Feedback and Control in Biological Circuit Design

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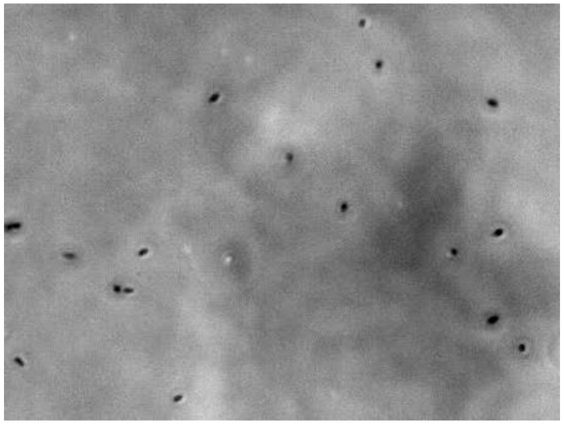
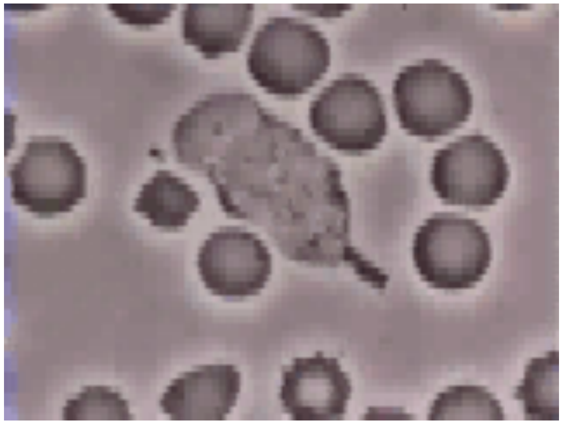
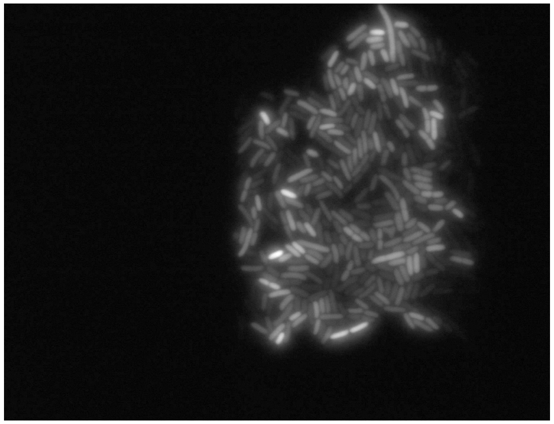
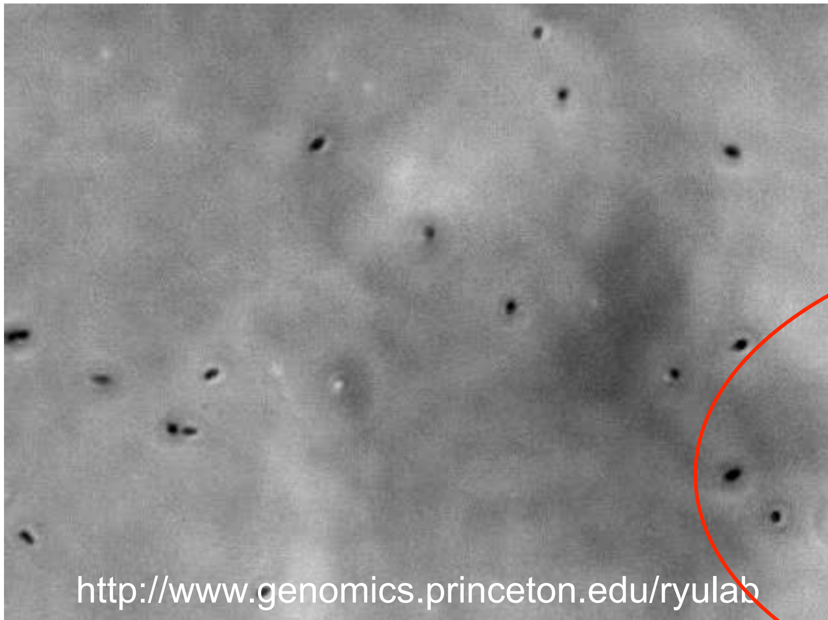
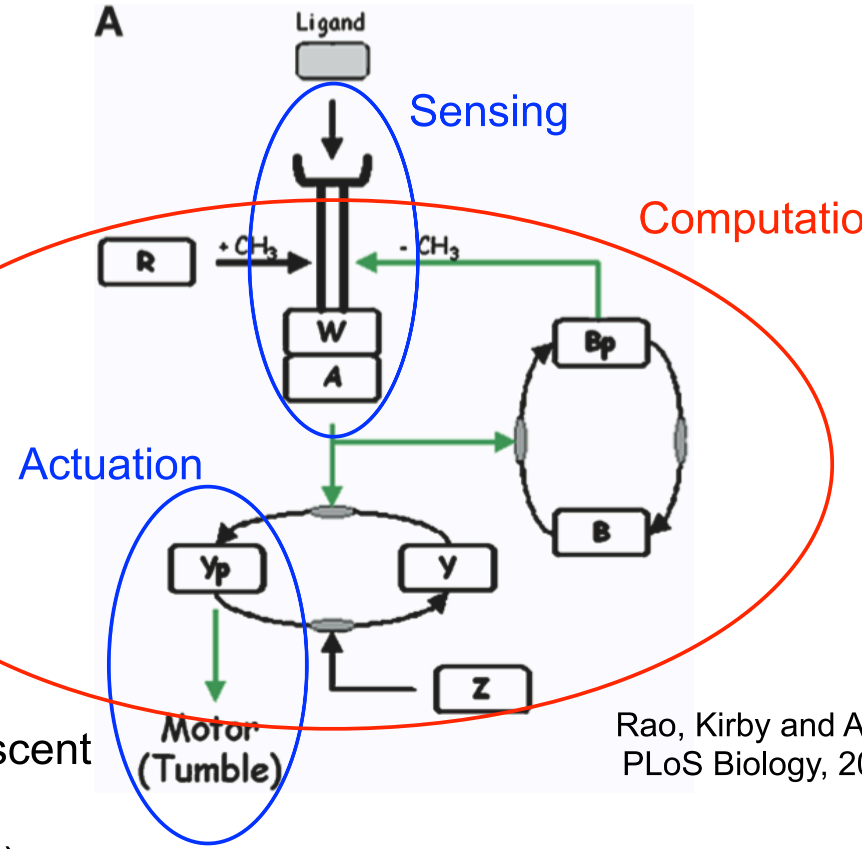




Example: Chemotaxis





Rao, Kirby and Arkin
PLoS Biology, 2004

Implements key principles of feedback

- Dynamics allow exploration, gradient ascent
- Robust with respect to nutrient levels (adaptation via methylation of receptors)

Can we do better?

- Make use of modular sensors and actuators
- Modify dynamics to provide different types of behavior, robustness properties, ...

Design of Biomolecular Feedback Systems

I. Biological circuit design (synthetic biology)

II. System Identification in Cells

- Regulatory activity revealed by dynamic correlations in gene expression noise (Mary Dunlop [UC Berkeley/U. Vermont])
- Joint work with Michael Elowitz

III. Robustness to Uncertainty

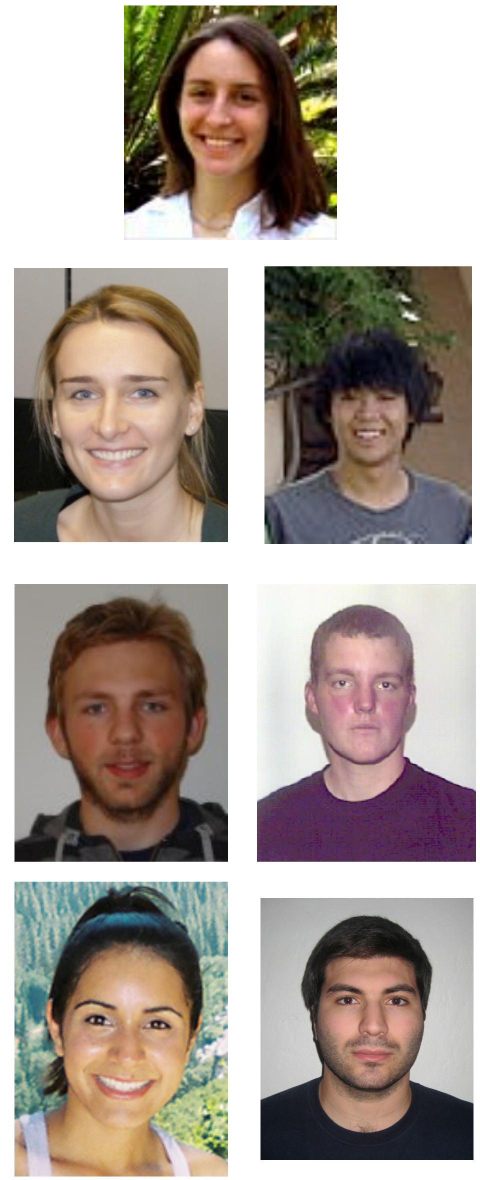
- *In vitro* rate regulators (Elisa Franco, Fei Chen)
- Joint work with Erik Winfree

