Design of Bio-molecular Feedback Systems

Domitilla Del Vecchio
EECS, University of Michigan
MechE, MIT

CDC, Shanghai, 2009

Outline

- **Part 1:** Overview of synthetic biology and simple modules
- **Part 2:** The challenge of composing modules together
- **Part 3:** Fabrication technology

Part 1

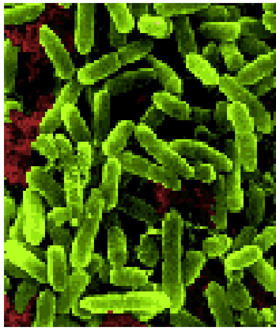
Overview of synthetic biology and simple modules

- History and basic technology
- Simple modules

Why designing 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

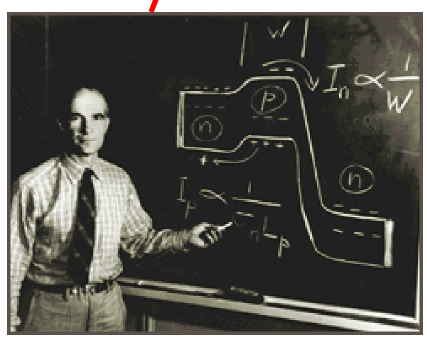
Electronic Engineering

Electrical Engineering	Vacuum Tube era	Transistor era	To Electronic computers

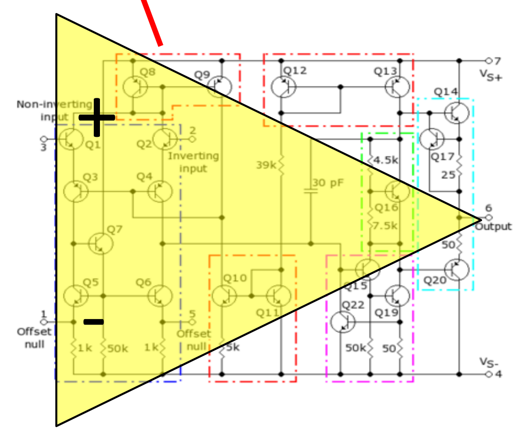
1904 — 1948 — 1964

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

Fleming invented the diode (a two-terminal device)

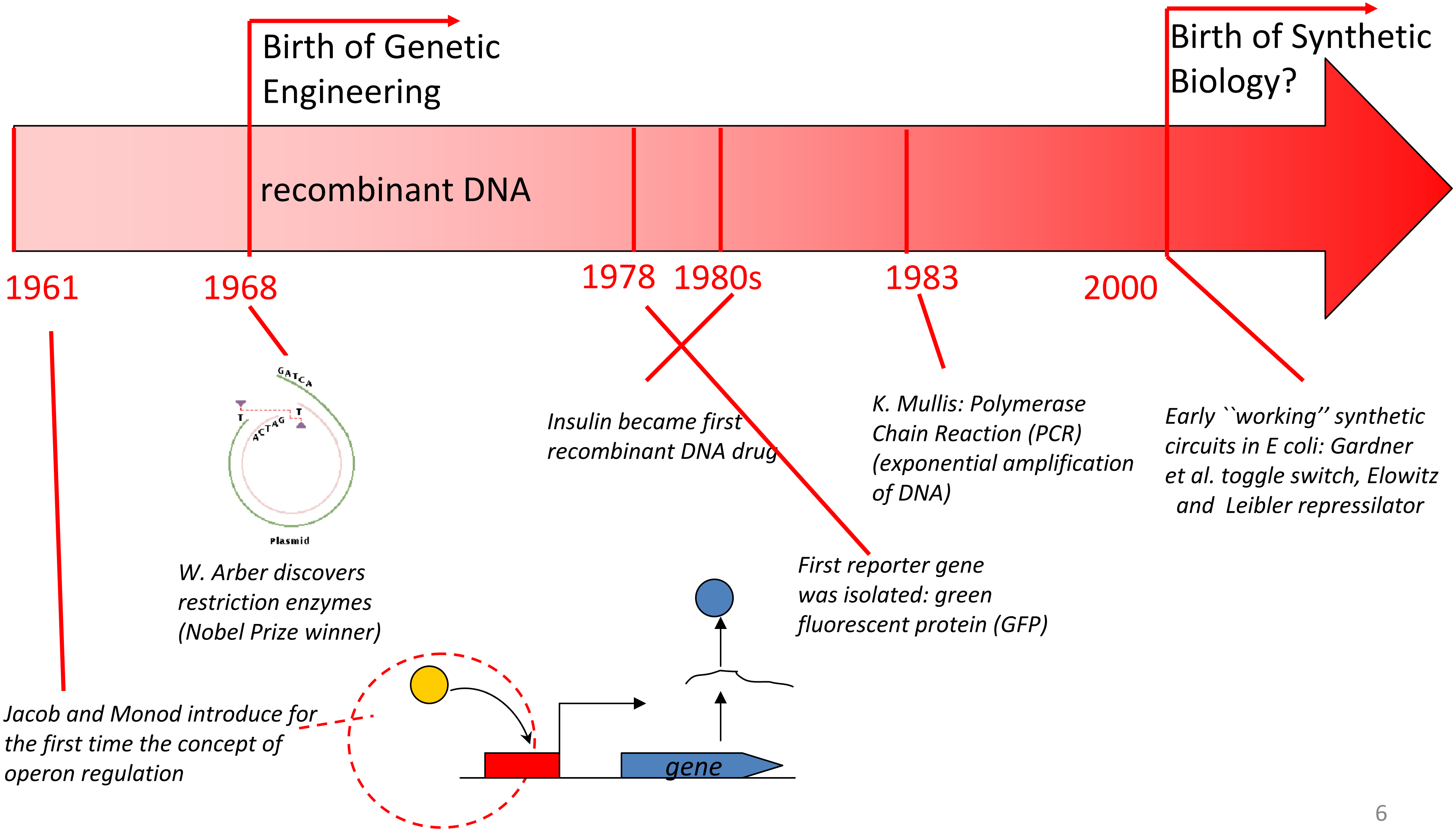


William Shockley explains how the bipolar junction transistor works (BJT) December 1947, Bell Laboratories (Nobel Prize in Physics in 1956)



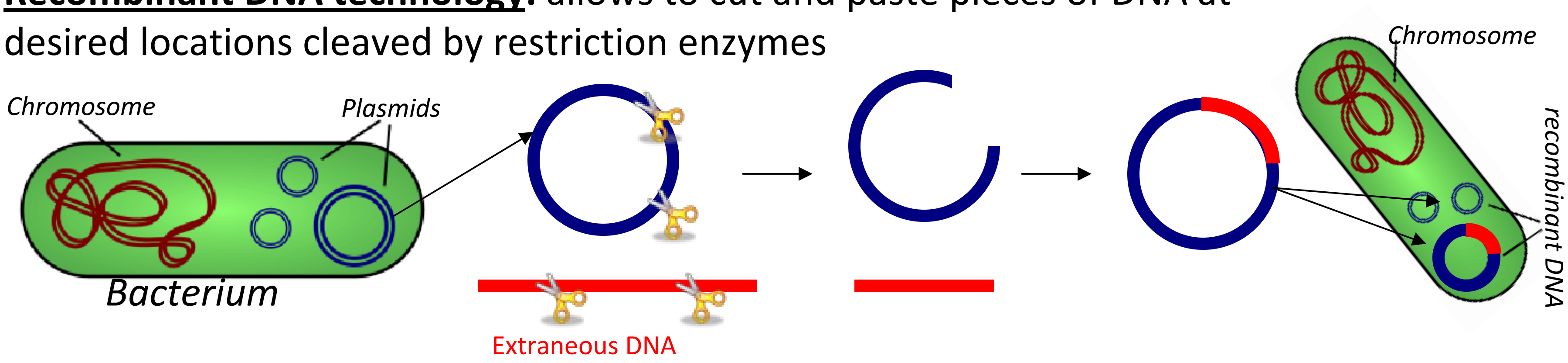
Operational Amplifier (OPAMP)
1964 Wildar at Fairchild Semiconductor

Synthetic biology: A historical perspective

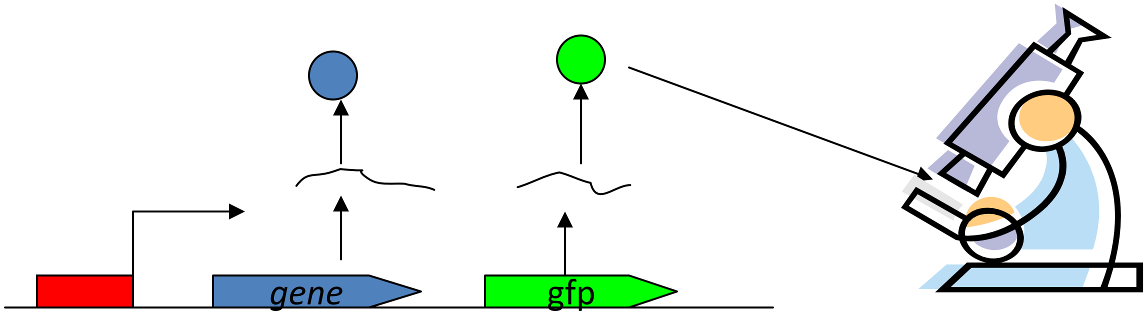


Key enabling technology

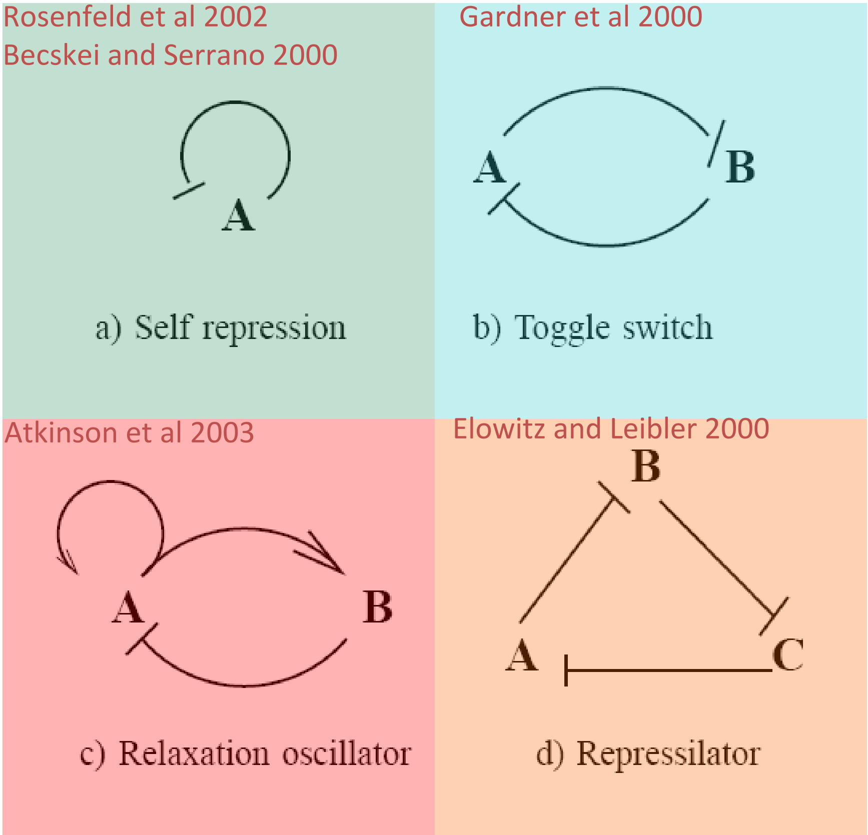
Recombinant DNA technology: allows to cut and paste pieces of DNA at desired locations cleaved by restriction enzymes



