Biomolecular Breadboards for Prototyping and Debugging Synthetic Biocircuits

UCSF

Richard M. Murray
California Institute of Technology

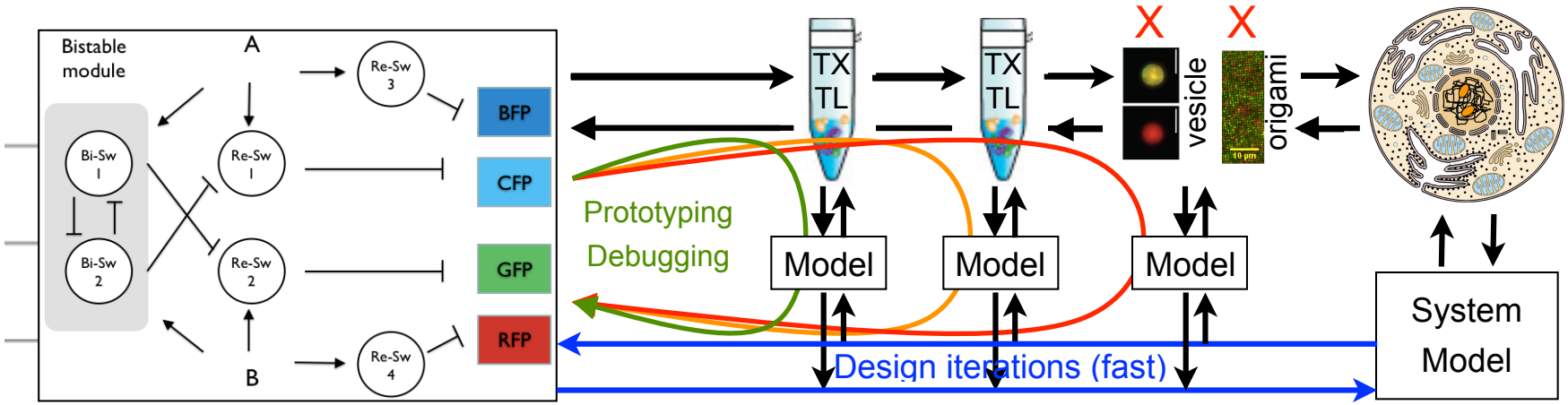
Paul Rothemund, Caltech
Vincent Noireaux, U. Minnesota
Adam Abate, UCSF

DARPA Living Foundries Project Meeting
11-12 June 2014

Outline

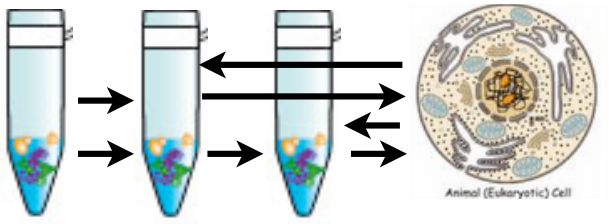
I. Overview of ultimate goals and potential impact
II. Technical overview of accomplishments (previous + current)
III. Next steps, technical hurdles, transition opportunities

Project Overview



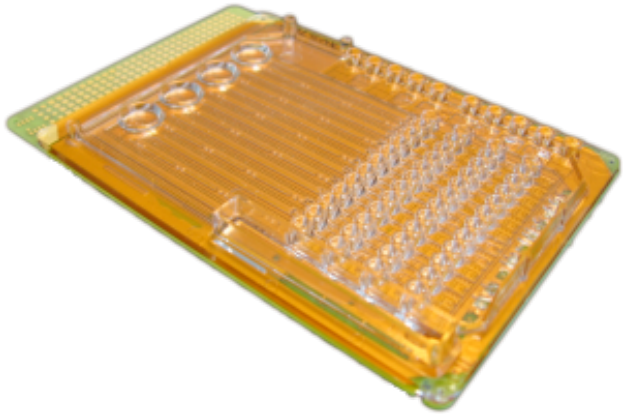
Task 2.1: TX-TL prototyping and debugging

- Linear DNA assembly with 1 day cycle time
- Rapidly modulate component concentrations
- TX-TL modeling toolbox
- Implement circuits *in vivo*



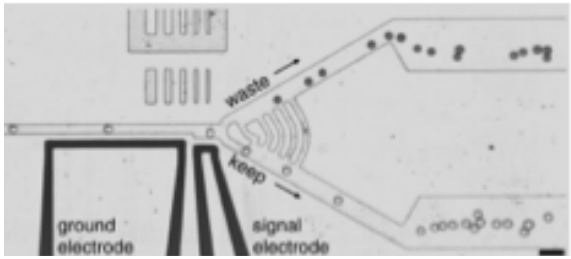
Task 2.6: Droplet-based automation

- Automated protocols
- Impedance and fluorescence properties
- Chemical transformation



Task 2.7: Ultrahigh throughput prototyping

- Screen 10^6 circuit combinations/day,
- Use data for modeling and circuit optimization
- Test on biosynthesis pathways



Sun et al, JoVE 2013
Sun et al, ACS Syn Bio, 2013

Overall Project Goals and Objectives

Develop, demonstrate, document, and disseminate two new "biomolecular breadboards" that provide engineers with 10-100X improvement in time required to conceive, design and implement working biomolecular circuits

Program metric	Current	Phase I	Phase II
1. Time required from synthesized DNA sequences to measurement of circuit performance (on cell-free breadboards)	1-2 wk	3 days ✓	1 day ✓
2. Time required to build a novel, modest complexity (6-8 unique promoter) circuit - existing design, novel components	3-6 mo	1 mo ✓	1 wk **2-4 wks**
3. Number of circuits that can be tested simultaneously, varying component concentration and/or cell-free toolkit parameters	5	25 ✓	100 ✓
4. Number of genes and regulatory parts characterized, modeled and available for use in cell-free circuits (and artificial cells)	2	5 ✓	20 ✓
5. Number of circuit combinations that can be screened per day, varying component concentration and/or genetic elements	5	N/A	10 **by 12/14**

