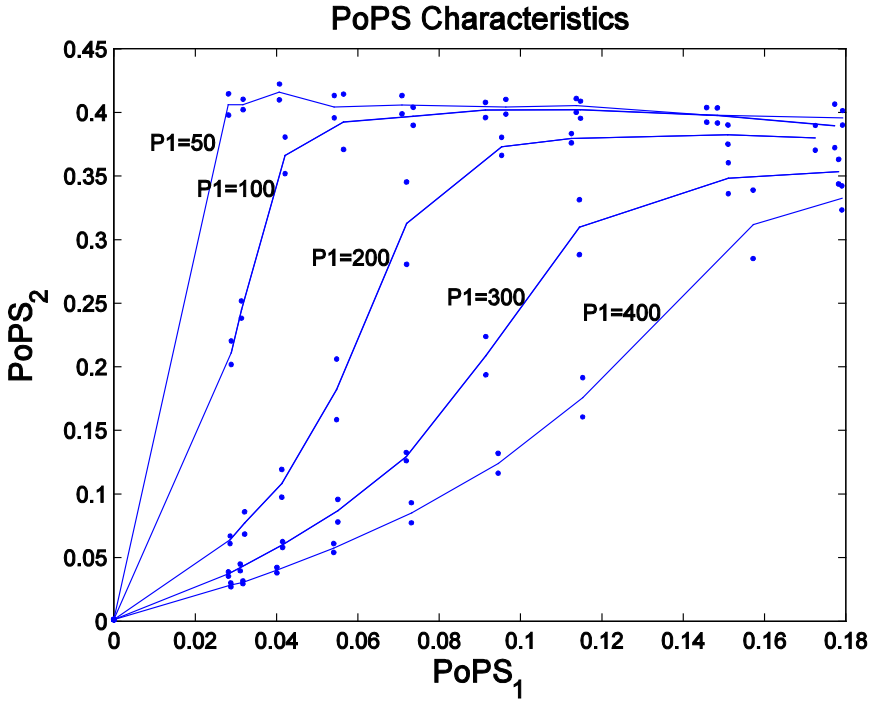
Characteristics

In silico experiments through SSA

(Varadarajan and Del Vecchio, 2009)



