Feedback and Control in Biological Circuit Design (Synthetic Biology)

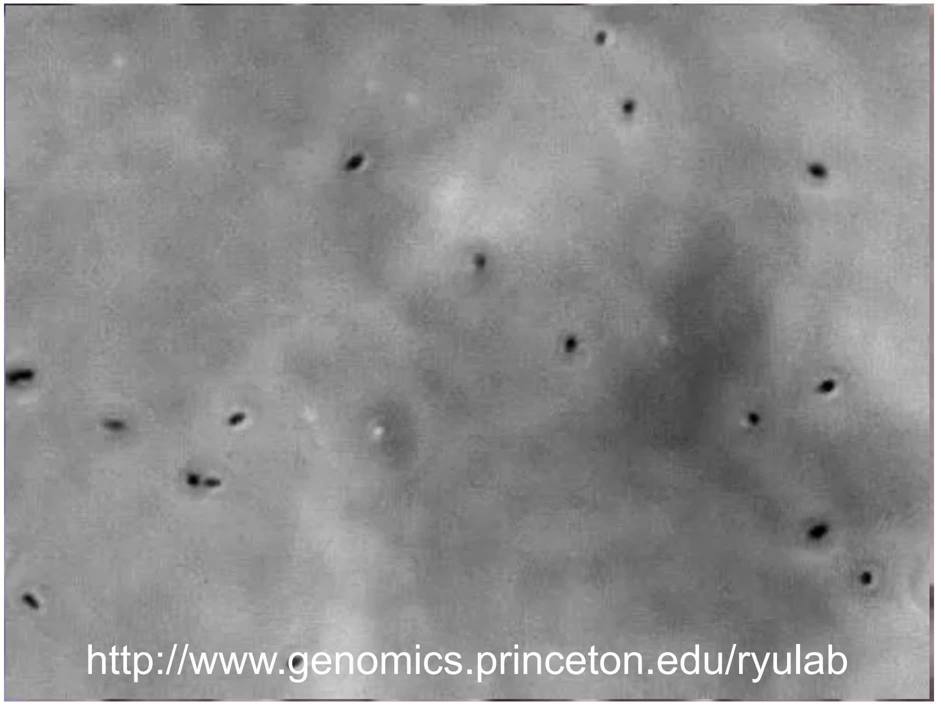
Richard M. Murray
Control & Dynamical Systems / Bioengineering
California Institute of Technology

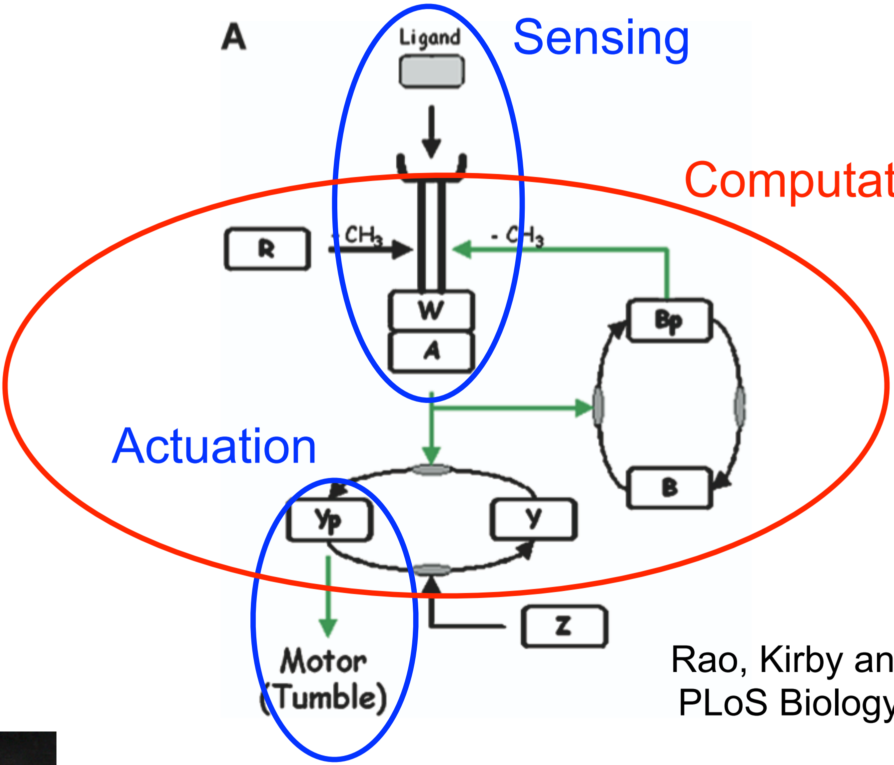
34th Chinese Control Conference and SICE Annual Conference 2015
30 July 2015

Emzo de los Santos (BE)   Victoria Hsiao (BE)
Yutaka Hori (CMS)   Zach Sun (MD/PhD)   Enoch Yeung (CDS)

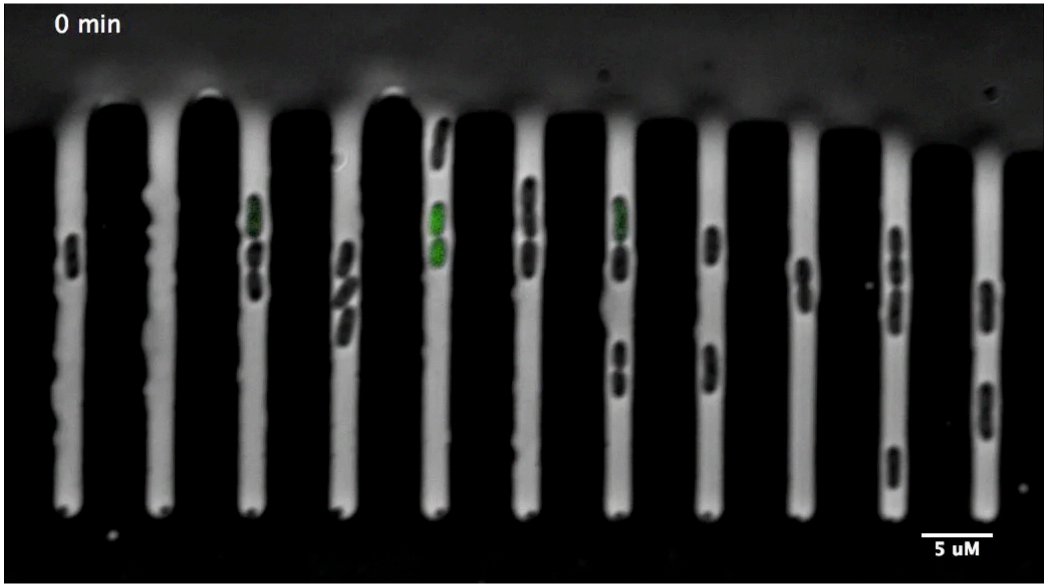
Henrike Niederholtmeyer, Sebastian Maerkl (EPFL)