Documentation: **http://openwetware.org/wiki/Biomolecular_Breadboards**

- Z. Z. Sun et al, Protocols for Implementing an Escherichia Coli Based TX-TL Cell-Free Expression System for Synthetic Biology. Journal of Visualized Experiments, 2013
- Z. Z. Sun et al, Linear DNA for rapid prototyping of synthetic biological circuits in an Escherichia coli based TX-TL cell-free system. *ACS Synthetic Biology*, 2013
- Takahashi et al, Rapidly Characterizing the Fast Dynamics of RNA Genetic Circuitry with Cell-Free Transcription −Translation (TX-TL) Systems, *ACS Synthetic Biology,* 2014 [SIM module from CSHL 2013 course]
- D. Seigal-Gaskins *et al.*, Resource usage and gene circuit performance characterization in a cell-free 'breadboard', *ACS Synthetic Biology*, 2014 (to appear)

Sample TX-TL Based Design Process (Phase I)

Stage 0: modeling with TX-TL modeling toolbox

- Desired function + specs → set of possible designs

Stage 1: prototyping with linear DNA (0.5-1 day/cycle)

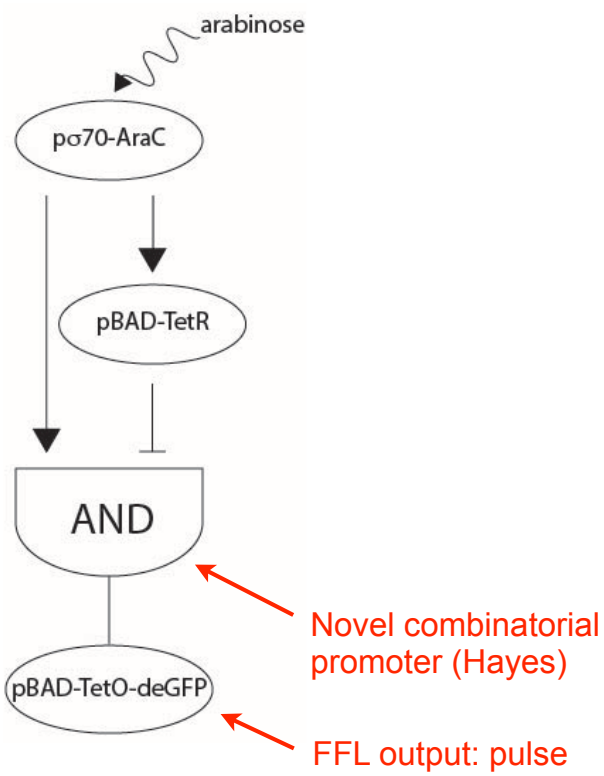
- Components from std library or PCR extension (no cloning)
- Compare w/ models; insure we can model what we see
- Downselect 4-8 designs to test in plasmids

Stage 2: prototyping with plasmid DNA (2 days/cycle)

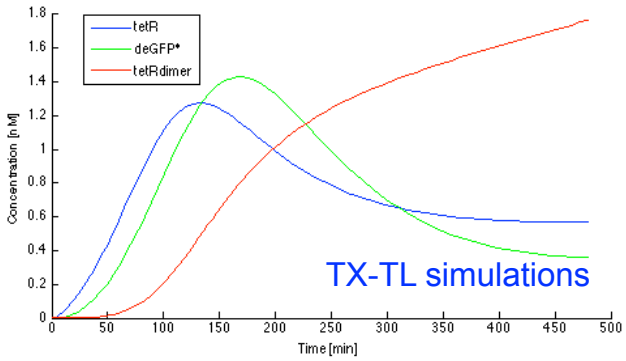
- Clone into plasmid(s), using std sequences/protocols
- Verify operation in TX-TL, incl copy number variability
- Match results to models and linear DNA

Stage 3: validate in cells (2-3 days/test)

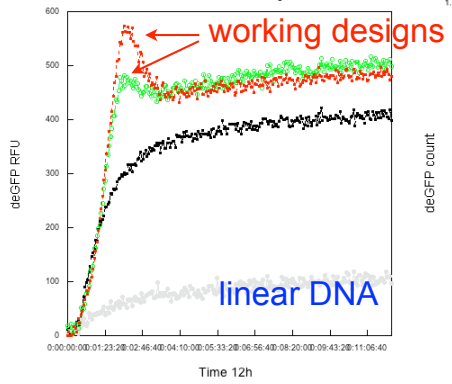
- Test top constructs from plasmid-based TX-TL assay