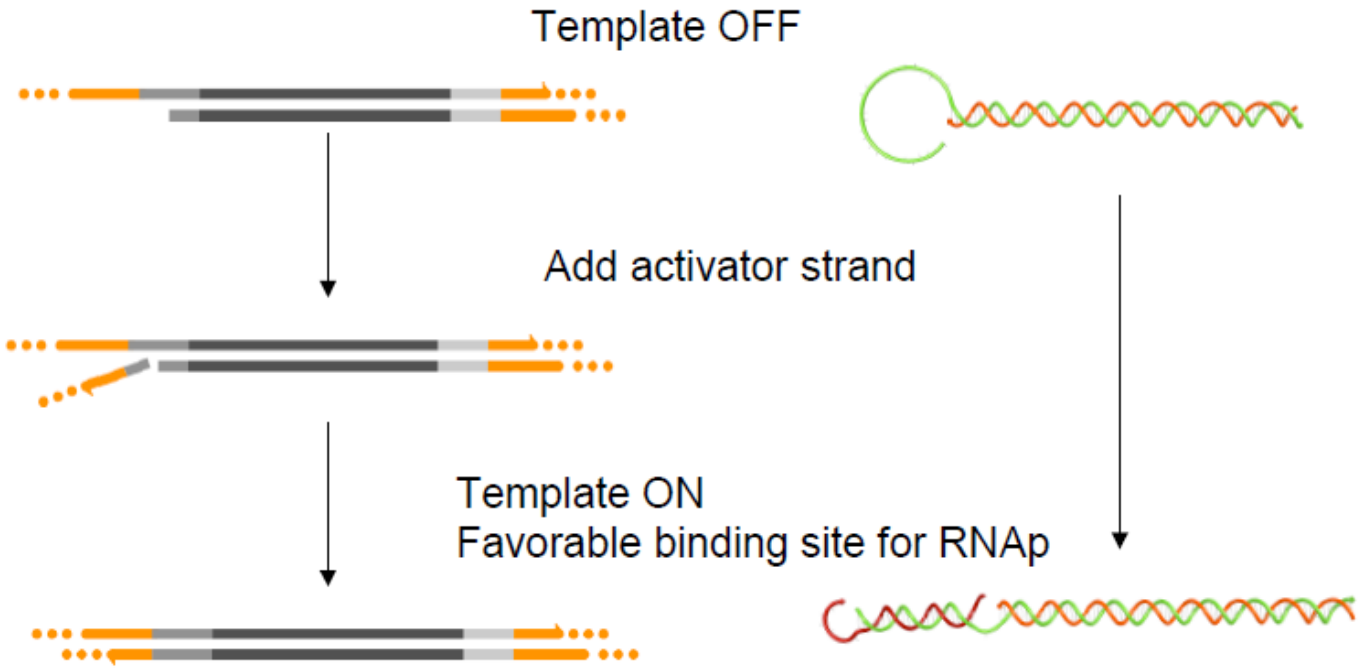




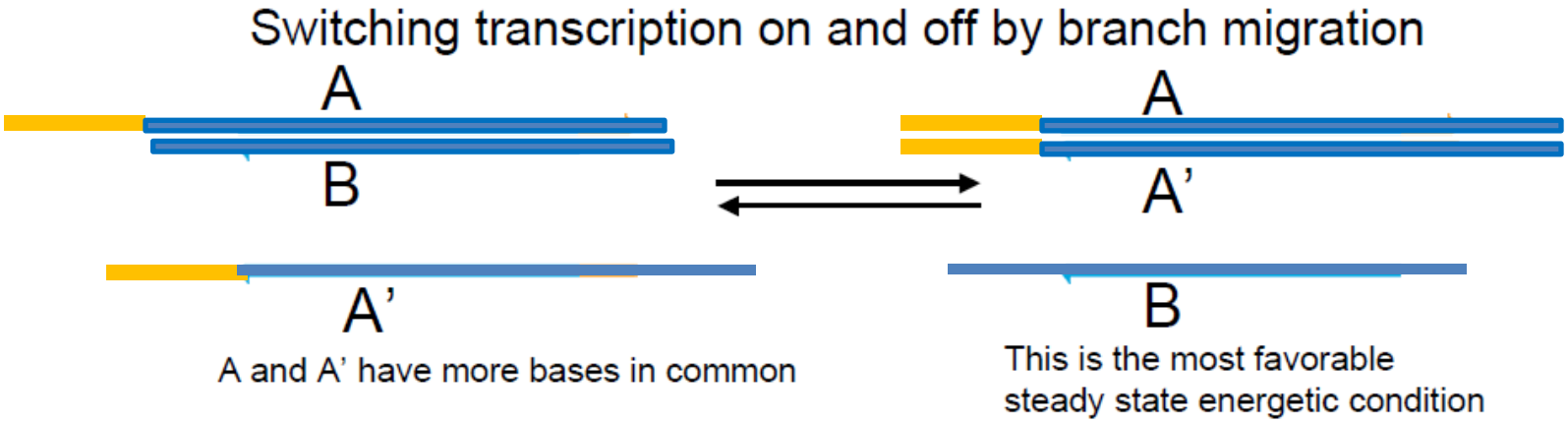
Findings: Input/output PoPS gain in the linear region is 2

In vitro transcriptional circuits: circuits with no proteins

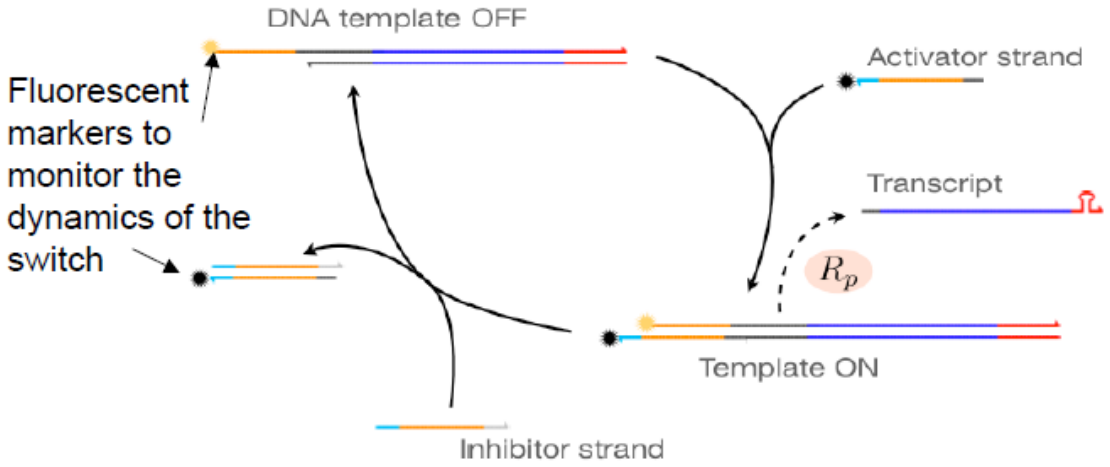
Transctiption is regulated with nucleic acids only (no proteins)