IV. Design of Dynamics

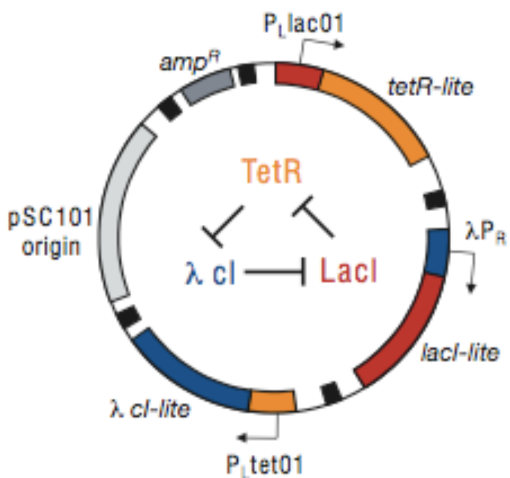
- Using time-delays to tune *in vivo* oscillators (Johan Ugander, Arthur Prindle)

V. Control Design (?)

- Some thoughts on a "framework" for design of biochemical feedback systems



Biological Circuit Design (Synthetic Biology)

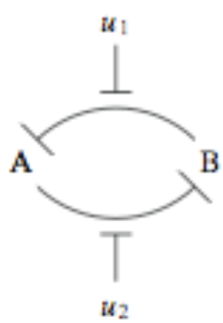


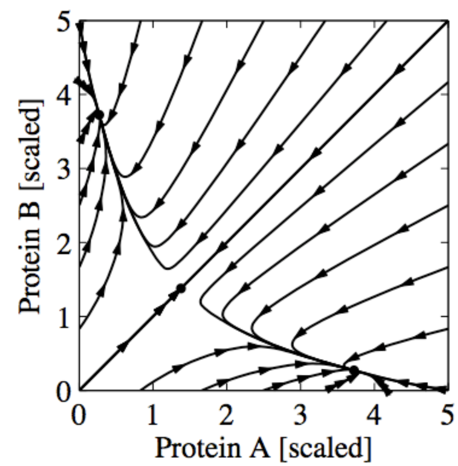
Repressilator (Elowitz & Leibler)

- Ring oscillator with three repressors in a cycle
- Provides oscillations at frequency comparable to cell cycle

Genetic Switch (Collins and others)

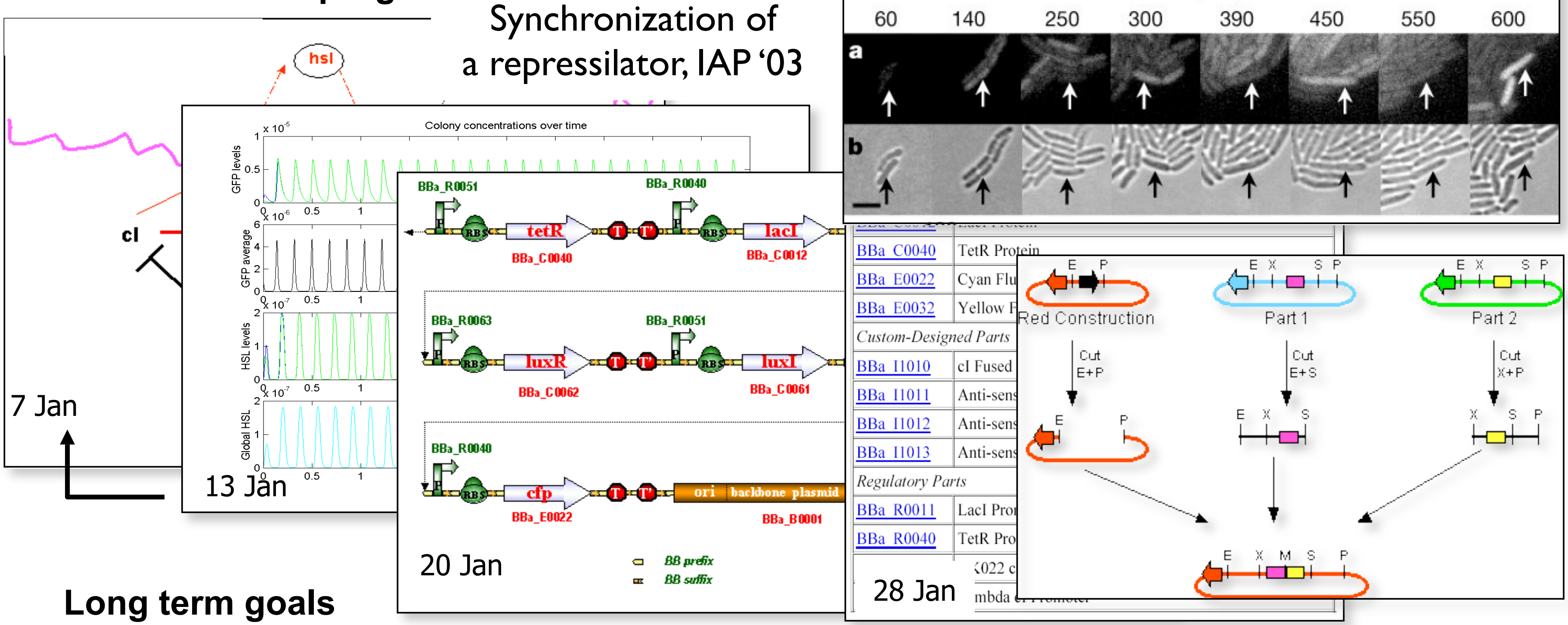
- Interconnect two genes via cross-repression
- Resulting circuit has two states: "(1,0)", "(0,1)"
- Can analyze robustness, speed of response





Modular Synthetic Biology

MIT Bio-Bricks program



Long term goals

- Better understanding of biological function
- New devices for interfacing with biological systems (diagnosis, medication)
- Novel biological processes: biofuels production, bio-remediation

State of the Art

- DNA synthesis: < $1 per base pair (simple circuit: 5000 bp), 6-10 weeks delivery time
- Alternative: manual cloning to put together existing components (eg, bio-bricks)

Toward a Control Theory for Synthetic Biology

Differences from traditional systems

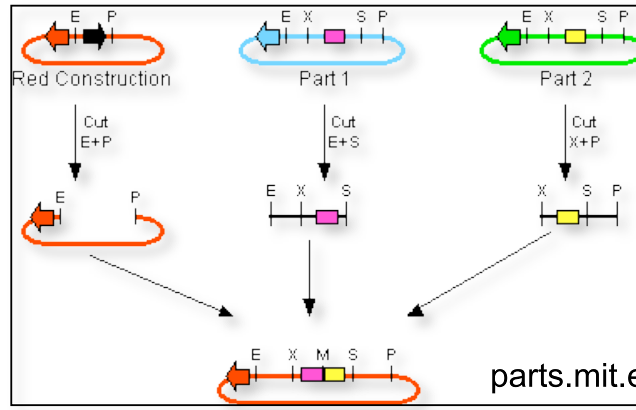
- Complexity - biological systems are much more complicated than engineered systems
- Communications - signal representations are very different (spikes, proteins, etc)
- Uncertainty - very large uncertainty in components; don't match current tools
- Evolvability - mutation, selection, etc

(Engineered) Modularity would be very useful

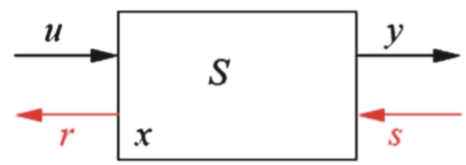
- To build complex systems, we need to be able to isolate subsystems (probably)
- Biobricks: modularity at DNA + device level
- Retroactivity (DDV et al): candidate methods for minimizing effects of loading by downstream devices

