APPLICATIONS TO BIOLOGY

Short Stories

All characters appearing in this work are purely fictitious.

Any resemblance to any person living or dead is purely coincidental.

All dialogues are fictitious and resemblance to real events is also purely coincidental.

...kind of

THE CAST

The control engineers

Domitilla Del Vecchio
Elisa Franco
Frank Doyle

The Biologists

Alexander Blake ..... played by Elisa Franco
William Smith ..... played by Frank Doyle
John White ..... played by Domitilla Del Vecchio

THE STORIES

Modularity in the Design of Biological Circuits in Living Cells

The control engineer..... Domitilla Del Vecchio
The biologist (Alexander Blake)..... Elisa Franco

The 'Wind Tunnel' idea for Engineering Biological Circuits

The control engineer..... Elisa Franco
The biologist (William Smith)..... Frank Doyle

Biological Clocks: From Natural to Engineered ones

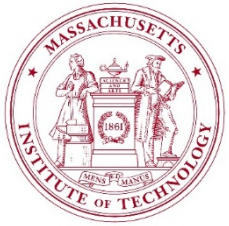
The control engineer..... Frank Doyle
The biologist (John White)..... Domitilla Del Vecchio

Modular Design of Biological Circuits in Living Cells

Domitilla Del Vecchio

Mechanical Engineering

MIT



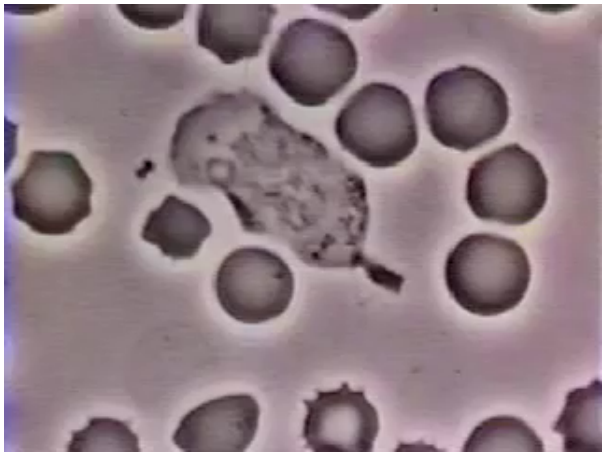
CDS@20, August 2014

Why to design biological circuits?

ALTERNATIVE ENERGY
(bio-fuels)
Engineering bacteria that…
- Produce hydrogen or ethanol
- Transform waste into energy

COMPUTING APPLICATIONS
(molecular computing)

MEDICAL APPLICATIONS
(targeted drug delivery)



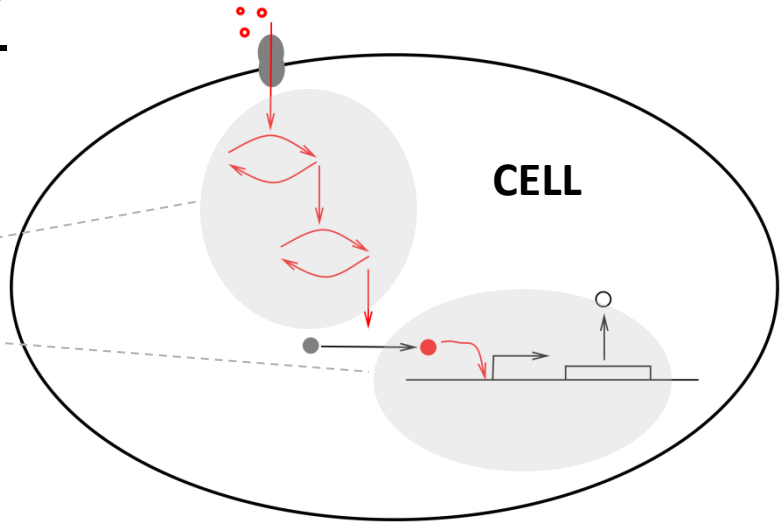
BIO-SENSING
(detecting pathogens or toxins)

What is a biological circuit?

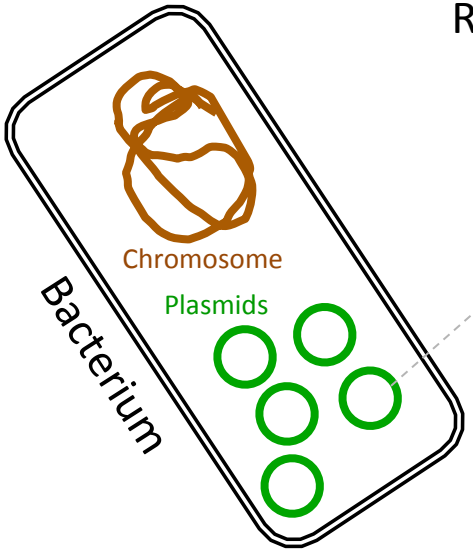
It is a network of activation and repression interactions between genes and proteins

Examples: Signal transduction networks
Gene transcription networks

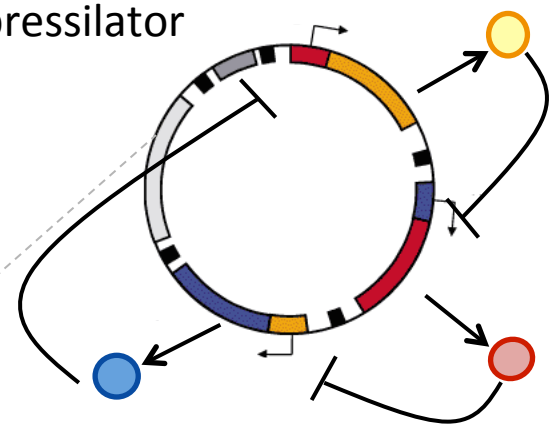
Signal carrier: protein amounts



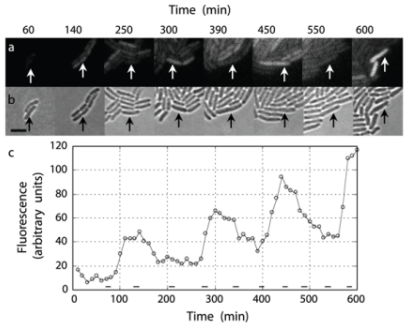
How to construct biological circuits: Synthetic biology



Repressilator

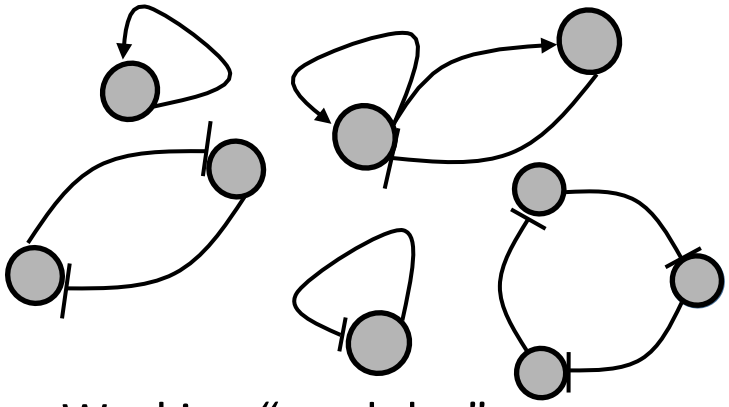


(Elowitz and Leibler, *Nature* 2000)