Implementing activation and repression

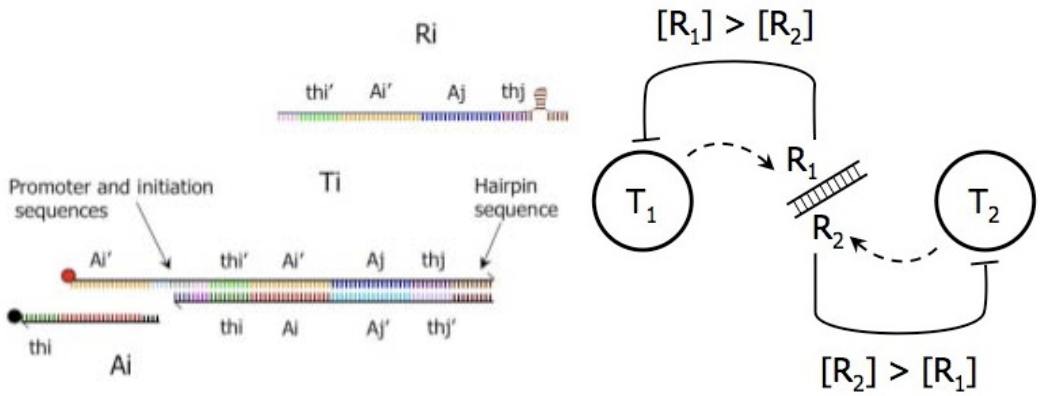


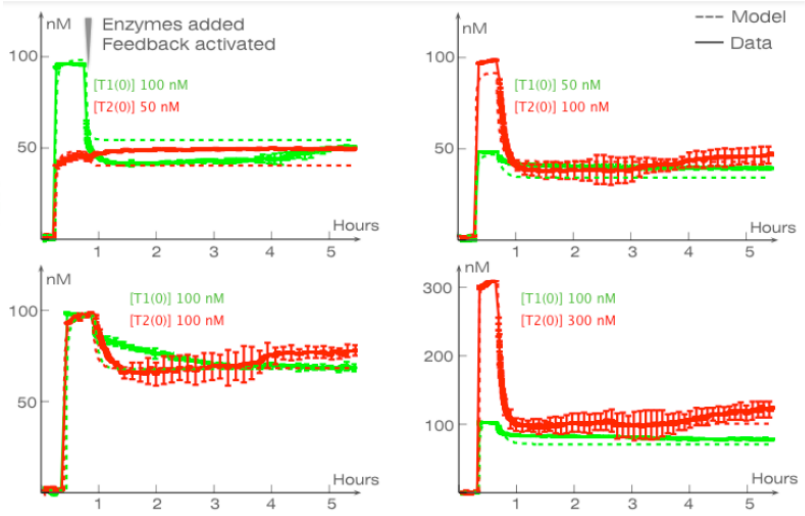
We can use this principle to turn ON and OFF the synthetic templates



An in-vitro circuit: Rate regulator

- Idea: produce two chemicals at same rates
  - Common operation for metabolic networks - maintain stoichiometry
  - Implemented using *in vitro* technology (test tubes instead of cells)





- Molecular programming for in vitro systems
  - Exploit Watson-Crick base pair binding (A-T, C-G)
  - Can "compile" functional specifications into RNA and DNA sequences
  - Circuits are biocompatible ⇒ some hope of embedding into cells