Stochasticity and robustness are critical

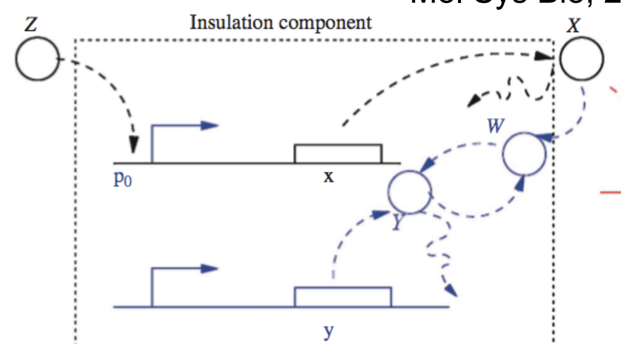
- Program time-evolving distributions to achieve desired function
- Make use of heterogeneous redundancy to provide robustness (?)



parts.mit.edu



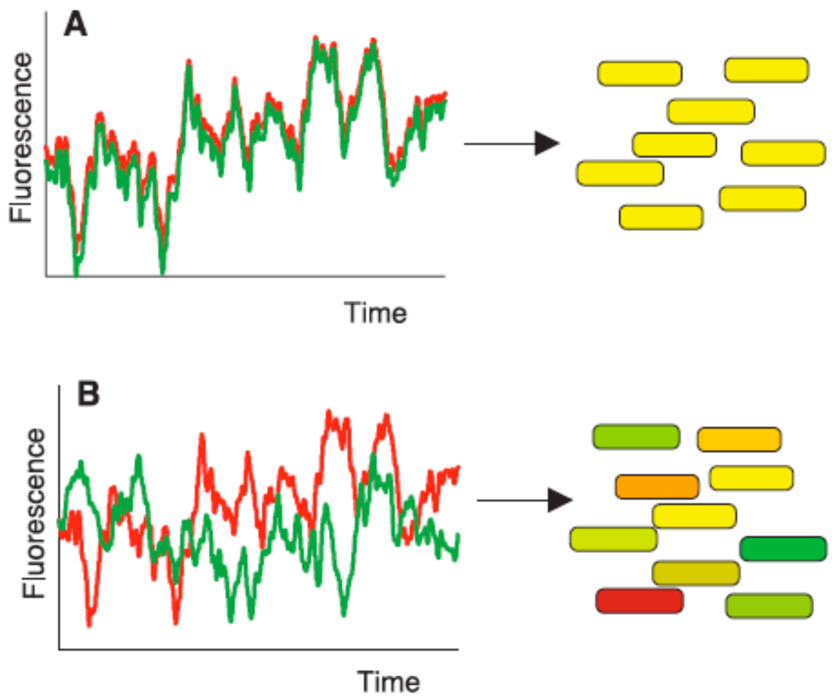
Del Vecchio et al,
Mol Sys Bio, 2008



Cell Noise (Elowitz et al, 2002)

Noise in cells

- Experiments by Elowitz, Levine, Siggia, Swain. *Science* 2002
- Put RFP and GFP under identical promoters; *should* get yellow
- Results: get range of colors



Extrinsic Noise:

- global to a single cell, but varies from one cell to the next (e.g. cell volume, plasmid copy number)

Intrinsic Noise:

- inherent stochasticity in gene expression (e.g. what order reactions occur in)

$$\dot{x}_i = E(t) \cdot f_i(x_i, I_i(t))$$

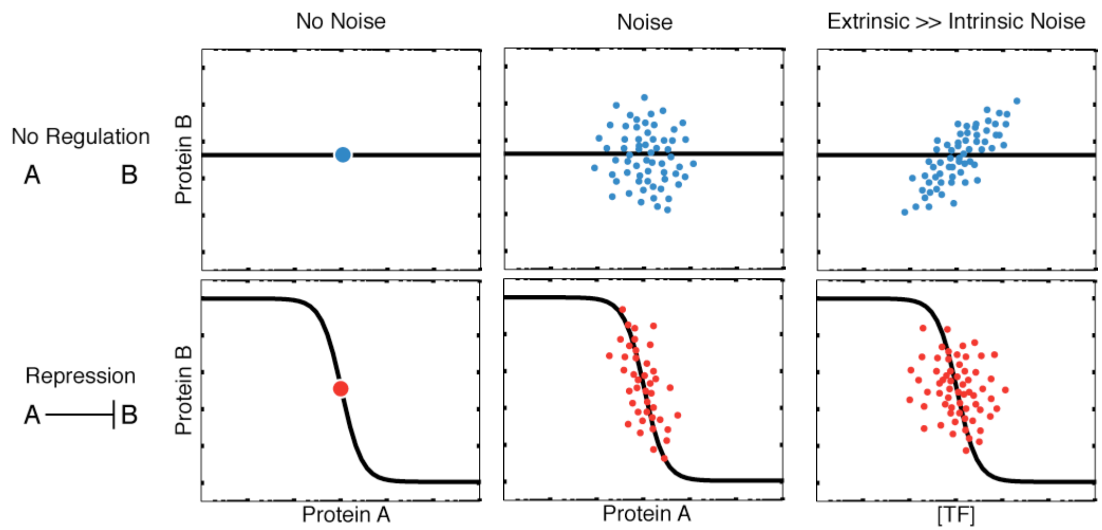
System Identification Using Cell Noise

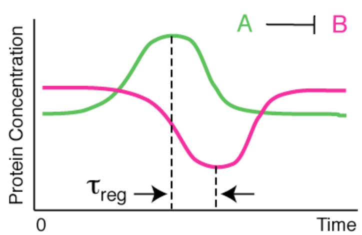
Traditional systems identification

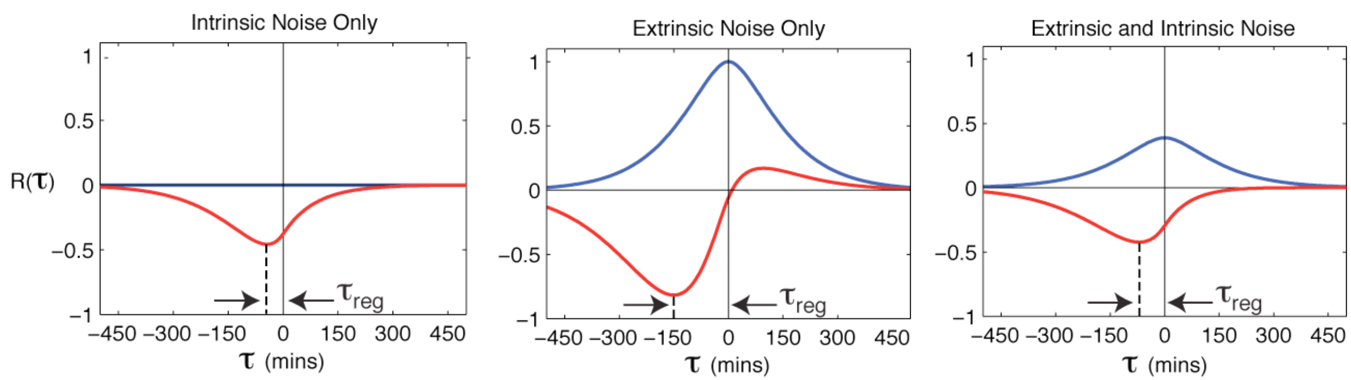
- Engineering: forced response. Difficult to do in in vivo (eg, sinusoids are tricky)
- Biology: gene knockouts; steady state measurements using gene arrays

Idea: use noise as a forcing function

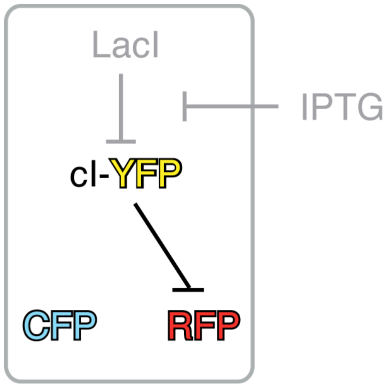
- Steady state distributions are not enough if extrinsic noise is present
- Need to use correlation data instead