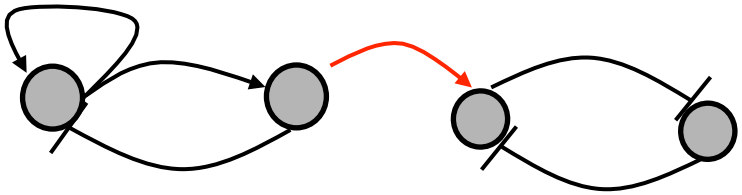
Fluorescence quantifies protein concentration

Bottom-up design of complicated systems is a major goal



Working “modules”

Bottom-up circuit design



Outline

ANALYSIS

Modularity and retroactivity

DESIGN

Insulation devices to attenuate retroactivity

- **DDV:** <<I would like to use your clock to create a multi- module system. We can start by connecting the clock to a slave that must be timed [DDV shows this on slide] We can use the activator as the output of the clock and use it as an input to the downstream system and...>>

- **WB:** <<Wait, wait, wait! What is an input? And an output? What the heck are you talking about?>>

- **DDV:** <<The activator is a species that we can use as a driving input for...>>

- **WB:**<<Again, input! What is an input? Input assumes that you are creating boundaries somewhere in the system. The activator is just a protein!!! I am lost!>>

- **DDV**:<<Ok, ok, let's try like this. I mean A is an input to the downstream system if it also activates some gene in the downstream system and... >>

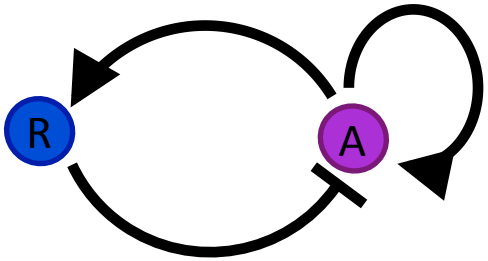
- **WB:**<<Ah, I see. You just want to add a gene with a promoter activated by A. [These engineers are weird...and what terminology they use...inputs! Ah!] Well, I bet if we do this, the clock will not work anymore.>>

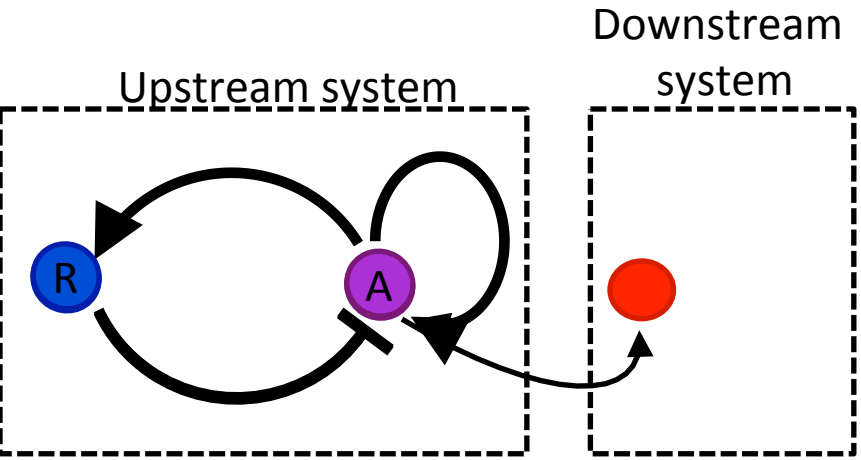
- **DDV:**<<What? This does not make any sense! A is just an input to the downstream module>>

- **WB:**<<Module, what is a module??? There are no boundaries in biological systems. Everything is just a mix of things. If I add more stuff to the clock, why should it still work??? Ahhh, you engineers! You think biology is like engineering and want to tell us how to handle biological systems? [laughing!] You have no idea what you are talking about. Go back home kiddo! Go back to work on your Electrical Circuits!!!>>

Does the clock really fail when 'connected'?

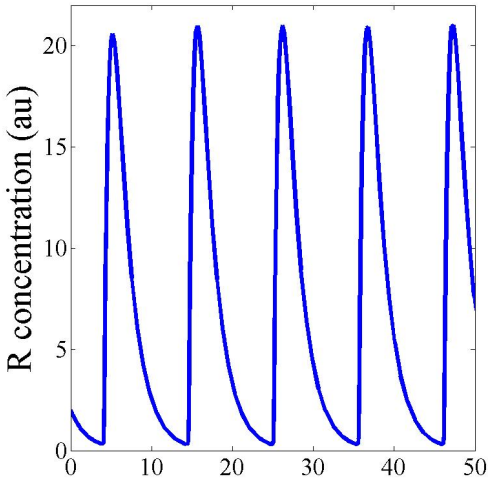
(Atkinson et al, *Cell* 2003)

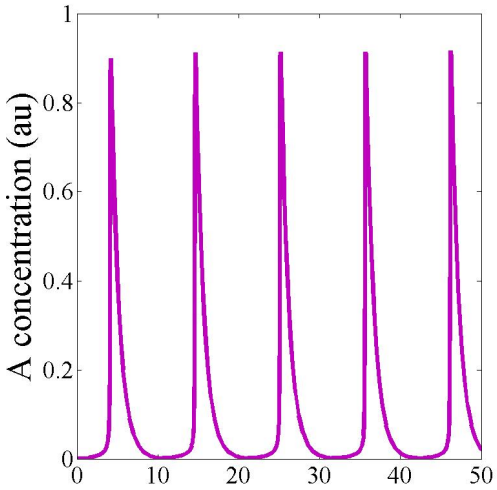


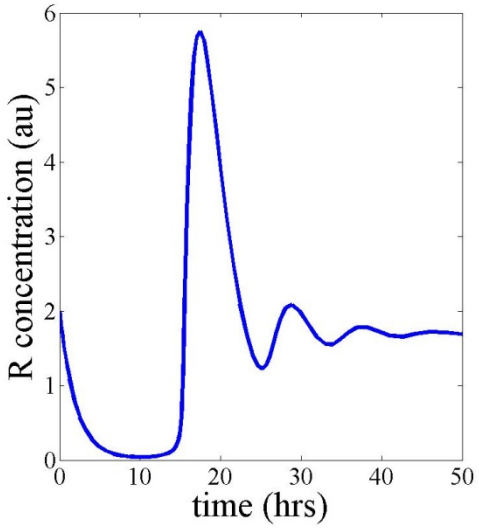


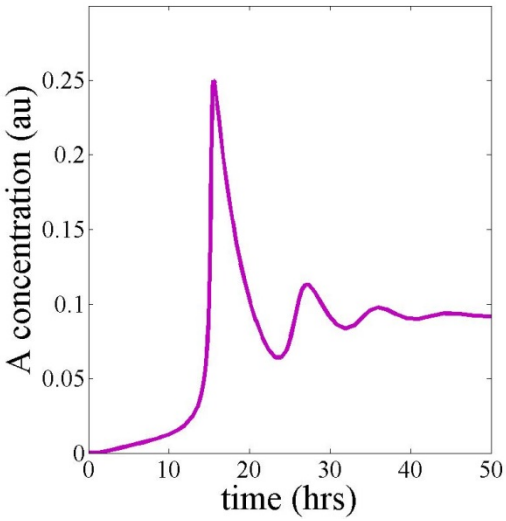
Retroactivity

(loading in biological circuits)





