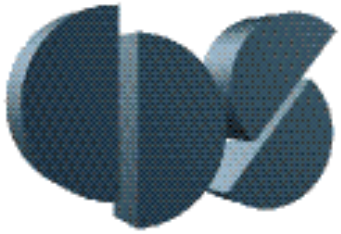
Biomolecular Breadboards for Prototyping and Debugging Synthetic Biocircuits



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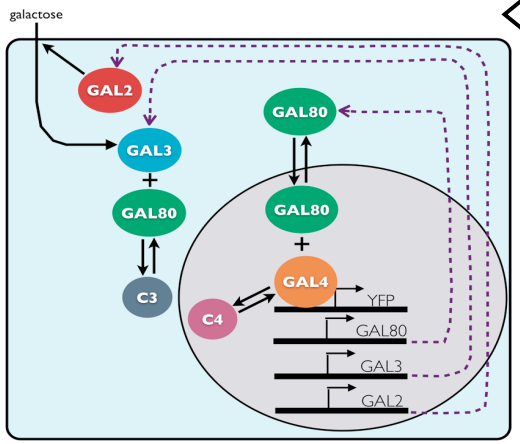
Analysis & Design of Biomolecular Feedback Systems

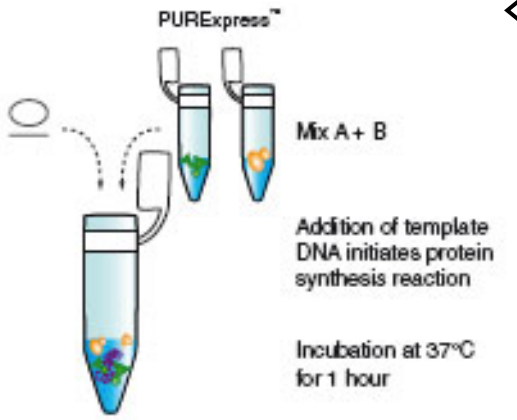
Systems Theory

- Role of feedback
- System identification
- Effects of crosstalk



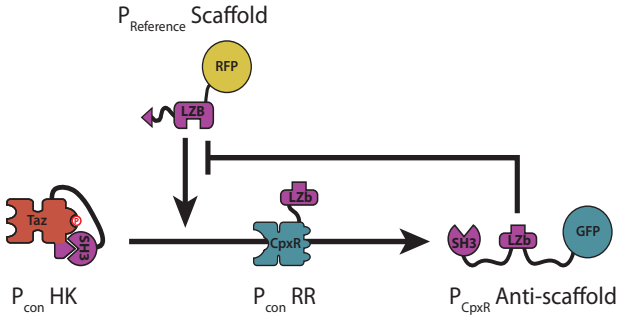
In Vitro Testbeds

- RNA-based genelets
- Biomolecular breadboards
- Droplet-based automation



Biocircuit Design

- Fast feedback mechanisms
- Heterogeneous redundancy
- Systematic design methods



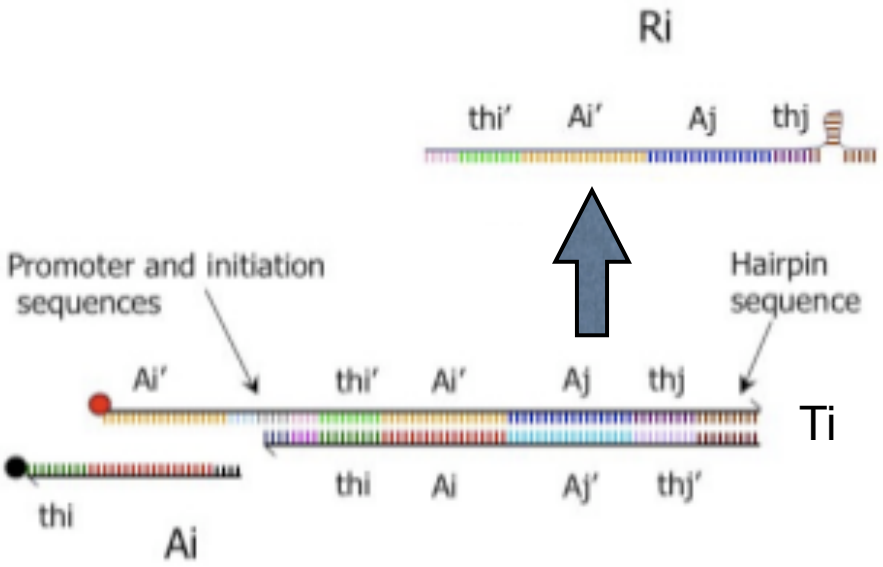
Some results to date

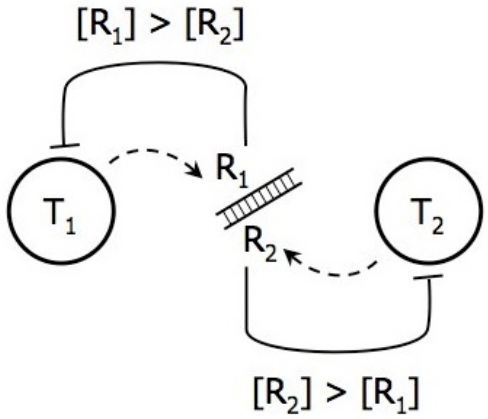
- Role of (multiple) feedback loops in generating bimodality, robust response
- Design of rate and concentration regulation circuits (genelets + scaffold proteins)
- Time-delay as a mechanism for “design of dynamics” (mainly theory, so far)
- Prototyping/debugging using cell-free extracts (modeling/experiments, droplets/bulk)
- Effects of resource limits (TX-TL experiments, simulations + theory)
- Temperature dependence and temperature compensation

Genelet Circuits: In Vitro Rate Regulator

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)





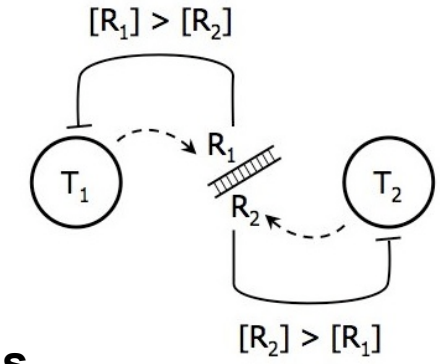
Molecular programming for in vitro systems

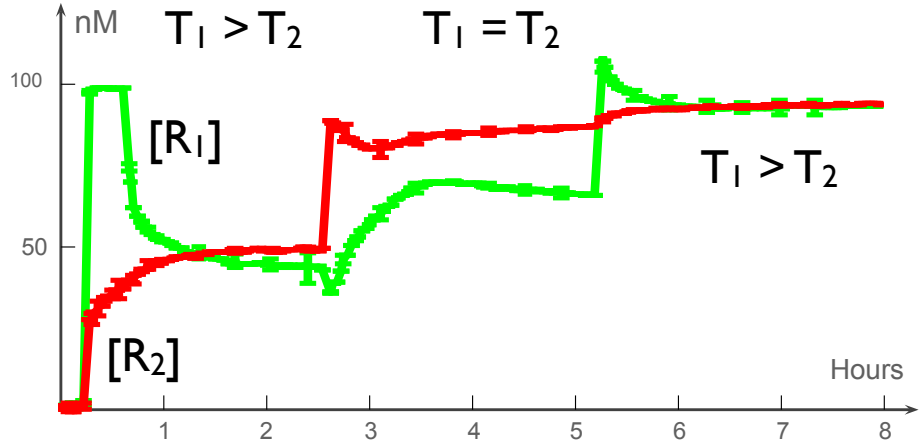
- Exploit Watson-Crick base pair binding (A-T, C-G)
- Can "compile" functional specifications into RNA and DNA sequences
- Circuits are biocompatible ⇒ some hope of embedding into cells