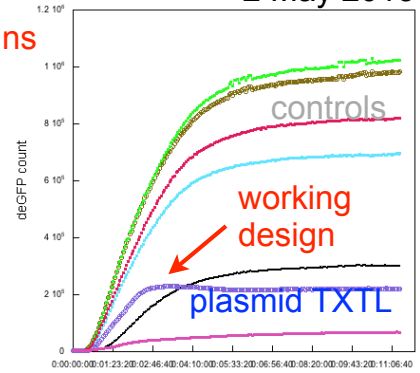
Project start: 15 Apr 2013



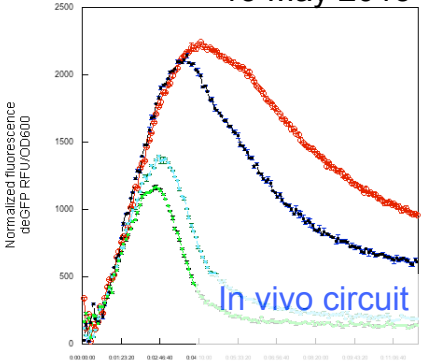
28 Apr 2013



2 May 2013



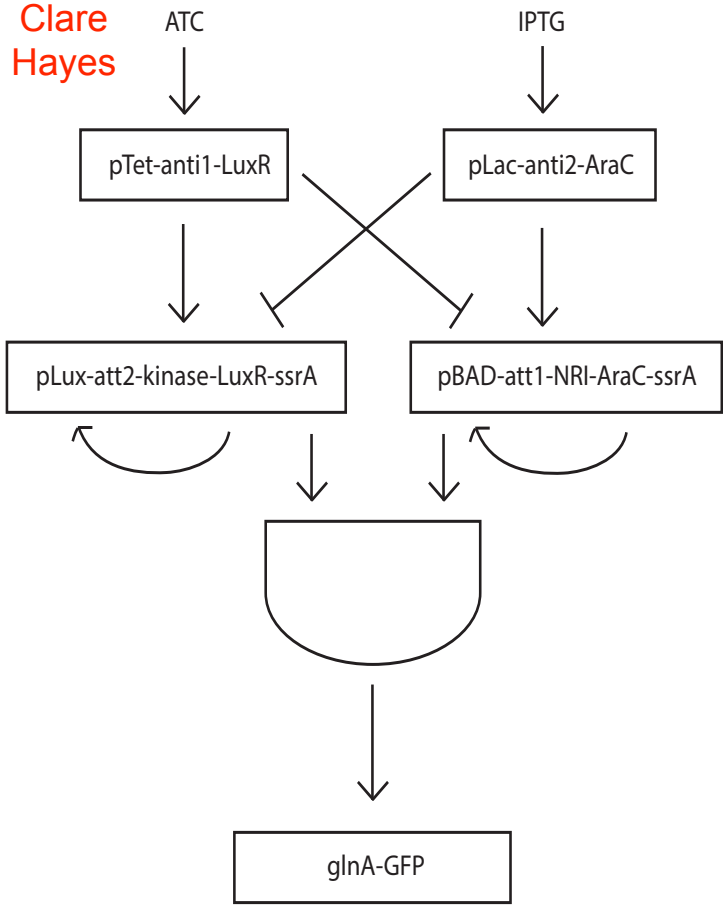
13 May 2013



Current Work: 6-8 Element Circuit Prototyping

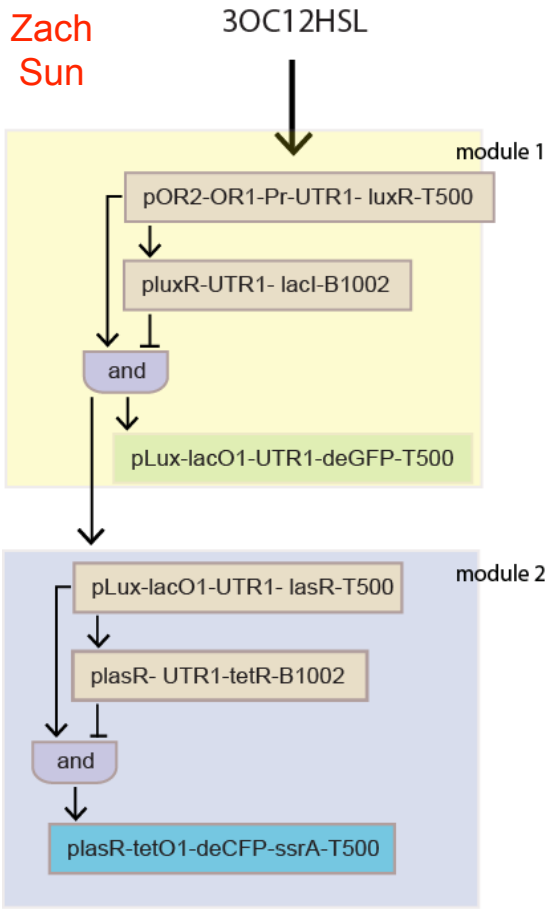
Latching XOR gate

Clare Hayes



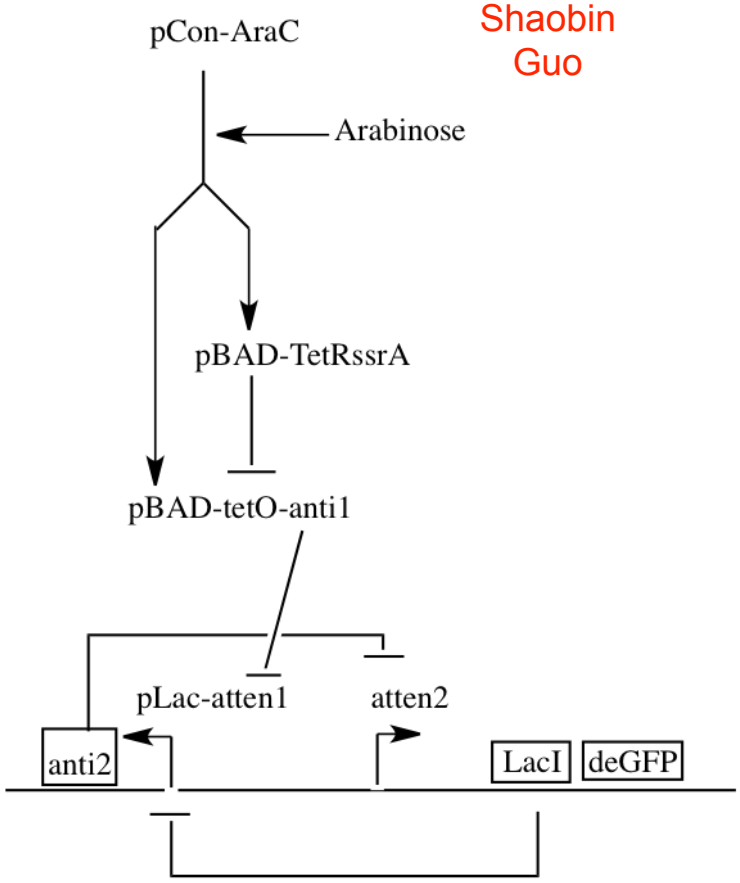
Sequential FFL circuit

Zach Sun



Fold change detector

Shaobin Guo



Difficulties encountered led to missing second milestone target date (15 May)

- Repetitive sequence and hairpins in TX-TL designs lead to cloning problems