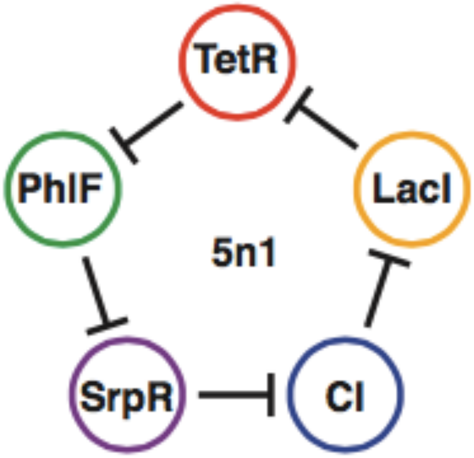
Feedback Systems in Biology


http://www.genomics.princeton.edu/ryulab



Rao, Kirby and Arkin
PLoS Biology, 2004

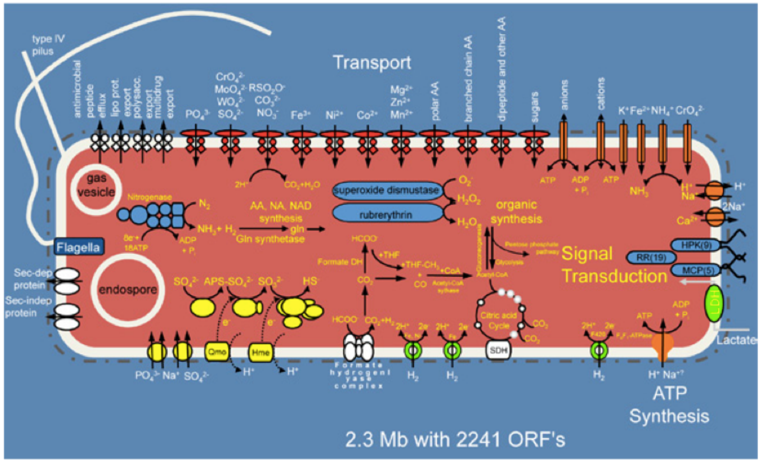


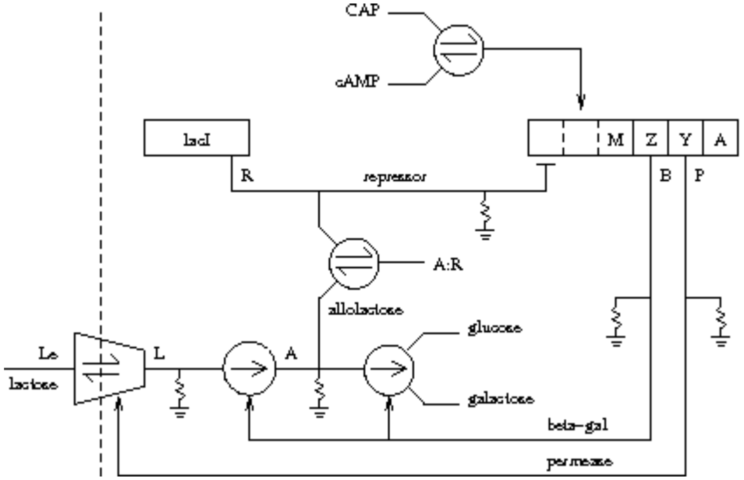


Outline

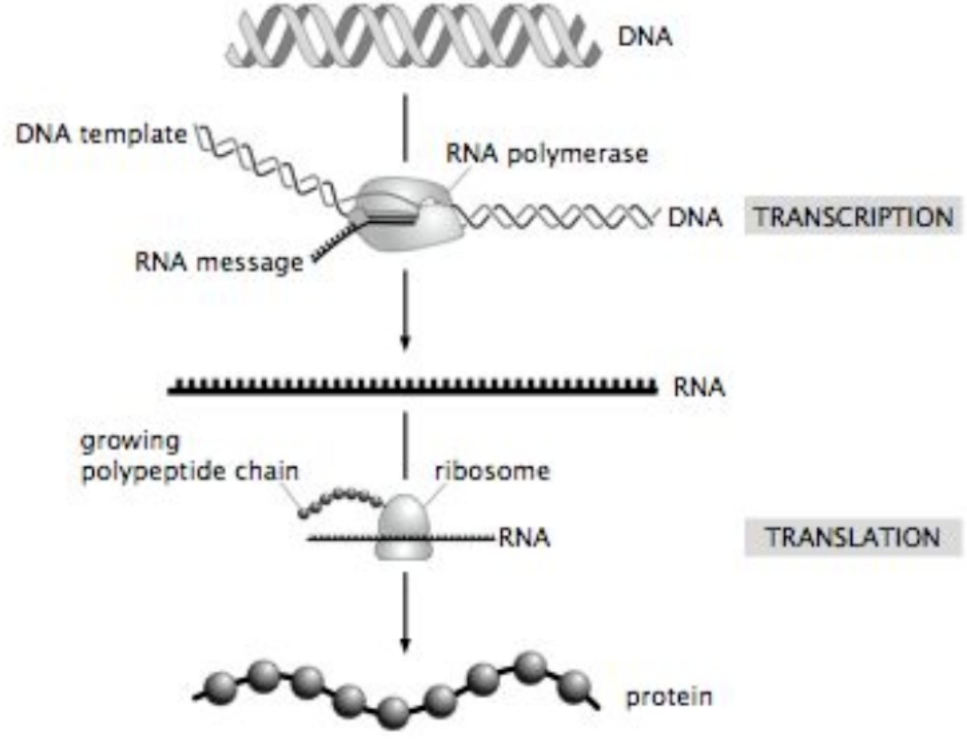
Goal for the talk: explain what synthetic biology is about and the role that systems and control theory might plan

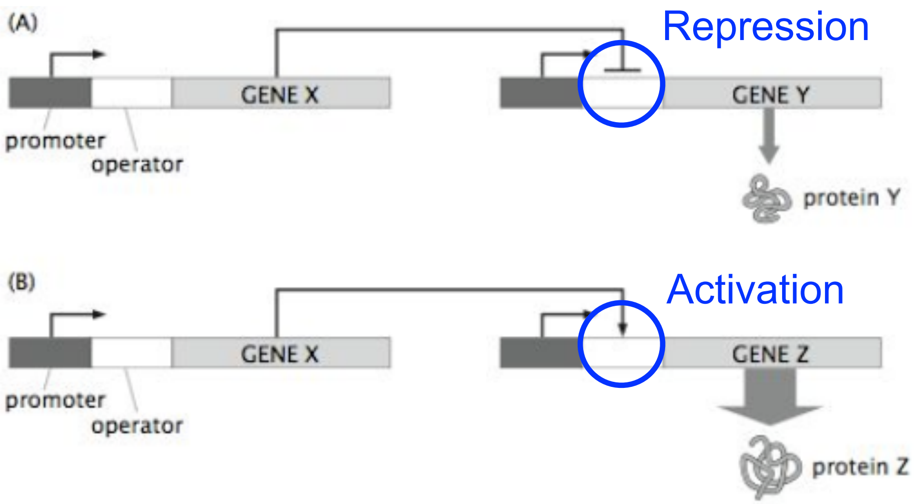
1. **Brief tutorial on biology and synthetic biology**
2. **Some recent results on design of biomolecular feedback circuits**
   - Concentration tracking (Hsiao, de los Santos et al, ACS Syn Bio, 2014)
   - Biomolecular event detectors (Hsiao, Hori et al, work in progress)
   - Biomolecular oscillators (Niederholt-meyer, Sun et al, submitted)
3. **Opportunities for the systems and control community**





Central Dogma: DNA to Proteins





Transcription: DNA to mRNA

- Double stranded DNA contains nucleotide sequence (A, C, T, G) on a sugar (deoxyribose) backbone
- Watson-Crick pairing: A:T, C:G
- RNA polymerase transcribes DNA sequence to RNA sequence (A, C, U, G sequence on a ribose backbond)