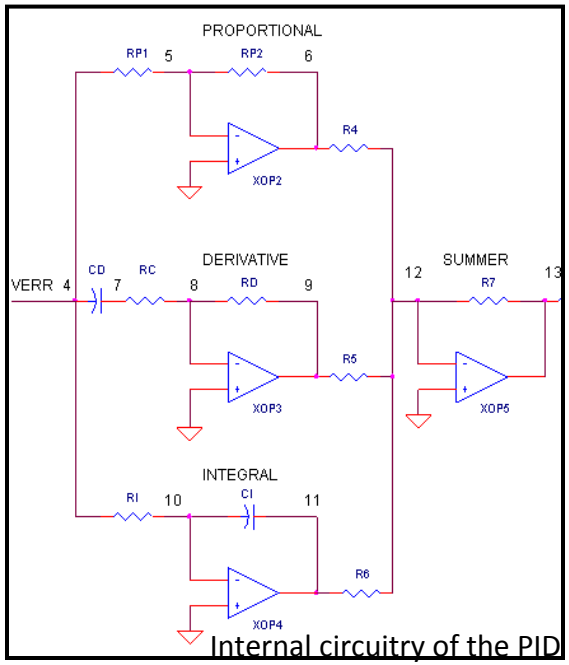




As a result, we fail to transmit the periodic signal to the downstream system

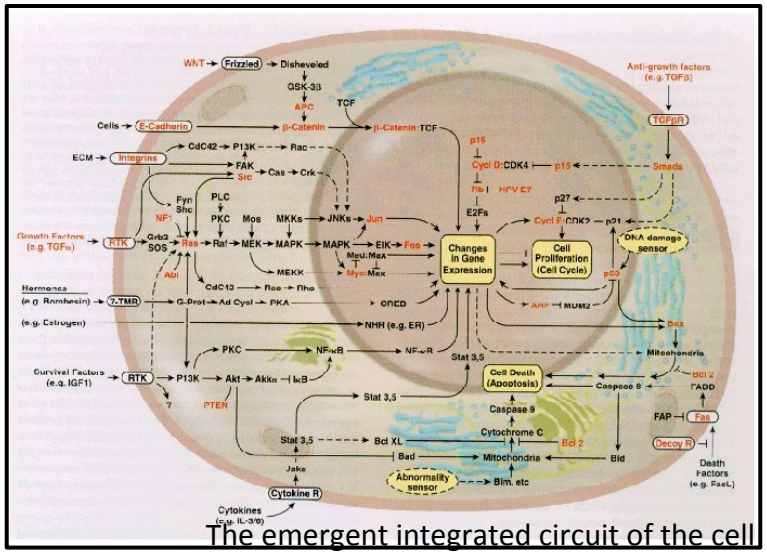
Modularity

Modularity guarantees that the input/output behavior of a system does not depend on the context (surrounding systems)



Internal circuitry of the PID

In electrical circuits, a module's behavior does not depend on the modules it connects to

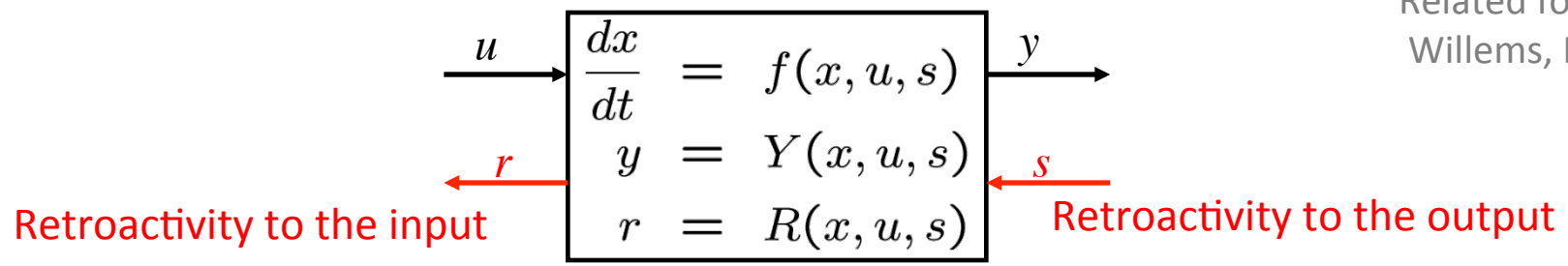


The emergent integrated circuit of the cell

Functional modules recur also in biological networks (e.g. Alon (2007)), but unfortunately, their functionality is often context dependent

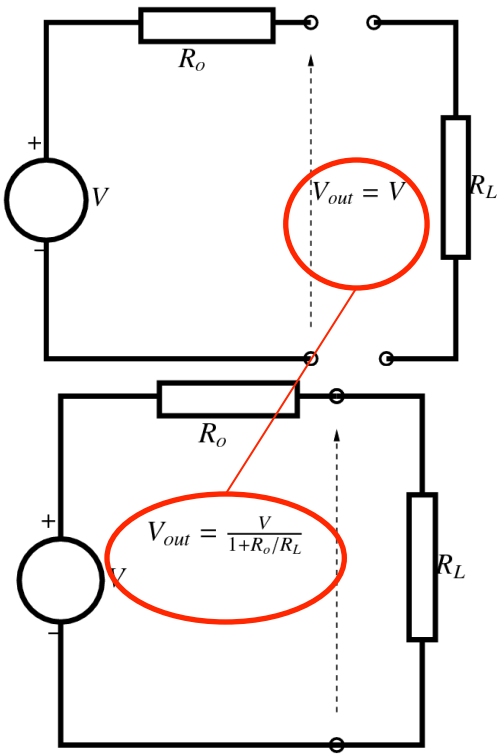
A "system concept" to explicitly model retroactivity