Rate Regulator Results

In vitro experiments

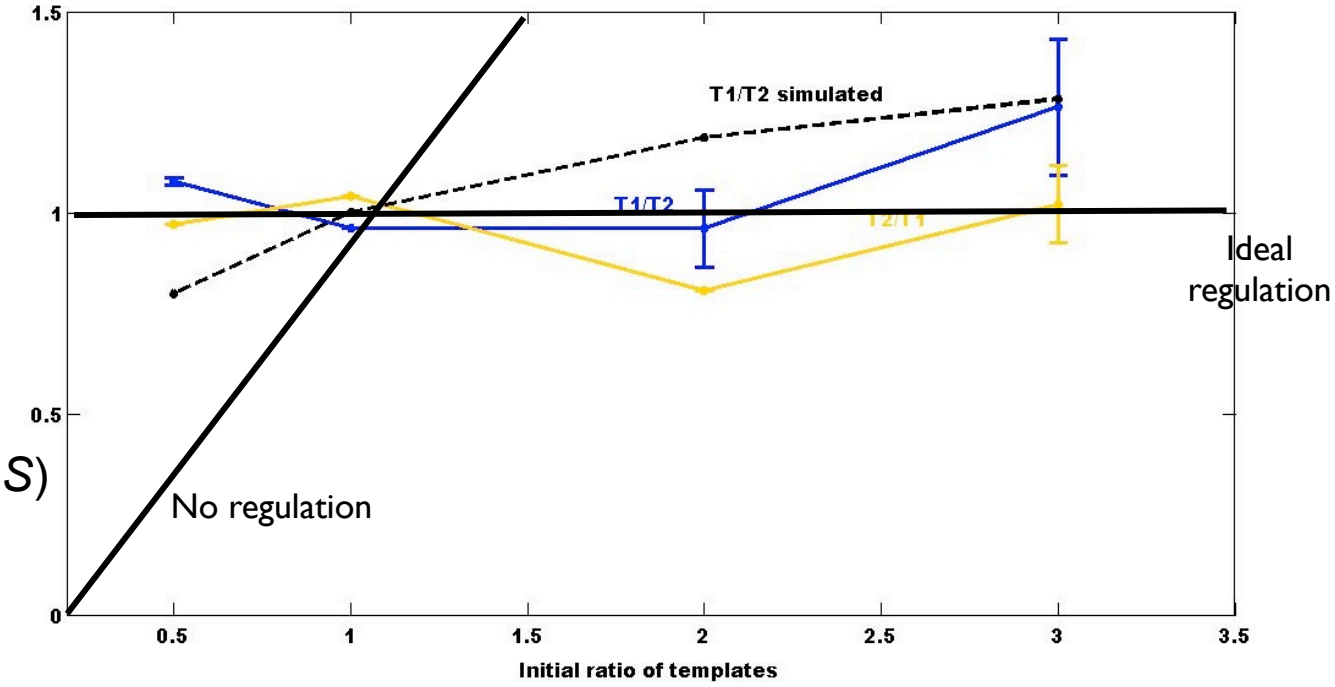
- Add templates + enzymes to test tube
- Use fluorophors to measure amount of repression





Rate regulator functions correctly

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

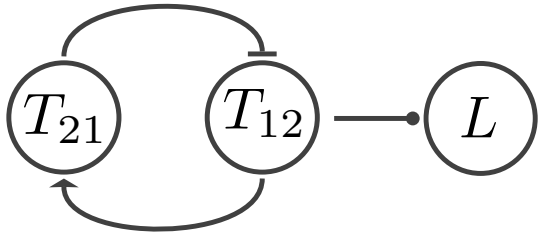


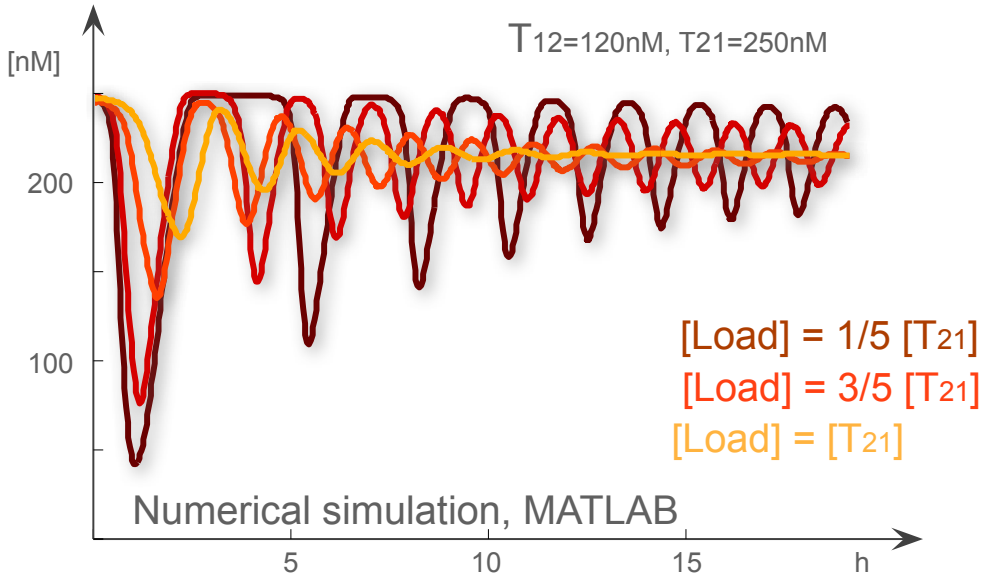
Extensions (ongoing)

- Coupling/loading (*PNAS*)
- Sensing/actuation
- Integral feedback (via feedforward "loops")

Improving Modularity

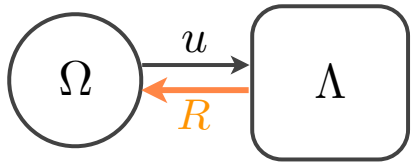
Effects of loading



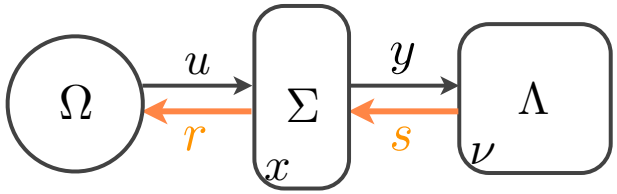


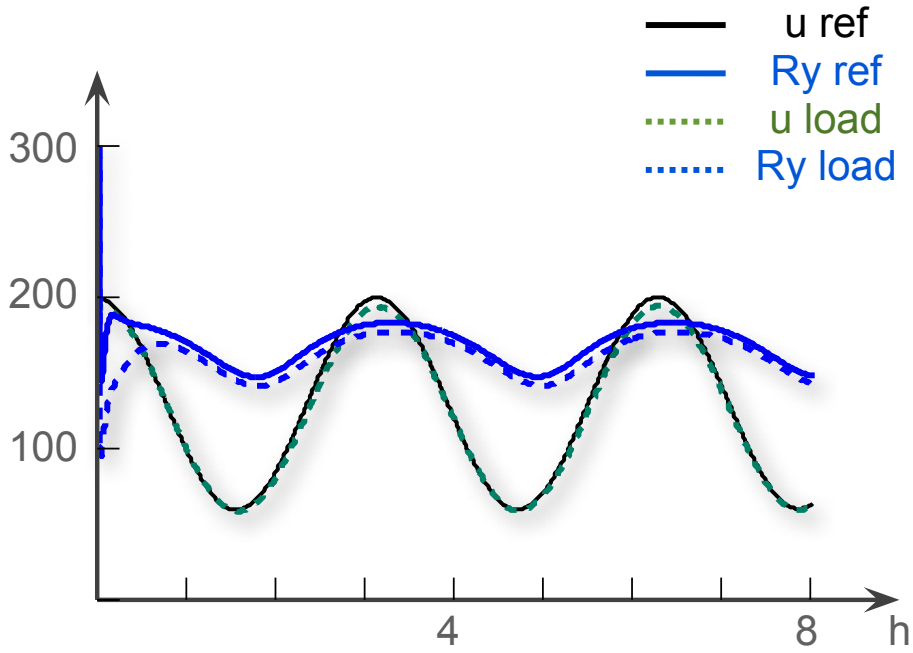
Modeling approach: retroactivity (Del Vecchio and Sontag, 2008)