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*



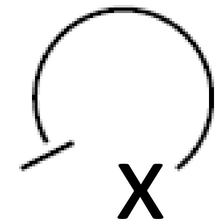
Monostable modules

Bistable modules

Relaxation oscillators

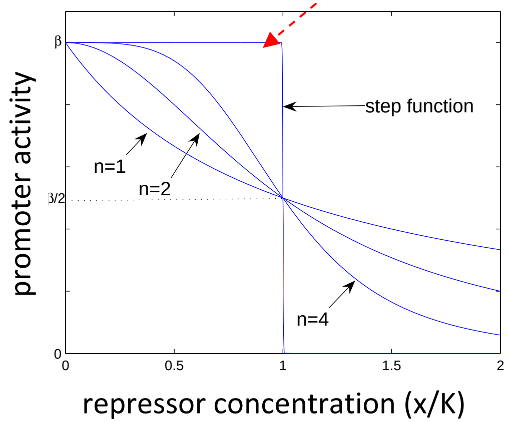
Loop oscillators

A self repressed gene:Dynamics

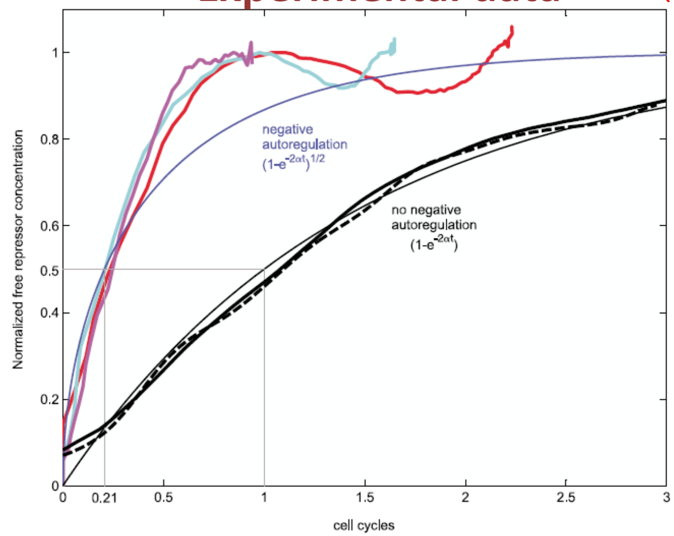


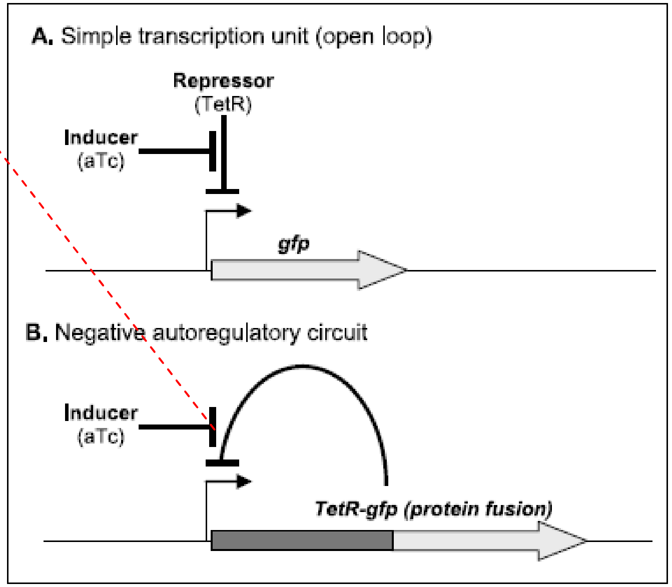
negative feedback

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



Experimental data





For n=1:

Without negative feedback

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

With negative feedback

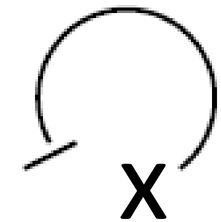
$$\frac{x_2(t)}{x_2^{st}} = \sqrt{1 - e^{-2\alpha t}}, \qquad \beta_2/\alpha \gg k$$

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

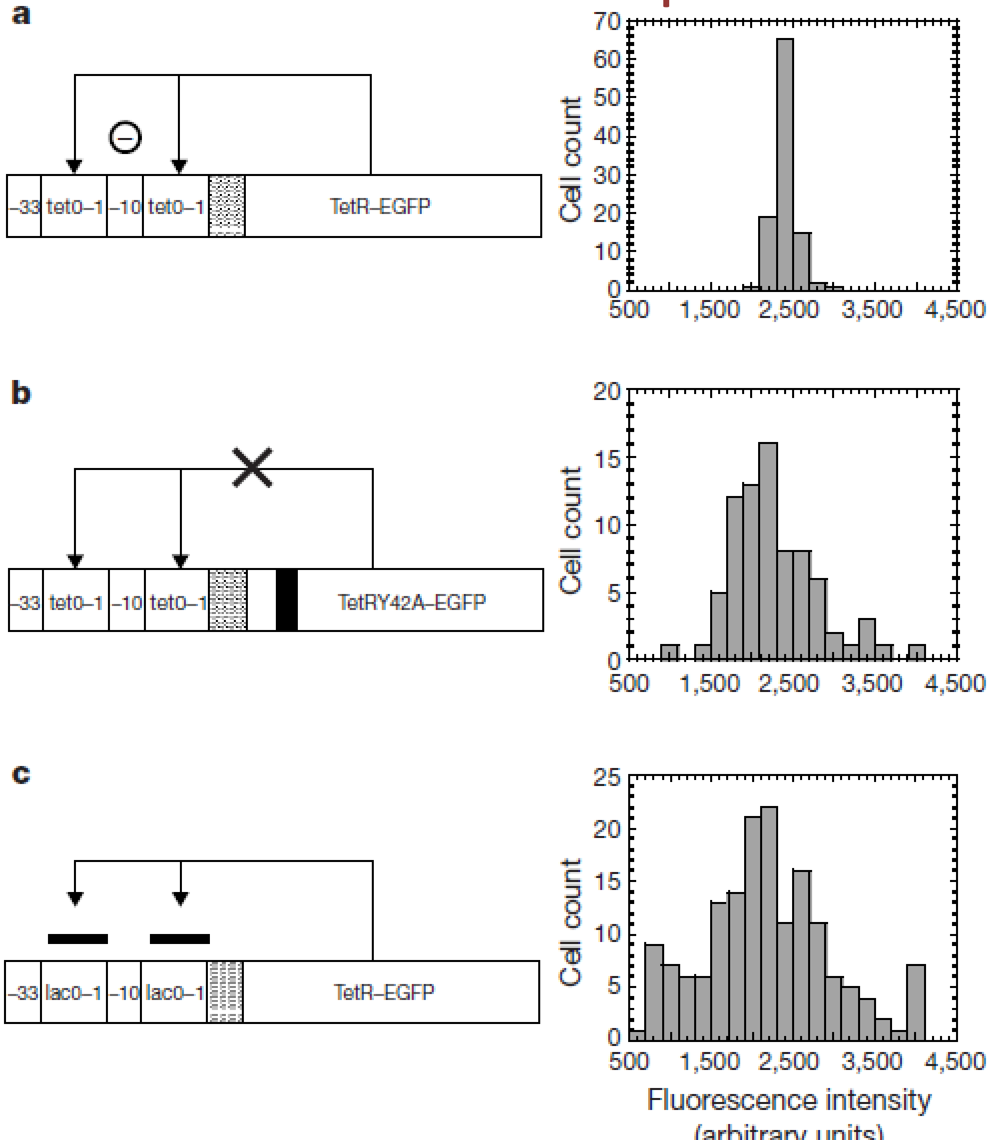
$$x_2^{st} = \sqrt{k\beta_2/\alpha}$$

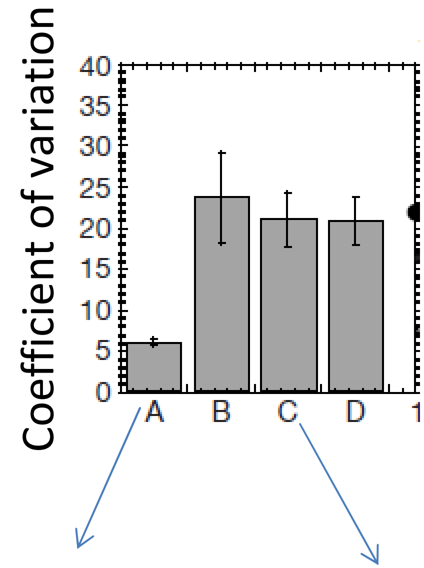
Negative feedback speeds up the response time (Rosenfeld et al 2002)

A self repressed gene: robustness



Experimental data



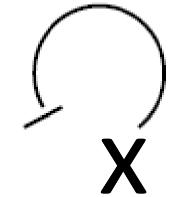


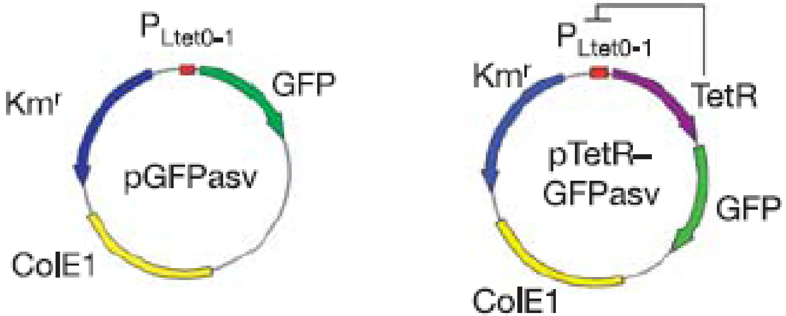
autoregulated

unregulated

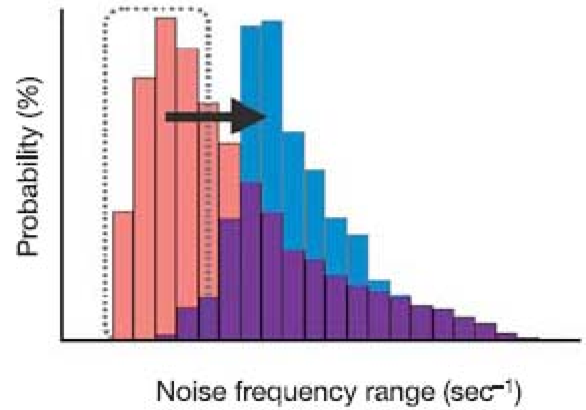
Becskei and Serrano, Nature 2000:
Negative autoregulation decreases noise

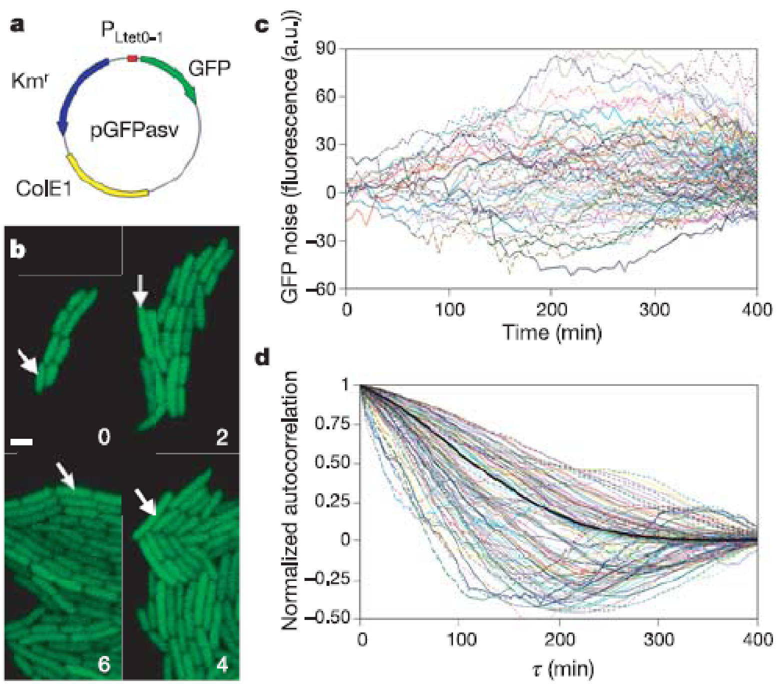
Self repressed gene: Frequency analysis



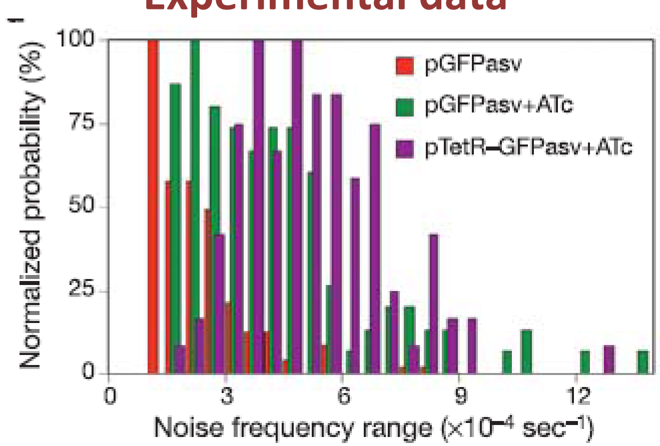


Simulation data (SSA)



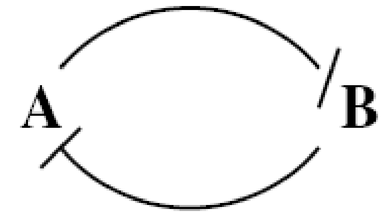


Experimental data

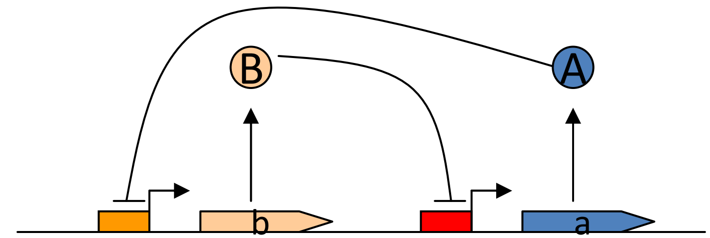


Austin et al. Nature 2006: Negative autoregulation shifts frequency content to high frequency