- Strong RBS's used in TX-TL for good gene expression cause loading issues in cells
- Matching *in vitro* to *in vivo* require multiple *in vivo* iterations ⇒ slower DBT cycle

Next: TX-TL prototyping of more complex circuits

8-16 promoter circuits designed and implemented by undergrads

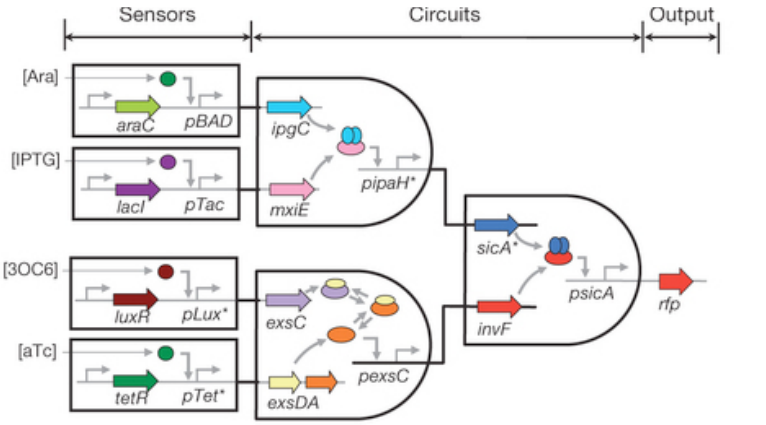
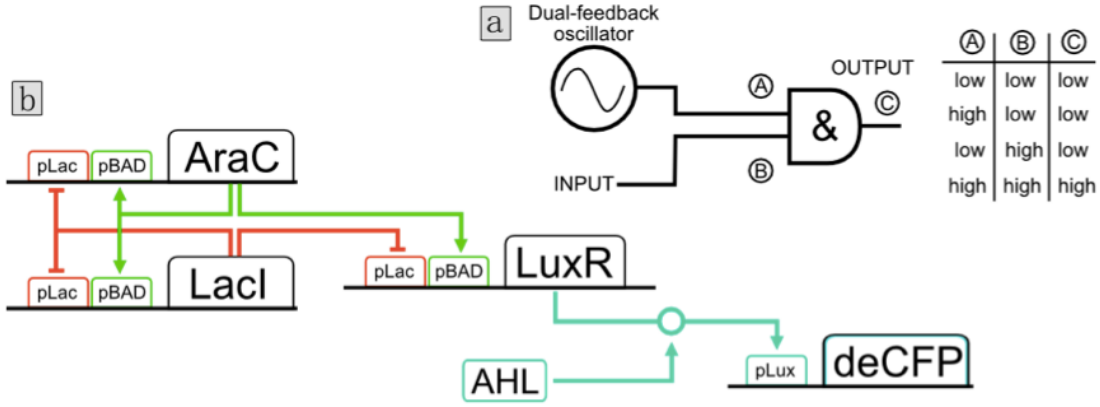
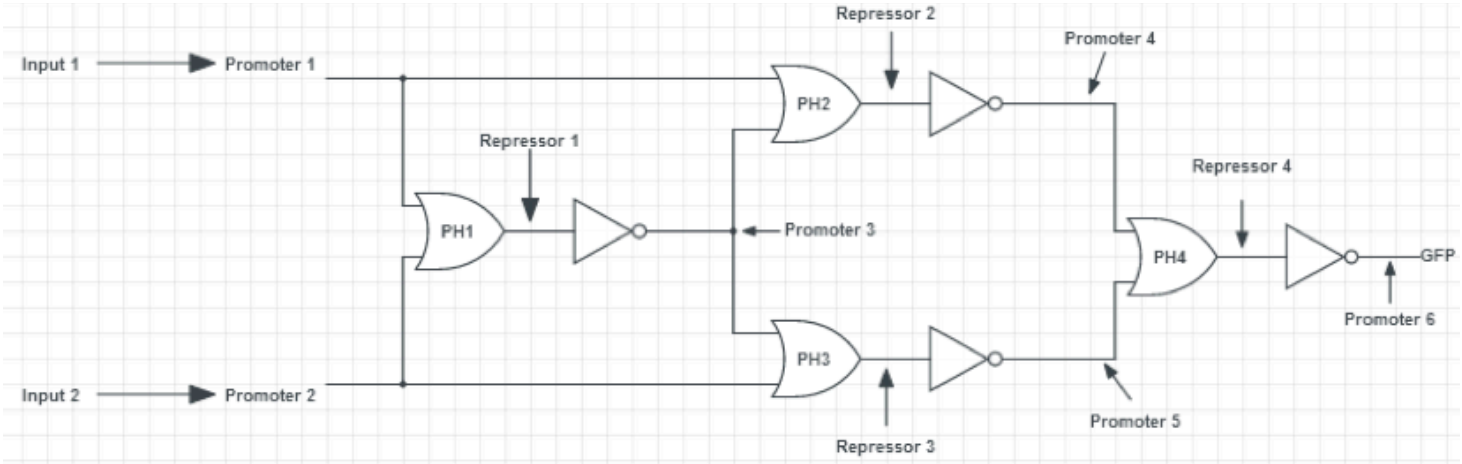


Figure 2. *4-input AND gate* [7]. The circuit to be tested in this project will be based on this design.