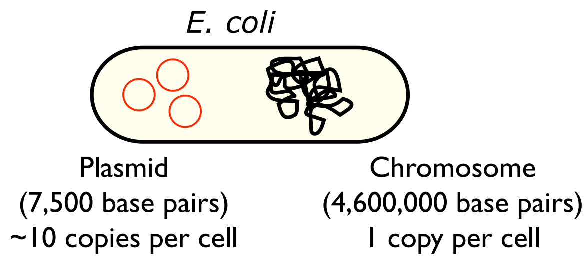


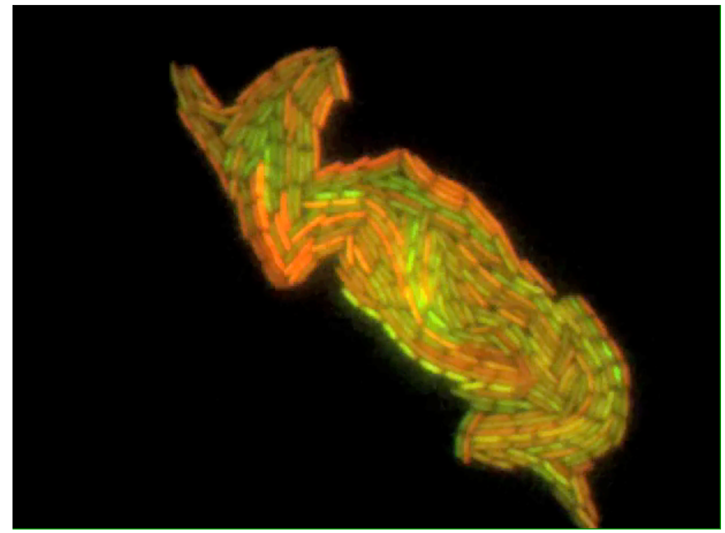


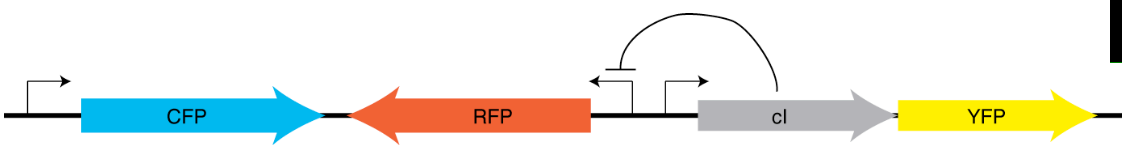


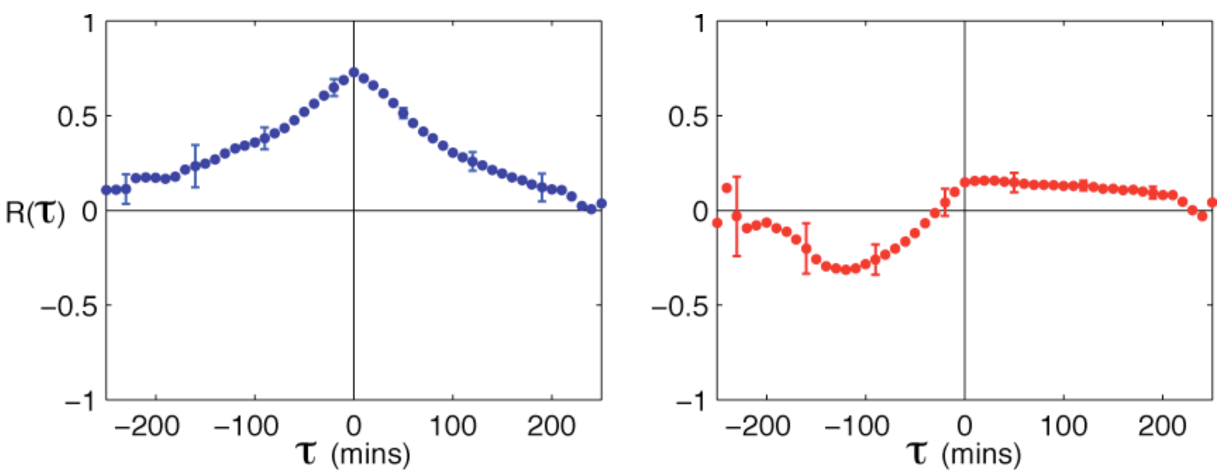
System ID of a Synthetic Circuit (Dunlop, Elowitz & M)







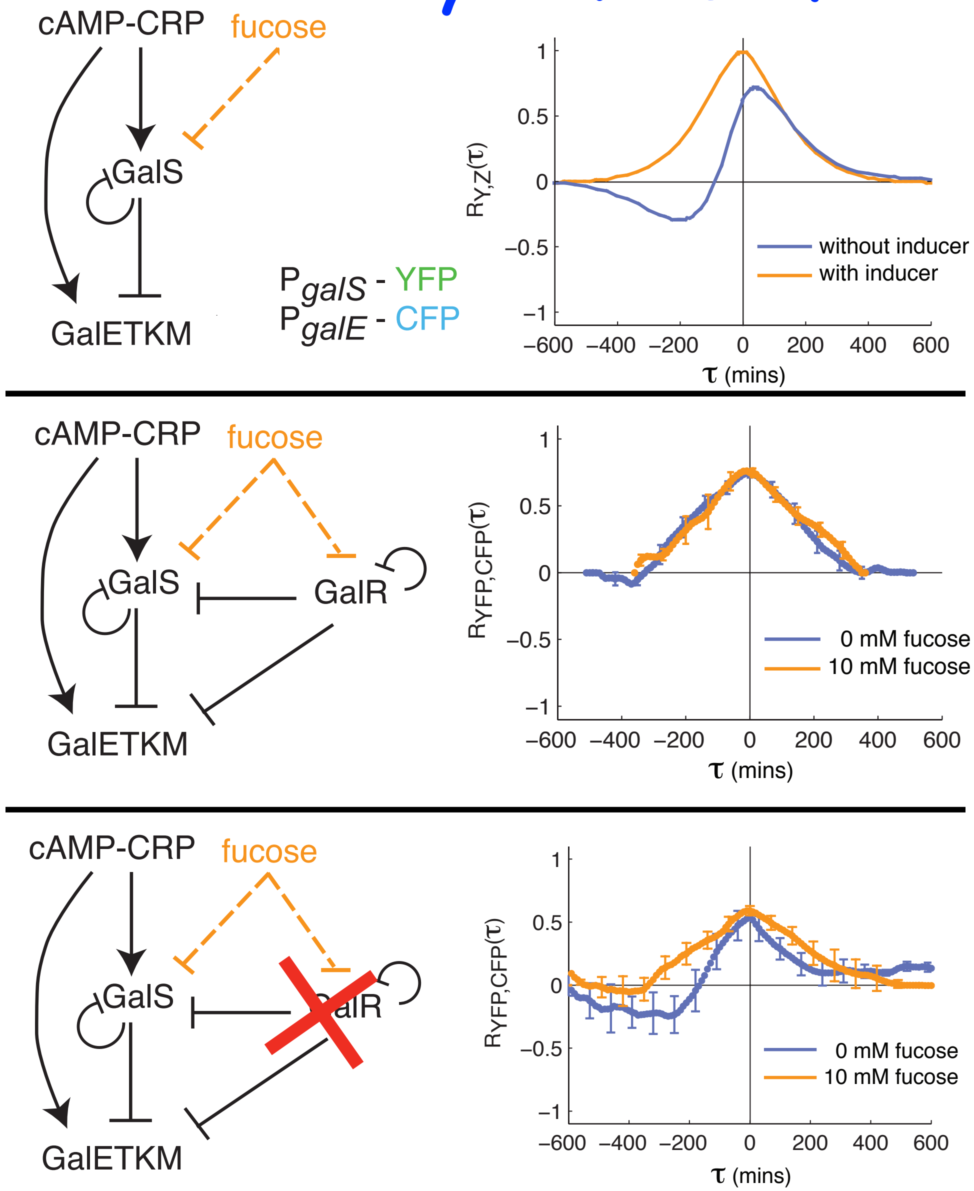




Results to date

- Synthetic circuit demonstrates viability of approach
- Implemented on natural circuit (using promoter fusion)

System ID of an in vivo circuit



Galactose regulation in *E. coli*

- GalE regulated by CRP via a feedforward loop
- GalR represses feedforward loop when fucose is present
- Promoter fusions measure GalS and GalE concentrations

System ID shows FFL is not active

- Addition of fucose shows no change in correlations => GalS is not actively regulating GalE