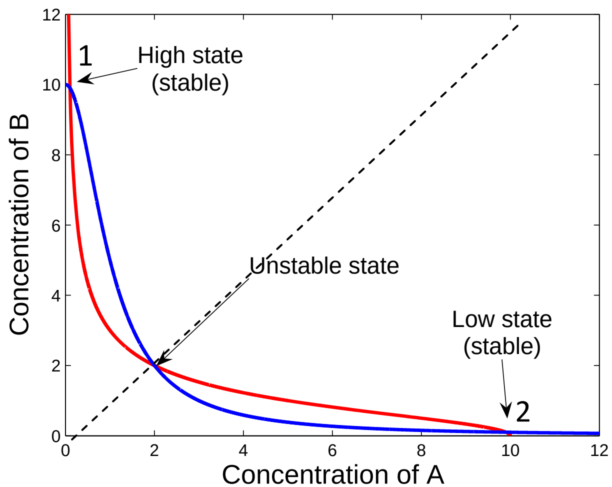
Toggle switch

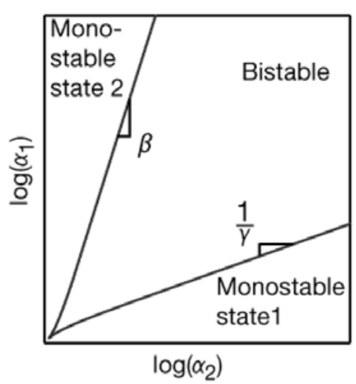


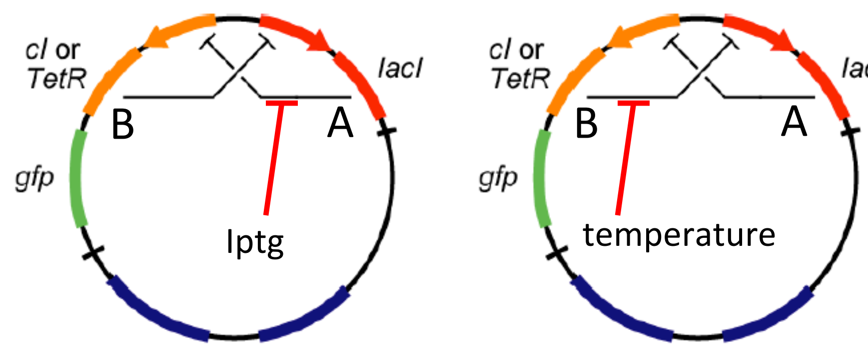
$$\frac{dA}{dt} = \frac{\beta}{1+(B/K_1)^n} - \alpha_1 A$$
$$\frac{dB}{dt} = \frac{\gamma}{1+(A/K_2)^n} - \alpha_2 B$$

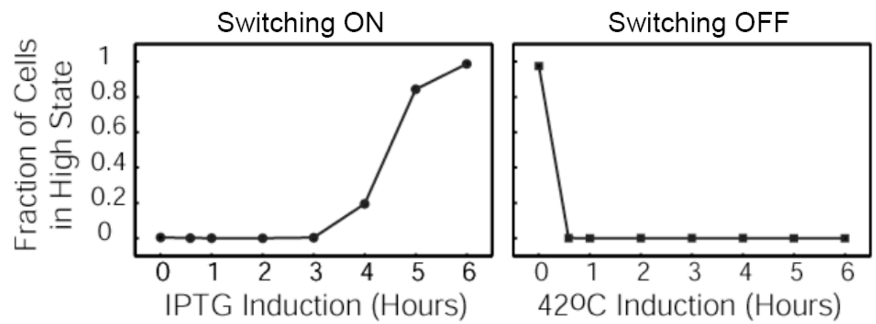


Symmetric design

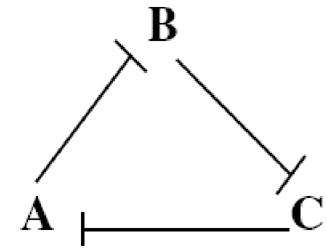


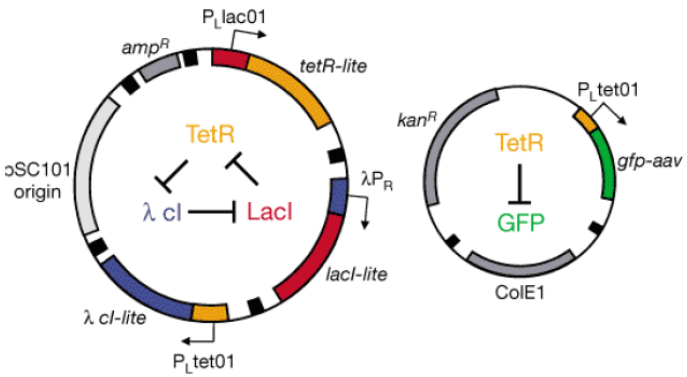




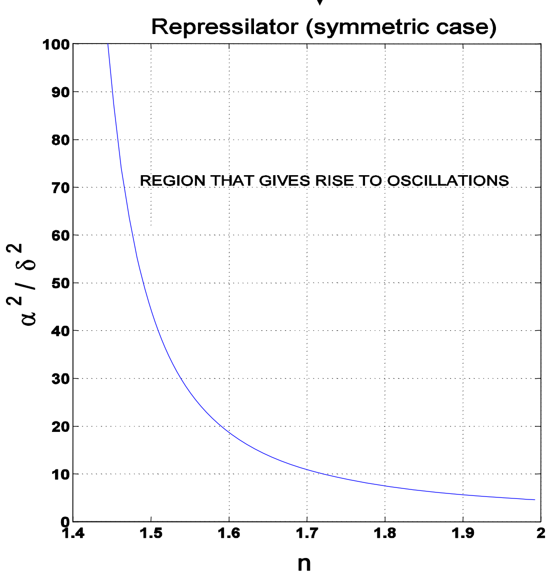


Loop oscillators: The repressilator



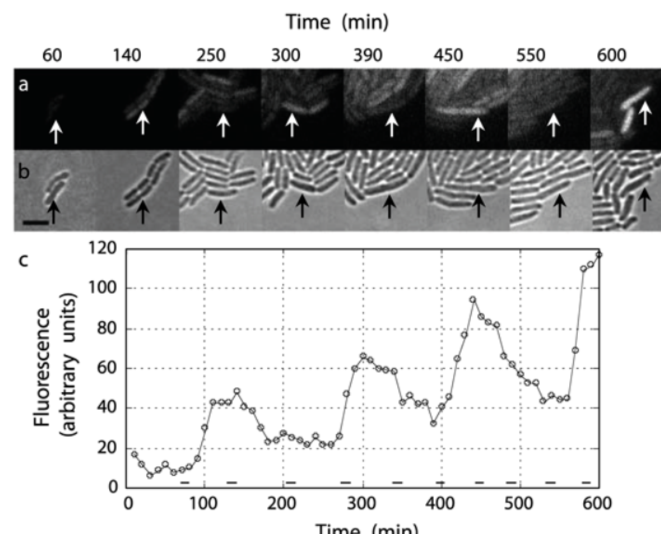


(Hastings 1977, Mallet-Paret 1990)



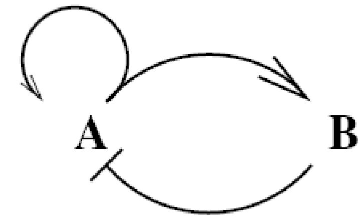
$$f(p) = \frac{\alpha^2}{1+p^n}$$

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



Elowitz and Leibler, Nature 2000

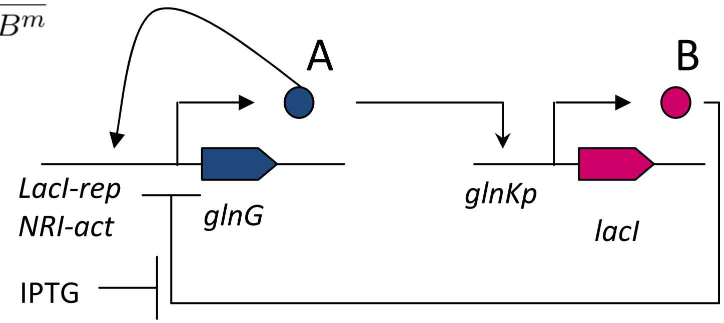
Relaxation oscillators: Atkinson et al. clock



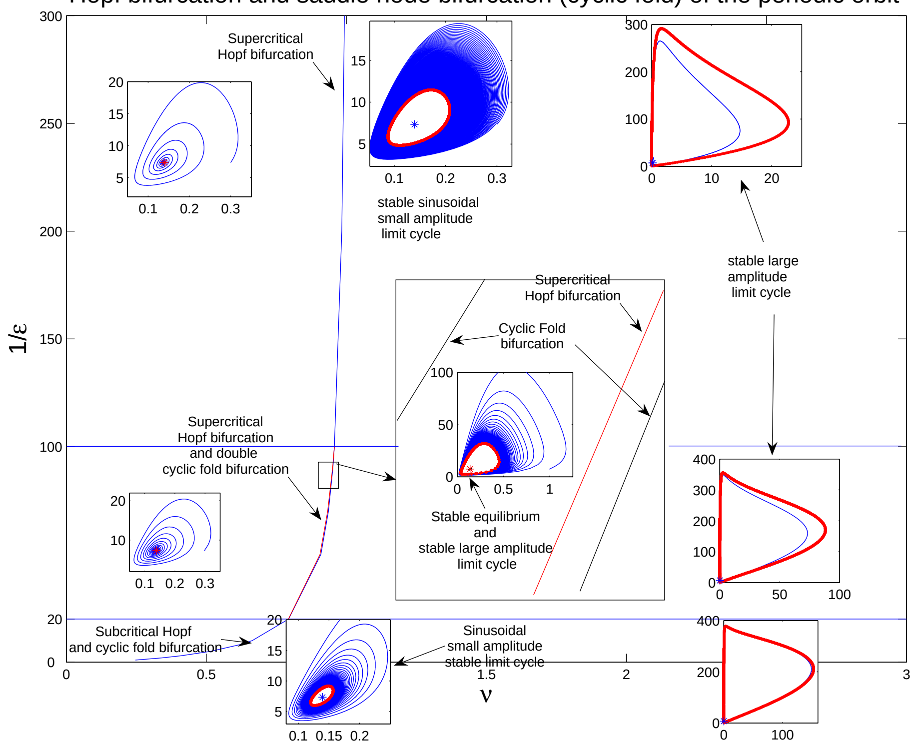
$$\frac{dr_A}{dt} = -\frac{\delta_1}{\epsilon} r_A + F_1(A,B)$$
$$\frac{dA}{dt} = \nu\left(-\delta_A A + \frac{k_1}{\epsilon} r_A\right)$$
$$\frac{dr_B}{dt} = -\frac{\delta_2}{\epsilon} r_B + F_2(A)$$
$$\frac{dB}{dt} = -\delta_B B + \frac{k_2}{\epsilon} r_B,$$

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

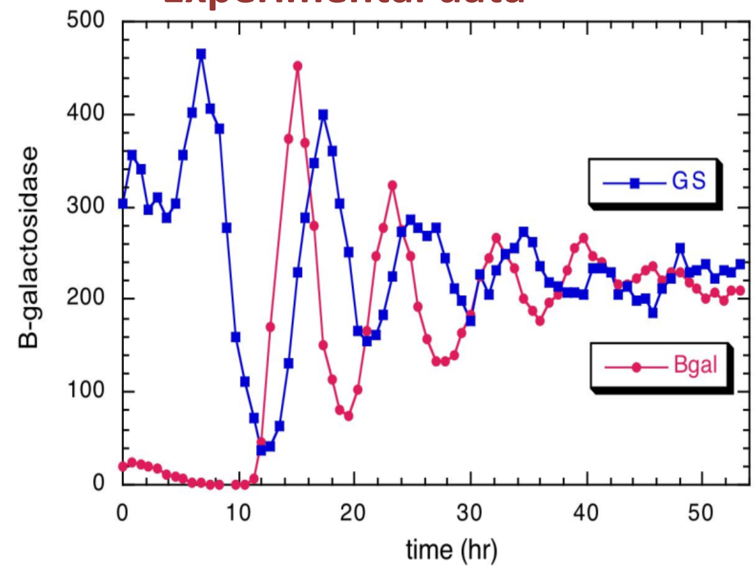
$$F_2(A) = \frac{K_2 A^n + K_{B0}}{1 + \gamma_3 A^n}.$$



Hopf bifurcation and saddle node bifurcation (cyclic fold) of the periodic orbit



Experimental data



(Courtesy of Ninfa Lab at Umich)