Stage 0: modeling with TX-TL modeling toolbox

Stage 1: prototyping with linear DNA

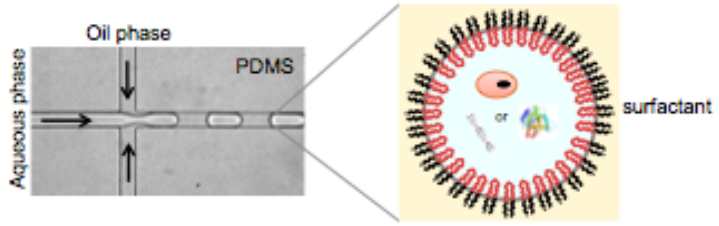
Stage 2: prototyping with plasmid DNA

Stage 3: validate in cells (2-3 days/test)

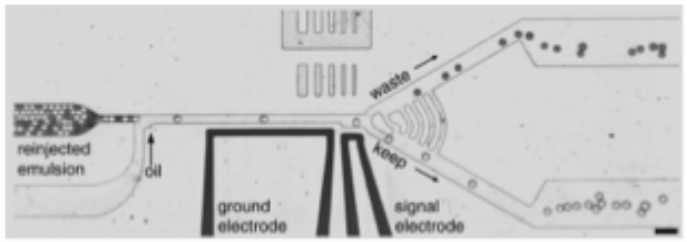
High Throughput Microfluidics (w/ A. Abate)

High throughput microfluidic platform

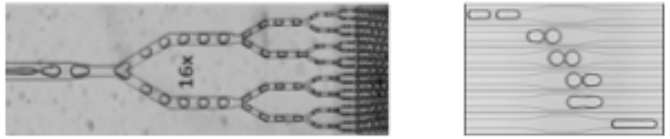
- Droplet generation



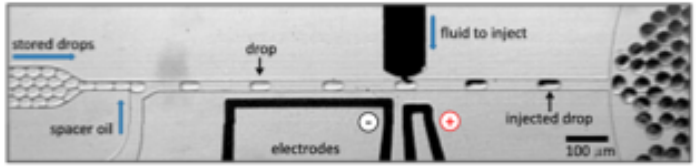
- Sorting



- Splitting



- Picoinjection



Goal: rapid design space exploration

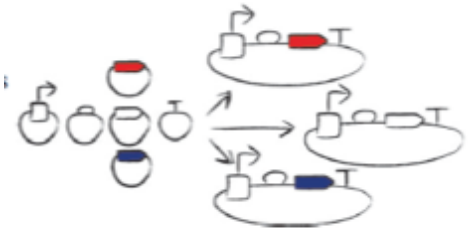
- Screen 10^6 circuit combination per day, varying component concentration and/or genetic elements
- Make use of data for mathematical modeling and circuit optimization
- Test on biosynthesis pathways

