Biomolecular Breadboards for Prototyping and Debugging Synthetic Biocircuits

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Outline

I. Project overview
II. Technical results: TX-TL, biochemical wires, artificial cells
III. Next steps
IV. Summary of collaborations

Project Goals and Approach

Goal: 10-100X reduction in cycle time

- Breadboard-based prototyping
- Predictive models
- Parallel testing

Cell-free (TX-TL)

- E. coli cytoplasm
- Linear DNA (PCR)
- Cheap: $0.03/ul

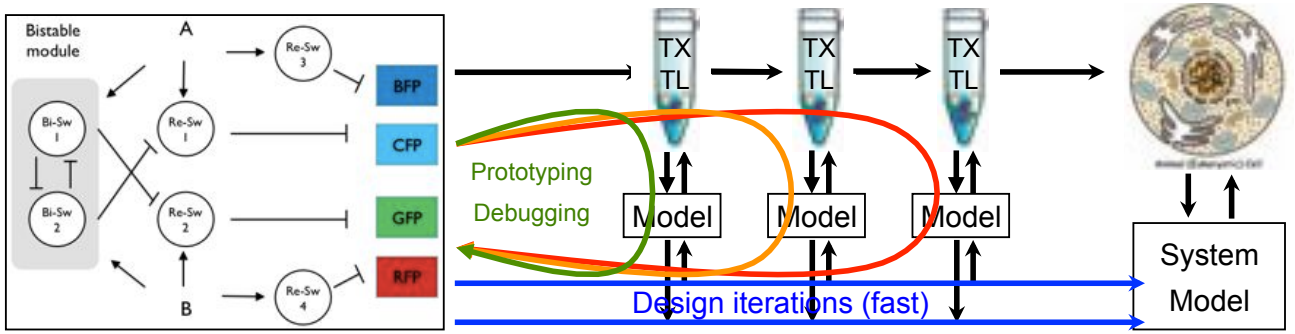
Biochemical wires

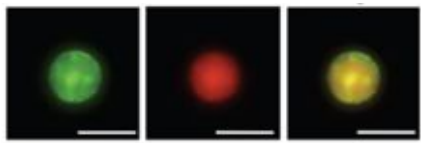
- Spatially localized reactions
- 10 um origami

Artificial cell

- Spatial constrained reactions
- 1-1000 fL volumes

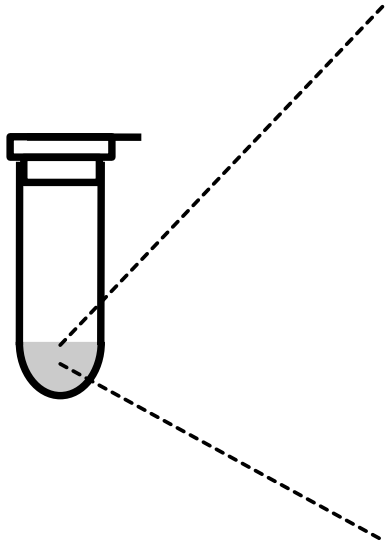
Program metric	Current	Phase I	Phase II
1. Time required to go from synthesized DNA sequences to measurement of circuit performance (on cell-free and origami breadboards)	1-2 wk	3 days	1 day
2. Time required to build a novel, modest complexity (6–8 unique pro- moter) circuit (existing design, novel components)	3-6 mo	1 mo	1 wk
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	10