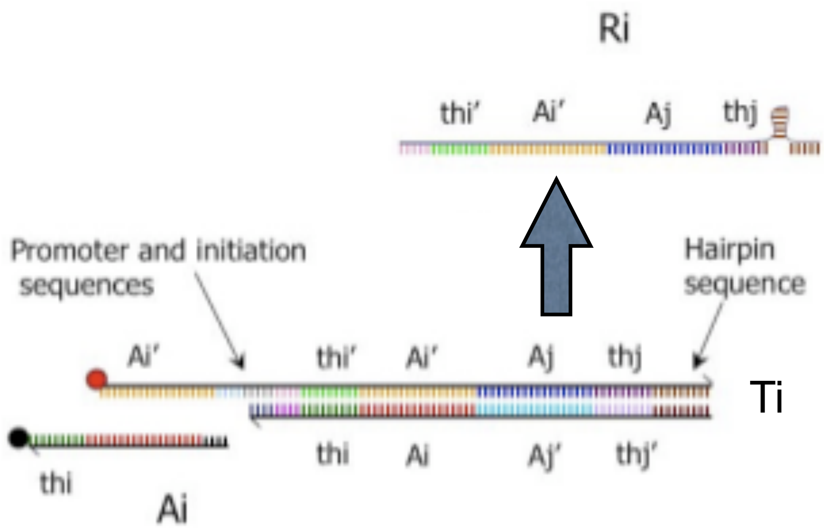
Hypothesis: GalR repression dominant

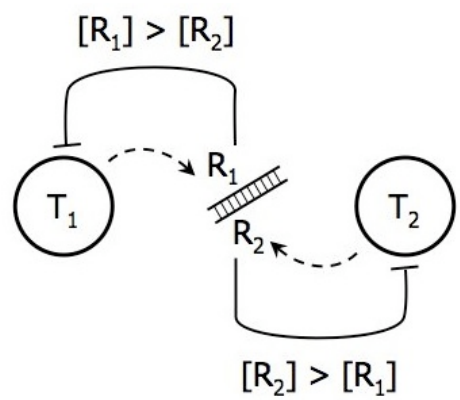
- If repression by GalR is large, GalS is always “off” => no connection
- Removal of GalR recovers expected correlations

In Vitro Rate Regulator (Franco, Winfree & M)

Idea for a circuit: 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

Rate Regulator Results

In vitro experiments

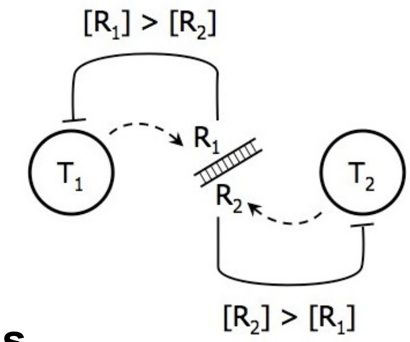
- Add templates + enzymes to test tube
- Use fluorophors to measure amount of repression

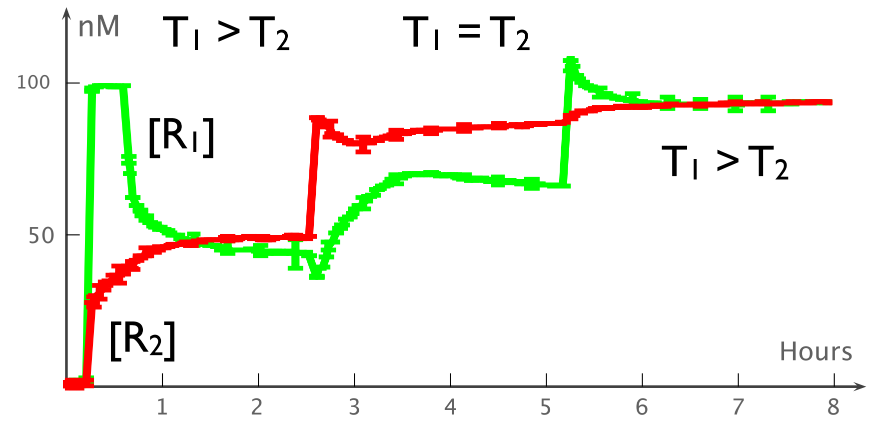
Rate regulator functions correctly

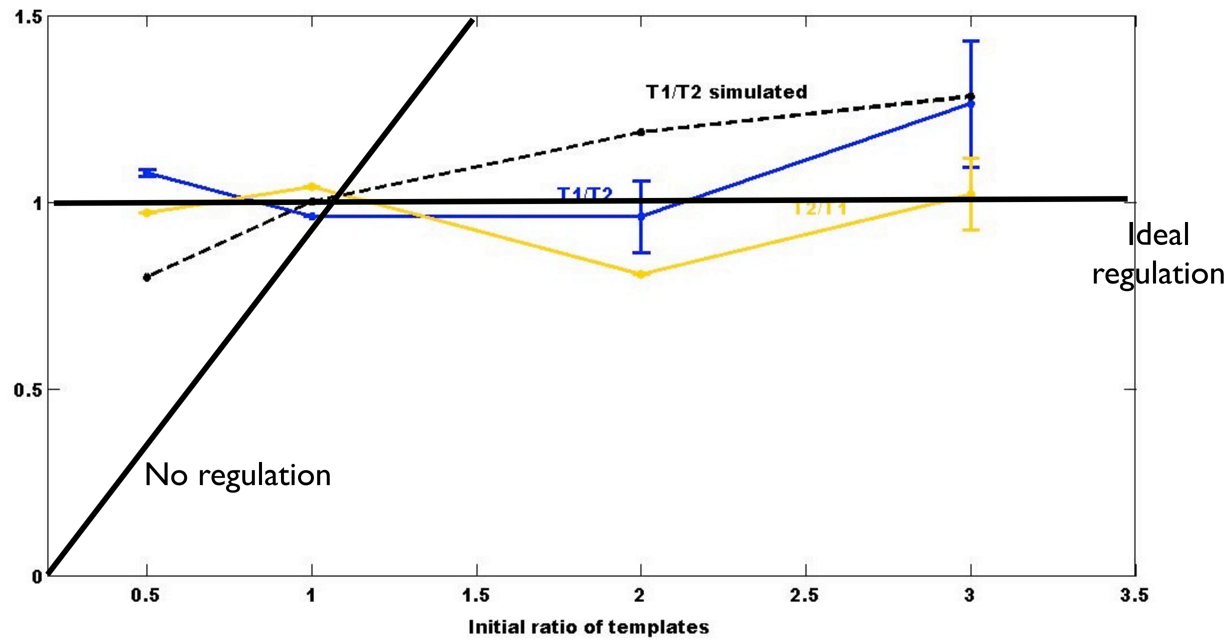
- When T1 is high, get more repression of T1 (to bring R1, R2 into balance)
- Can also use cross activation

Next steps

- Loading effects
- Sensing/actuation
- Integral feedback (Fei)