Exploration methods

- Component combinatorics

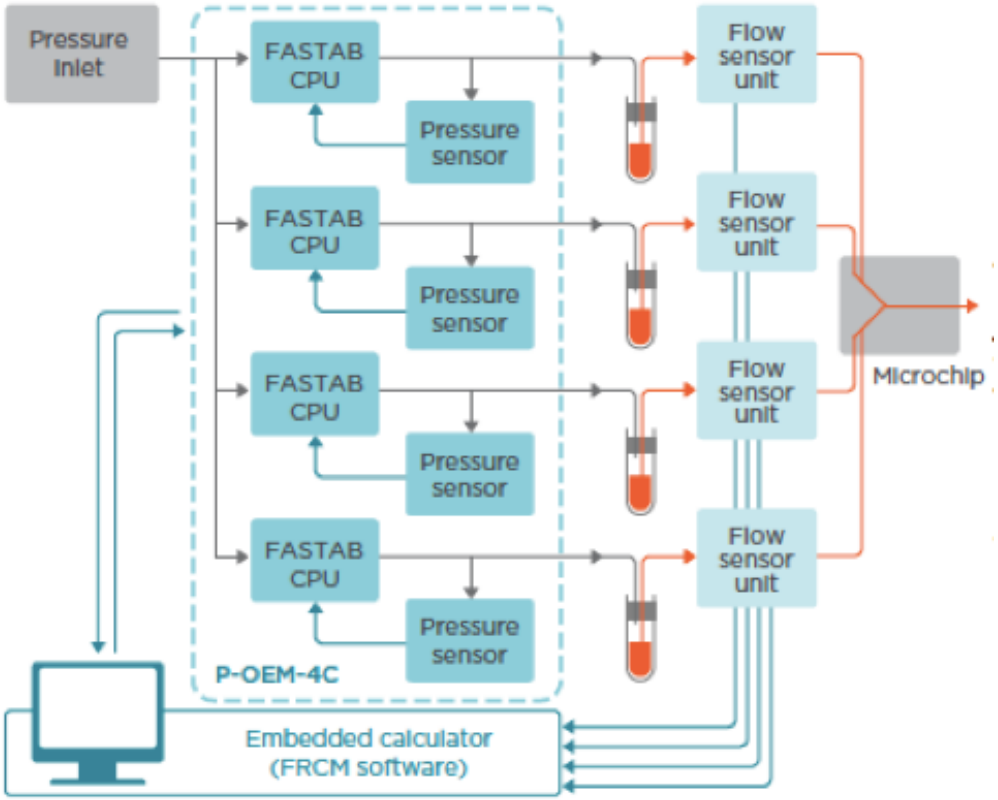
1X 5X 3X

- TX-TL based genetic libraries



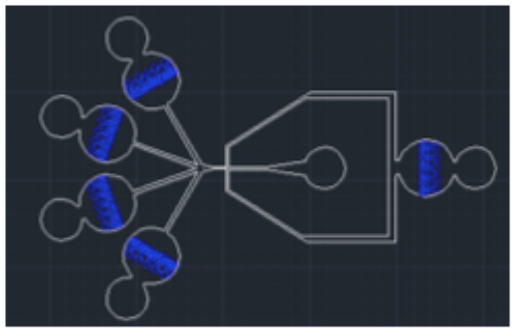
Challenges: measurement, combinatoric mixing, droplet ID, system ID, theory

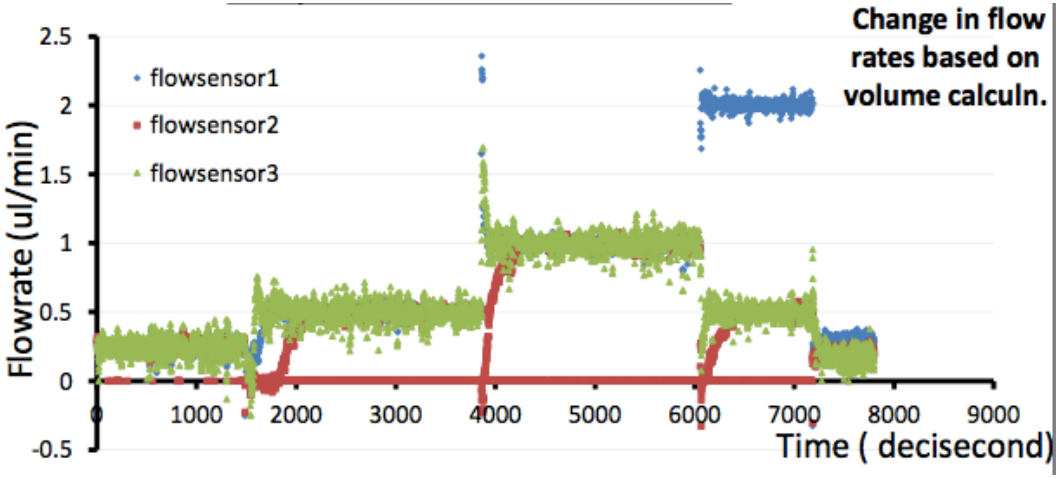
High Throughput Microfluidics (UCSF)



Building four-input multiplexer

- Capable of mixing 4 different inputs in combinatorial ratios
- Keep track of concentrations either by timing of droplets or by use of dyes
- Test on relevant biosynthetic pathway
  - System ID and optimization
  - Direct design space exploration