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



- Use "insulator" to isolate

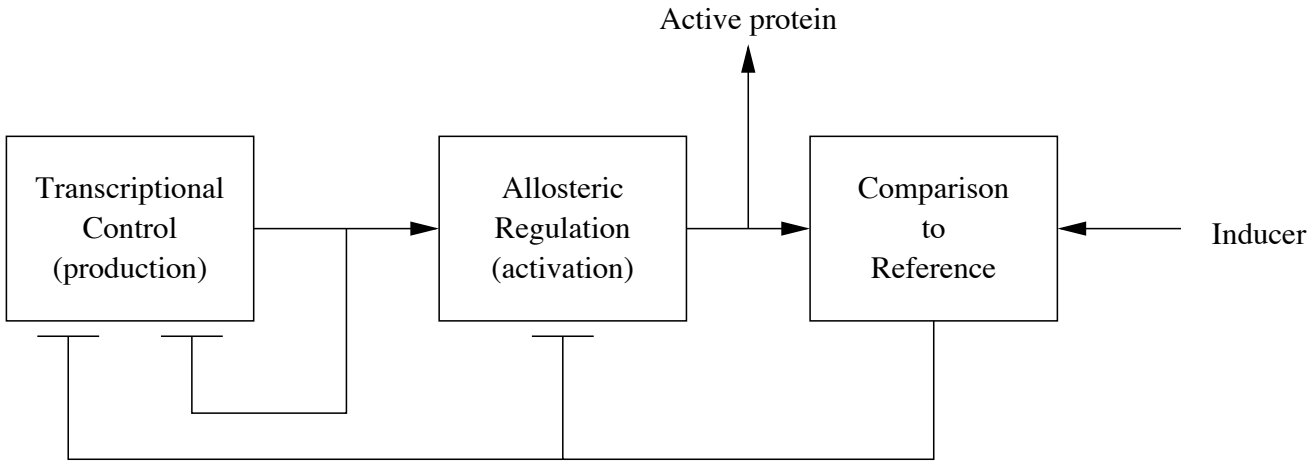


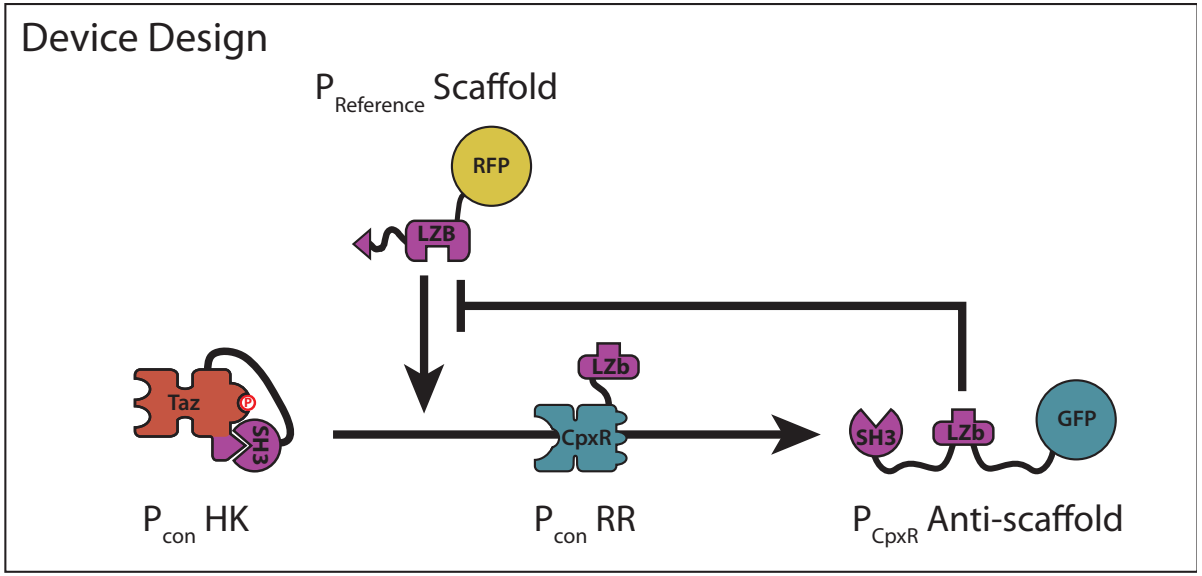


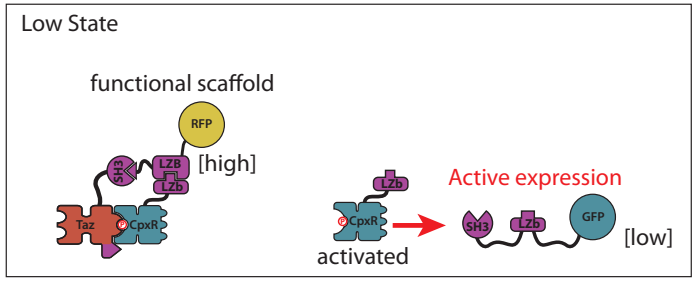
From Genelets to Genes

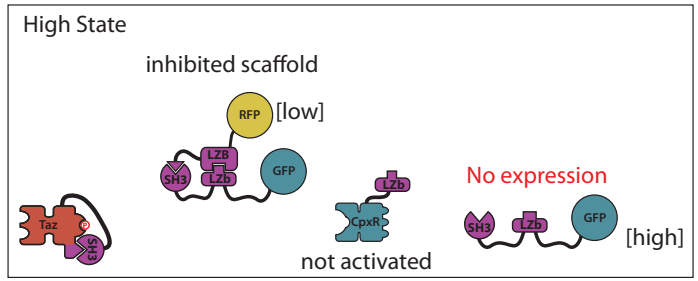
Moving to *in vivo* circuits

- Explore heterogeneous redundancy
- Use allosteric regulation for rapid response (similar to chemotaxis)
- Transcriptional feedback for low frequency gain
- *Need a way to compare signal with a reference*









Concentration Regulation via Scaffold Proteins (Hsiao)

Basic idea

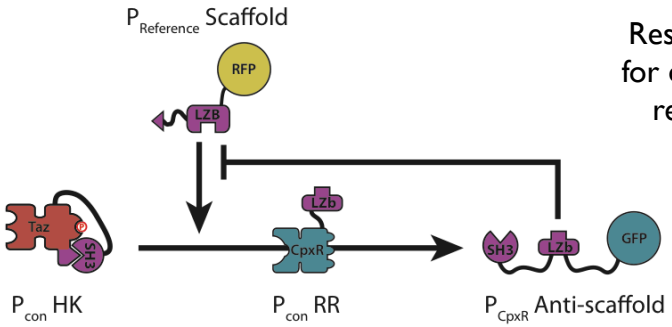
- Use scaffold domains to modulate activity of histidine kinase and response regulator proteins
- Use anti-scaffold feedback to modulate activity level and regulate concentration of output to match input

Results to date

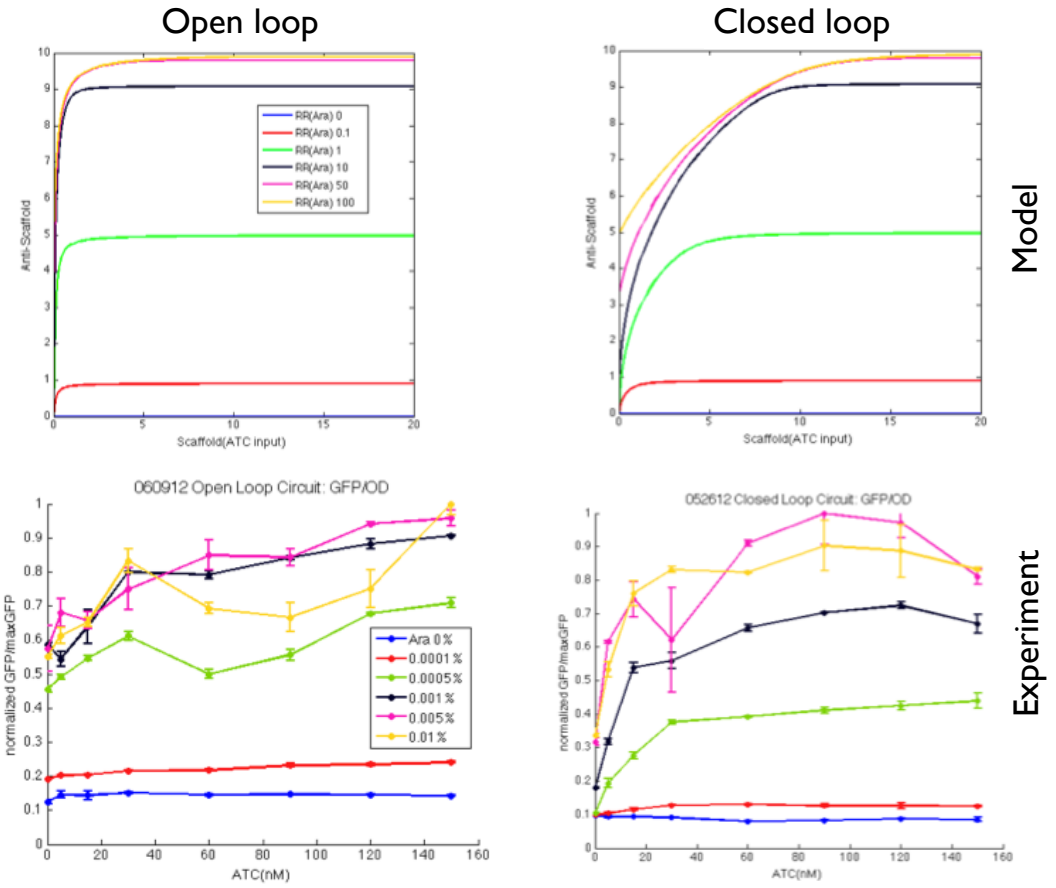
- Models + experiments demonstrate ability to modulate output (GFP) concentration to better match input (RFP) concentration
- Finalize quantitative comparison and explore design tradeoffs