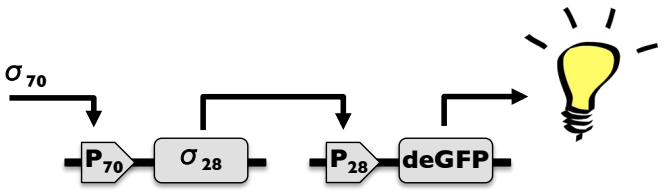


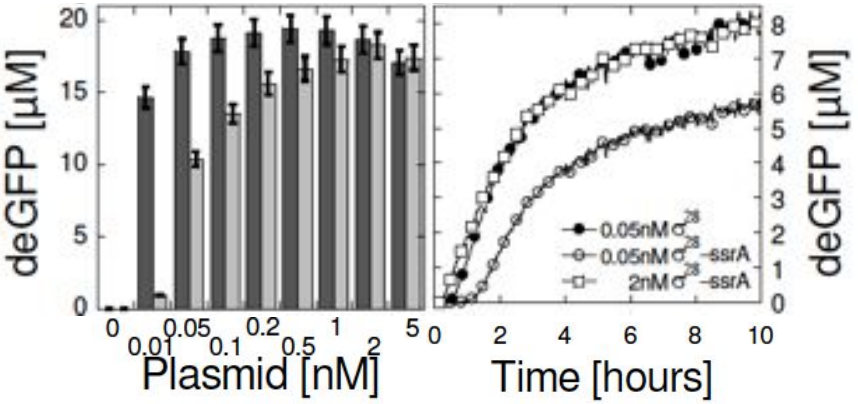
http://www.openwetware.org/wiki/breadboards

E. Coli cell-free TX-TL system

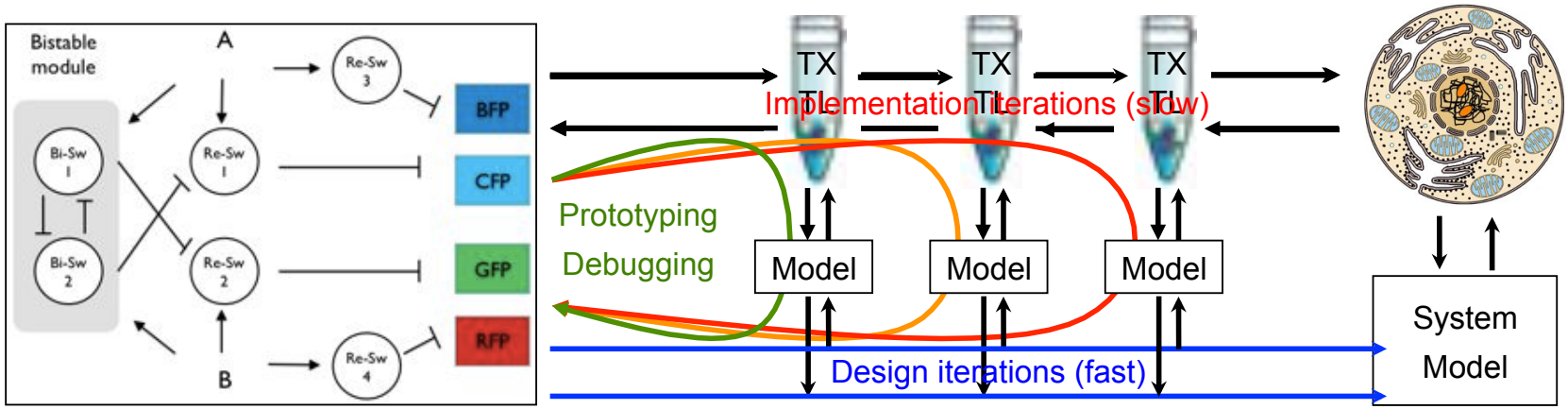


- Lysate: E. coli extract.
  No endogenous information
  TX: housekeeping core + σ70
  TL: ribosomes, cofactors….
- Reaction buffer: 30-40 components.
- DNA: plasmids and PCR prepared in lab.





Task 1.1: Cell-Free Circuits Breadboard



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

80% • Q2: Demonstrate breadboard on 2 circuits (eg, switch, IFFL), document iteration time

50% • Q3: Post complete protocols + variations (degradation, energy, ...) + validated models

10% • Q4: Demonstrate design of 6-8 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