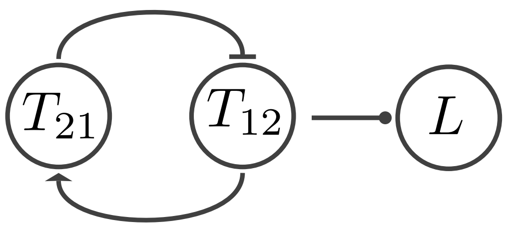


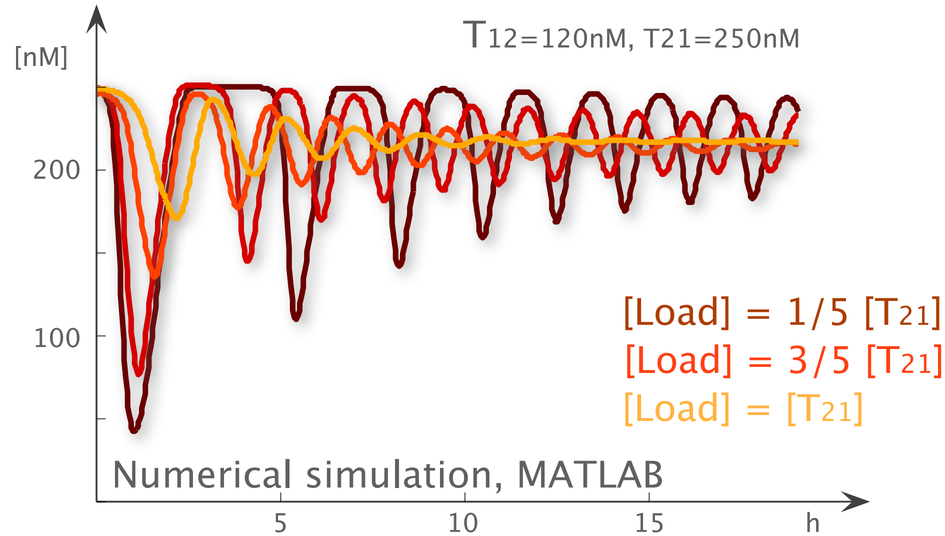




Improving Modularity

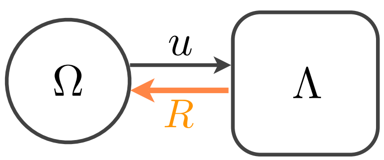
Effects of loading



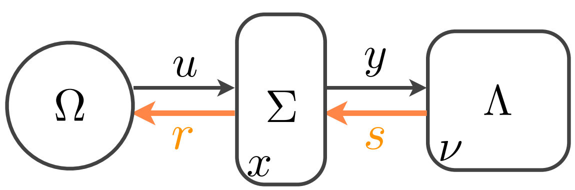


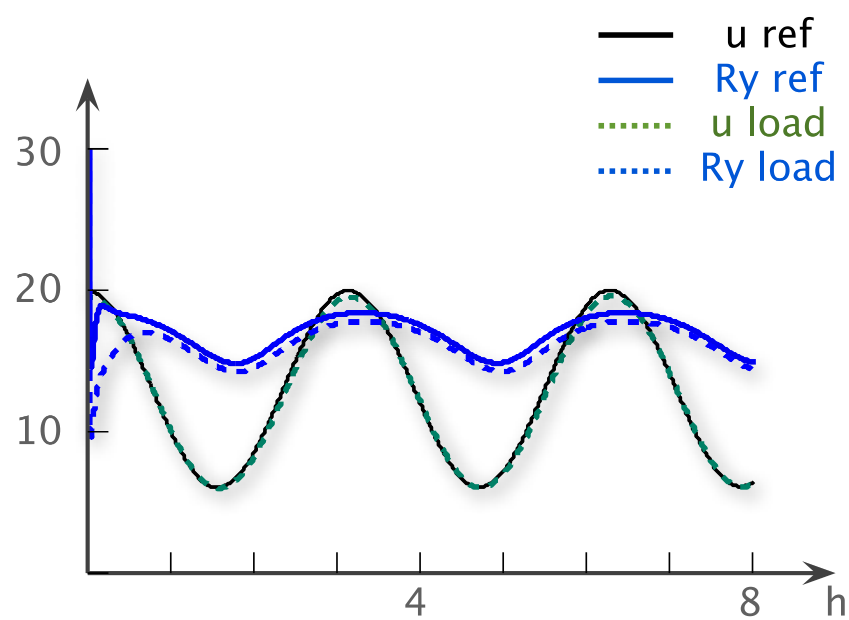
Modeling approach: retroactivity (Del Vecchio and Sontag, 2008)

- Keep track of how much down-stream load affects circuit

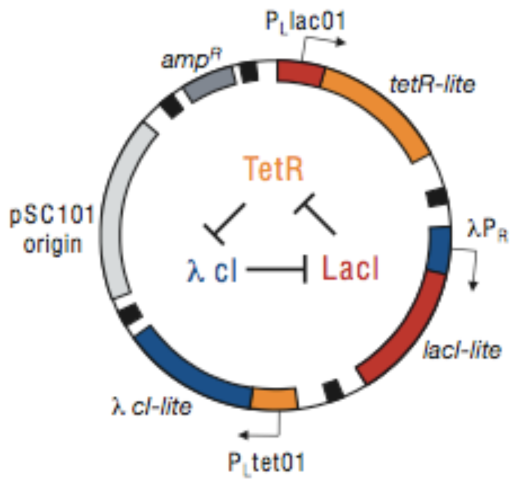


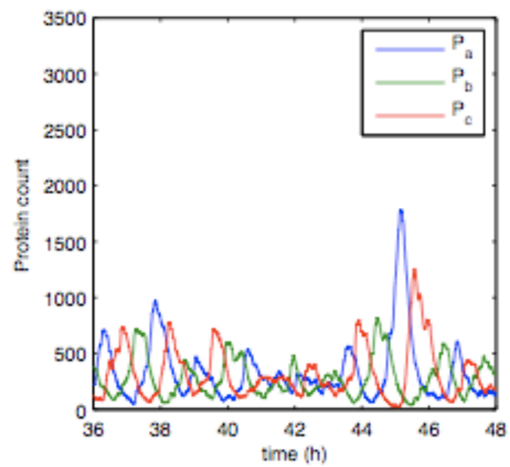
- Use “insulator” to isolate

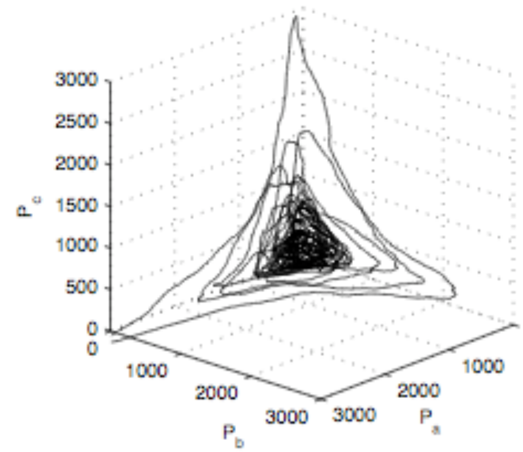




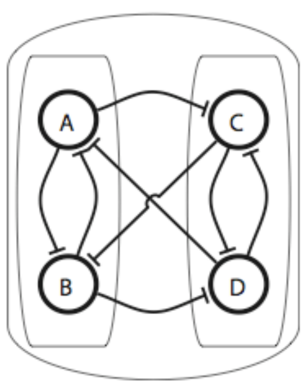
Improving the Performance of Oscillators