Future possibilities

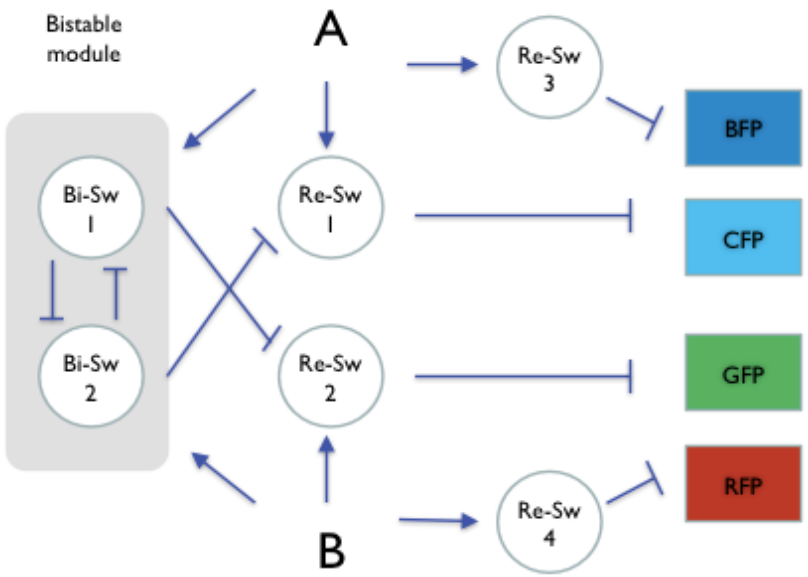
- Protein domain provide mechanism for programmable circuits
- Starting point for biomolecular event detectors (comparators)



Response regulator circuit for detecting and regulating relative concentration.



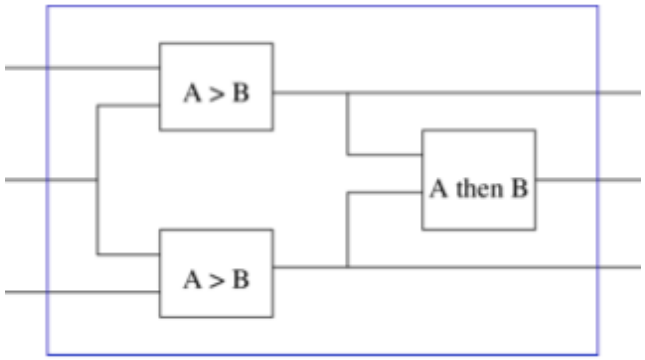
Future State: Event Detectors (In Vitro & In Vivo)



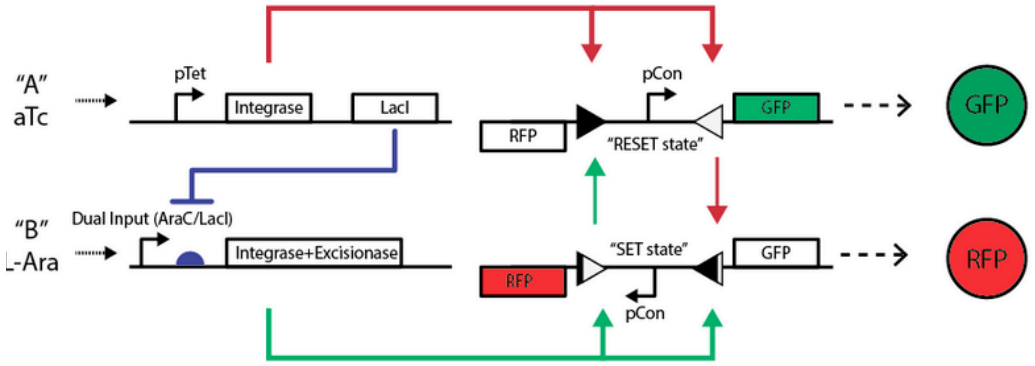
Input	Output
No input	BFP, CFP, GFP, RFP
A	GFP, RFP
B	BFP, CFP
A then B	GFP
B then A	CFP

Approach

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



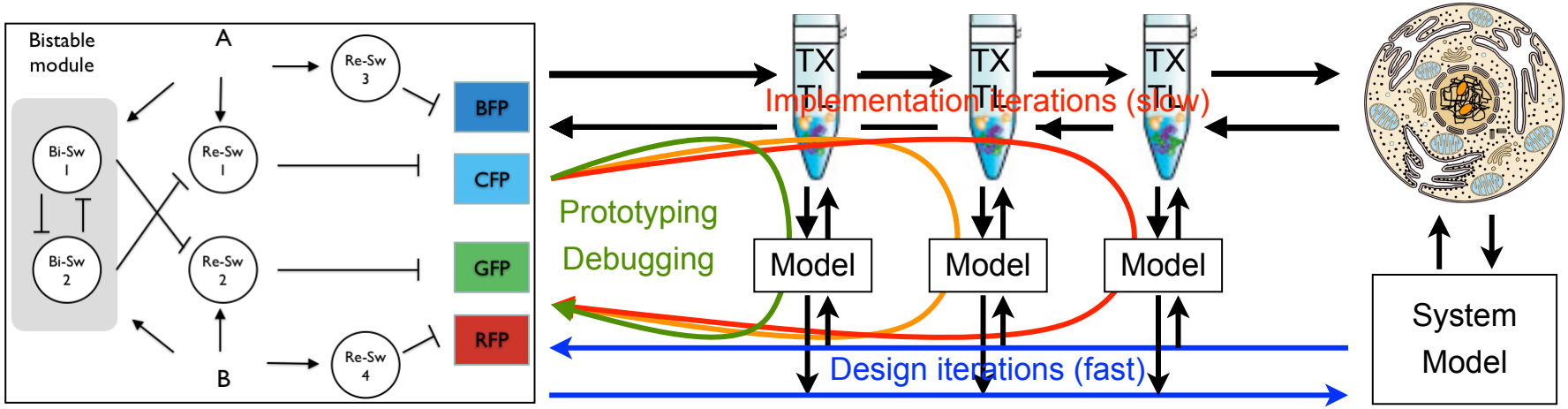
Interconnection of modules
to detect more complex events



GFP if A is always less than B
RFP if A is greater than B

Sun et al, JoVE 2013 (a)

Cell-Free Biomolecular Breadboards



Key characteristics of the cell-free breadboard (Noireaux et al)

- Inexpensive and fast: ~$0.03/ul for reactions; typical reactions run for 4-6 hours
- Easy to use: works with many plasmids or linear DNA (PCR products!)
  - Can adjust concentration to explore copy number/expression strength quickly
- Flexible environment: adjust energy level, pH, temperature, degradation

Milestones/demo for Phase I http://www.openwetware.org/wiki/breadboards

- Q1: Post protocol on web, along with controls + summary of costs
- Q2: Demonstrate breadboard on 2 circuits (eg, switch, IFFL), document iteration time
- Q3: Post complete protocols + variations (degradation, energy, ...) + validated models
- Q4: Demonstrate design of 3-6 promoter circuit with 3 day cycle time, 1 month total

Phase II demo: 8-16 promoter circuit, 100 variations, 1 day cycle time, 1 week total

Sample TX-TL Based Design Process

S0: modeling (minutes/cycle, systematic design & analysis)

- Desired function + specs → set of possible designs (circuits) + sensitivity analysis
- Goal at this stage is to determine what circuits to test in TX-TL and predict outputs

S1: linear DNA (4-8 cycles @ 2 cycles/day, 24-96 variants)