(Cell population measurements)

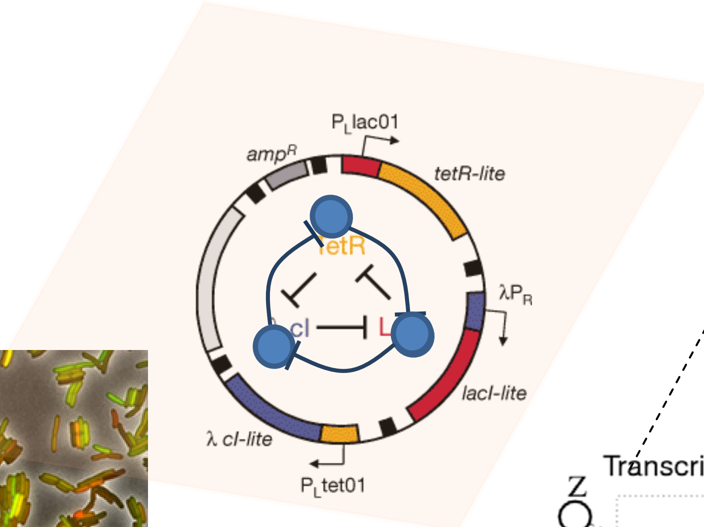
Atkinson et al. Cell 2003

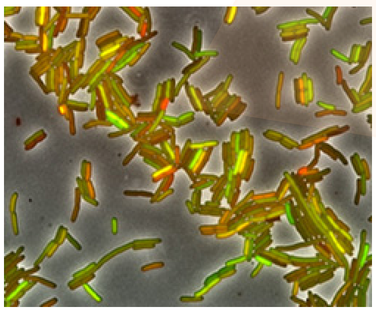
Part 2

The challenge of composing modules together

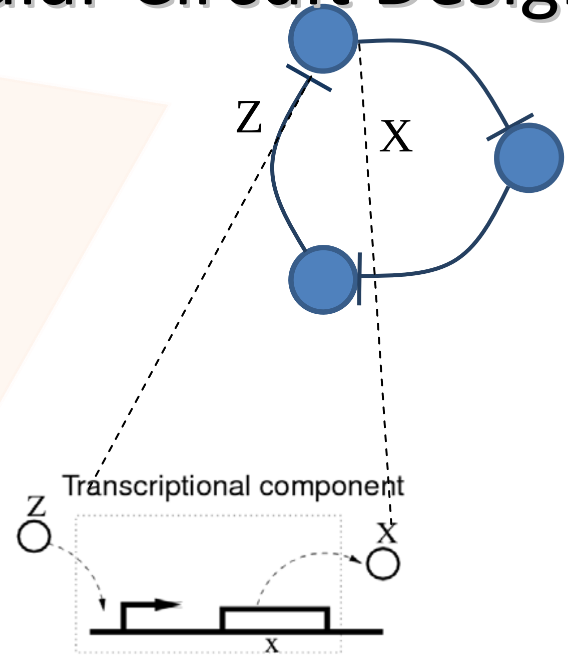
- **Retroactivity phenomenon and its modeling**
- **Insulation devices**
- **Implementation examples**

Synthetic Biology: Enabling Technology for Bio-molecular Circuit Design



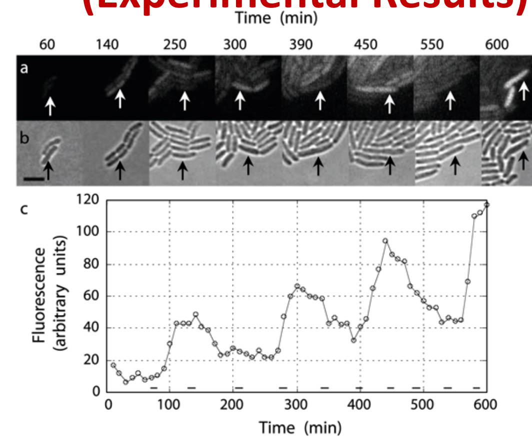


Courtesy of Elowitz Lab

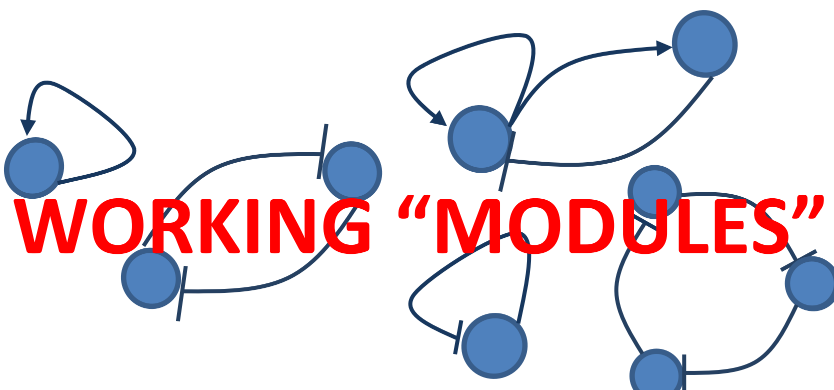


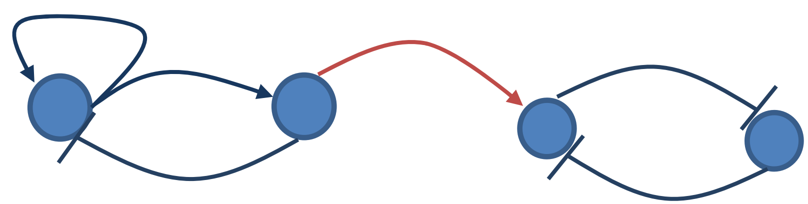
Repressilator

(Experimental Results)



(Elowitz and Leibler, *Nature* 2000)

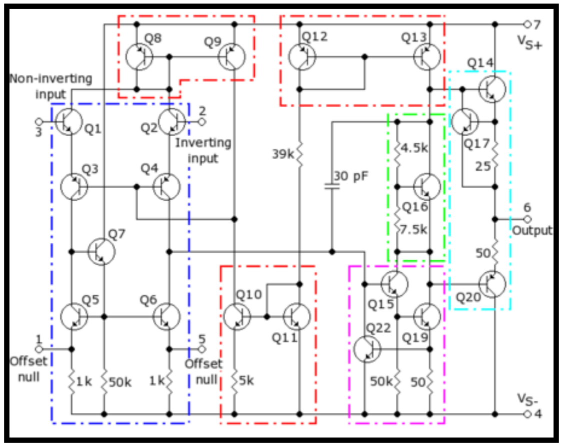




WORKING "MODULES"

NOT WORKING INTERCONNECTIONS !

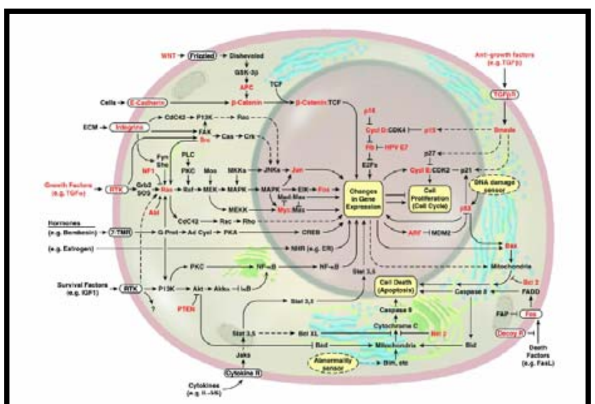
Modularity: A fundamental property



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.
<u>Electronics and Control Systems Engineering</u> 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)]

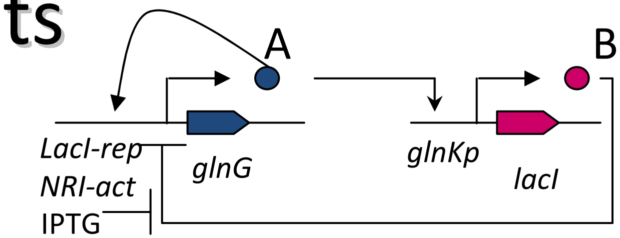
Functional modules seem to recur also in <u>biological networks</u> (e.g. Alon (2007)). But…

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?

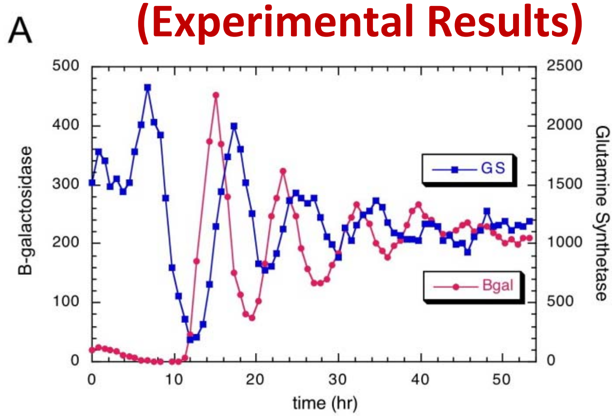
Modularity is **not** a Natural Property of Bio-molecular Circuits

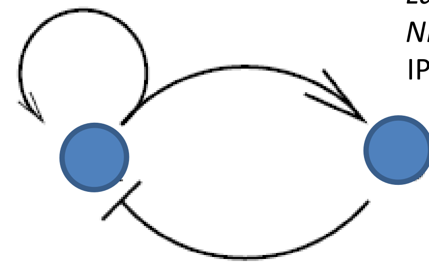


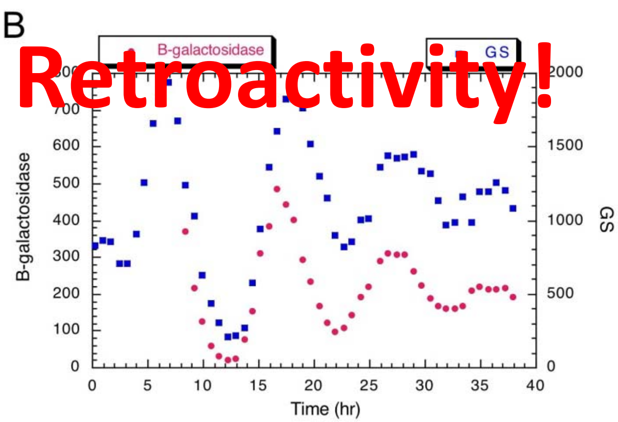
(Atkinson et al, *Cell* 2003)

Activator/Repressor Clock

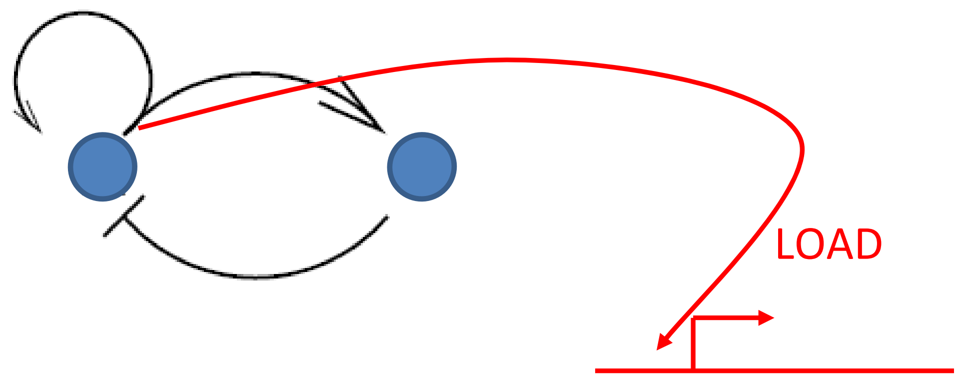
(Experimental Results)







Retroactivity!