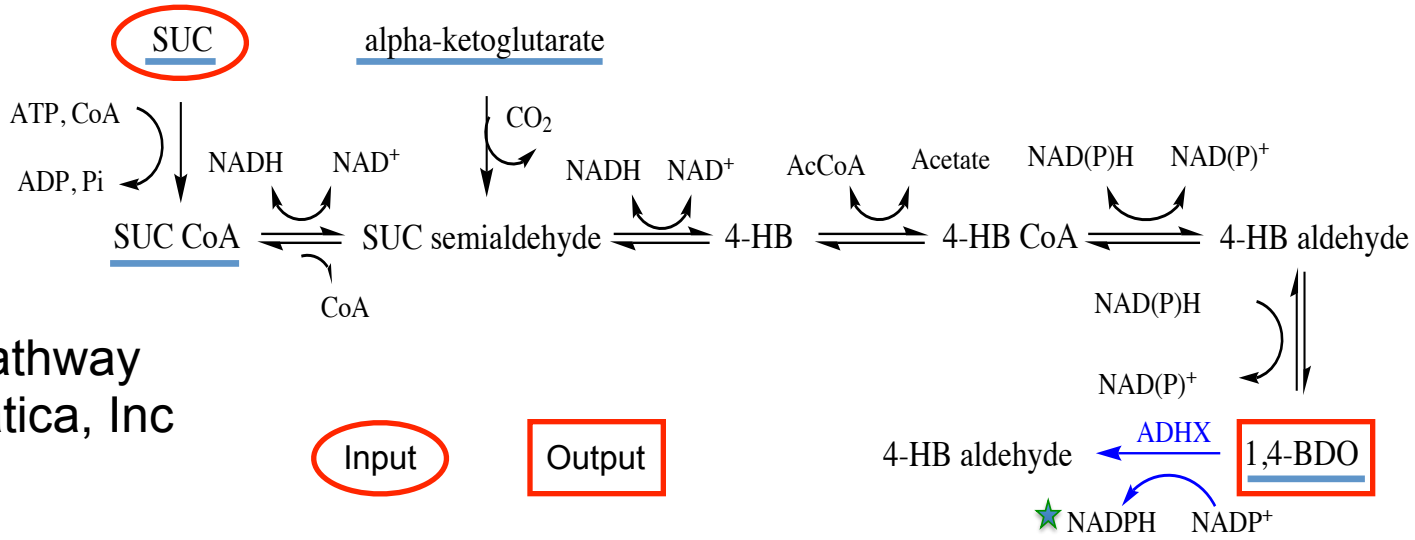




Application: 1,4-BDO pathway exploration

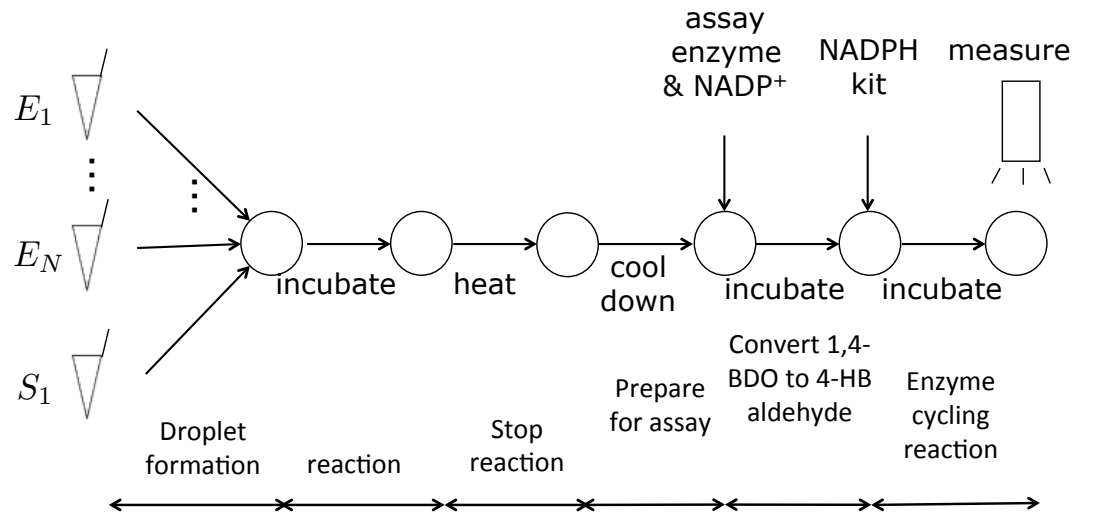
1,4-BDO

- High-value chemical used in plastics, elastic fibers, and solvents (~$33/L)
- E. coli biosynthesis pathway optimized by Genomatica, Inc (San Diego)



Design space exploration

- Use ultra-high throughput micro-fluidics with TX-TL to optimize pathway
- BDO sensing via engineered enzyme (from Genomatica)



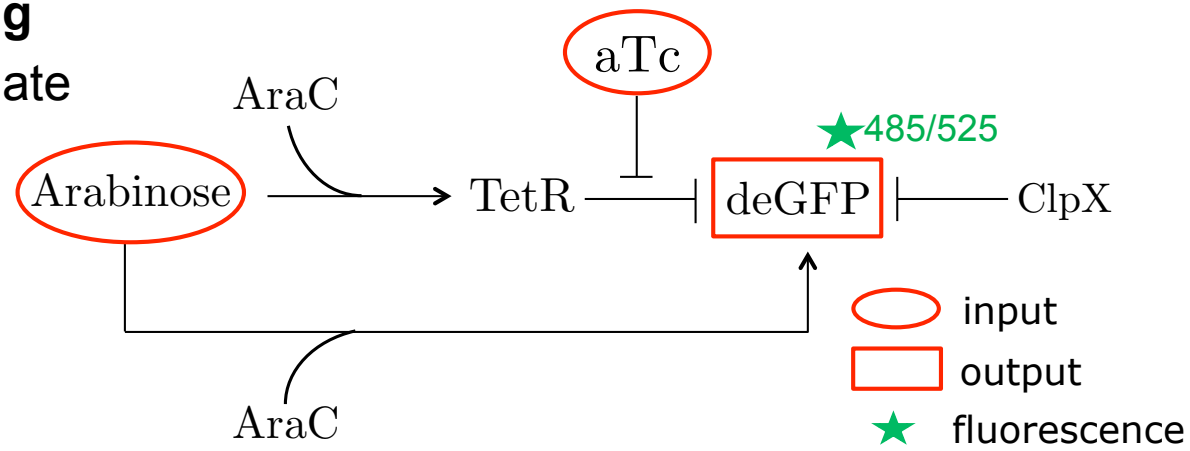
Challenges

- Compatability of process steps with TX-TL and microfluidics
- Interaction of pathway (and co-factors) with native pathways

Modeling and System ID

Good progress in TX-TL modeling

- Testing IFFL as pathway surrogate
- Able to capture FFL performance across operation conditions with accuracy of ~10%