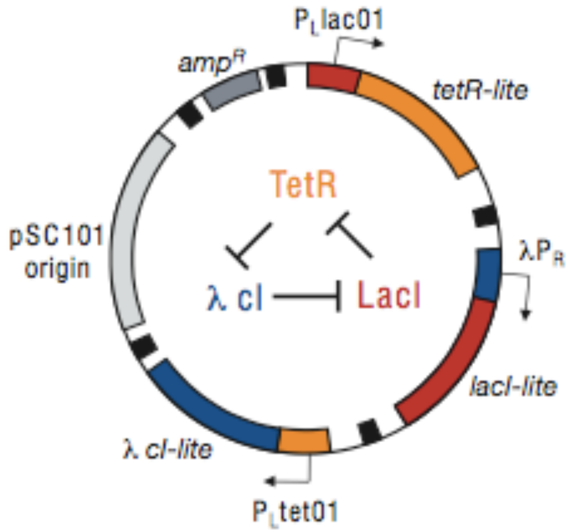
Translation: mRNA to protein

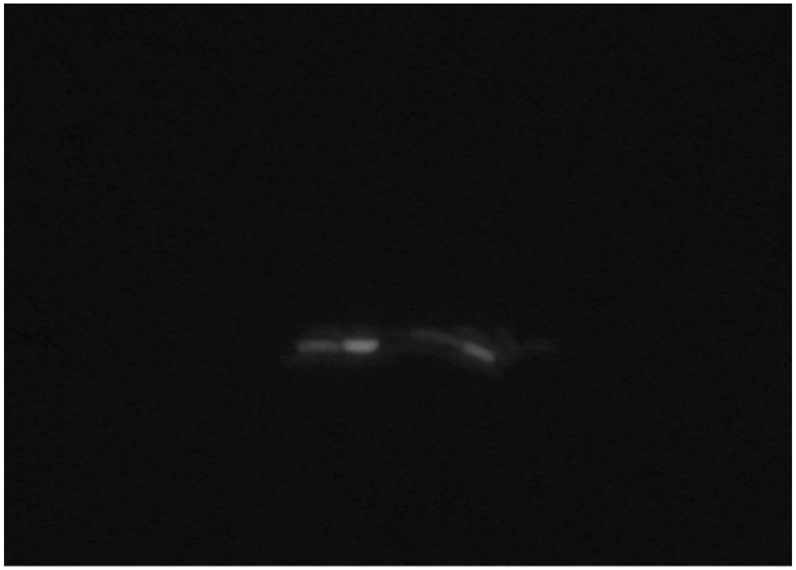
- mRNA is translated by ribosomes into a chain of amino acids using the genetic code (3 bp code for 1 aa)
- Amino acid chain (polypeptide chain) folds into a protein

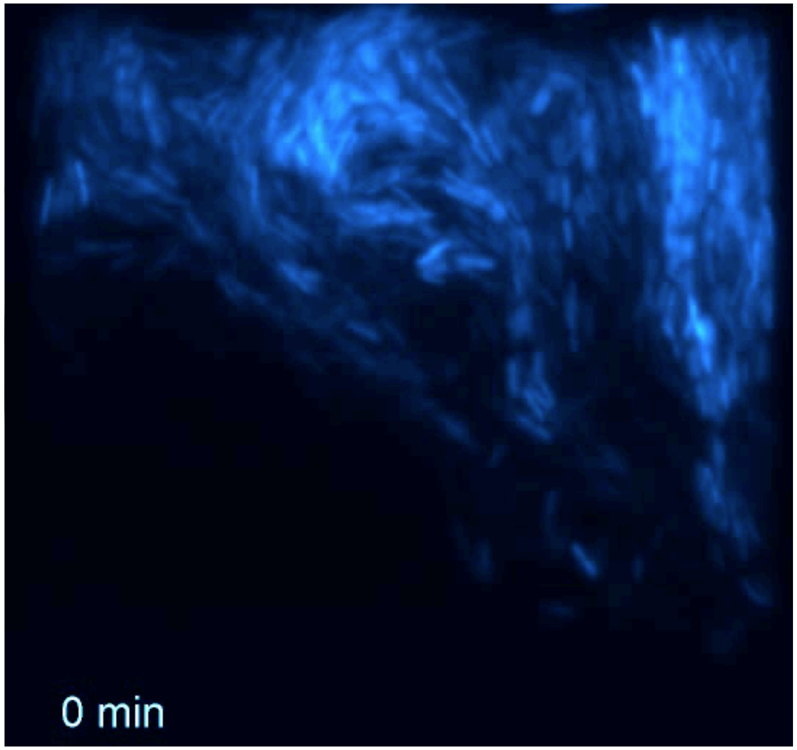
Regulation: control of gene expression

- Proteins bind to DNA, RNA and proteins to modulate gene expression
- Repression: X turns off expression of Y
- Activation: X turns on expression of Z

Biological Circuit Design (Synthetic Biology)







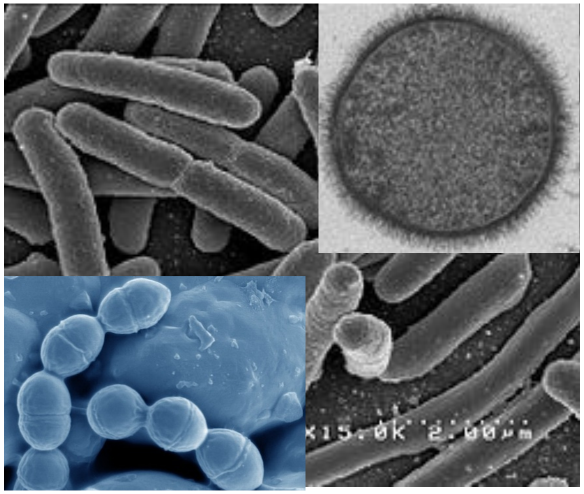
Repressilator (Elowitz & Leibler; 2000)

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

Synchronized oscillators (Danino, Mondragon-Palomino et al, 2010)

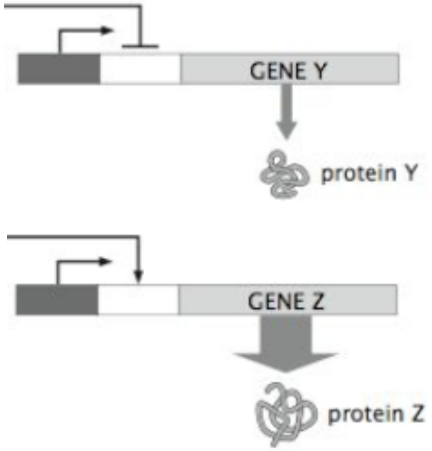
- Coupled oscillators by using cell-cell signaling
- Used relaxation style oscilator built on coupled +/- feedback loops + delay