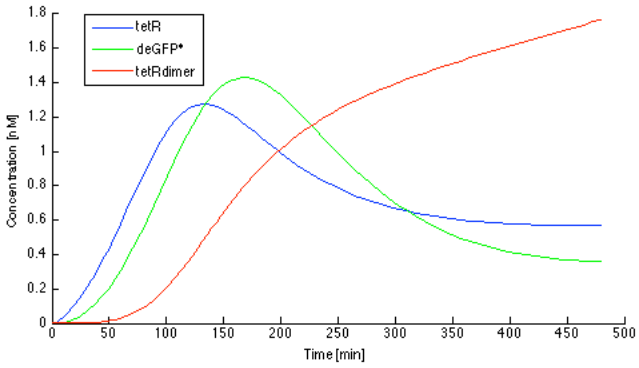
- Components from std library or PCR extension (no cloning)
- Test in TX-TL with GamS, ClpX. Try multiple circuits + vary ratios of copy numbers (based on achievable copy #'s)
- Compare w/ models; insure we can model what we see
- Goal: downselect 4-8 designs to test in plasmids

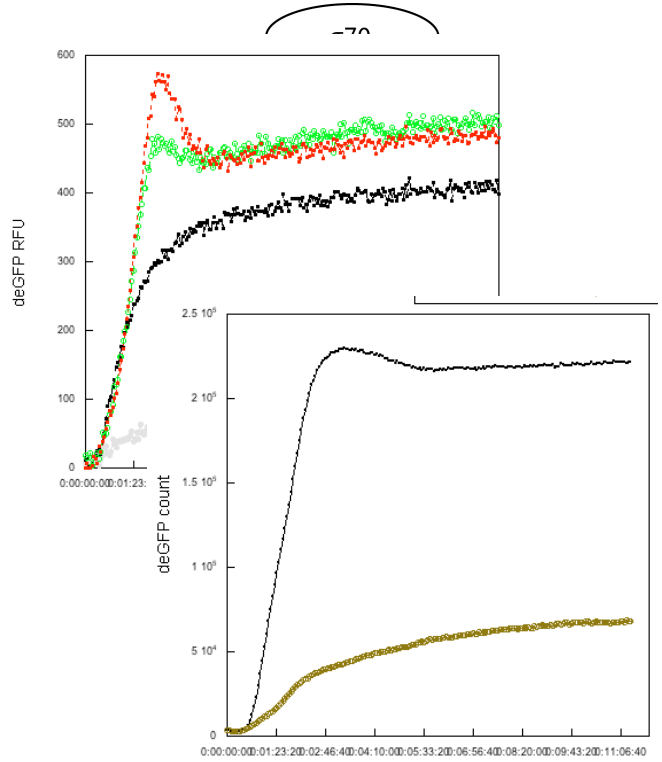
S2: plasmids (2-4 cycles @ 2 days/cycle, 8-24 variants)

- Clone into plasmid(s), using std sequences/protocols
- Verify operation in TX-TL, incl copy number variability
- Test robustness in multiple extracts w/ varying conditions
- Match results to S0 models and S1 linear DNA

S3: validate in cells (1 cycle, 4 days, 1-4 variants)

- Test top constructs from plasmid-based TX-TL assay



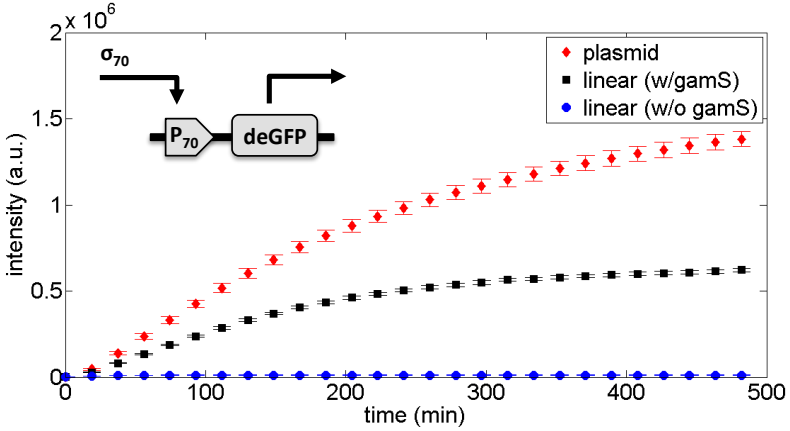


TX-TL Core Processes

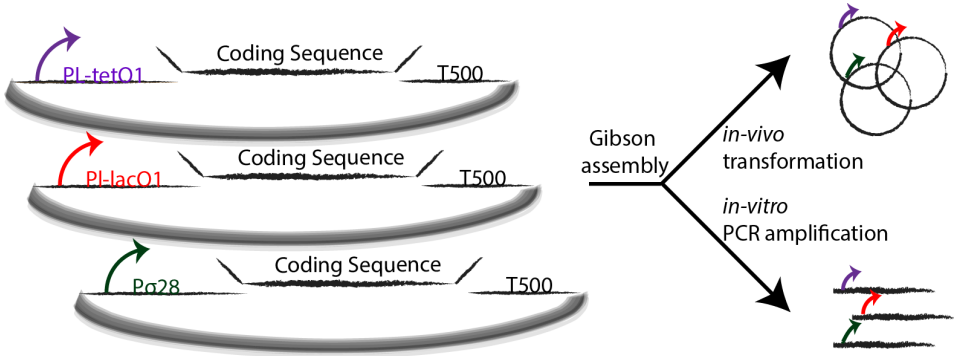
Zachary Sun, Vincent Noireaux

Rapid prototyping using linear DNA

- Use PCR products with GamS to get expression levels of ~60% of plasmid

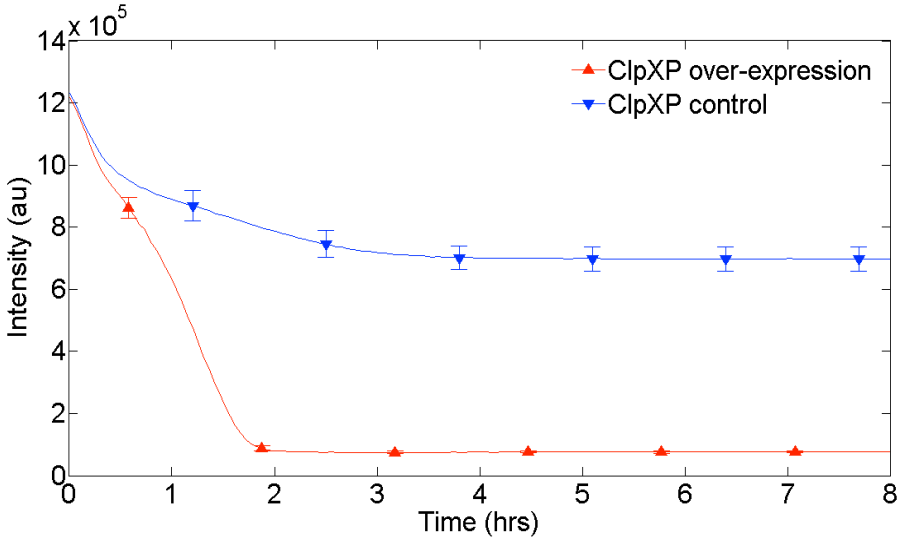


- Allows rapid assembly of constructs
  - PCR extension for simple circuits
  - IDT gBlocks + isothermal ass'y