Courtesy of Ninfa Lab at Umich

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

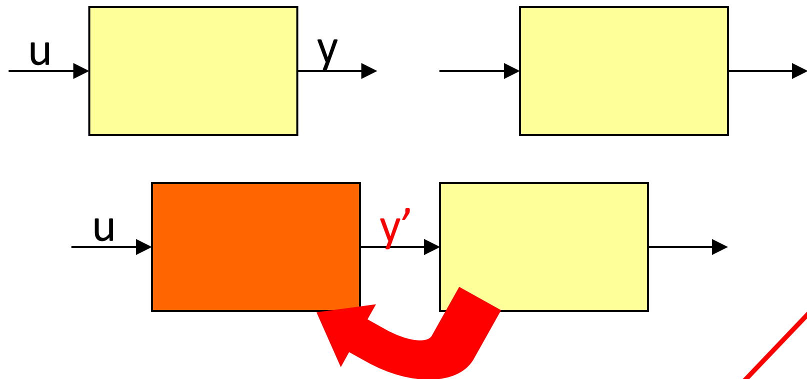
Part 2

The challenge of composing modules together

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

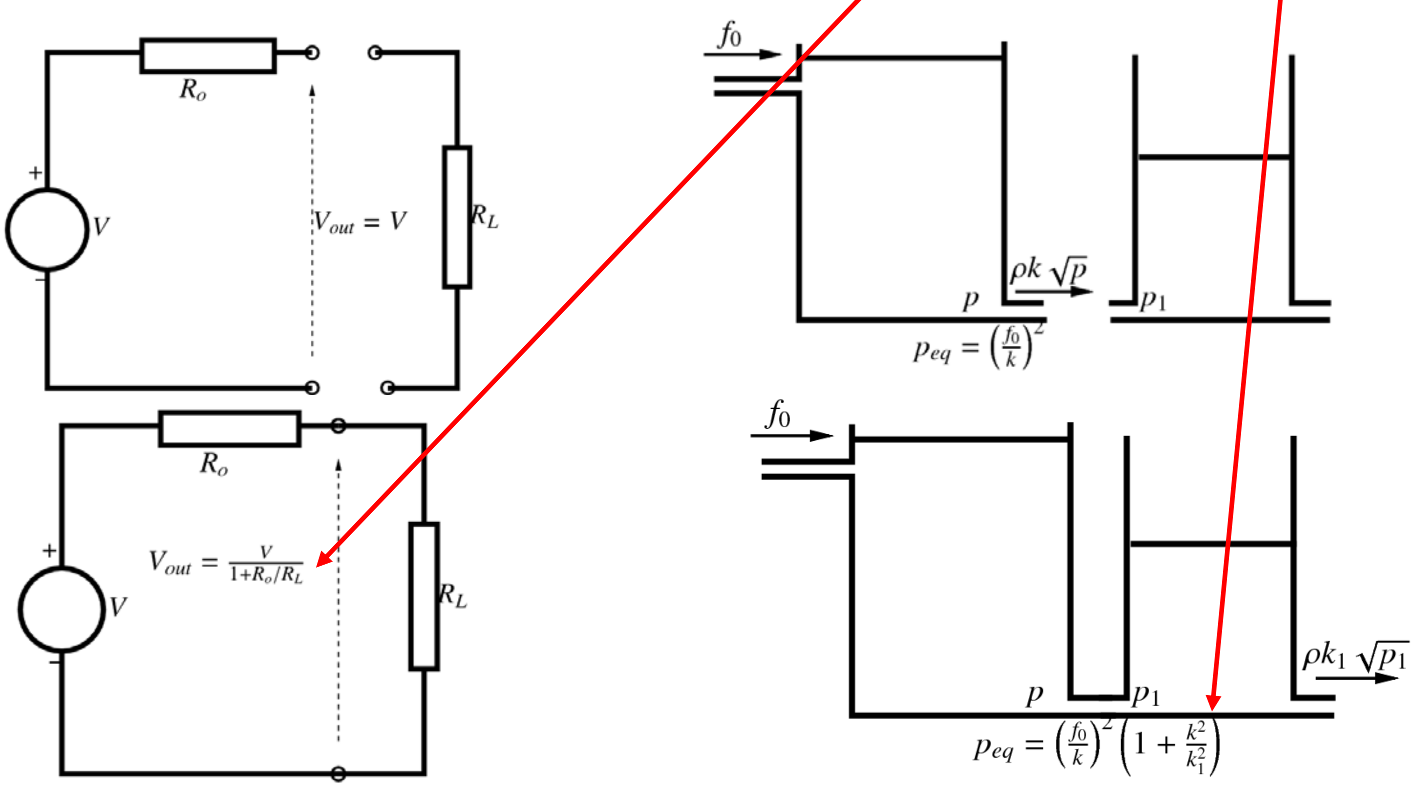
A systems theory with retroactivity

Basic Idea:

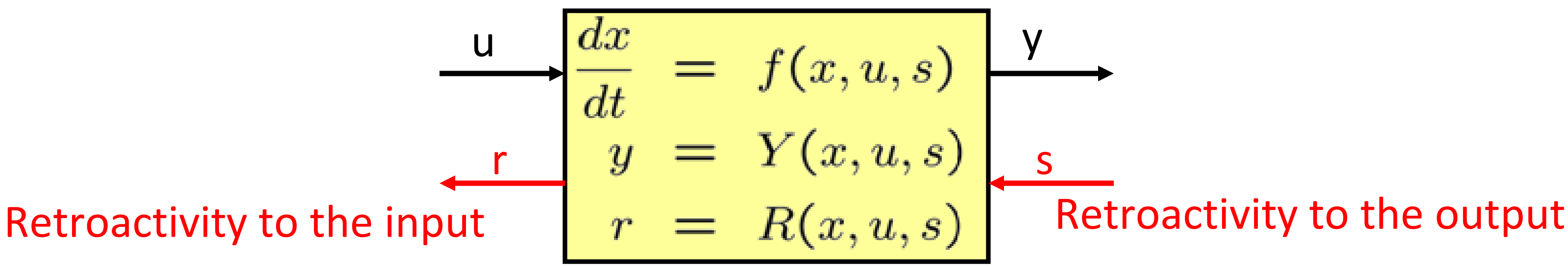


The interconnection changes the behavior of the upstream system

Familiar Examples:

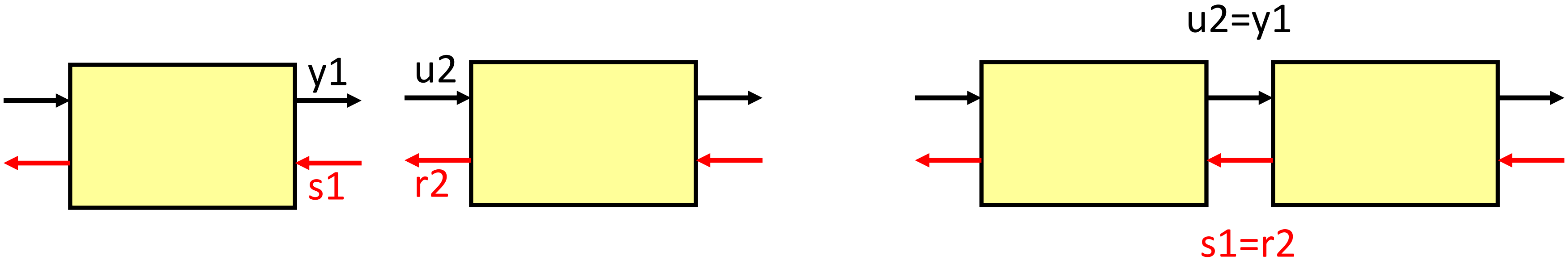


A systems theory with retroactivity

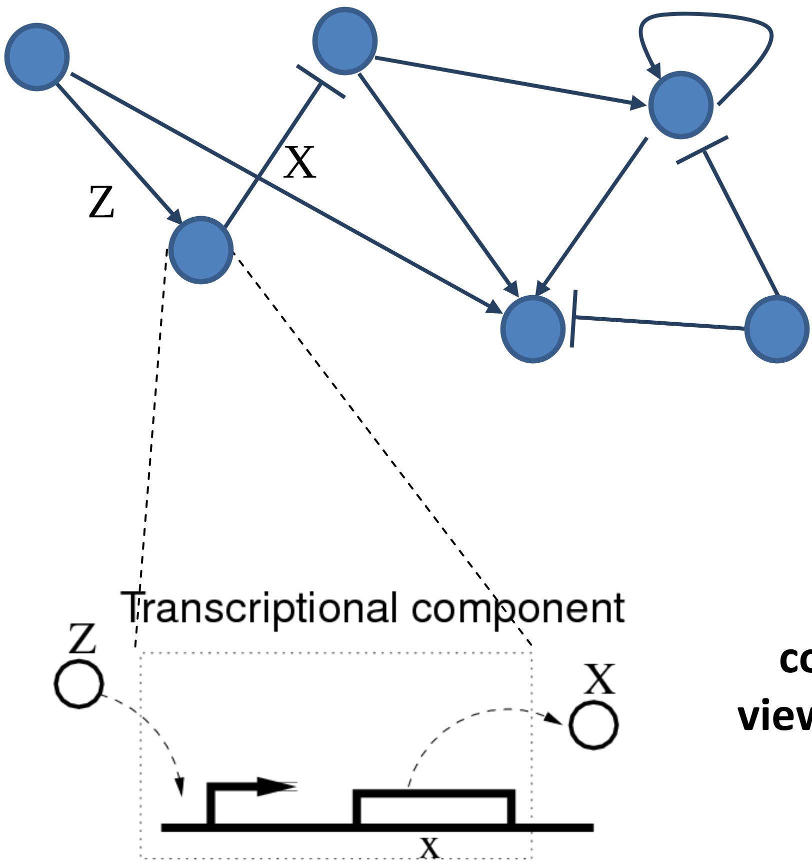


<u>Def:</u> The I/O model of the **isolated system** is obtained when s=0 and when r is not an additional output

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



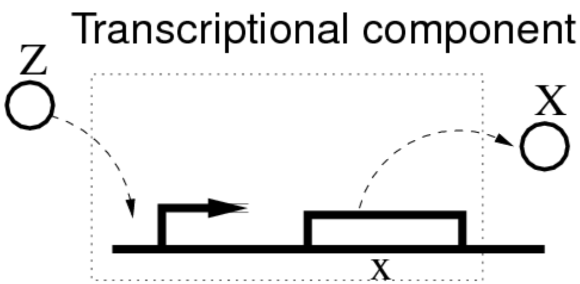
Gene regulatory circuitry: A network of transcriptional modules



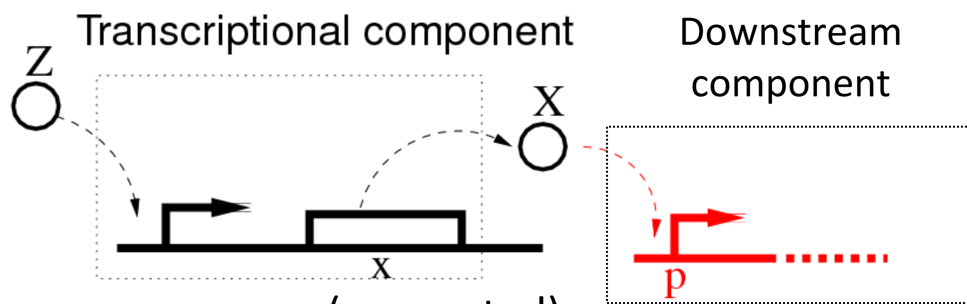
A transcriptional component is typically viewed as an input/output module

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

Retroactivity in transcriptional networks has dramatic effects on the dynamics



(isolated)



(connected)

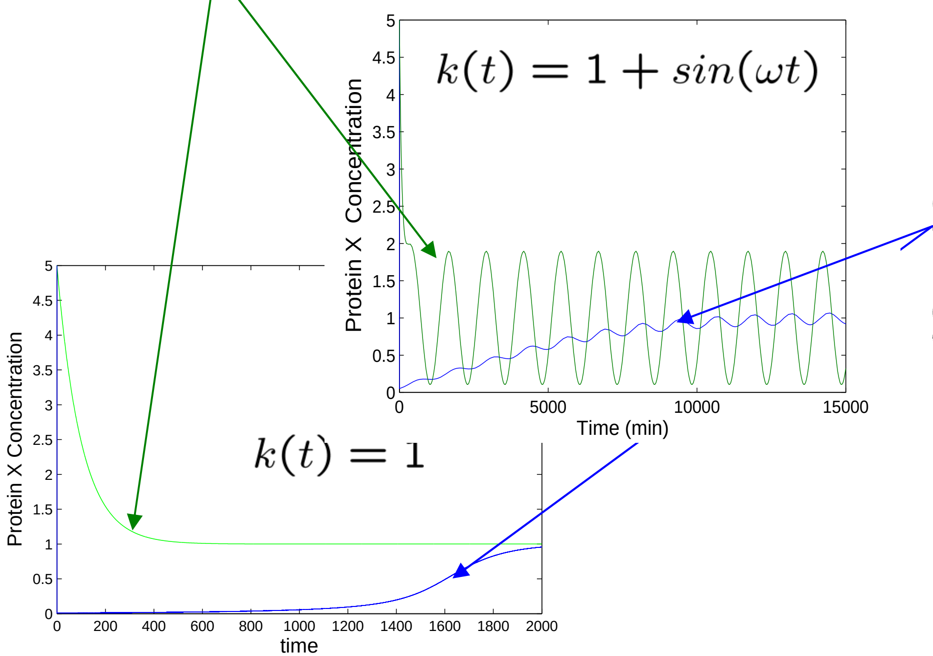
$$\frac{dX}{dt} = k(t) - \delta X$$

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

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

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

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



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)