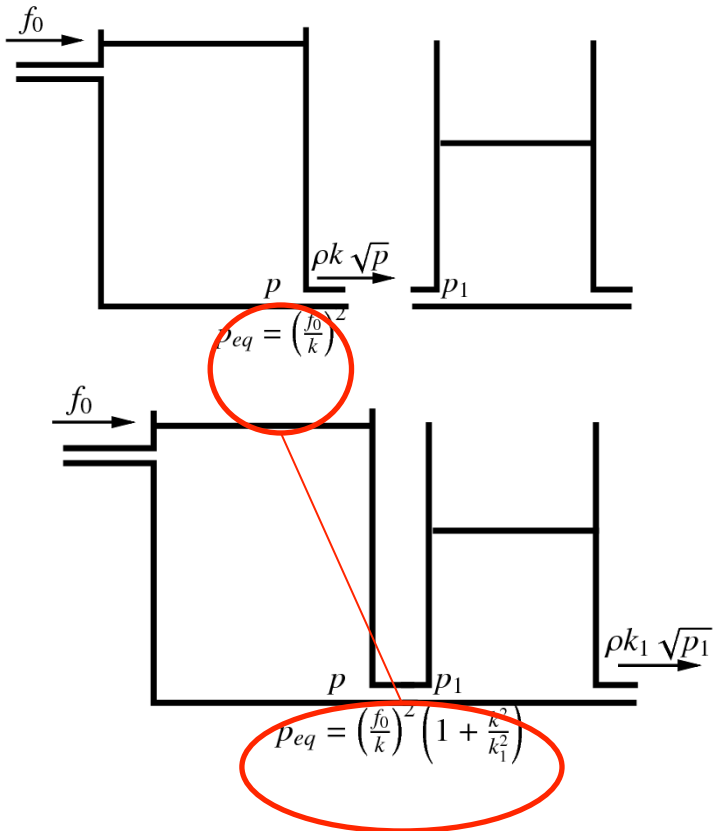
Related formalisms:
Willems, Paynter



Def: The I/O model of the **isolated system** is obtained when s=0

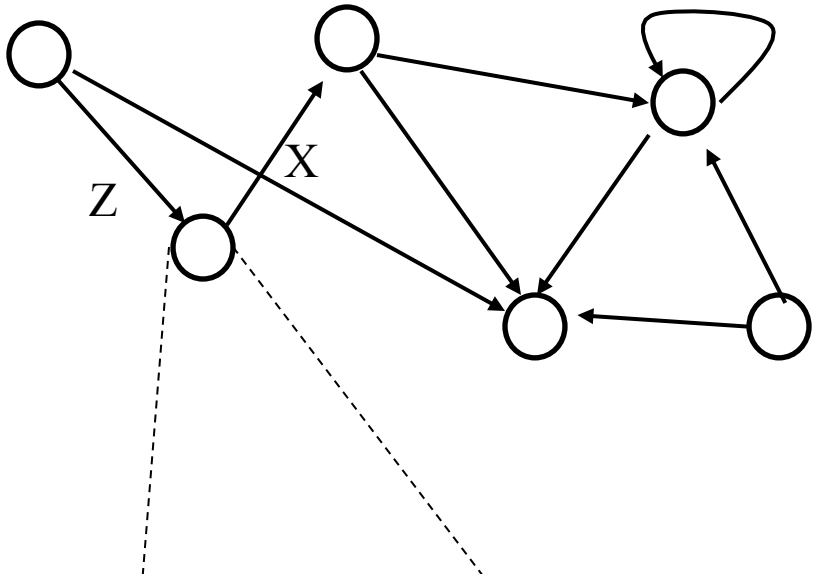
Familiar Examples:

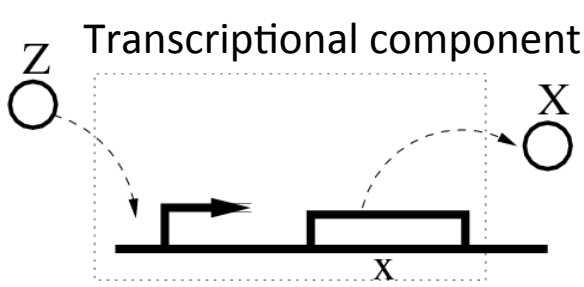




Del Vecchio and Sontag, *EJC* 2009

A genetic circuit is composed of interconnected transcriptional components

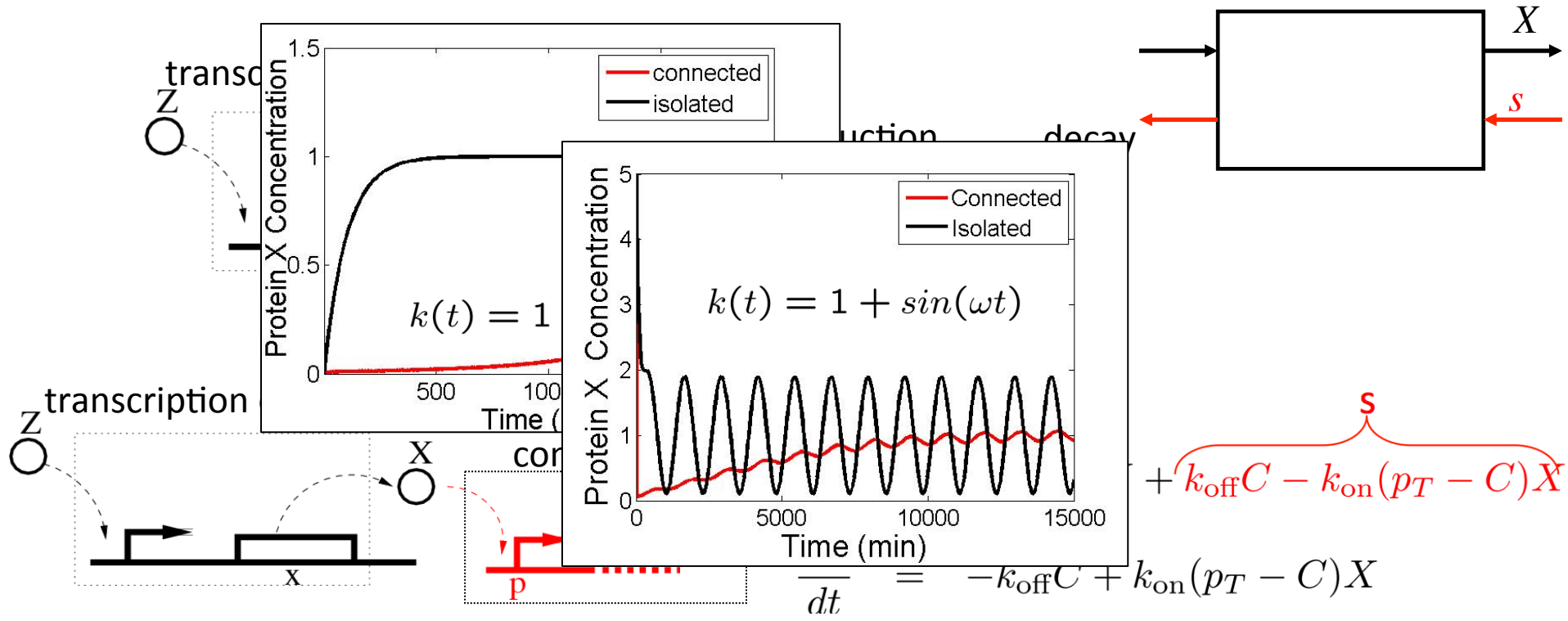




A transcriptional component is typically viewed as an input/output module

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

Retroactivity effects in a transcriptional module



total amount of site p

$$\mathcal{R}(X) = \frac{p_T/k_d}{(X/k_d + 1)^2}$$

dissociation constant

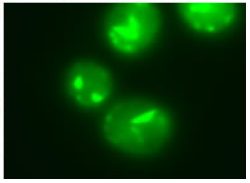
Use sing pert

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

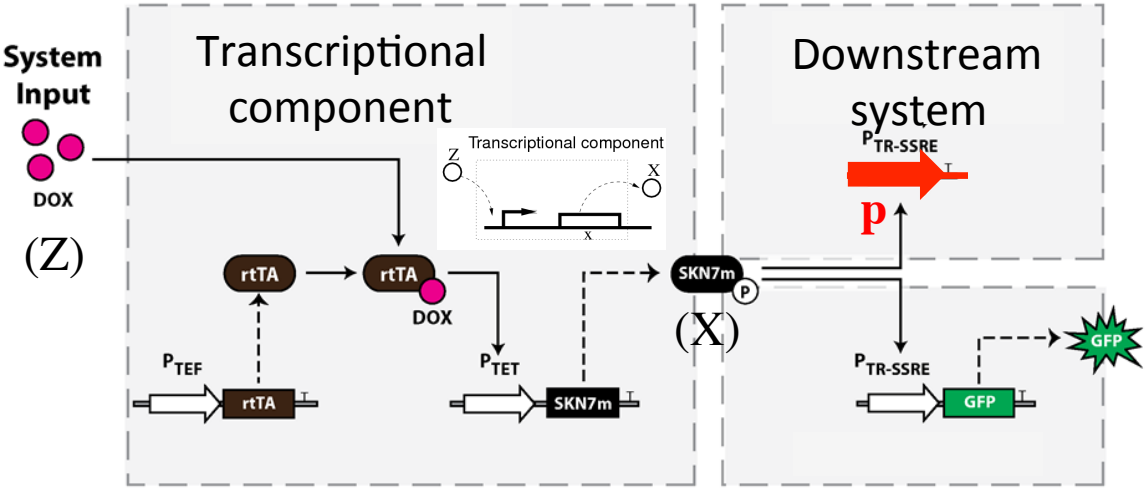
retroactivity measure

D. Del Vecchio, A. J. Ninfa, and E. D. Sontag, *Molecular Systems Biology,* 2008

A. Gyorgy and D. Del Vecchio, *PLOS Comput Biol* 2014 (Thevenin's'like thm for arbitrary networks)

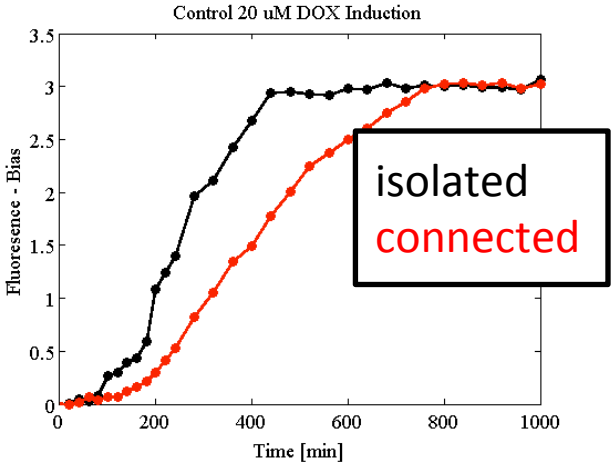


In vivo experiments (yeast)

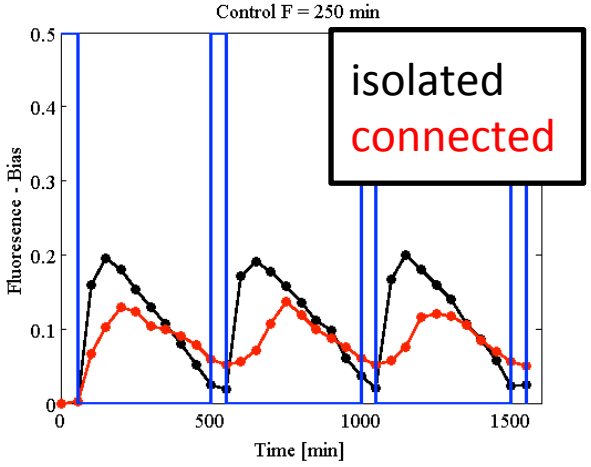


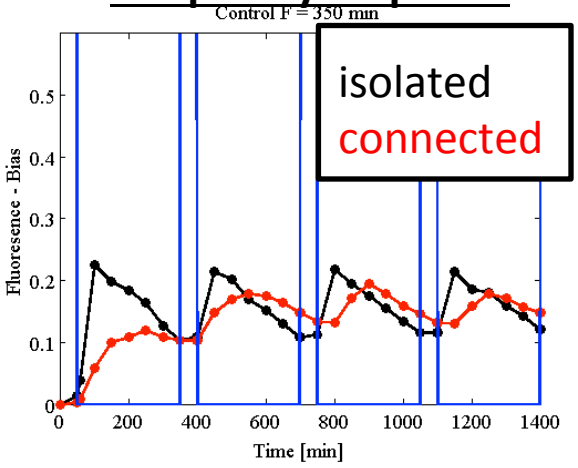
With Mishra, Rivera, and Weiss

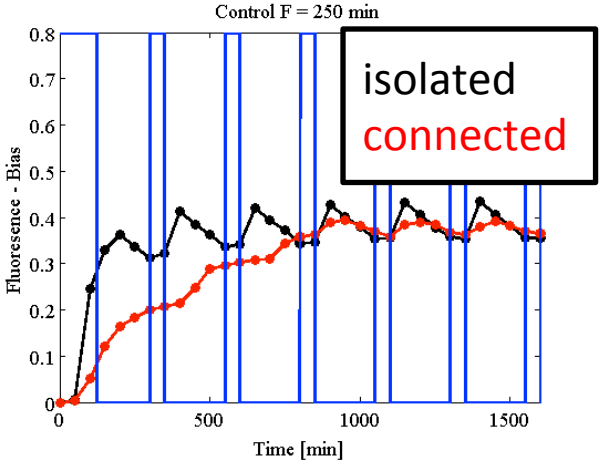
Step response



Frequency response







Retroactivity slows down the response of a transcriptional component (reduces bandwidth)

Has also been shown: in transcriptional systems in *E.coli* (Jayanthi et al. *ACS Synth Bio*, 2013)
in signaling systems *in vitro* (Jiang et al. *Science Signaling*, 2011)

Outline

ANALYSIS

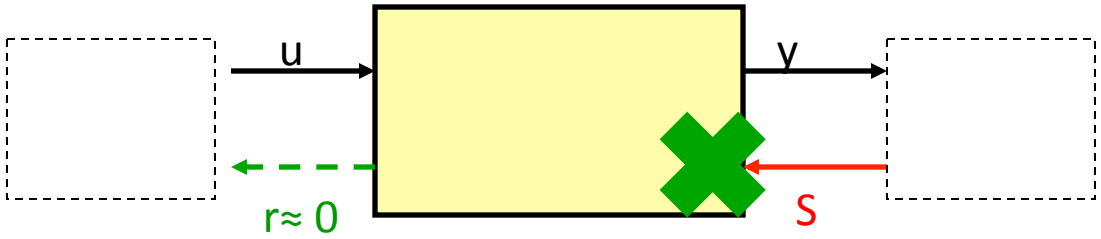
Modularity and retroactivity

DESIGN

Insulation devices to attenuate retroactivity

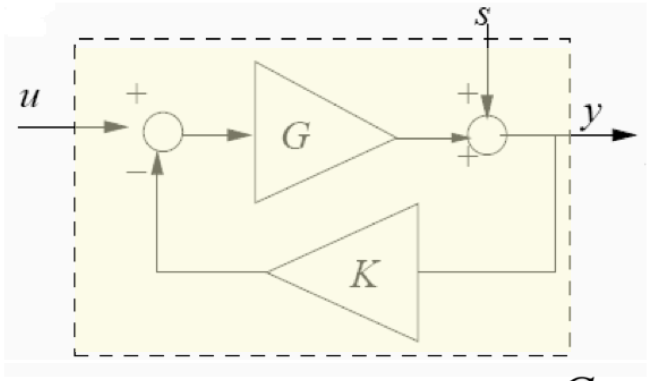
Insulation devices for attenuating retroactivity

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.