Protein degradation

- Use clpXP machinery to degrade tagged proteins

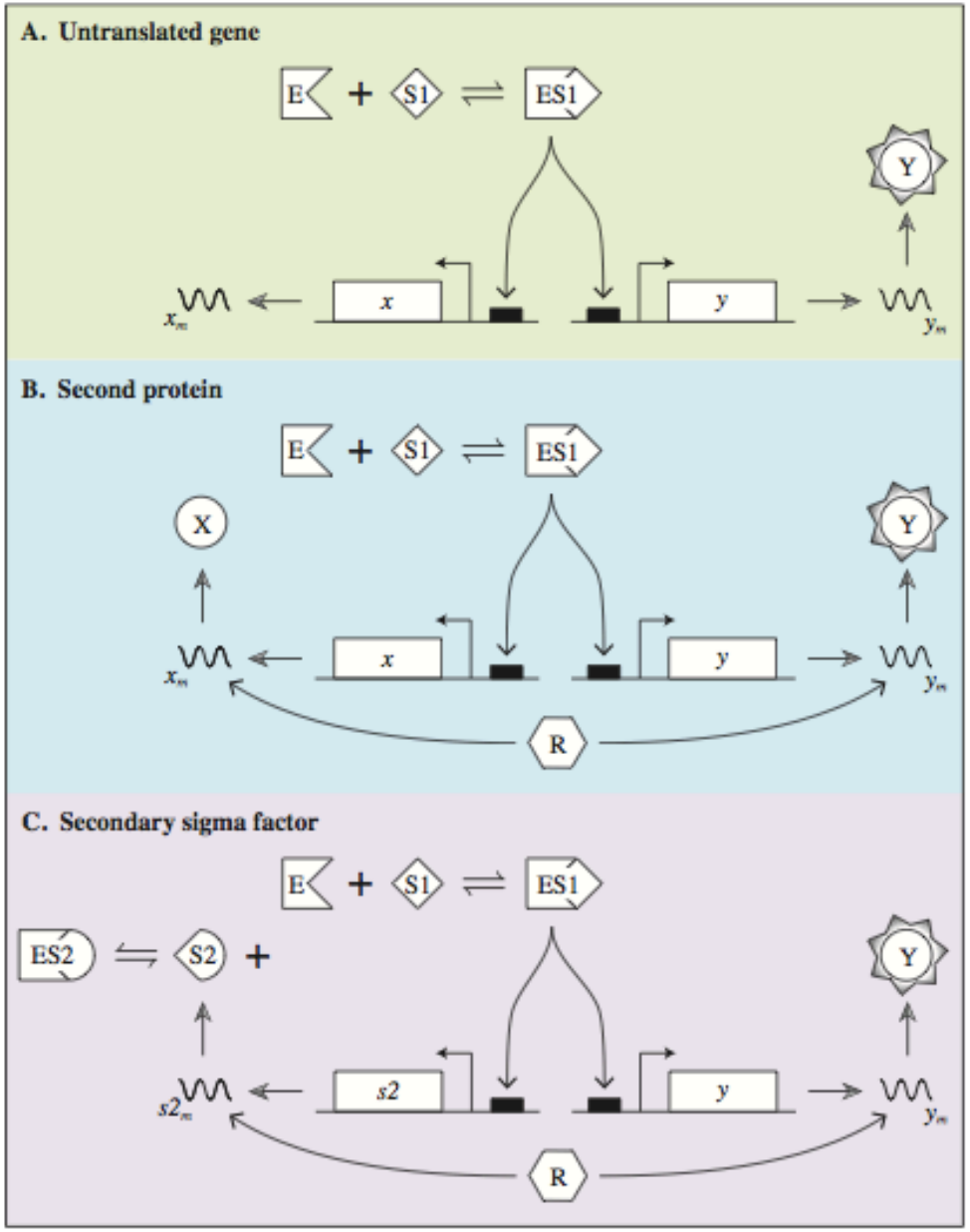


Tested components

- RNA polymerases: E. coli*, T7
- Activators: sigma28*
- Repressors: tetR*, lacI*
- Reporters: deGFP*, MG, mSpinach
- DNA/RNA/protein deg: gamS*, clpXP*

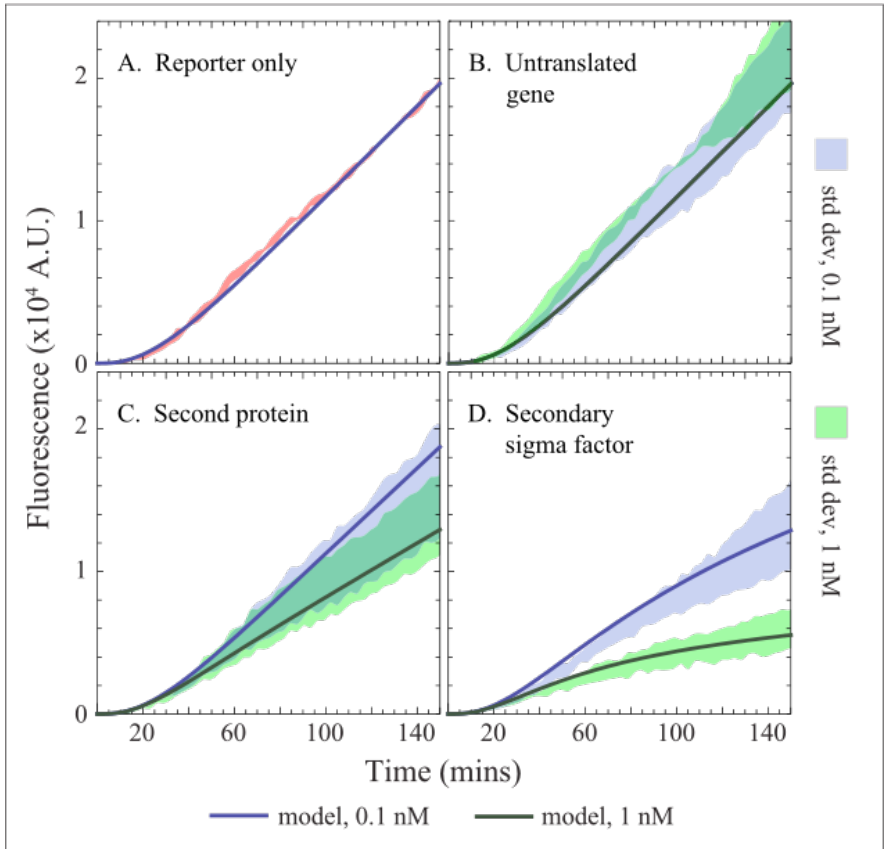
* preliminary models also available

Effects of Resource Limits



Which resources are limited?

- No evident transcriptional limits [B]
- Limited protein resources (AA, ATP) generate significant coupling [C]
- Sigma factors sequester RNAP [D]