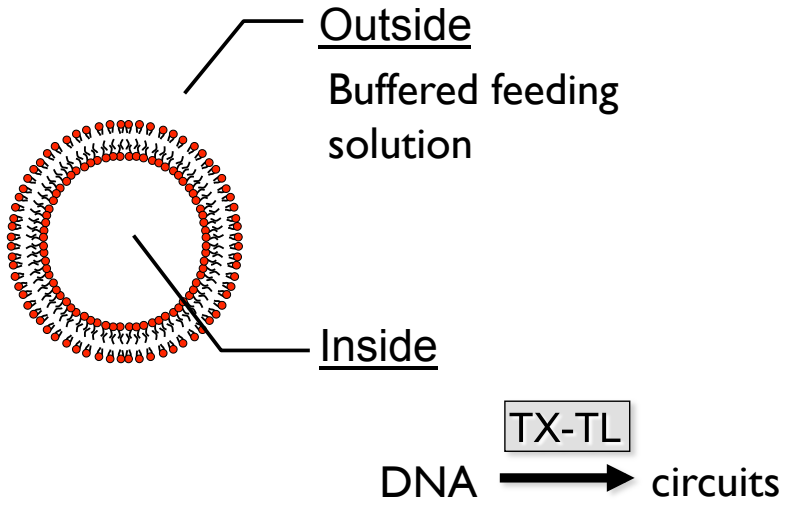
Task 1.2: Artificial Cells

Demonstrate the feasibility of a programmable synthetic phospholipid vesicle system with elementary synthetic gene circuits:

- Create cell-sized (1-50 μm diameter) synthetic phospholipid vesicles containing TX-TL system and genetically encoded circuits.
- External inducers (tetracycline, arabinose, ...) will diffuse through the membrane and activate the circuits or repress expression of fluorescent protein reporters.

Approach

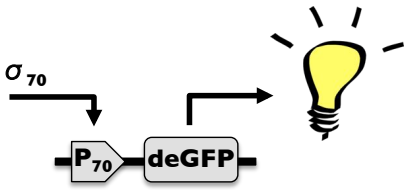
- Demonstrate stable synthetic liposomes capable of hosting transcriptional activation and repression units.
- Demonstrate activation and repression units that can be turned on and off using inducers diffusing through the membrane (arabinose, lactose, tetracycline, …..).

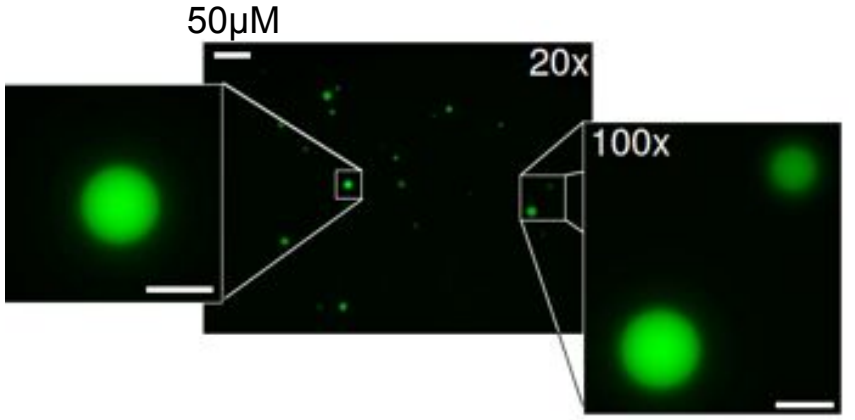


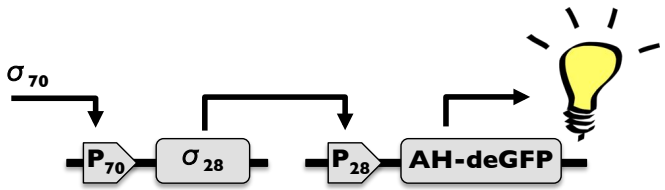
Extensions

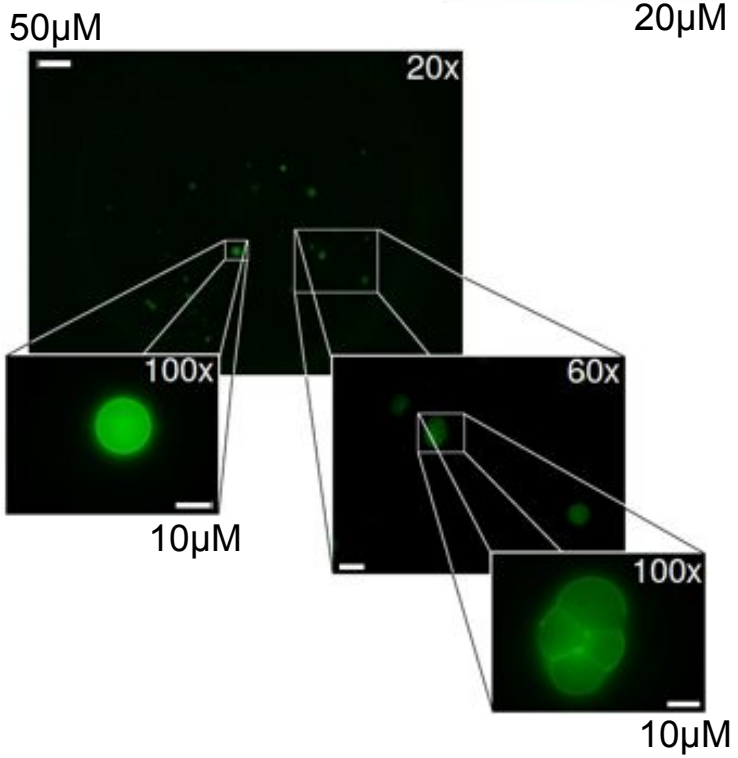
- Changing the phospholipid composition. Now PC, objective: add 1 or 2 other lipids. The protocol of vesicle preparation will be adapted if needed.
- Understand the importance of the phospholipid composition for pattern formation, self-organized systems at the membrane.