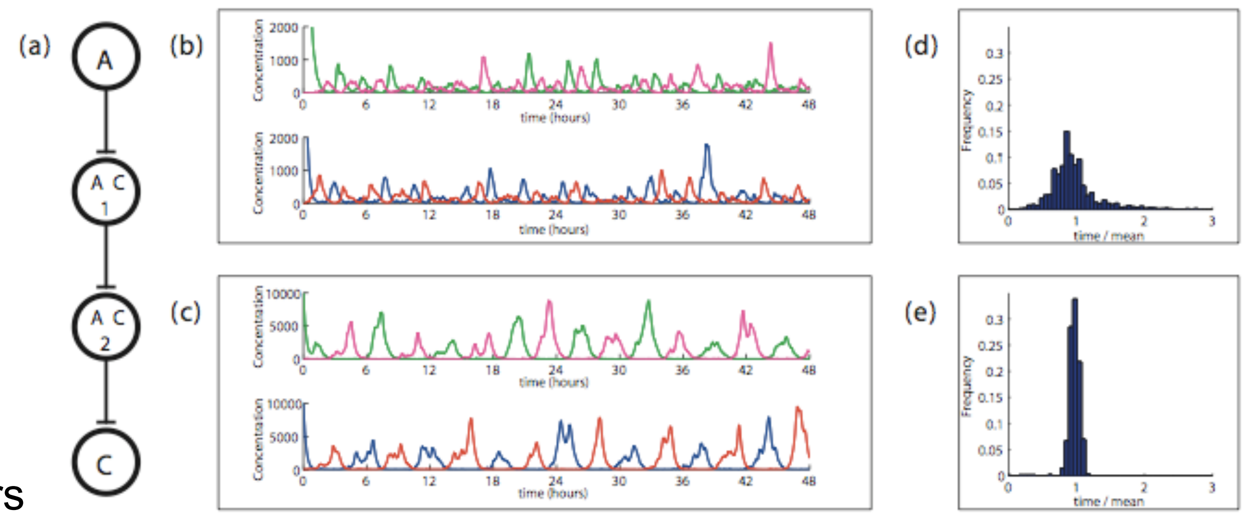






Toggelator





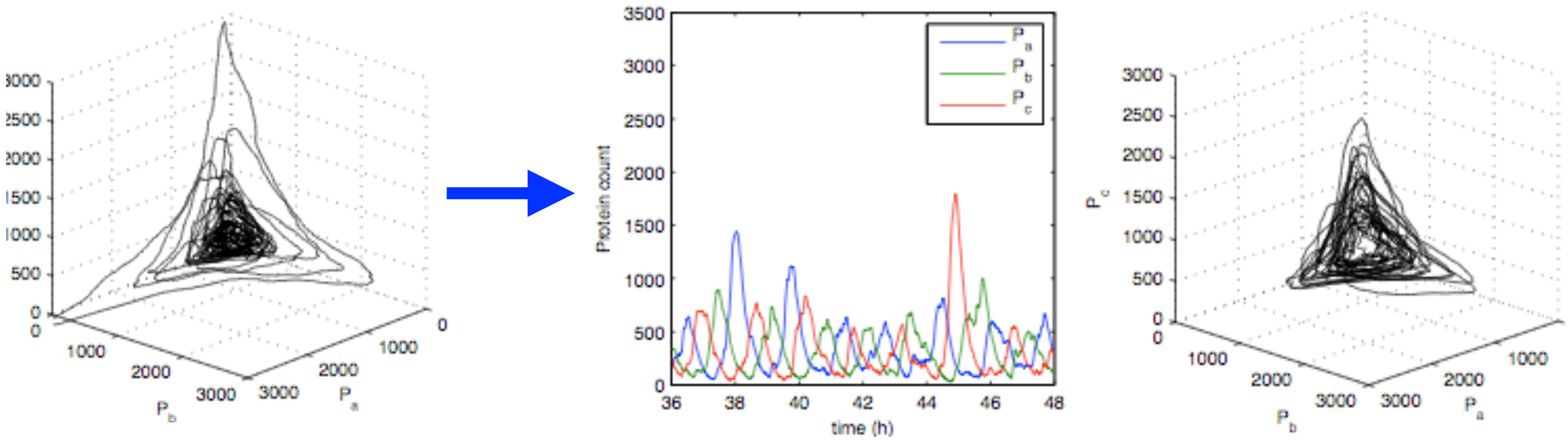
- Coupled oscillators
- Add additional "delay" (ACi)

Ugander, Dunlop & M, ACC 07

Improving Oscillator Performance by Adding Delay

Transcription & Translation	Promoter region	Decay
$GeneA_{on}(t) \xrightarrow{k_1} GeneA_{on}(t+\tau_{ps}) + M_A(t+\tau_1)$ $GeneA_{off}(t) \xrightarrow{\epsilon \cdot k_1} GeneA_{off}(t+\tau_{ps}) + M_A(t+\tau_1)$ $M_A(t) \xrightarrow{k_2} M_A(t+\tau_{rbs}) + P_A(t+\tau_2)$	$GeneA_{on}(t) + P_C(t) \xrightarrow{k_3} GeneA_{off}(t)$ $GeneA_{off}(t) \xrightarrow{k_4} GeneA_{on}(t) + P_C(t)$	$P_A(t) \xrightarrow{k_5} \emptyset$ $M_A(t) \xrightarrow{k_6} \emptyset$
$GeneB_{on}(t) \xrightarrow{k_1} GeneB_{on}(t+\tau_{ps}) + M_B(t+\tau_1)$ $GeneB_{off}(t) \xrightarrow{\epsilon \cdot k_1} GeneB_{off}(t+\tau_{ps}) + M_B(t+\tau_1)$ $M_B(t) \xrightarrow{k_2} M_B(t+\tau_{rbs}) + P_B(t+\tau_2)$	$GeneB_{on}(t) + P_A(t) \xrightarrow{k_3} GeneB_{off}(t)$ $GeneB_{off}(t) \xrightarrow{k_4} GeneB_{on}(t) + P_A(t)$	$P_B(t) \xrightarrow{k_5} \emptyset$ $M_B(t) \xrightarrow{k_6} \emptyset$

Junk Delay:

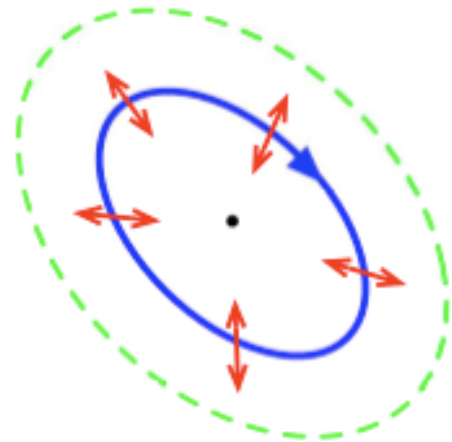


Reduced Order Analysis

$$\dot{P}_A = -\beta P_A(t) + \alpha\left(\frac{1}{1+P_C(t-\tau)^2}\right) + \alpha_0$$

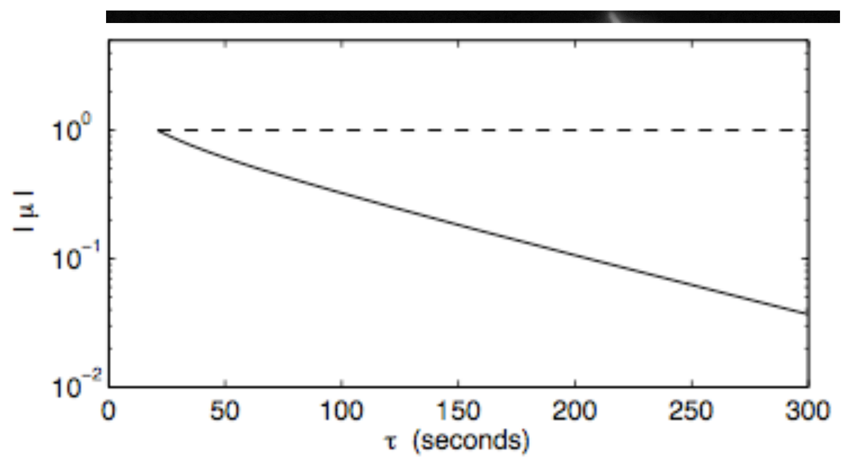
$$\dot{P}_B = -\beta P_B(t) + \alpha\left(\frac{1}{1+P_A(t-\tau)^2}\right) + \alpha_0$$

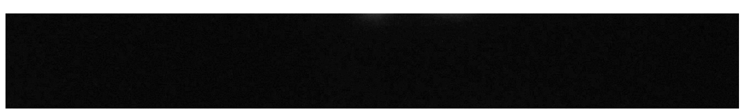
$$\dot{P}_C = -\beta P_C(t) + \alpha\left(\frac{1}{1+P_B(t-\tau)^2}\right) + \alpha_0$$



$$\frac{d\mathbf{x}}{dt} = A_0^*(t)\mathbf{x}(t) + \sum_{i=1}^{d} A_i^*(t)\mathbf{x}(t-\tau_i)$$

$$\mathcal{M} : C_0([-\tau, 0], \mathbb{R}^n) \mapsto C_0([T-\tau, T], \mathbb{R}^n)$$