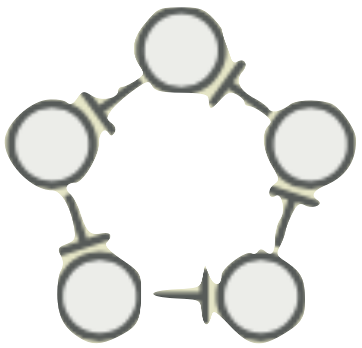
How Synthetic Biology Works



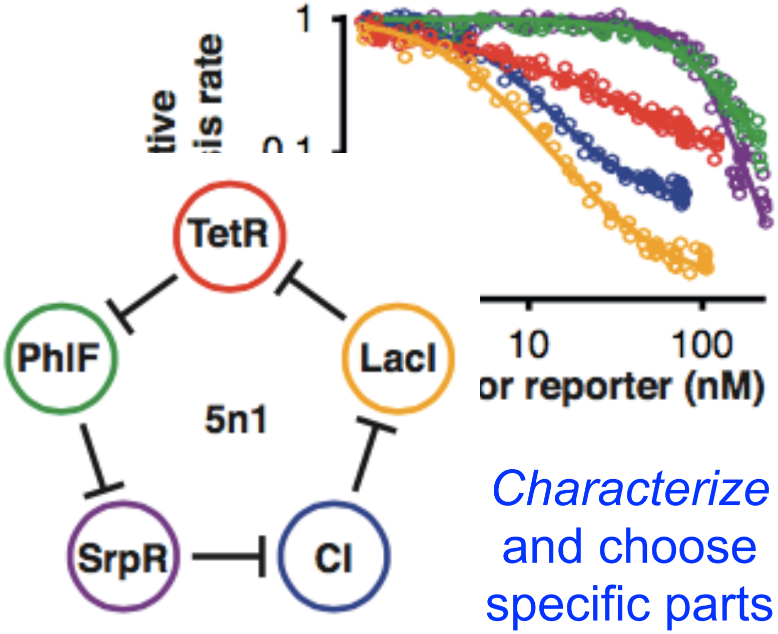
Natural organisms

Mine “parts”



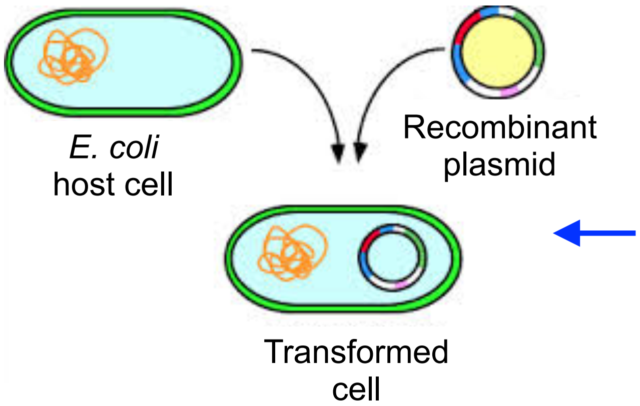


Conceive new circuits



Characterize and choose specific parts to use in circuit

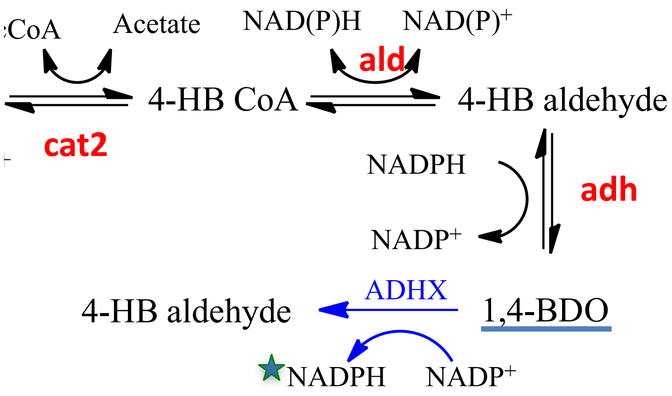
Synthesize circuit using DNA synthesis ($0.05/bp)



Transform circuit (plasmid) into cells

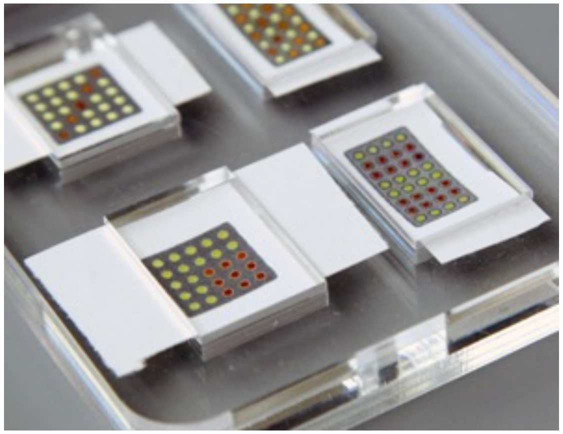
Synthetic Biology Applications

Materials Synthesis



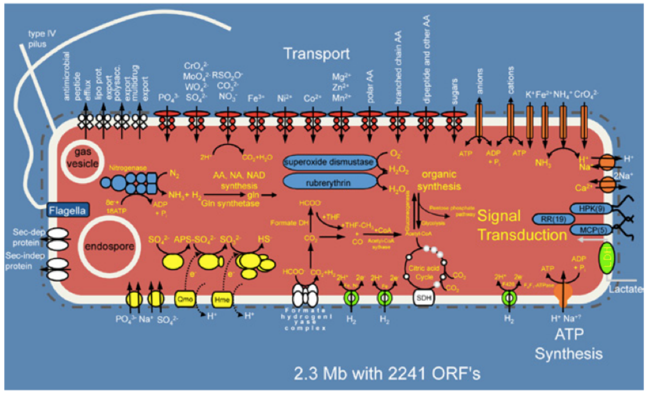
- Conversion of input resources to output products in modular way

Event Detectors



- Detection of environmental signals at the molecular scale

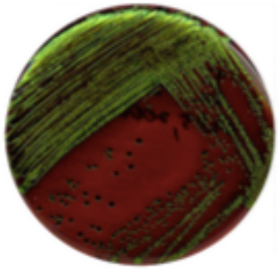
Artificial Cells



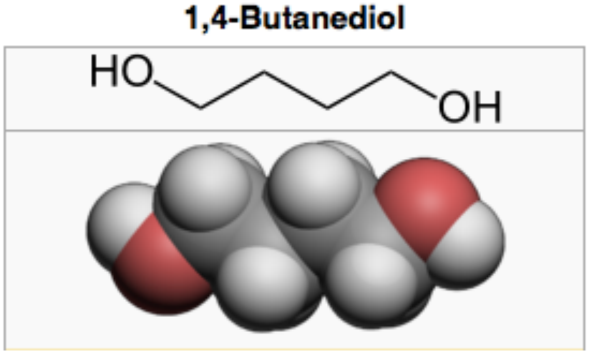
- Self-contained nanoscale biomolecular *machines* (circuits, subsystems, etc)

Potential Markets for Synthetic Biology Products

- Bio-based chemicals ("green" chemistry): $4B
- Bio-defense (molecular detection): $7B
- Therapeutics (health/medicine): $140B
- Nanoscale robotics: $0 (today…)