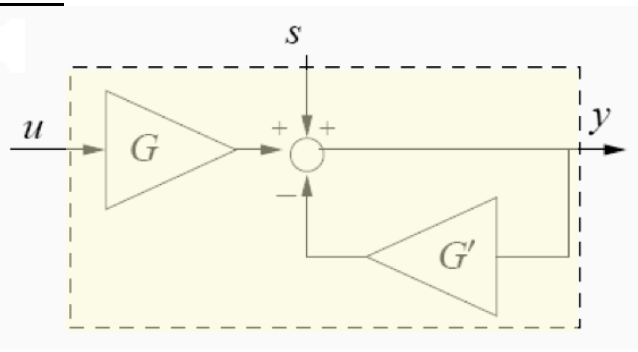


1. The retroactivity to the input is approx zero: r≈0
2. The retroactivity to the output s is attenuated

Principle 1: Retroactivity attenuation through high-gain feedback



$G' = KG$



as $G \to \infty$

$$y = G(u - Ky) + s \;\Rightarrow\; y = u\frac{G}{1+KG} + \frac{s}{1+KG} \to 0$$

Connected approximated system

$$\dot{\bar{X}} = (k(t) - \delta\bar{X})\left(\frac{1}{1+R(X)}\right)$$

Isolated system

$$\dot{X} = k(t) - \delta X$$

Apply large input amplification G and large output feedback G'

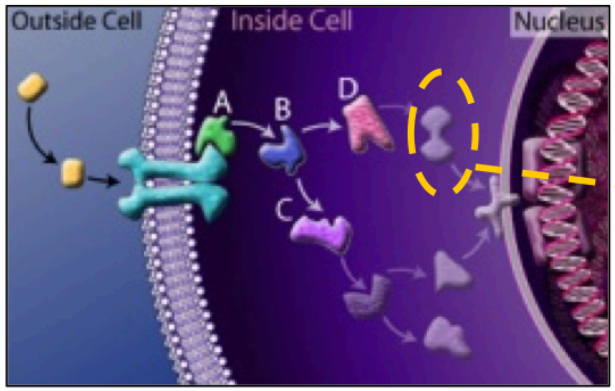
$$\dot{\bar{X}} = (Gk(t) - G'\bar{X})\left(\frac{1}{1+R(X)}\right)$$

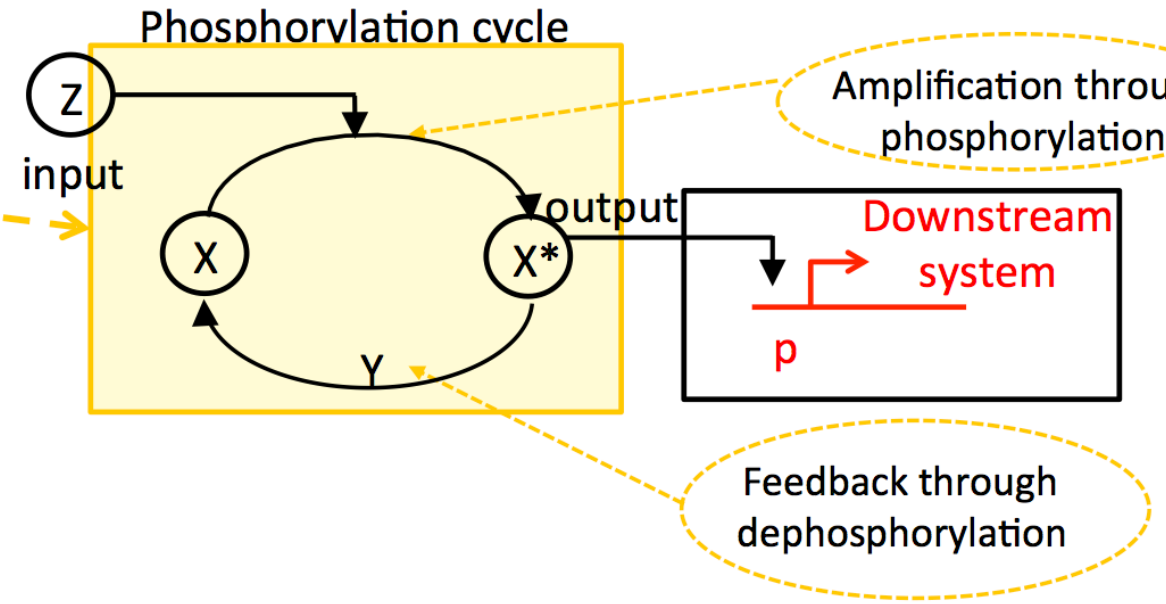
$$\dot{X} = Gk(t) - G'X$$

For $\dot{X} = G(t)(k(t) - KX)$ with $G(t) \geq G_0 > 0$ and $\dot{k}(t)$ bounded: $\left|X(t) - \frac{k(t)}{K}\right| \leq c_0 e^{-KG_0 t} + \frac{c_1}{G_0}$

as G grows, the signals $\bar{X}(t)$ and $X(t)$ become close to each other

Principle 1: implementation with phosphorylation

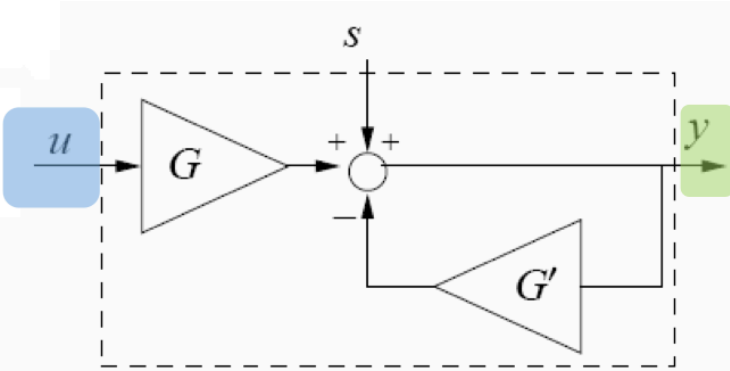




Using a simple one-step reaction model for
the phosphorylation and dephosphorylation reactions:

$$\dot{X}^* = (k_1 X_T \left(1 - \frac{X^*}{X_T}\right) Z(t) - k_2 Y X^*) \left(\frac{1}{1 + R(X^*)}\right)$$

G **G'**



A phosphorylation cycle functions as an insulation device for large amounts of X and Y

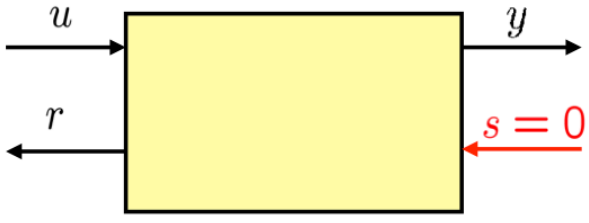
Del Vecchio, Ninfa, and Sontag, *MSB*, 2008

SCRIPT (2008): DDV and William Blake (WB)—Proposing the construction of insulation devices

- **DDV**: <<You were right that the clock would not work. Retroactivity can be responsible for that, but I think I have found a solution using covalent modification cycles as buffers between the clock and the downstream system [DDV shows slide and explains the principle through feedback]>>
- **WB:** <<Oh, that is interesting and counterintuitive to me. I want to understand this more! What model did you use for phosphorylation for coming up with your equations?>>
- **DDV:** <<A one-step reaction for both the forward and backward phosphorylation reactions>>
- **WB:** <<Mmm, one step enzymatic reactions? Do you have any idea of how complex are in reality the molecular mechanisms involved in most enzymatic reactions? Here is the diagram of a covalent modification "cycle" that we could purify and reconstruct in vitro. Our lab has been studying this for many years [WB shows the slide with the PII-UMP complicated diagram]. As you can see, not only we have all the complexes bound to substrates, but we have multii-site modification reactions, and many combinations in which the units of the protein can be bound to load/modified. What are your G and your k in this system??? >>
- **DDV:** <<Well, I am not sure about that given that there is no distinct cycle, but the basic idea should still work since you still have forward and backward reactions that you can make strong…>>
- **WB:**<<Well, reconstructing this system in vitro is going to take a year at least, generating the time-varying data that you'd like is going to take between one and two additional years as we need to find a way to remove the stimulation. There is no way I am going to spend the next three years working on an experiment based on the intuition provided by a…. TOY MODEL! Ah! You engineers… biology is so much more complex than you think…go back kiddo, go back to work on your toy models of electrical circuits.>>