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

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

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 $k_{on} = k_{off}/k_d$

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

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

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

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

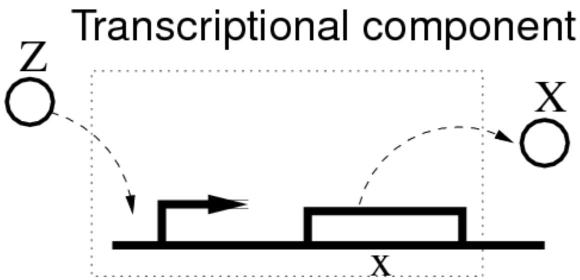
$\bar{s} = 0 \Leftrightarrow \frac{d\gamma(\bar{y})}{d\bar{y}} = 0 \longrightarrow$ we take $\frac{d\gamma(\bar{y})}{d\bar{y}}$ as a measure of retroactivity

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

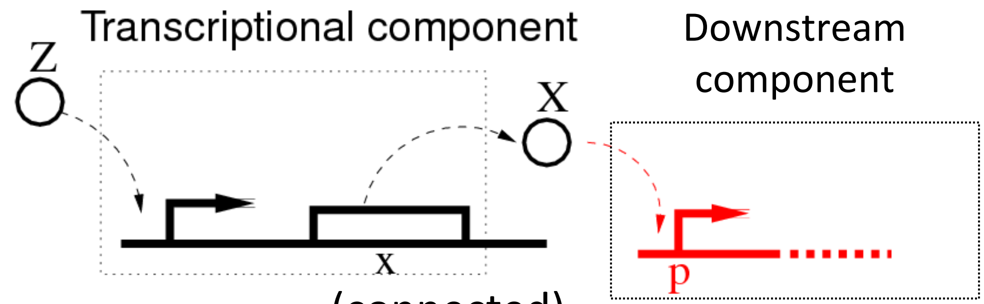
The value of the retroactivity measure for the interconnection through transcriptional regulation

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

Effect of R(X) on the dynamics



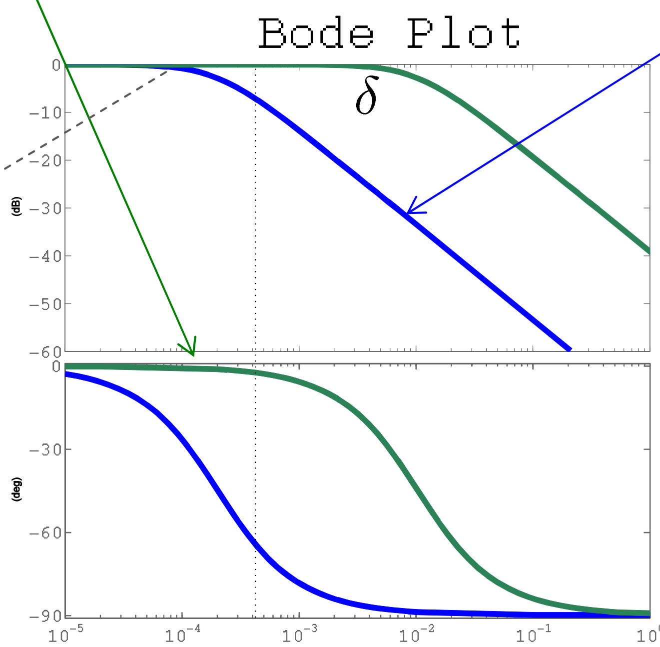
(isolated)



(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

Is this finding experimentally relevant?

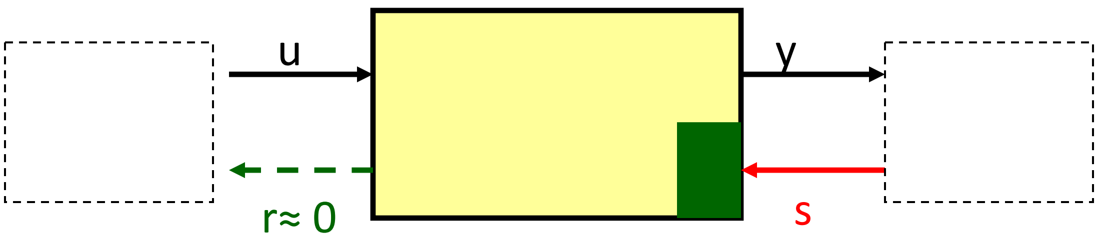
Part 2

The challenge of composing modules together

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

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.



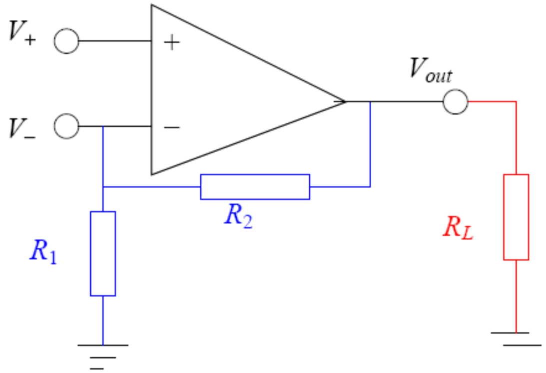
1. The retroactivity to the input is approx zero: r≈0

2. The retroactivity to the output s is attenuated

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

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

Non-inverting amplifier:



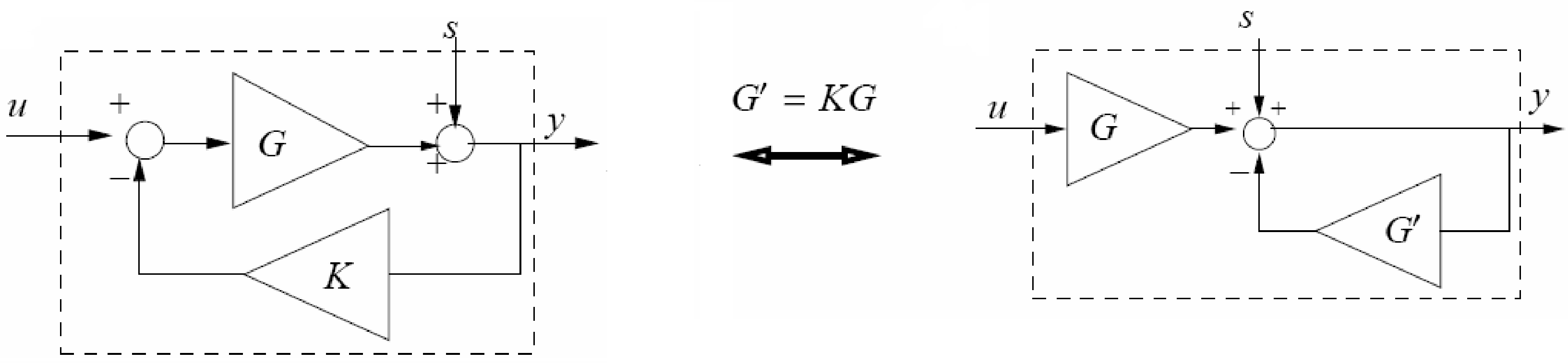
$$V_{out} = G(V_+ - V_-)$$

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

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

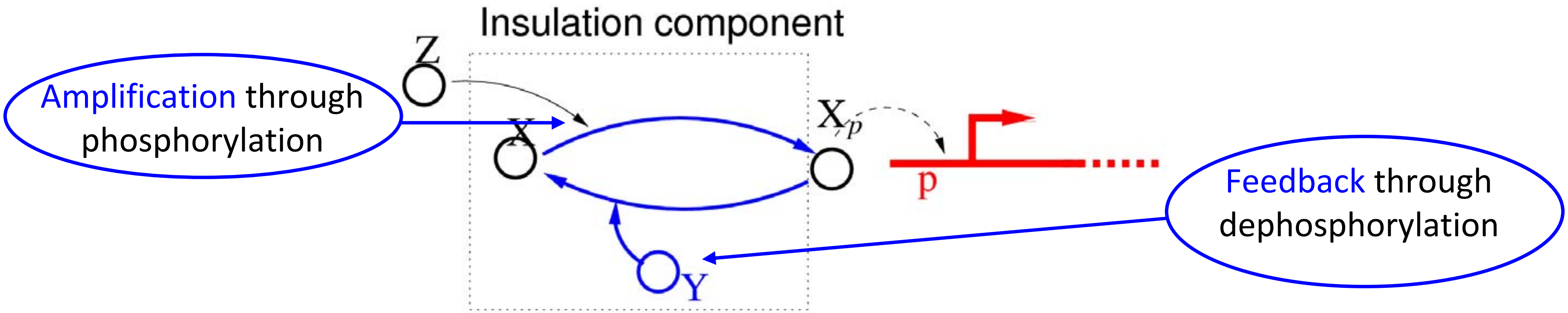
in which $G(t) \geq G_0 > 0$ and $|u'(t)| \leq V$ uniformly in t. Then,

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

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?

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



$$Z + X \xrightarrow{k_1} Z + X_p, \quad Y + X_p \xrightarrow{k_2} Y + X$$

with conservation law $X + X_p + C = X_{TOT}$

$$\frac{dX_p}{dt} = k_1 X_{TOT} Z(t) \left(1 - \frac{X_p}{X_{TOT}} - \boxed{\frac{C}{X_{TOT}}}\right) - k_2 Y X_p + \boxed{k_{off} C - k_{on} X_p (p_{TOT} - C)}$$

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

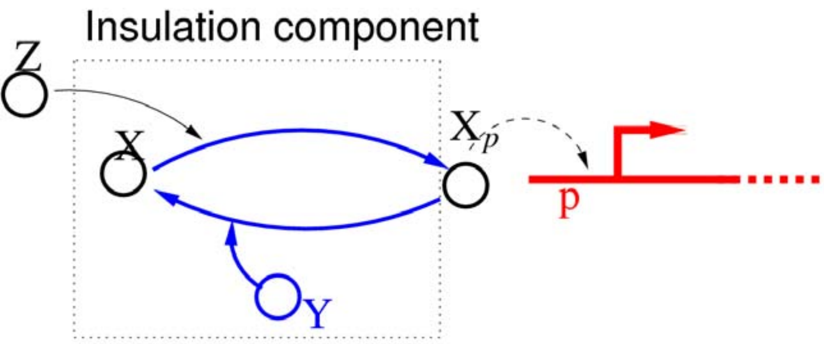
s

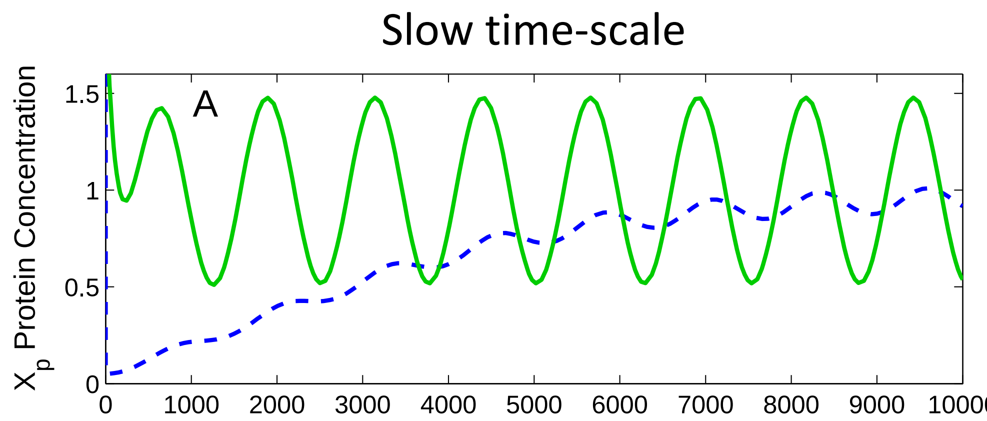
$$\epsilon := k_1 X_{TOT}/k_{off}, \; p_{TOT} \ll X_{TOT}, \; X_p \ll X_{TOT}$$

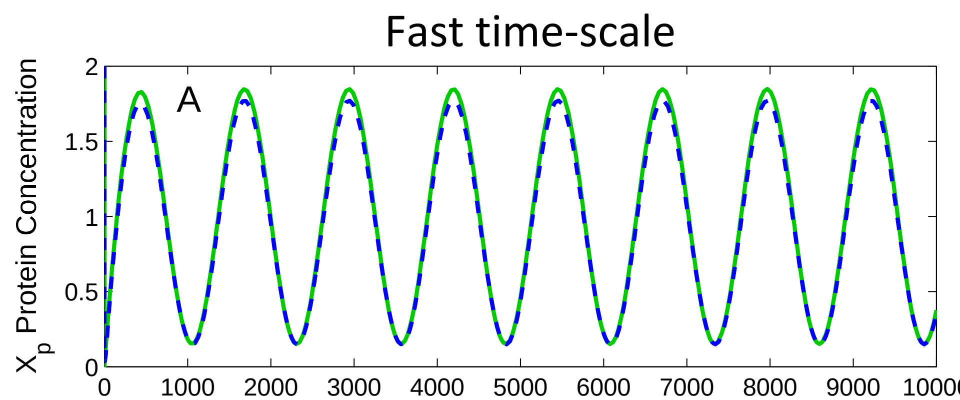
$$\frac{d\bar{X}_p}{dt} = (GZ(t) - G'\bar{X}_p)(1 - \mathcal{R}(\bar{X}_p)) \qquad G = k_1 X_{TOT}, \; G' = k_2 Y$$

As G and G' grow, $\bar{X}_p(t)$ tends to $X_p(t)$ given by the isolated system

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

Del Vecchio and Jayanthi, CDC 2008

Part 4

Fabrication Technology

Parts, Devices, Systems

THE ABSTRACTION ADVANTAGE

Biological engineers can benefit from methods that made very large scale integrated (VLSI) electronics practical for the semiconductor industry. Standardization of technologies allowed chip engineers to specialize in circuit design or fabrication and to thereby manage complex problems at different levels of abstraction. Bio fab engineers can also cope with complexity by using abstraction hierarchies to hide unnecessary information. Thus, a bio fab designer working at the level of whole systems need worry only about which devices to include and how to connect them to perform the desired function without having to manufacture each device from scratch. Similarly, a device-level designer should know the functions and compatibility of individual parts within a device, whereas a parts-level engineer should understand how each part works internally but need not be able to synthesize its DNA raw material.