Materials Synthesis Example: 1,4-BDO

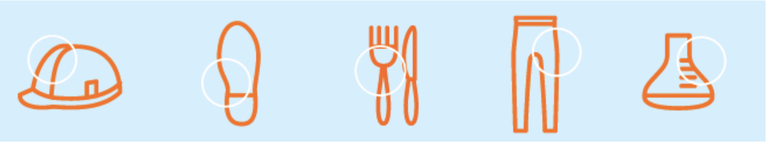


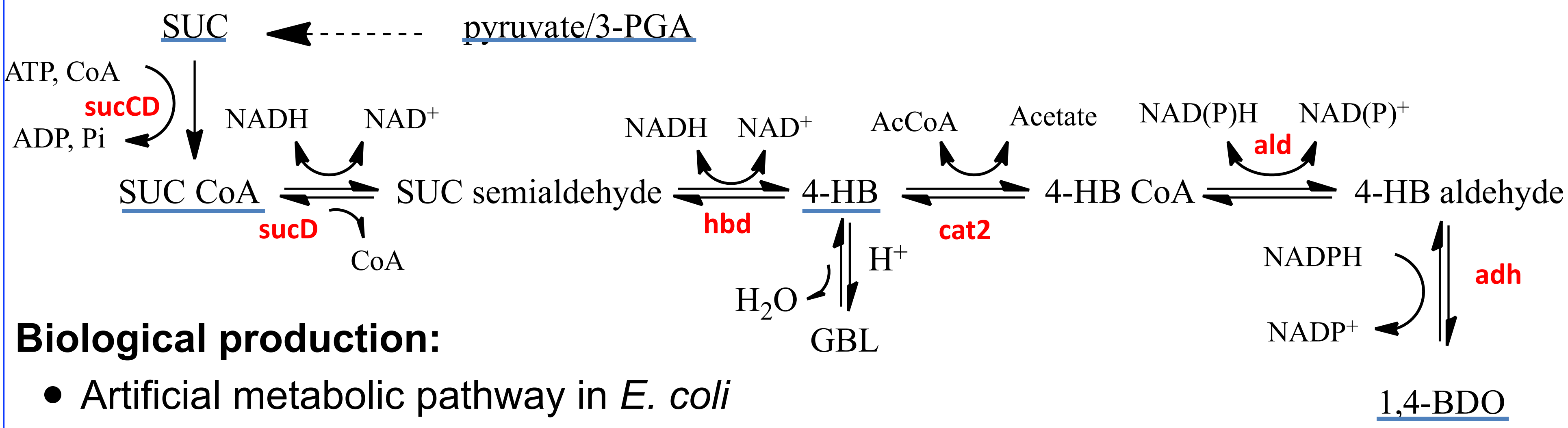
Industrial solvent used in manufacture plastics, elastic fibers and polyurethanes

- World production: one million metric tons per year
- Market price is about $2,000 USD per ton (2005)
- Sample usage: Spandex

Chemical production:

- Propylene oxide → allyl alcohol → 4-hydroxybutyraldehyde → 1,4-butanediol



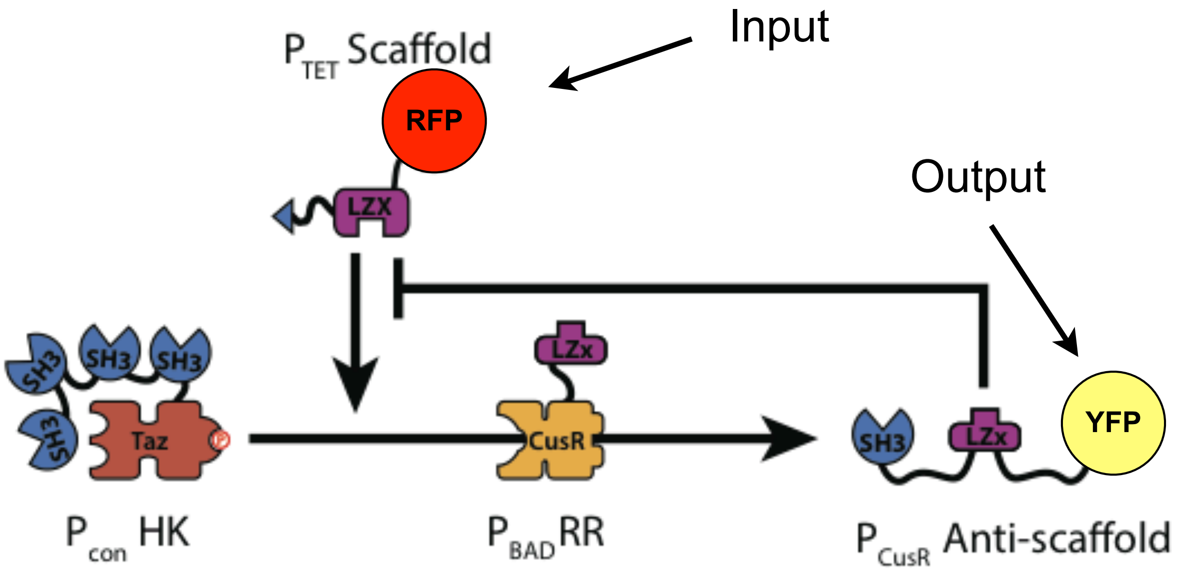


Biological production:

- Artificial metabolic pathway in *E. coli*
- Requires enzyme, strain, expression engineering

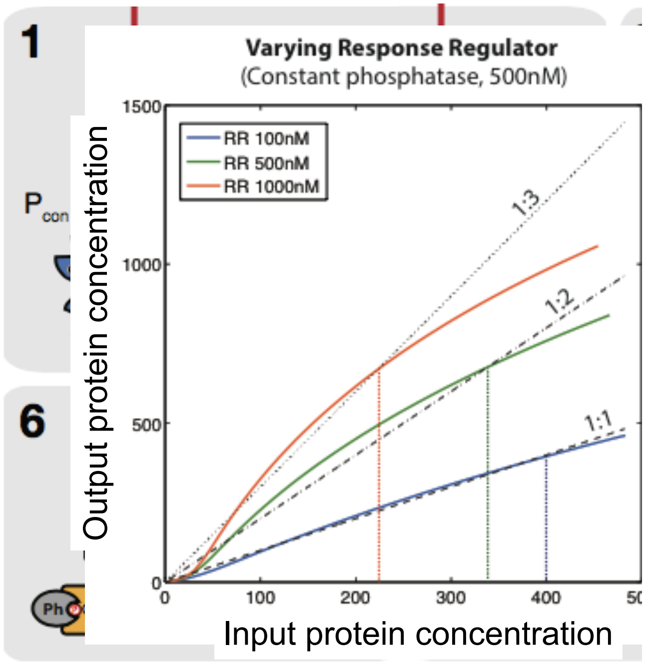
Hsiao and de los Santos et al, *ACS Synthetic Biology* 2014

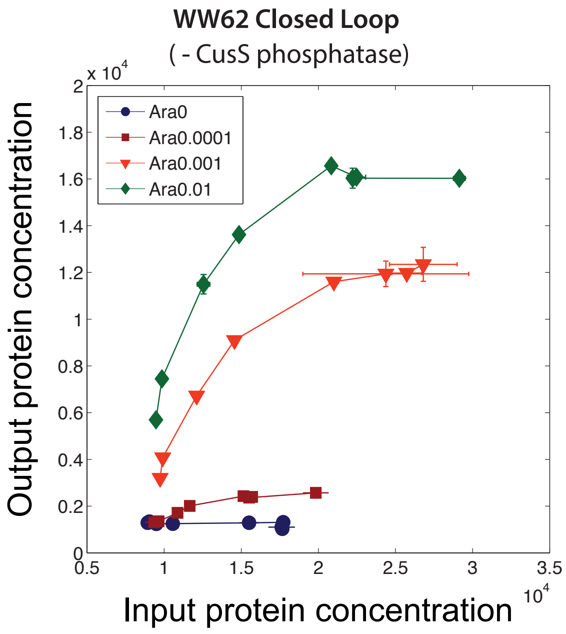
Concentration Regulation via Scaffold Proteins