$$\dot{x} = -d_1 x + \bar{c}_1$$

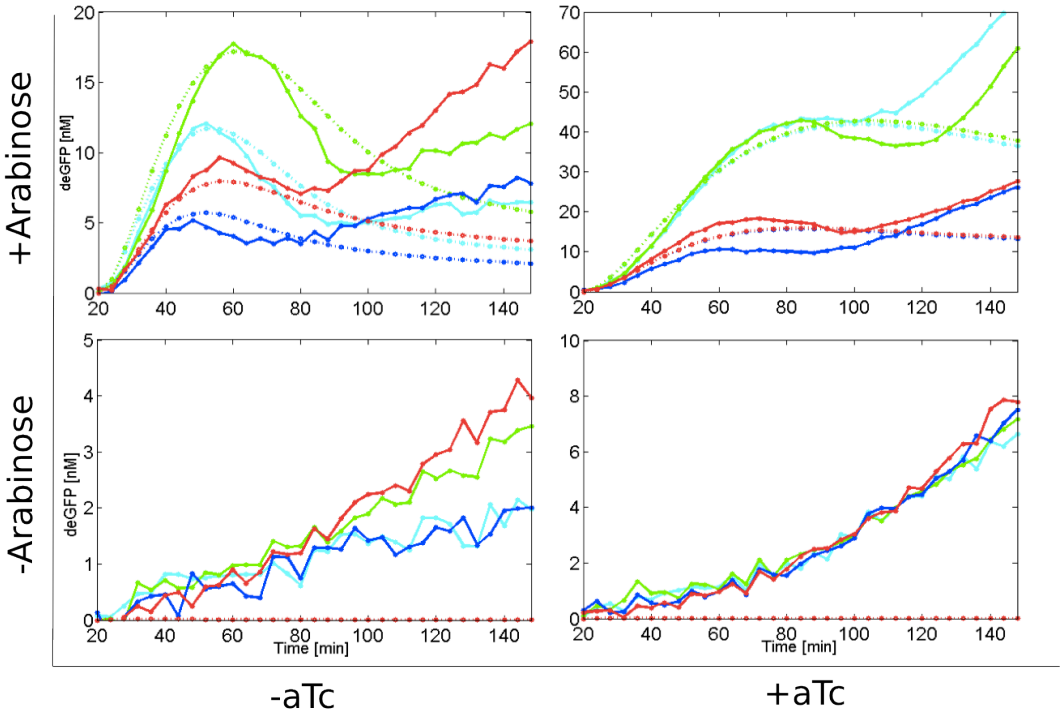
$$\dot{y} = -d_2 y + \bar{c}_2 \frac{x}{\bar{K}_1 + x} u_1 + \bar{b}$$

$$\dot{z} = -d_3 z - \bar{c}_\phi \frac{z}{K_\phi + z}\phi + \bar{c}_3 \frac{x}{(\bar{K}_5 + x) g(y, u_2)} u_1 + \bar{b}$$

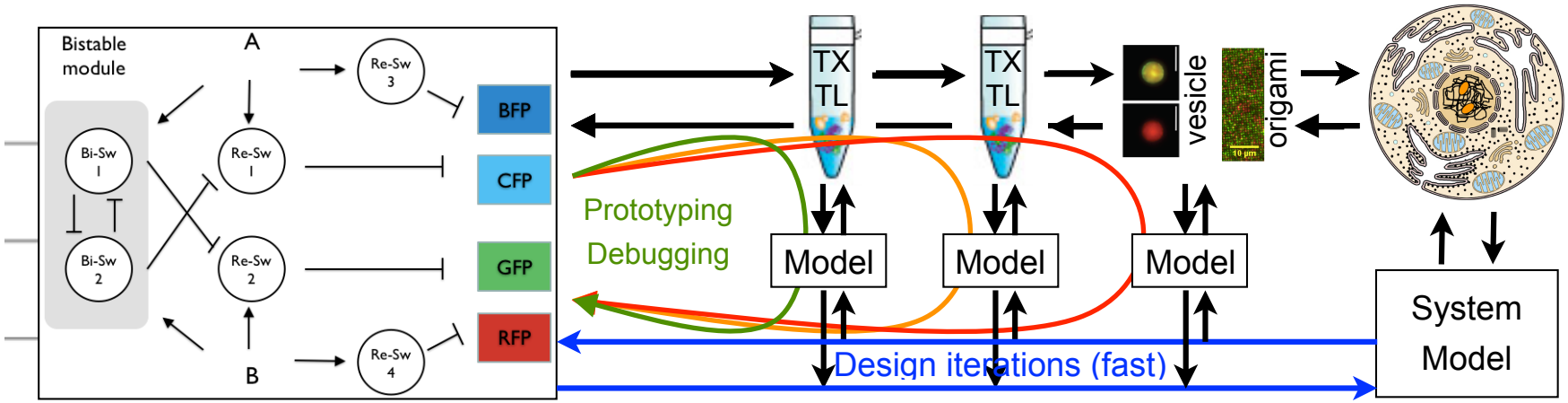
$$\dot{\phi} = -d_4 \phi + \bar{c}_4,$$

where $g(y, u_2) = \dfrac{-(\bar{K}_3 + u_2 - y) + \sqrt{(\bar{K}_3 + u_2 - y)^2 + 4y\bar{K}_3}}{2\bar{K}_7}$

u_1	Ratio of pBAD promoter induced by arabinose ($u_1 \in [0,1]$)
u_2	aTc concentration [nM]
K_1	MM const. for AraC:pBAD-TetR binding [nM]
K_3	Dissoc. const. for aTc:TetR binding [nM]
K_5	Dissoc. const. for (AraC):(pBAD-tetO-deGFP) [nM]
K_7	Dissoc. const. for (TetR):(pBAD-TetO-deGFP) [nM]
d_i	degradation rates [min^{-1}]
$\bar{c}_i$	(Maximal) expression rate × DNA concentration [nM/min]
$\bar{b}_i$	leakiness × DNA concentration [nM/min]



Next Steps, Transition Opportunities, Gaps



Collaborations and outreach

- CSHL SB course in Jul '13 and Jul '14
- Workshop (Jun '14) - 15 participants
- Current LF collaborations with Cornell, Northwestern, Princeton, UCSF, EPFL
- Genomatica collaboration for 1,4-BDO

Next steps

- 6-8 & 8-16 element circuit prototyping
  - Understand and solve key issues
- Chemical transformations in ALL
- High throughput pathway exploration

Transition opportunities

- Inexpensive design space exploration for circuits and biosynthesis pathways
  - Good interest from Genomatica
- Characterization and modeling of components, circuits, and systems
- Rapid design and prototyping by non-experts (courses, iGEM, etc)

Gaps

- Need more measurement capability
- Need to understand loading & context