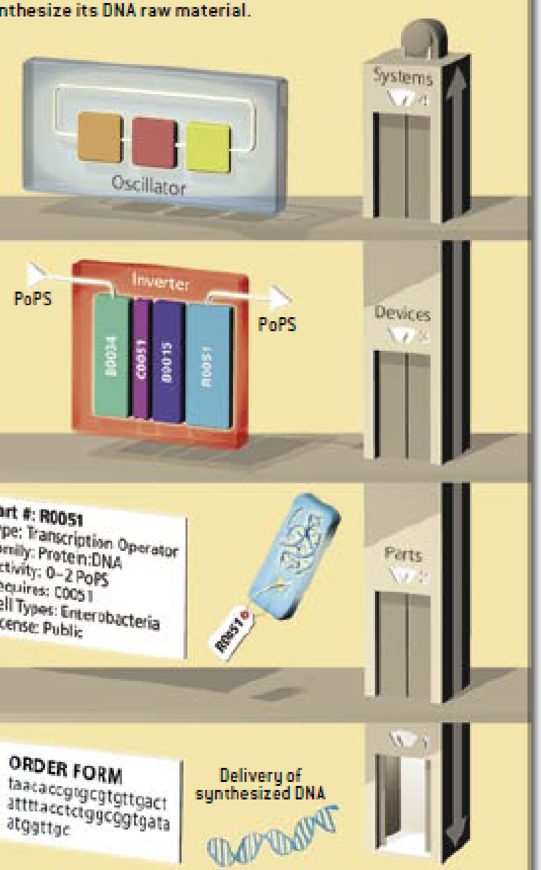
ABSTRACTION HIERARCHY

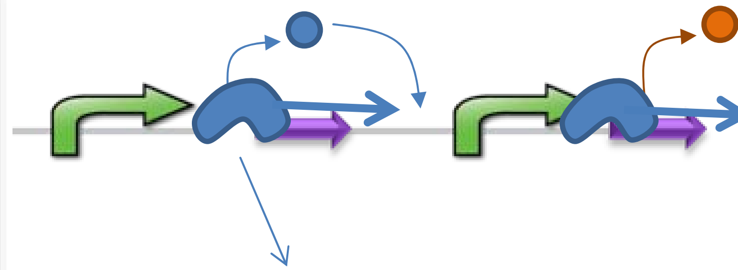
Systems
Combinations of biological devices that perform functions encoded by humans. A system of three inverters, for example, can operate as an oscillator.

Devices
Combinations of parts that perform discrete tasks. One inverter can take an input signal—for example, "HIGH"—and convert it to the opposite output signal, "LOW." A common signal carrier standard, polymerase per second (PoPS), allows devices to more easily be combined into systems.

Parts
Genetic material encoding biological functions. A transcription operator such as part #R0051, for example, is a piece of DNA that works with a matching binding protein (#C0051 in this case) to regulate gene activity. Off-the-shelf parts with clear specifications can be combined in a variety of devices.

DNA
Sequences for genetic parts. These can be specified by parts designers, manufactured off-site, then delivered. Fast synthesis technologies with low error rates make fabrication of custom DNA quick and reliable.



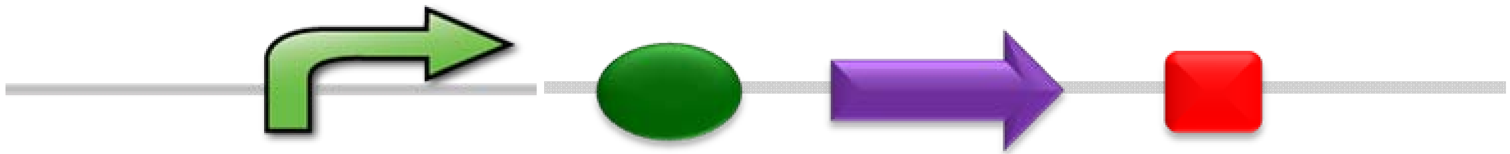


RnaPolymerase

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

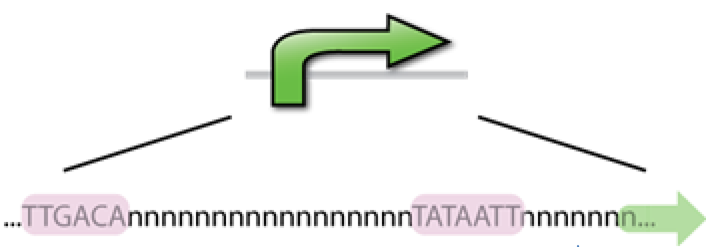
Library of standard Parts: Biobricks



Promoter **Ribosome Binding Site** **Protein Coding Sequence** **Terminator**

Catalog of parts & devices

Promoter



-35 -10

Region where RNAp binds to initiate transcription

Operator sites:
Regions where regulatory proteins bind

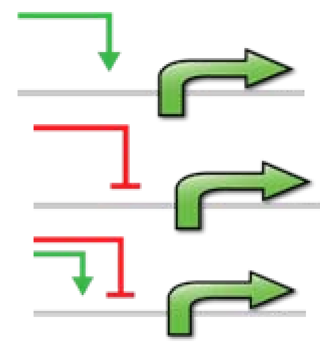
A promoter can be:

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

(2) Positively regulated

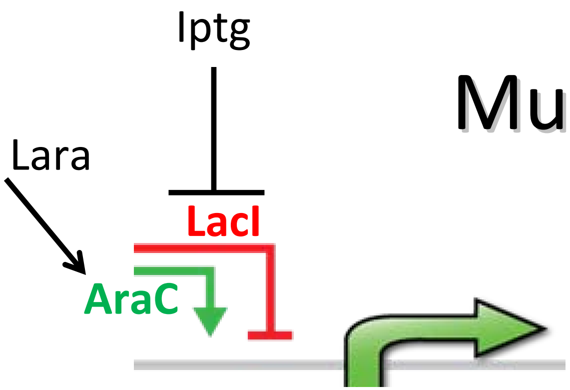
(3) Negatively regulated

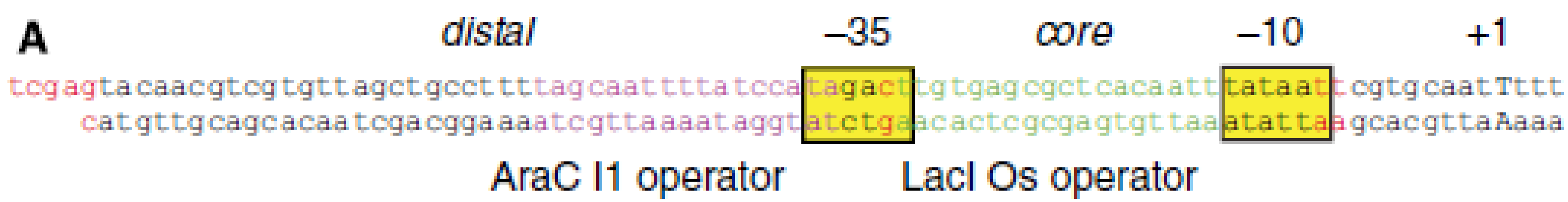
(4) Multi-regulated
Combinatorial promoters