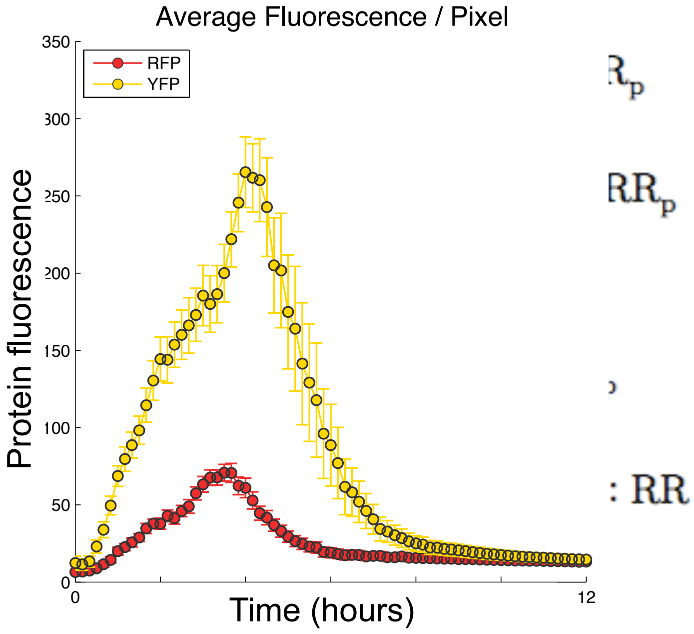


Circuit operation

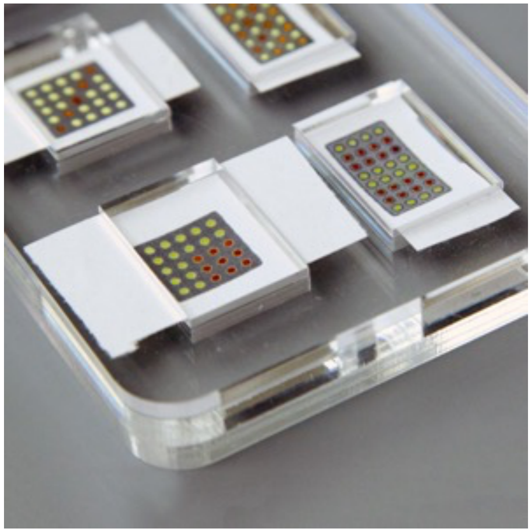
- Use scaffold domains to modulate activity of (non-cognate) histidine kinase and response regulator proteins
- Use anti-scaffold feedback to modulate activity level and regulate concentration of output to match input

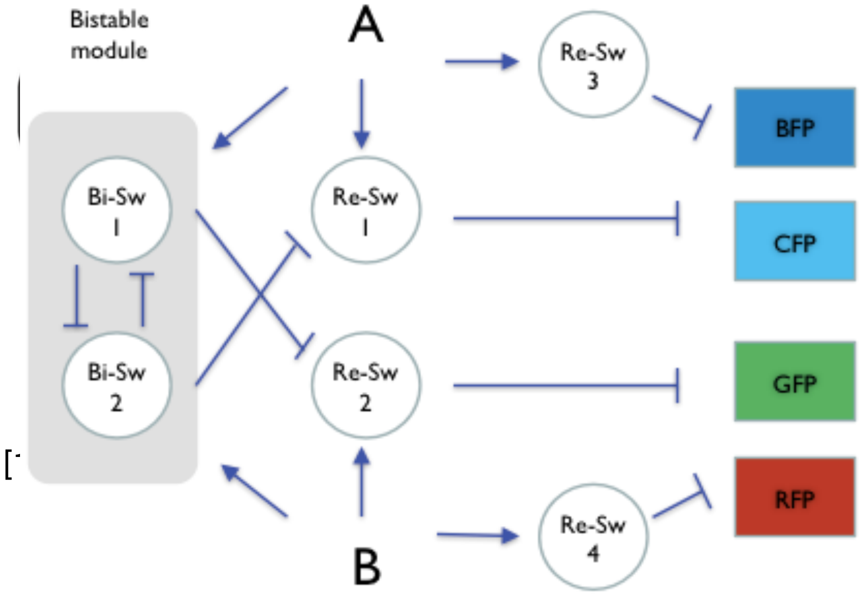




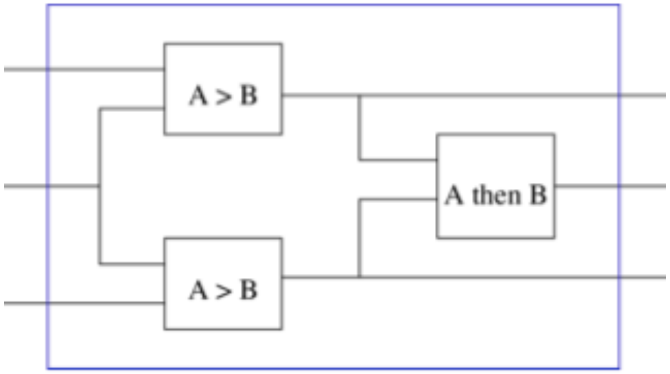


Biomolecular Event Detectors





Input	Output
No input	BFP, CFP, GFP, RFP
A	GFP, RFP
B	BFP, CFP
A then B	GFP
B then A	CFP



Interconnection of modules
to detect more complex events

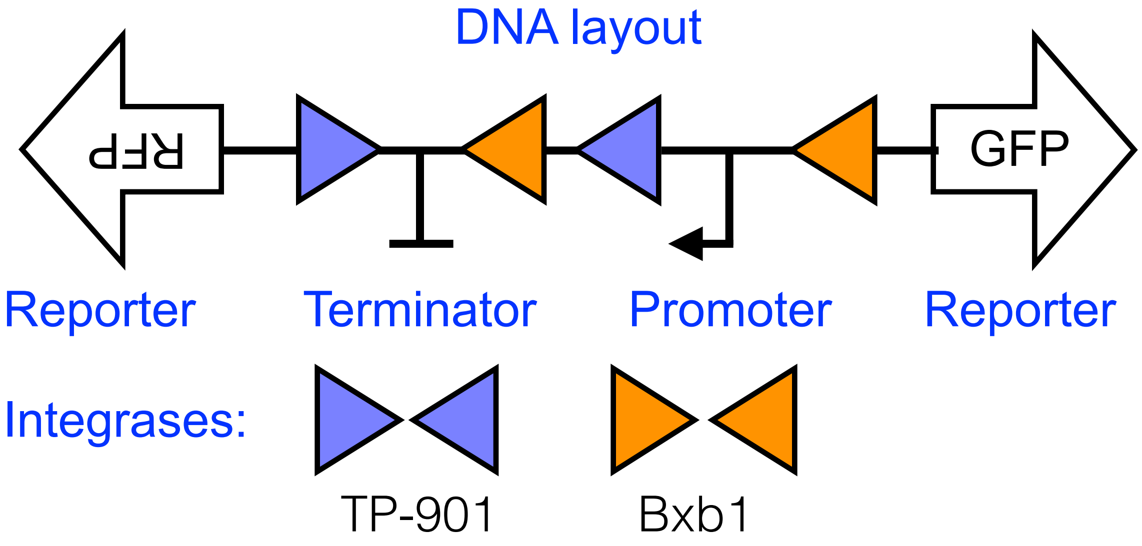
Approach

- Component technologies: signal detection, event memory, species comparison, logic functions
- Primitives: A > B, A followed by B, A > thresh, etc
- Interconnection framework: modular techniques for interconnecting components & detectors