Principle 2: Retroactivity attenuation based on time scale separation

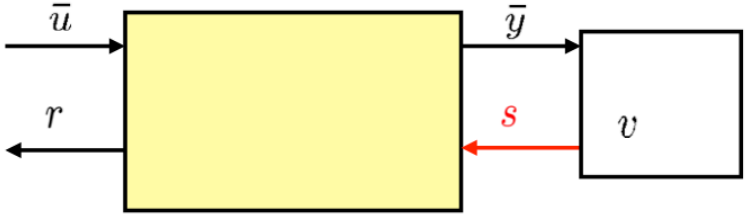
Isolated system



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

$$\dot{y} = G_1 f_1(u, y)$$

Connected system



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

$$\dot{\bar{y}} = G_1 f_1(\bar{u}, \bar{y}) + G_2 M s(\bar{y}, v)$$

large

Very large rates: $G_2 \gg 1$

$$\dot{v} = -G_2 N s(\bar{y}, v)$$

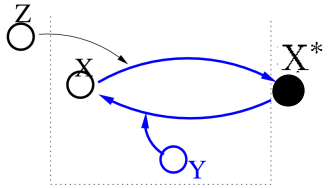
Fact: There are a matrix T and a non-singular matrix B such that

$$BM - TN = 0$$

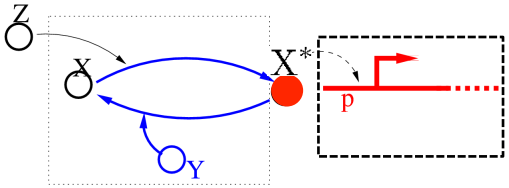
Theorem: As G_1 increases, signals $\bar{y}(t)$ and $y(t)$ approach each other independent of whether $G_2 \gg G_1$, $G_2 = O(G_1)$, or $G_2 \ll G_1$

Jayanthi and Del Vecchio, *IEEE Trans. Aut. Control*, 2011

Simulation results on a larger computational model

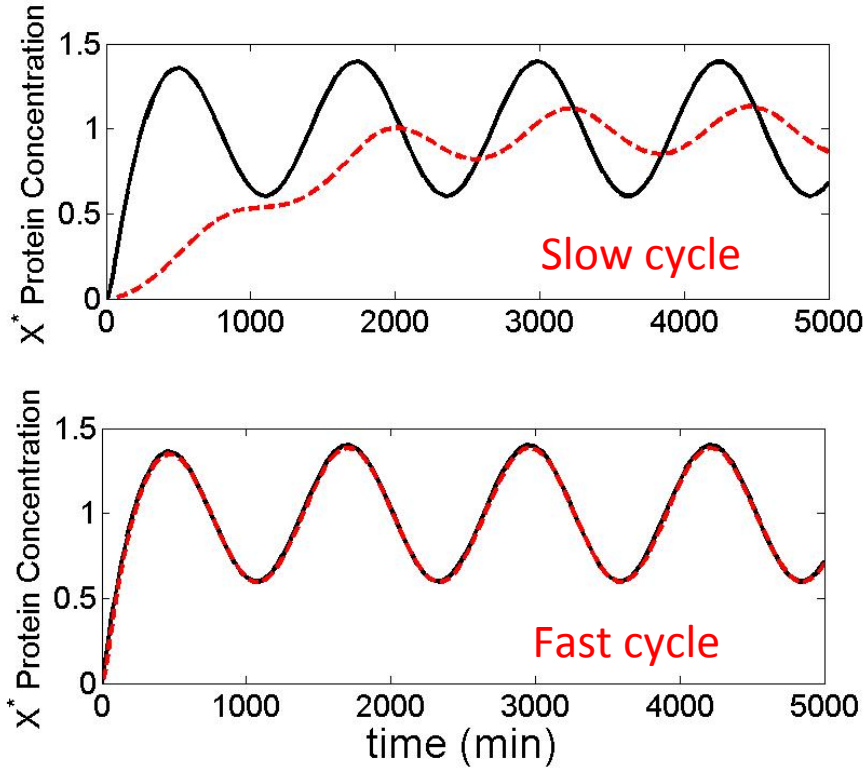


Isolated system



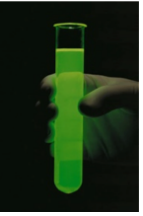
Connected system

Note: Insulation is attained with $p_T = 100 * X$



These systems can be extracted from natural networks and re-engineered to function as insulation devices for synthetic biology

Next: construct the insulation device in living cells and experimentally demonstrate that it attenuates retroactivity when placed between two modules

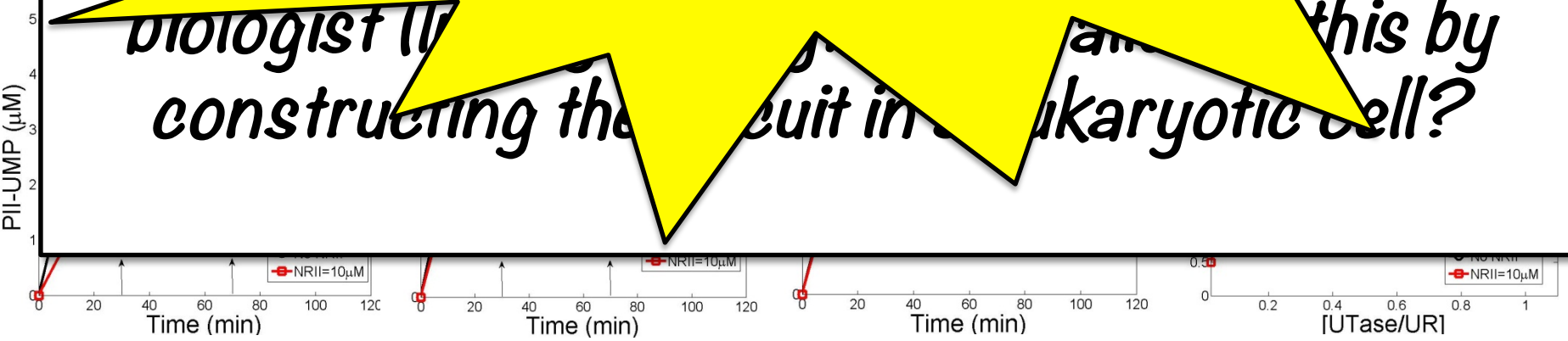


In 2010 Alexander Blake was finally persuaded to do the experiments *in vitro*…

Great! It took 4 years to have an expert in signal transduction validate my design in

5 years (2009-2014)