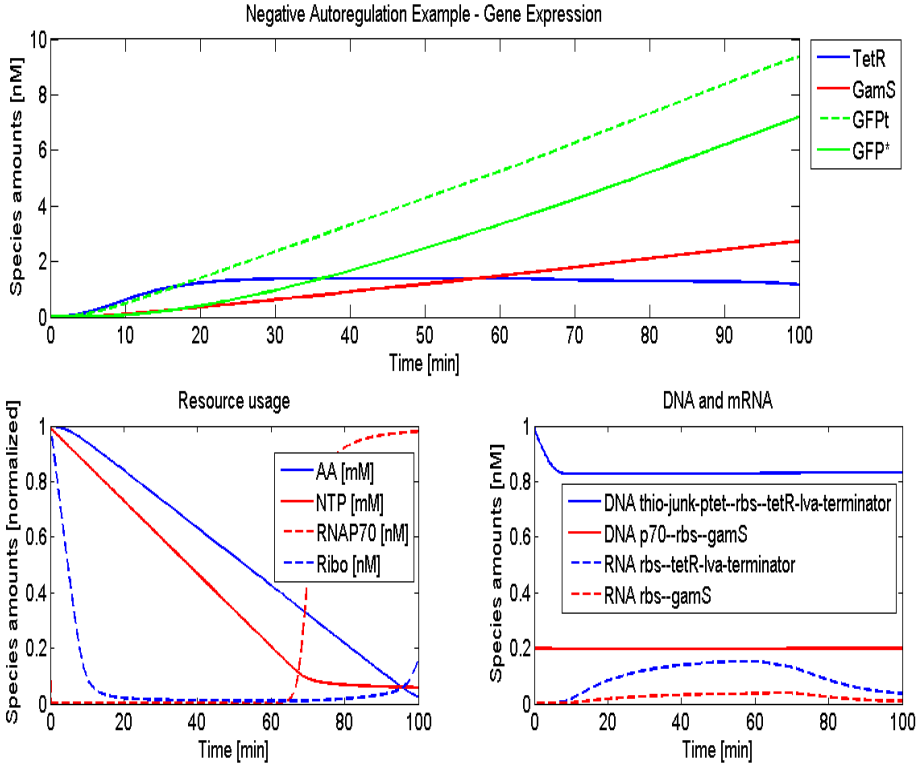


TX-TL Modeling

Zoltan Tuza, Vipul Singhal, Dan Siegal-Gaskins

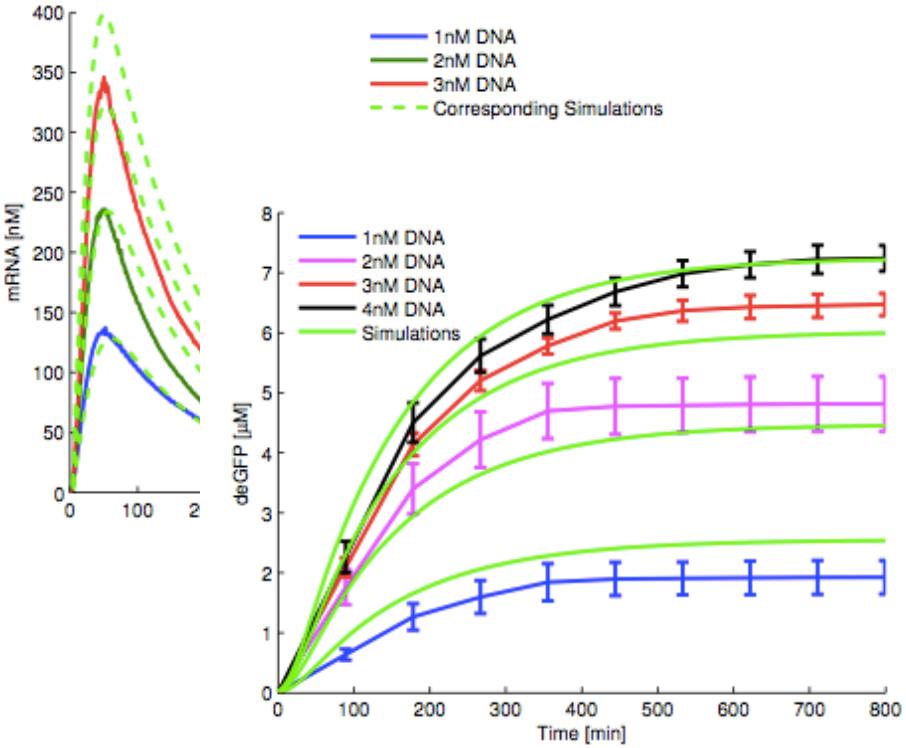
MATLAB toolbox (sf.net/projects/TXTL)

```
% Set up the standard TXTL tubes
tube1 = txtl_extract('e1');
tube2 = txtl_buffer('b1');

% Set up a tube that will contain our DNA
tube3 = txtl_newtube('circuit');
dna_tetR = txtl_dna(tube3, 'ptet', 'rbs', 'tetR', 100, 'linear');
dna_gamS = txtl_dna(tube3, 'p70', 'rbs', 'gamS', 10, 'plasmid');

% Mix the contents of the individual tubes and add some inducer
well_a1 = txtl_combine([tube1, tube2, tube3], [6, 2, 2]);
txtl_addspecies(well_a1, 'aTc', 0.1);

% Run a simulation
[t_ode, x_ode, names] = sbiosimulate(well_a1);
```



Resource utilization effects

- Model+TXTL shows effects of fixed number of RNAPs and ribosomes
- Additional sigma factor gene introduces significant 'crosstalk', reduces output
- Calibrated models that match experimental results



External TX-TL Circuit Testing

Circuit testing

- Stage 0: you send us cells/plasmids
- Stage 1: we perform TX-TL runs, compare to in vivo, send back data
- Stage 2: we send you extract + buffer, you take the data
- Stage 3: we show you how to make extract (JoVE paper on its way)
- Stage 4: you use TX-TL on your own

Things that work

- Transcriptional circuits
- RNA-based circuits
- Phosphorylation circuits

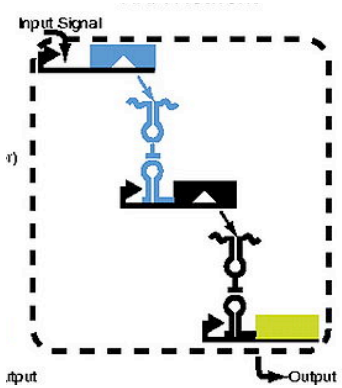
Things that haven't worked

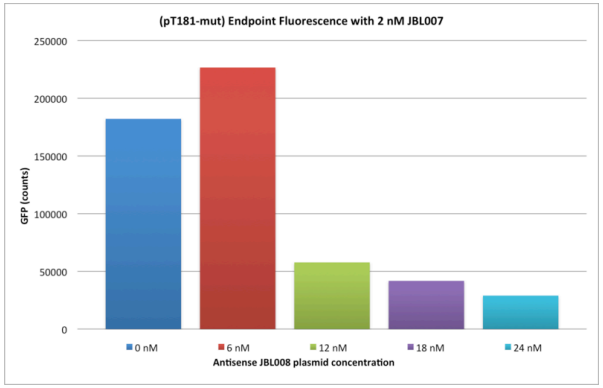
- Light-induced transcription factors
- Multi-layer cascades (resource lims)

CSHL synthetic biology course: Jul '13

- Will use TX-TL for RNA-based circuits

PI (+ contact)	Circuit/Technology	0 1 2 3 4
Lucks (CH)	RNA-sensing TFs	✓✓✓✓ -
Del Vecchio (EY)	Loading effects	✓✓ - - -
Temme (VH)	Orthogonal RNAPs	✓? - - -
Voigt (DSG)	4 input, 11 gene	✓x - - -
Tabor (JK)	Green light sensor	✓ ? - - -
Endy (VH)	DNA memory	✓ x - - -
Del Vecchio (SG)	Phospho-insulator	✓✓ - - -
Kortemme (EdIS)	Molecular sensors	✓○ - - -
Jewett (YW)	Butanediol pathway	✓○ - - -

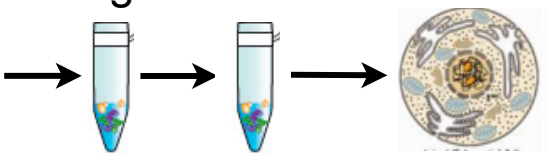




Biomolecular Breadboard Suite

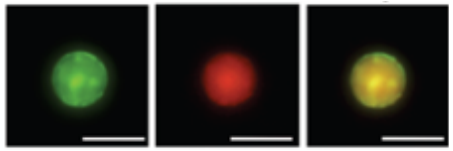
Cell-free breadboard

- Linear DNA assembly (build on work of others)
- Implement/test 6 circuits
- Document design cycle times (vs std cloning)
- Extract preparation video (→ JoVE)
- Predictive, modular models for switch, IFFL, neg fbk



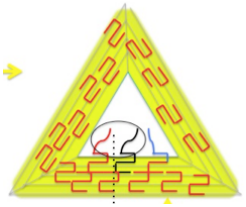
Artificial Cells

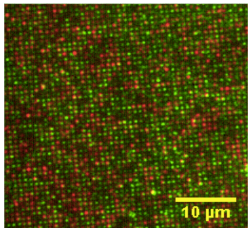
- Kinetics of expression inside vesicles
- Statistics of expression and induction (% of vesicles induced)
- Expression (and induction) as a function of vesicle size



Spatial Localization

- Control spatial location of DNA, RNA, proteins using DNA origami
- Explore effects of distance on hybridization, binding, scaffolding
- Demo'd transcription of bound DNA





Prototyping and debugging of *in vivo* and *in vitro* circuits

- Very little knowledge/infrastructure required to build in vitro circuits (try it!)
- Planning to have a workshop at Caltech in Sep 2013 for people who want to learn

Open source information

- TX-TL protocols, data, tools: http://www.openwetware.org/wiki/breadboards

Some Challenges and Research Directions (BFS)

Better understanding of uncertainty

- How do we capture observed behavior using structured models for (dynamic) uncertainty

Stochastic specifications and design tools

- How do we describe stochastic behavior in a systematic and useful way?
- How do we design stochastic behavior?
- What are the right design "knobs"?