Deployment: paper, hydrogels, cells…

Hsiao, Hori and M., bioRxiv, 2014

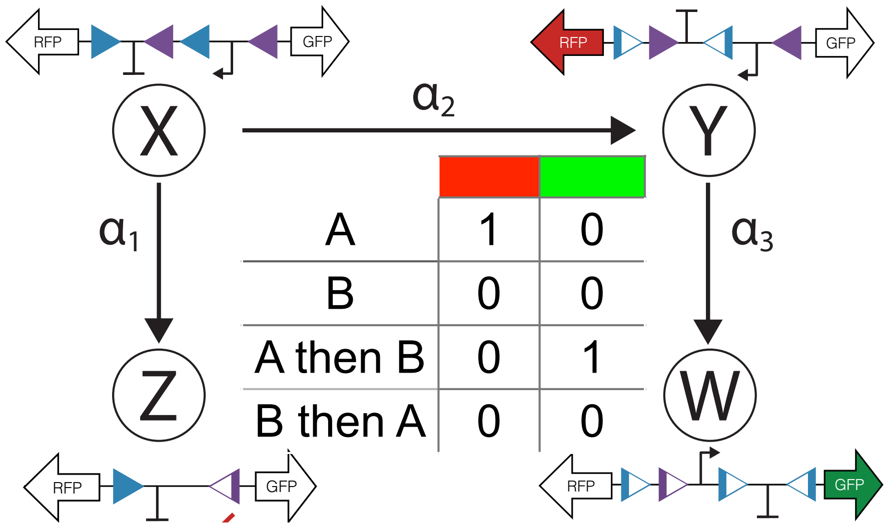
Event Ordering Detection (A then B)