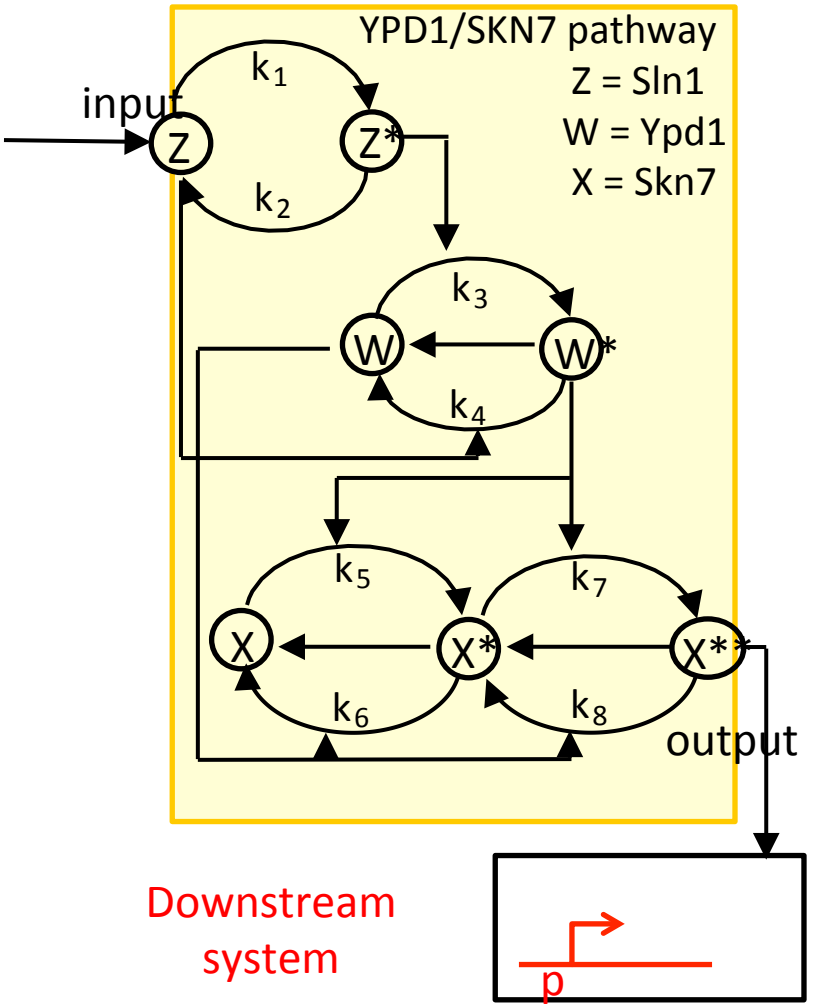
How lo… biologist (I… …this by constructing the circuit in a eukaryotic cell?

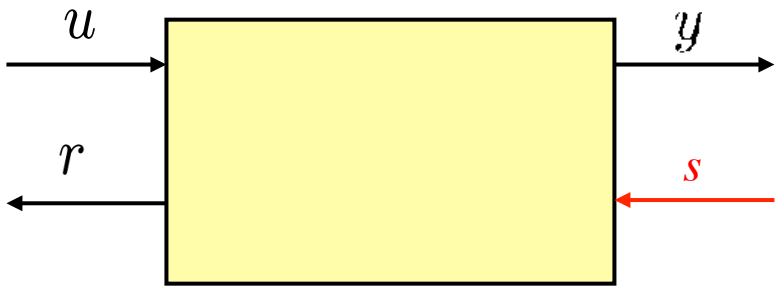


Covalent modification cycles can be re-engineered to function as insulation devices!

Jiang, Ventura, Merajver, Sontag, Del Vecchio, Ninfa, Del Vecchio, *Science Signaling*, 2011

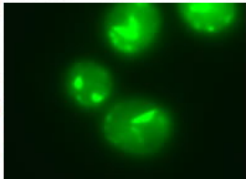
Our time-scale based mechanism was general enough to apply to complex systems *in vivo*



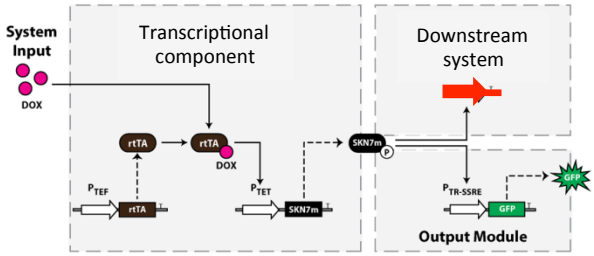


(model has about 30 state variables and more than 50 poorly known parameters)

Work with Ron Weiss (MIT)



Implementation of the insulation device *in vivo*



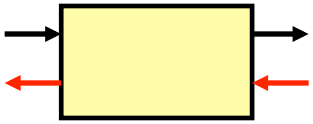
Quoting the biological engineering student who performed the experiments when he saw the first data:

<<I cannot believe what I see: It works! All this theory that looks like black magic is actually right? What a surprise! I now start to understand the difference between theory and modeling... >>

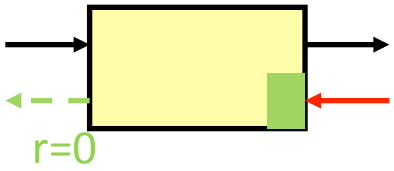
Mishra, Rivera, Del Vecchio* and Weiss*, *Conditionally Accepted Nature Biotech.*, 2014

Summary

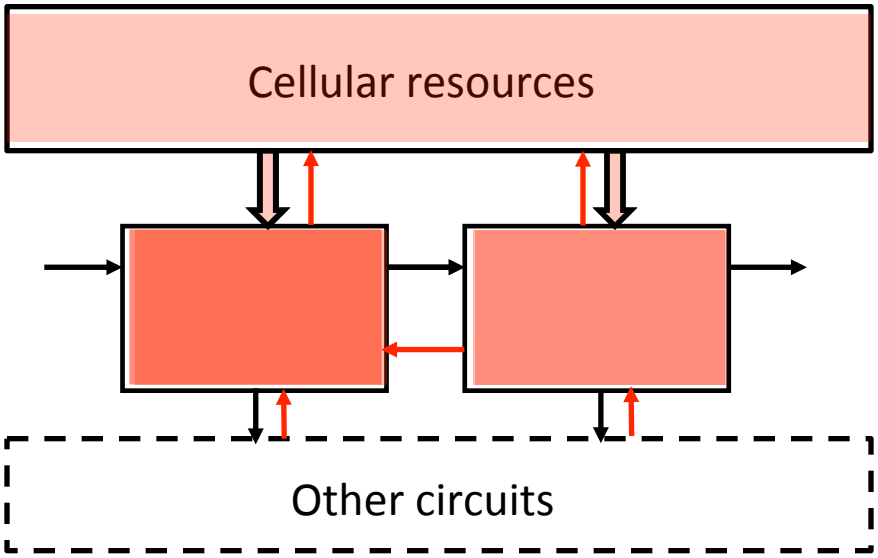
We have proposed a system concept with retroactivity to model impedance-like effects in biomolecular networks



We have introduced the notion of insulation device and introduced a mechanism for retroactivity attenuation based on time scale separation



The future



Retroactivity between modules is one source of context dependence

We have laid out the theoretical foundations to analyze and fix it

Modules also apply retroactivity to the "cellular system": creates subtle couplings

Modules often have "off-target" interactions

Interesting disturbance attenuation problems for nonlinear systems, which often cannot be solved through explicit feedback

Thanks to…

Ron Weiss (MIT)

Alex Ninfa (Umich)

Deepak Mishra (MIT)

Eduardo Sontag

AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
UNITED STATES AIR FORCE

NSF

Textbook

Biomolecular Feedback Systems

Princeton University Press, October 2014

by

Domitilla Del Vecchio and Richard Murray

draft available at

https://www.cds.caltech.edu/~murray/amwiki/BFS

Who is this?