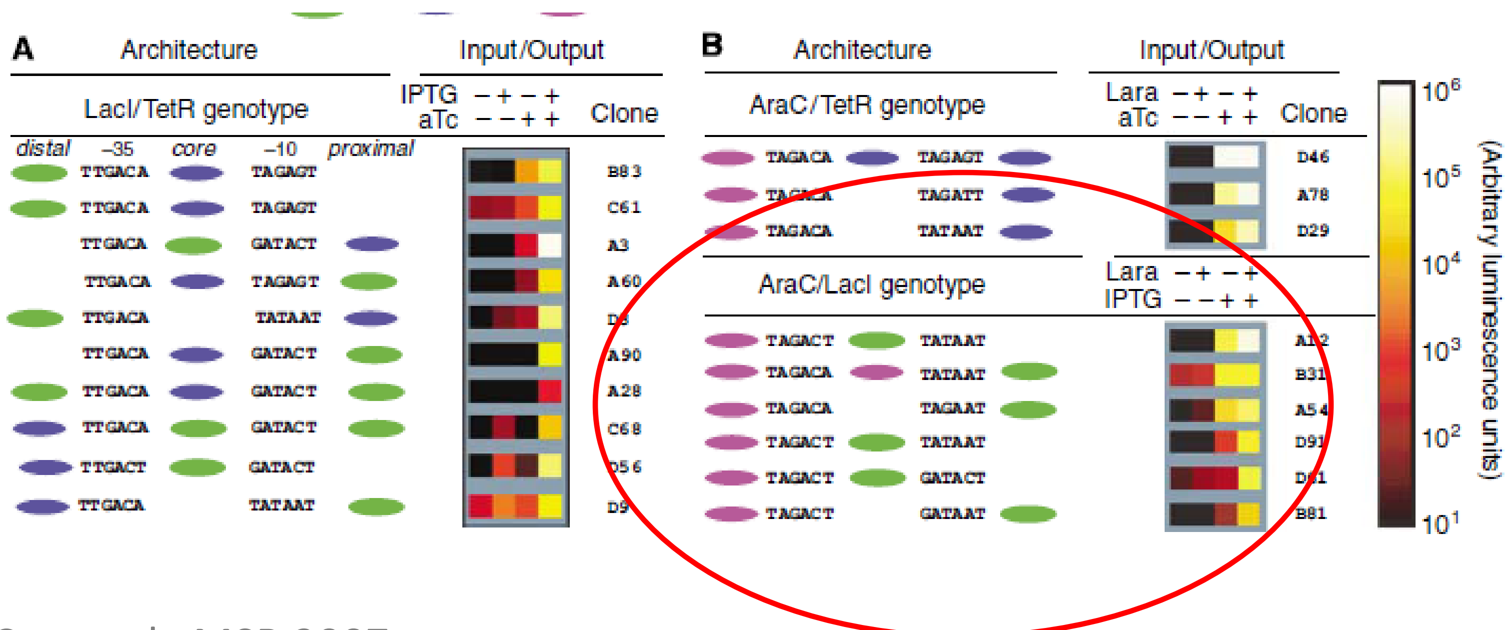


http://partsregistry.org/

Multi-regulated promoters

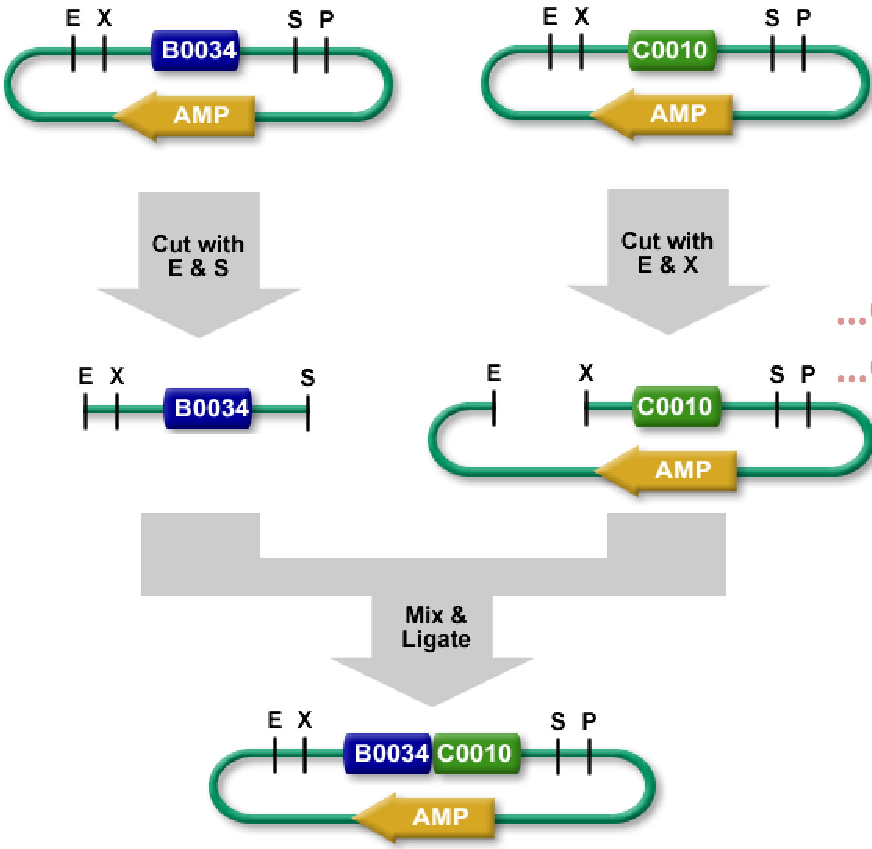






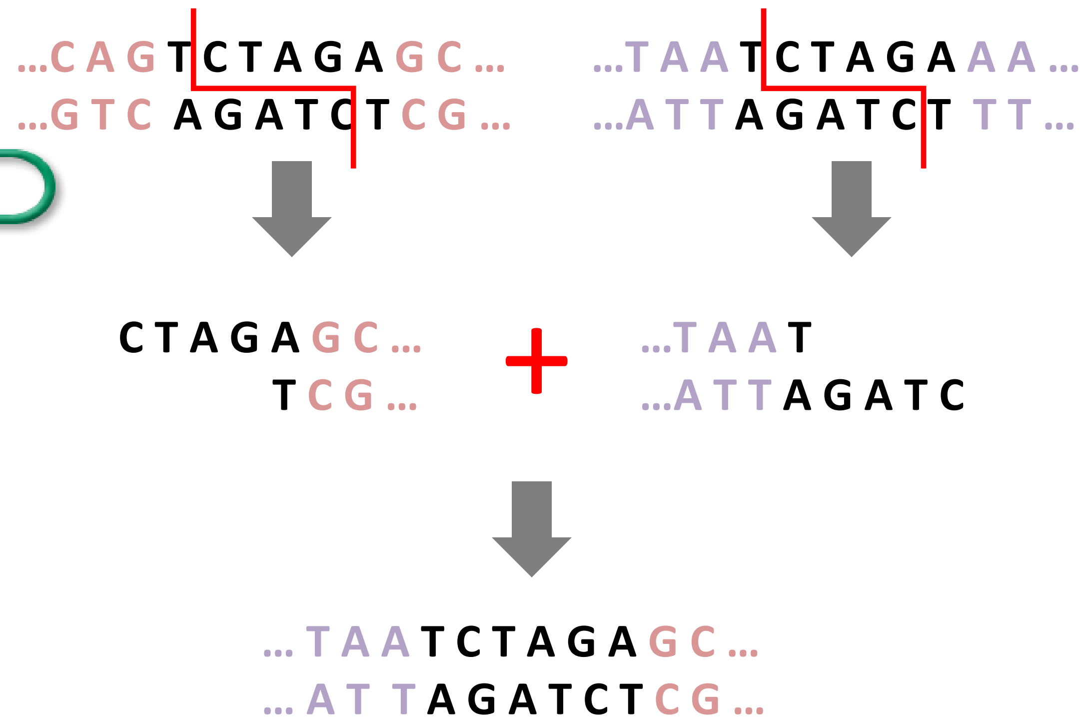
Cox et al. MSB 2007

Biobrick standard assembly



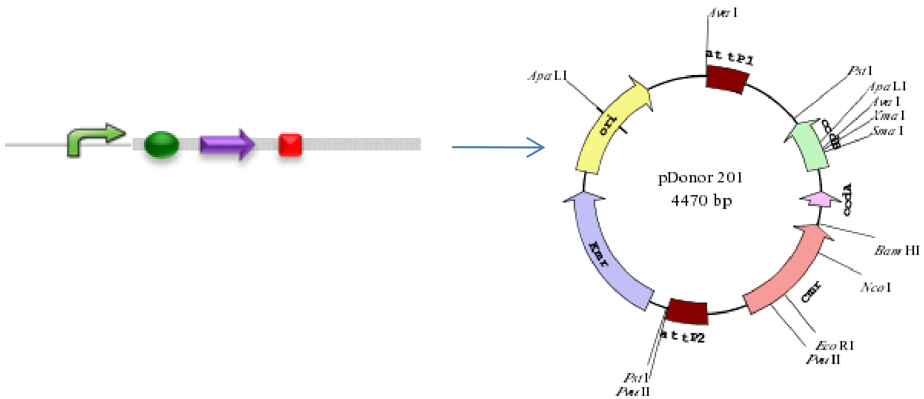
Restriction sites: E=EcoRI; X=XbaI
S=SpeI; P=PstI

Example: cutting with EcoRI

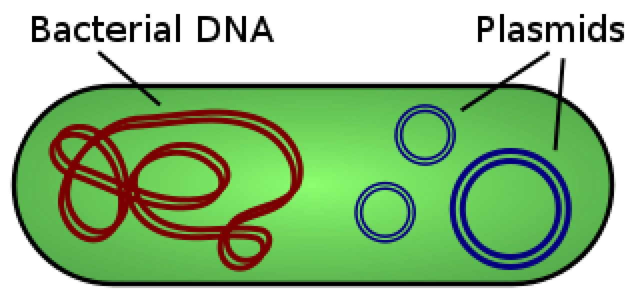


Any two biobricks can be combined in any order to form a new biobrick → Modular assembly

In vivo Implementation



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



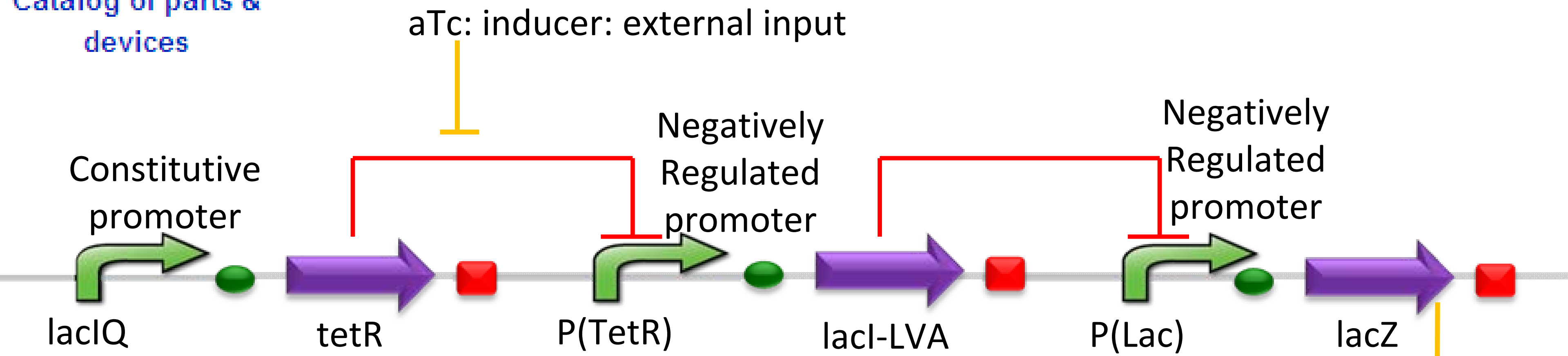
Transformation: process of inserting the plasmid within in the cell. It occurs by rendering the cells *competent,* (external membrane becomes permeable).

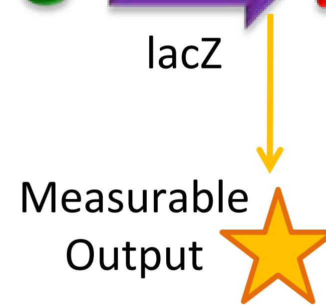
Inducers: signaling molecules that bind to repressors and disable them. The net effect is to start transcription. They can be added to the cell population to provide <u>input forcing</u> to the circuit

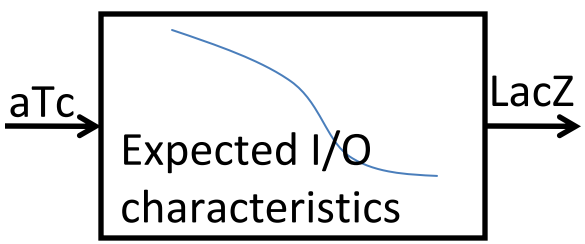
Reporter Genes: express proteins that produce an easily observable phenotype, for example, green fluorescent proteins, which causes cells to fluoresce green under blue Light. They are inserted after a gene of interest to measure its production rate. They Provide an easily <u>measurable output</u> to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

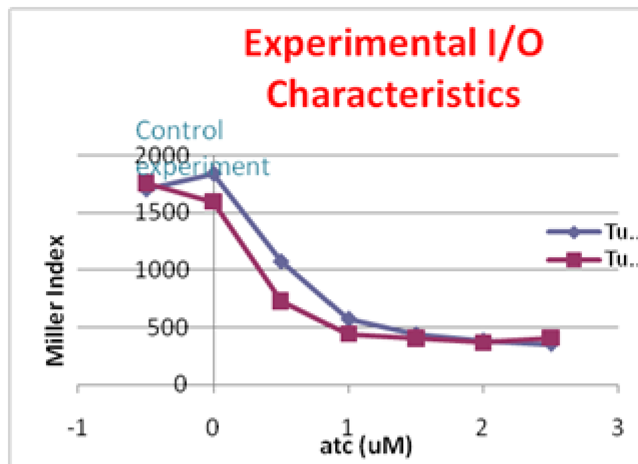
Example of a device: An inverter

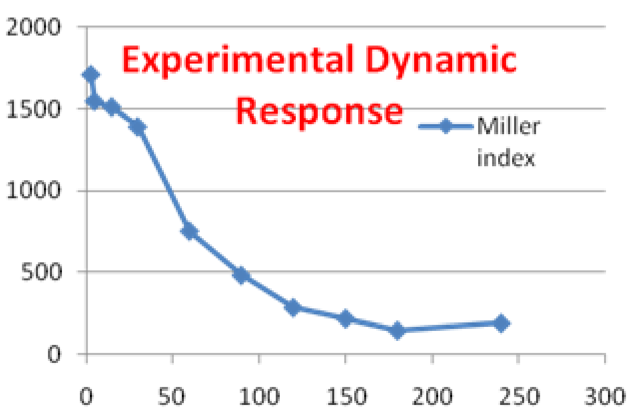
Catalog of parts & devices









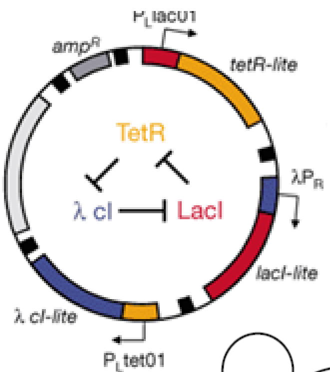


Expected dynamic response

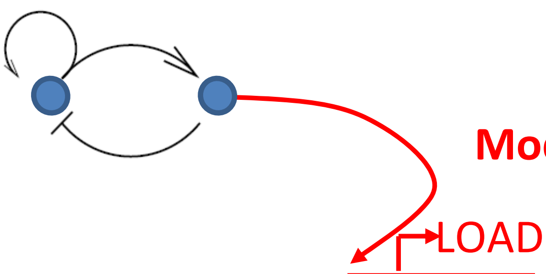
$$LacZ(t) \approx K * aTc * (1 - e^{-\alpha t})$$

Summary

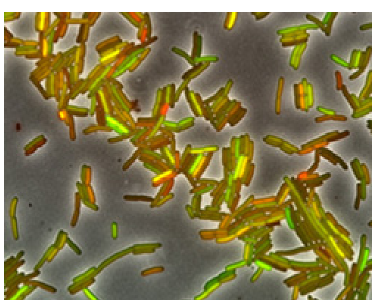
The ability of fabricating bio-molecular circuits has far reaching applications: medical and energy are two examples



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



Modular design is a grand challenge



Stochastic behavior is an integral part of these systems and must be explicitly considered for design

Acknowledgements

- Alex Ninfa
- Eduardo Sontag
- Peng Jiang
- Shridhar Jayanthi

- Funding: AFOSR

Wrap-up: Challenges and Opportunities for Control Theory

- Stochastic behavior: Bio-molecular systems are intrinsically stochastic
- Complexity : large state spaces, large number of parameters
- Modularity: tool for analysis/design, input/output descriptions
- Uncertainty: functional systems from uncertain components?
- Redundancy: a way to obtain robustness?
- Crosstalk: any circuit working in the cell environment uses up cellular resources
- …learning to communicate with biologists