DNA layout

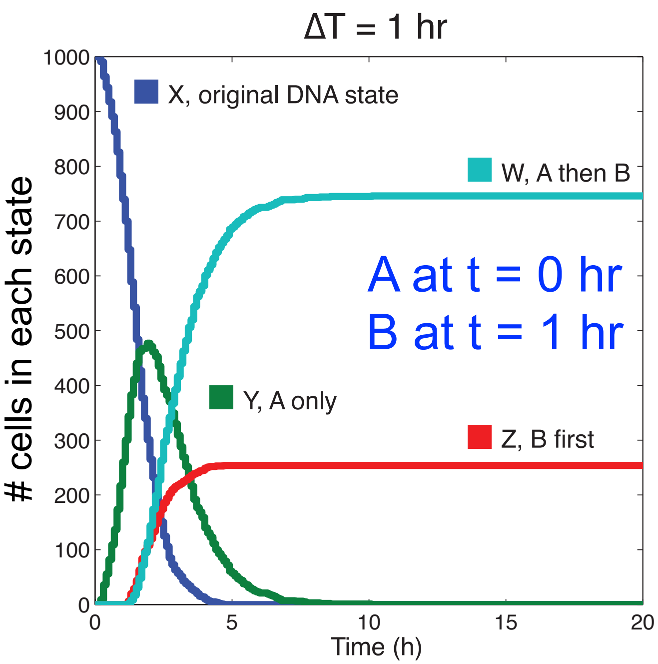
Reporter Terminator Promoter Reporter

Integrases: TP-901 Bxb1

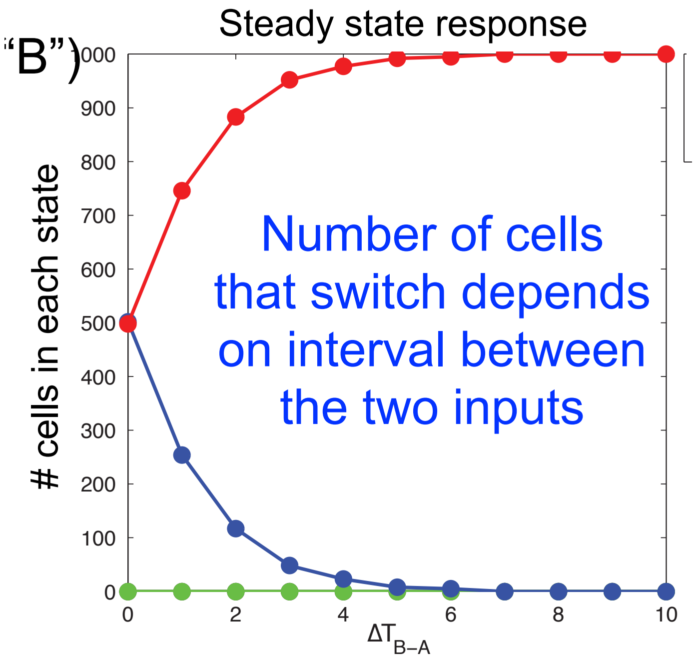


	🟥	🟩
A	1	0
B	0	0
A then B	0	1
B then A	0	0

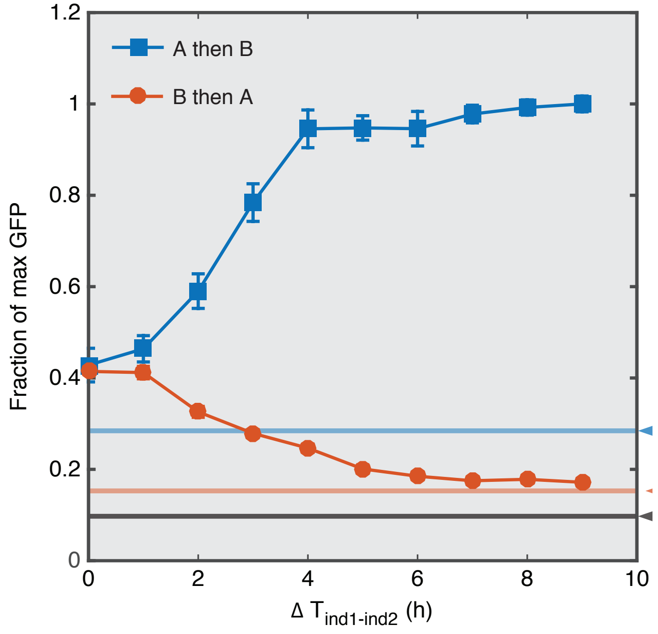
Markov process model



A at t = 0 hr
B at t = 1 hr

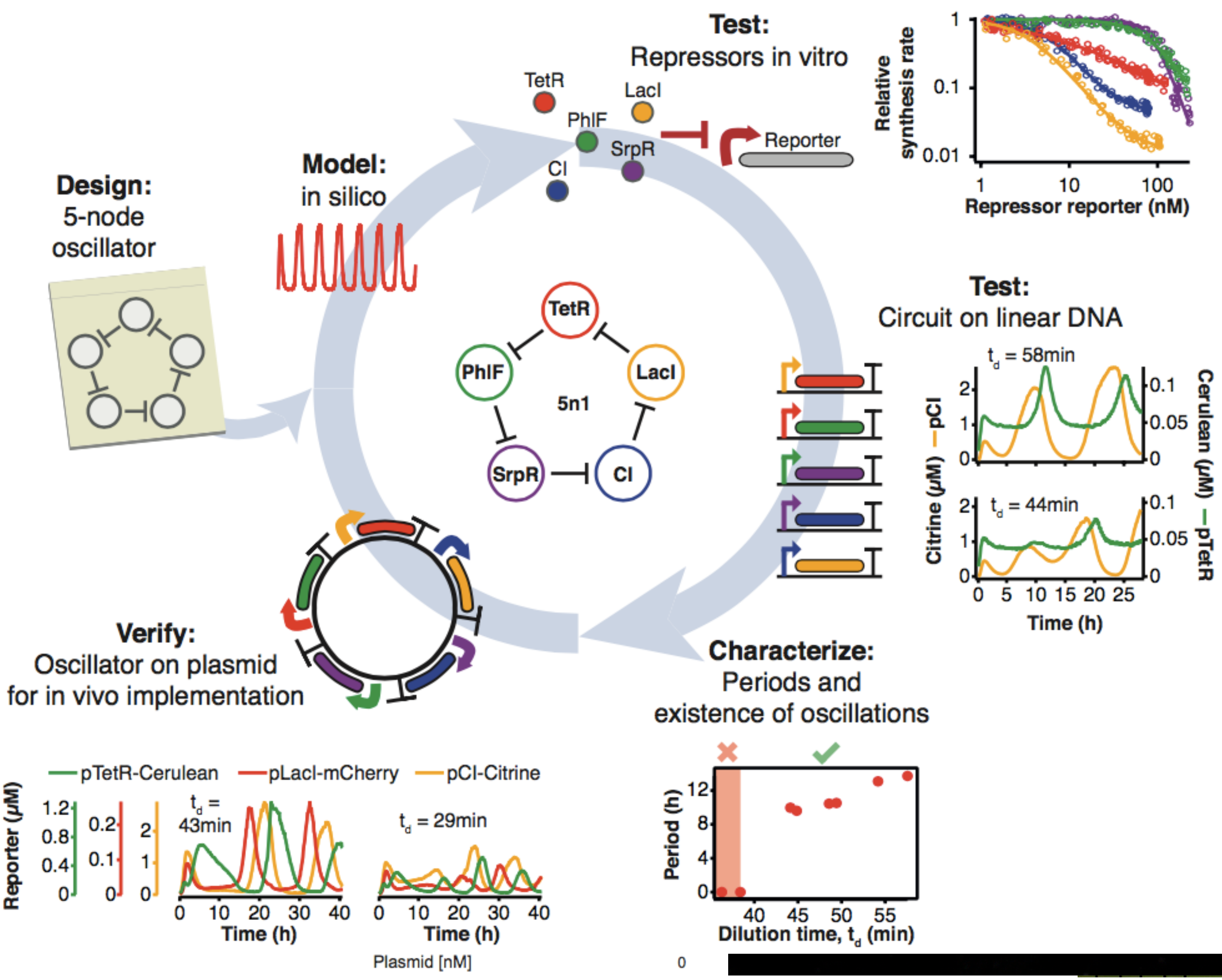


Number of cells that switch depends on interval between the two inputs



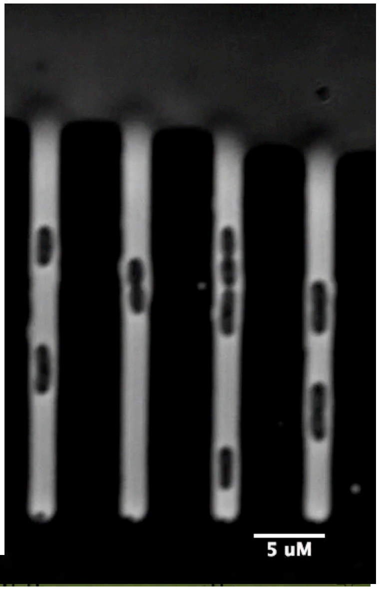
Niederholtmeyer and Sun et al., bioRxiv, 2015 [s]

5 Node Oscillator Design Cycle

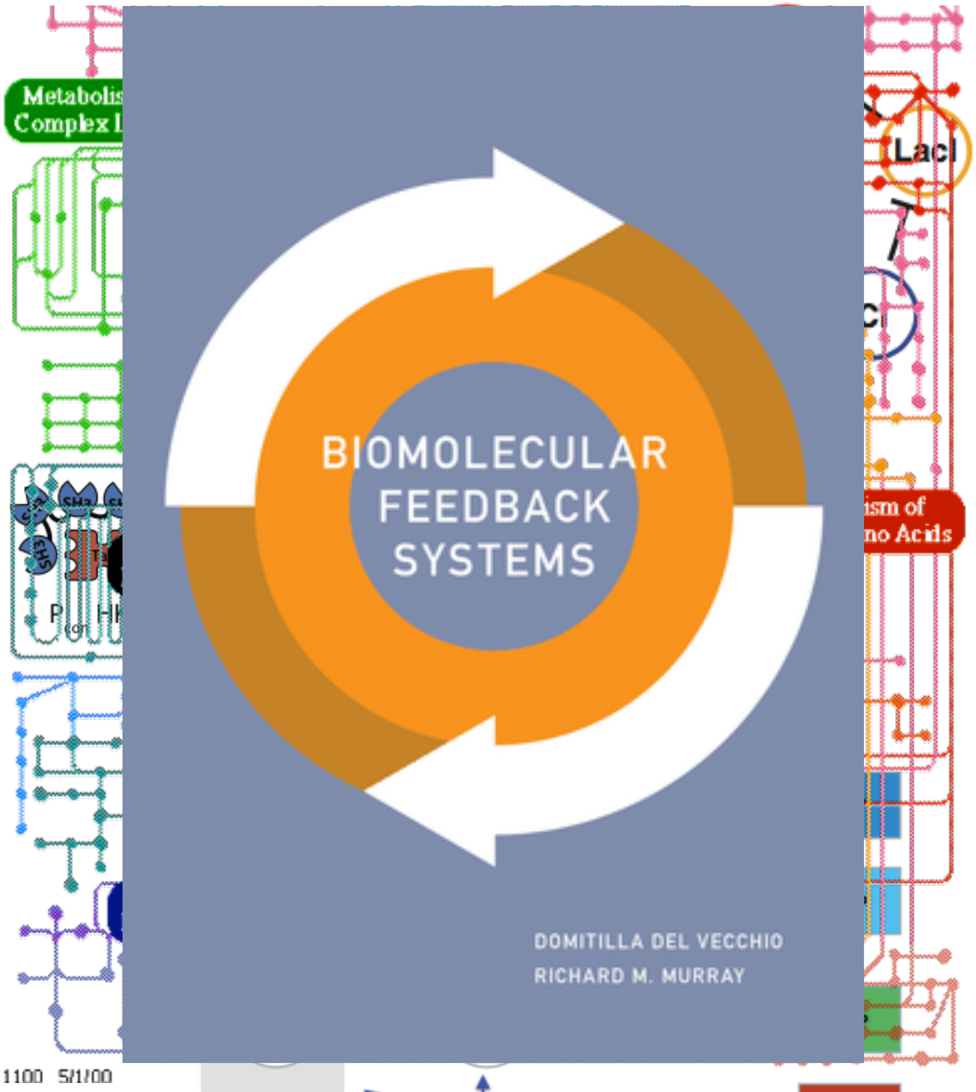


Timeline:

- 20 Jul: design start
- 24 Jul (4d): *in vitro* demo
- 23 Sep (2m): initial *in vivo*
- 30 Mar (6m): final *in vivo*
- 20 Apr (9m): submit/post



Control Theory for Biological Systems



http://www.cds.caltech.edu/~murray/BFS
(free PDF download for CCC/SICE'15)

What's different about biomolecular feedback systems

- Complexity
- Stochasticity
- Communications (and crosstalk)
- Resource limits
- Uncertainty (components and context)
- Evolvability

Potential application areas for tools from feedback control theory

- System identification
- Analysis (performance, robustness)
- Design (robustness, dynamics, interconnection, modularity)
- Fundamental limits

Many ways to get started!

- iGEM (undergraduate competition)
- CSHL Syn Bio course (2 wks, summer)