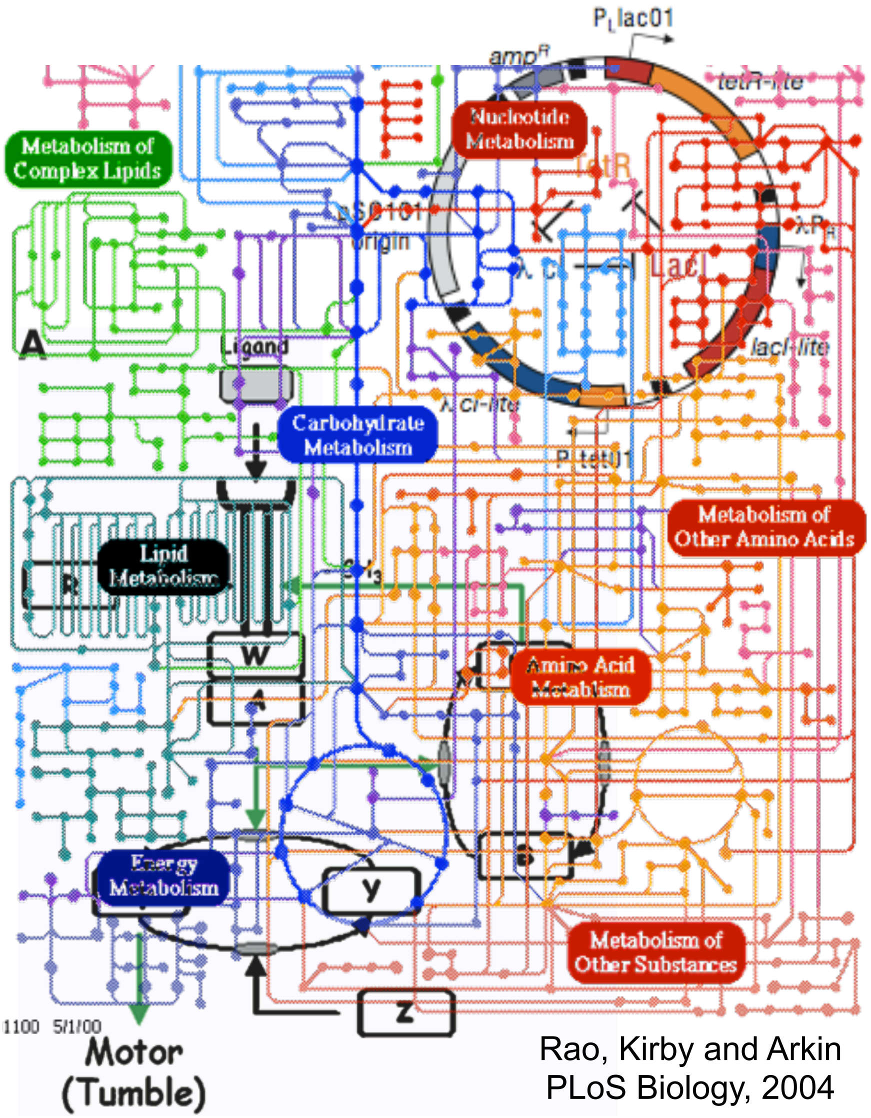




Curent work: *in vivo* experiments (Ben Prindle)

- Added delay elements to repressilator
- Related experiments: “A fast, robust and tunable synthetic gene oscillator”, Stricker et al (2008).

Control Theory for Biological Systems



Rao, Kirby and Arkin
PLoS Biology, 2004

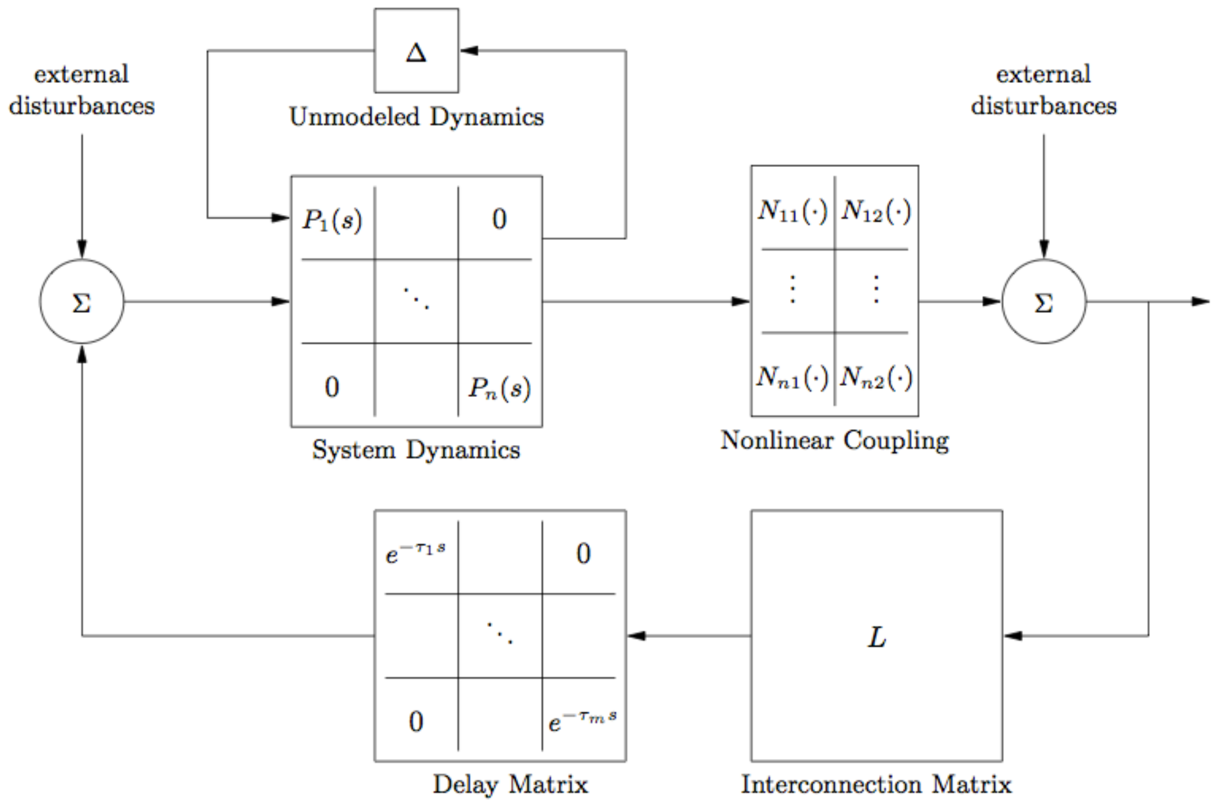
What's different about biological systems

- *Complexity* - biological systems are *much* more complicated than engineered systems
- *Communications* - signal representations are very different (spikes, proteins, etc)
- *Uncertainty* - very large uncertainty in components; don't match current tools
- *Evolvability* - mutation, selection, etc

Potential application areas for control tools

- *System ID* - what are the appropriate component abstractions and models?
- *Analysis* - what are key biological feedback mechanisms that lead to robust behavior?
- *Design* - how to we (re-)design biological systems to provided desired function?
- *Fundamental limits* - what are the limits of performance and robustness for a given biological network topology?

Design of Biomolecular Feedback Systems



Design the easy parts

- Interconnection matrix
- Time delay matrix

Design tools exist for pairwise combinations

- Linear + uncertain = robust control theory
- Linear + nonlinear = describing functions
- Linear + network = formation stabilization
- Linear + delay = Floquet analysis

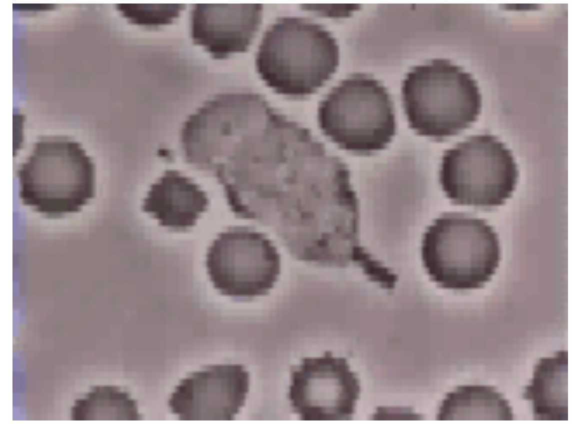
Open questions

- What is the class of feedback compensators we can obtain using L and τ ?
- How do we specify robustness and performance in highly stochastic settings?
- Can feedback be used to design robust dynamics that implements useful functionality?

Summary and Conclusions

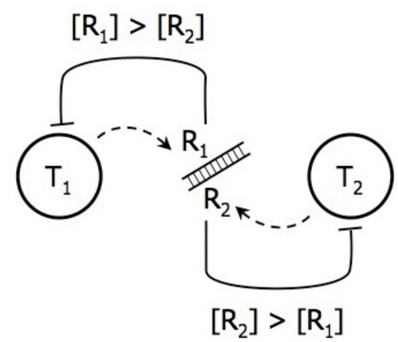
Initial steps in biological circuit design w/ feedback

- System ID - determine active network structure, in vivo
- Feedback circuits - rate regulation, modularity
- Design of dynamics using programmable time delays
- Networked control for biological systems



Next steps: Molecular Programming Project (MPP)

- 5 year goal: create the abstractions, languages and compilers for systematic design of molecular programs
- Explore applications in self-assembly, bio-technology
- Winfree (PI), Bruck, Klavins, M, Pierce, Rothemund



Reading:

- Regulatory activity revealed by dynamic correlations in gene expression noise. MJ Dunlop, RS Cox, JH Levine, RM Murray, MB Elowitz. *Nature Genetics*, 40:1493-1498, 2008
- Design and performance of in vitro transcription rate regulatory circuit. E Franco, RM Murray, CDC 2008 (+2009)
- Stochastic Sensitivity Analysis of Genetic Regulatory Networks, J. Ugander, MS thesis, 2008