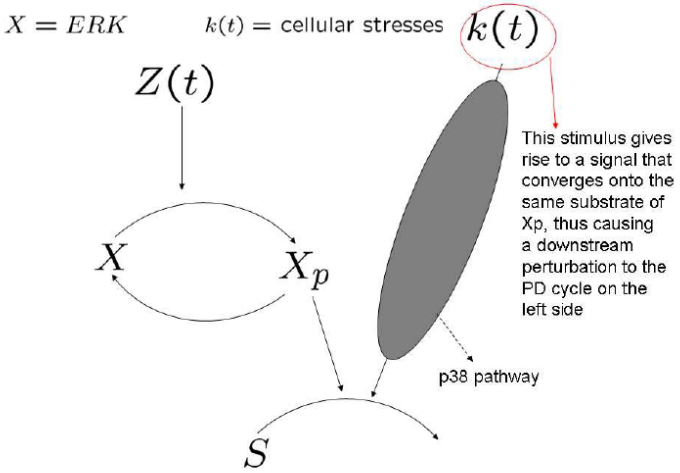
Franco, Winfree, and Murray, 2009

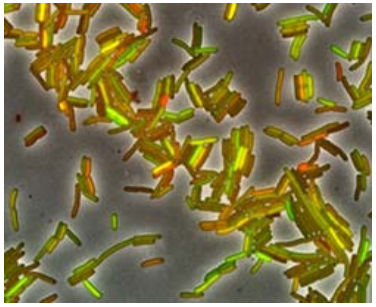
Comparing the two technologies

In vitro technology allows to run experiments in a fully controlled environment (the probe): Simulation data and experimental data usually agree

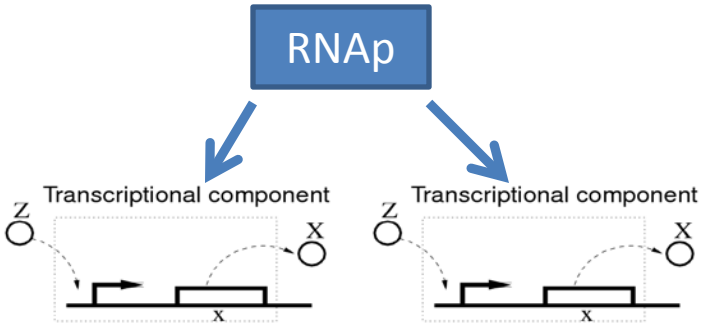
In vivo technology runs experiments in the cellular environment. Main challenges:

-**<u>Cross talk</u>**: the cell contains molecules that may interact with the circuit component
-**<u>Noise</u>**: the cellular environment is noisy and causes unpredictable stochastic fluctuations
-**<u>Competition for shared resources</u>**: the circuits that we introduce in the cell use cellular resources to work: ATC, RNAp, ribosomes, etc. They can thus severely impact the helthy behavior of the cell and as a consequence their own behavior





Courtesy of Elowitz Lab

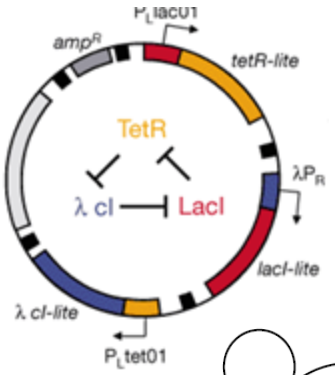


M Behar, HG Dohlman, and TC Elston. *PNAS 104(41):16146-51, 2007*

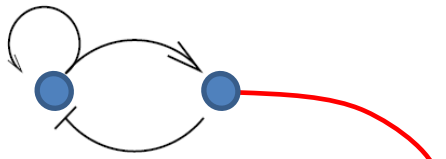
These are among the problems that make bio-molecular engineering *in vivo* fundamentally different from electrical engineering

Summary

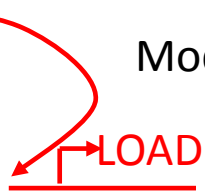
The ability of fabricating bio-molecular circuits has far reaching Applications: medical and energy are two examples

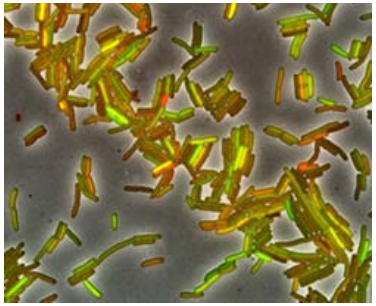


The technology for fabricating synthetic Bio-molecular circuits in cells is available



Modular design is still a challenge





Stochasticity is an integral part of these systems and must be Explicitly considered for design