Higher level design abstractions

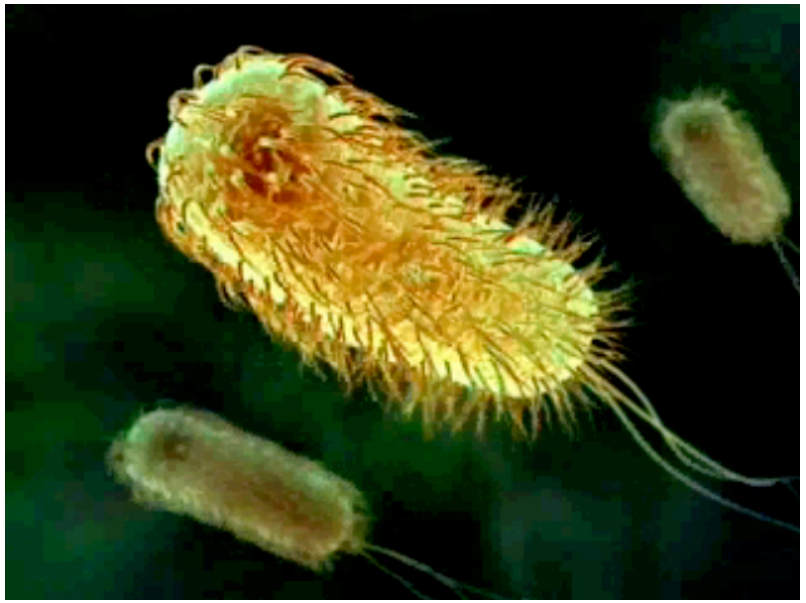
- What is the right "device-level" design abstractions (above strand diagrams)?

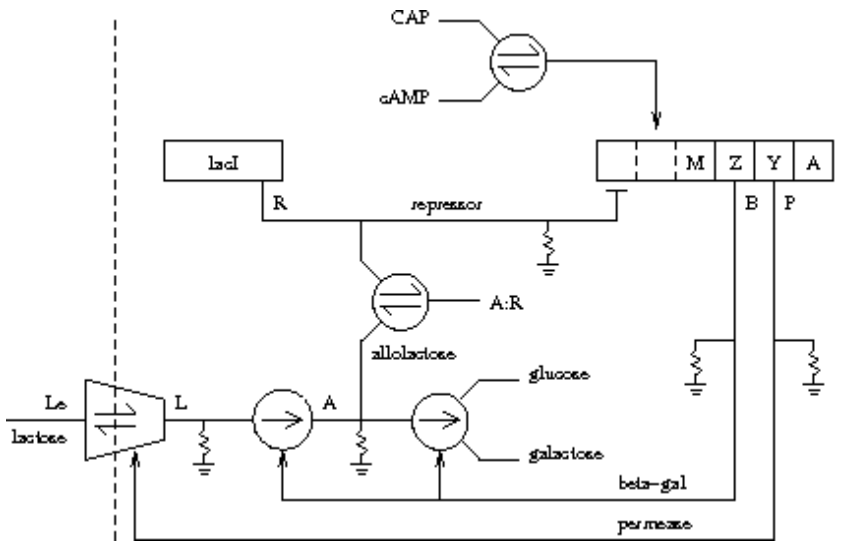
Redundant design strategies

- Start implementing non-minimal designs
- Analogy: stochastic memory storage

Scaling up: components → devices → systems

- How can we use in vitro "breadboarding" to design and implement complex systems

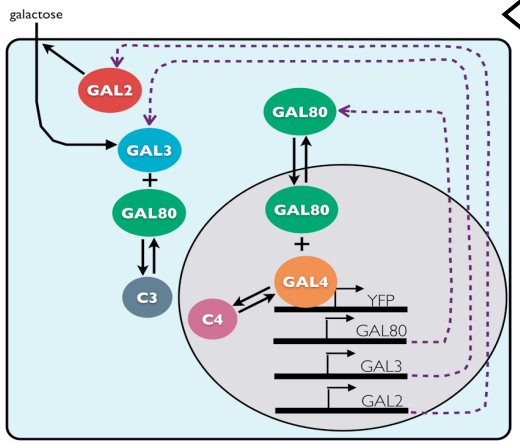




Analysis & Design of Biomolecular Feedback Systems

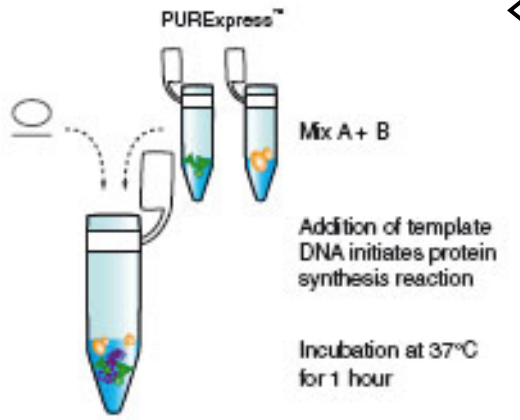
Systems Theory

- Role of feedback
- System identification
- Effects of crosstalk



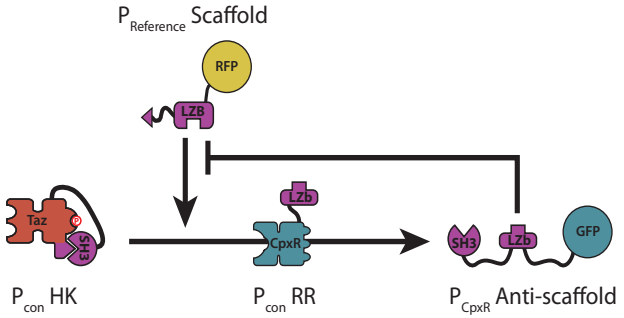
In Vitro Testbeds

- RNA-based genelets
- Biomolecular breadboards
- Droplet-based automation



Biocircuit Design

- Fast feedback mechanisms
- Heterogeneous redundancy
- Systematic design methods

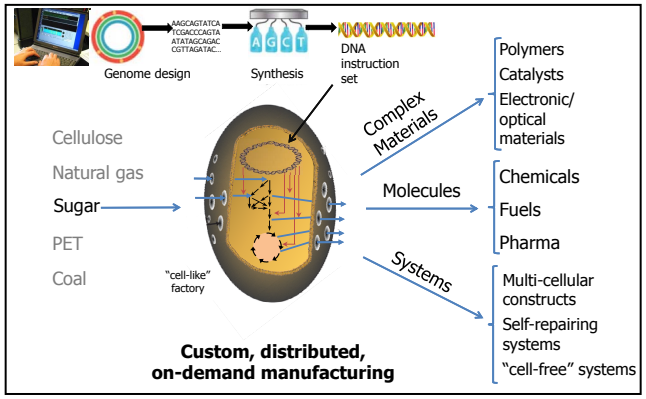


Some results to date

- Role of (multiple) feedback loops in generating bimodality, robust response
- Design of rate and concentration regulation circuits (genelets + scaffold proteins)
- Time-delay as a mechanism for “design of dynamics” (mainly theory, so far)
- Prototyping/debugging using cell-free extracts (modeling/experiments, droplets/bulk)
- Effects of resource limits (TX-TL experiments, simulations + theory)
- Temperature dependence and temperature compensation

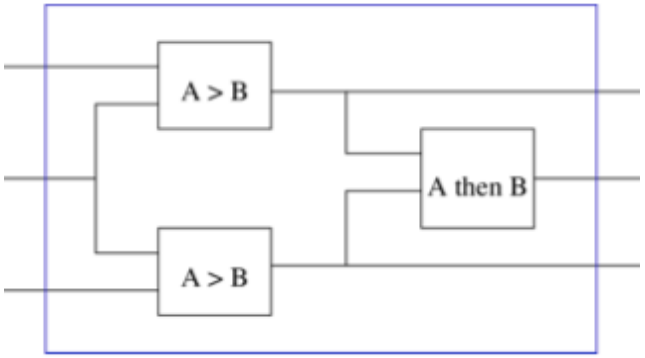
Synthetic Biology Applications

Living Foundries



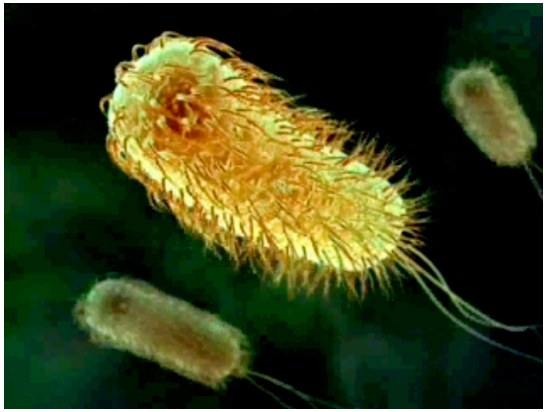
- Conversion of input resources to output products in modular way
- Control systems to regulate metabolic pathways and stress response
- Provide modularity and robustness, with ability to rapidly redesign pathway for new input/output pairs

Event Detectors



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

Artificial Cells



- Self-contained nanoscale biomolecular machines
- Subsystems: chassis, power supply, sensing (internal and external), actuation, regulation
- All components should be synthetic and programmed (compiled)