Cell-free expression inside vesicles





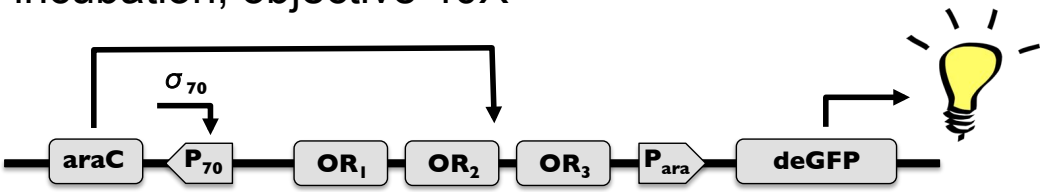




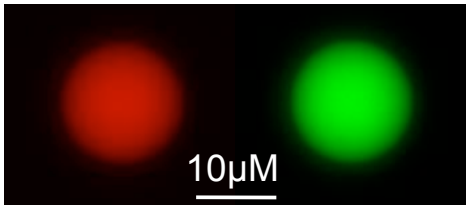
Inducible expression inside vesicles

Observations: 8 hours incubation, objective 40X

• Arabinose system:

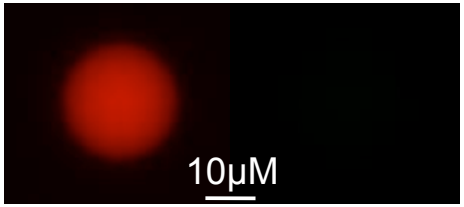


BSA-TRITC deGFP



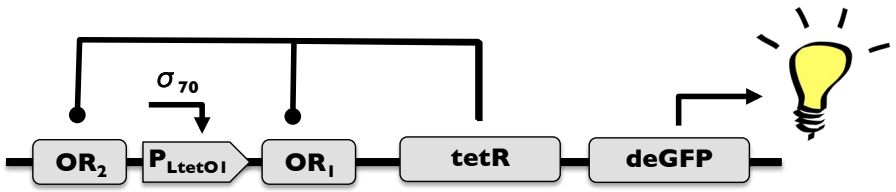
+ 10mM arabinose in feeding

BSA-TRITC deGFP

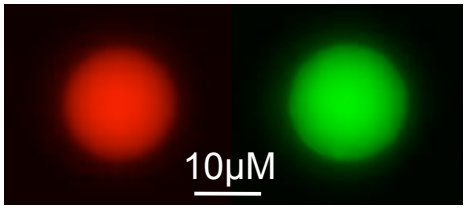


+ 0mM arabinose in feeding

• Tetracycline system:

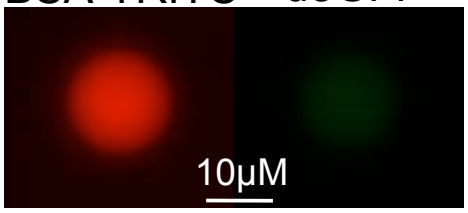


BSA-TRITC deGFP



+ 20μM tetracycline in feeding

BSA-TRITC deGFP

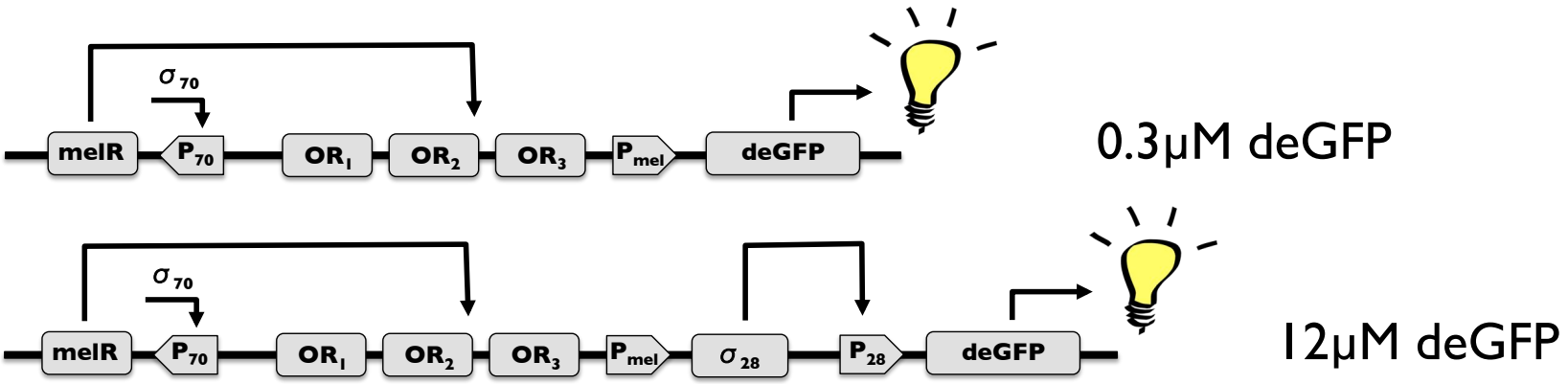


+ 0μM tetracycline in feeding

Interaction of ATc with membrane

Inducible expression inside vesicles

- Melibiose system: analogous to arabinose system



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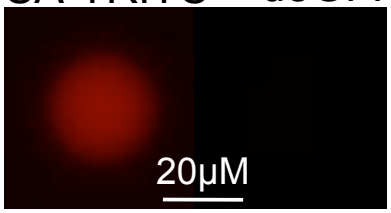
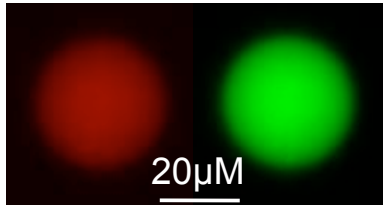


+ 0mM melibiose
in feeding

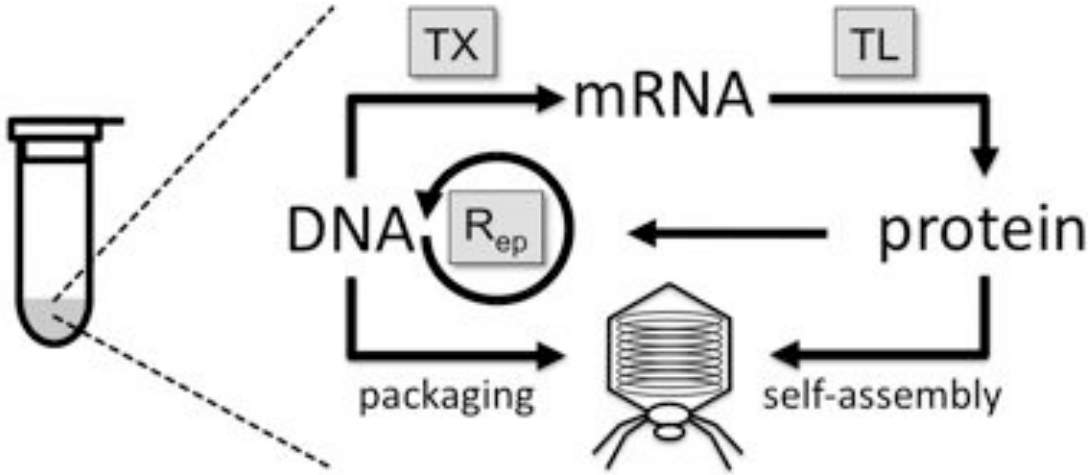
BSA-TRITC deGFP

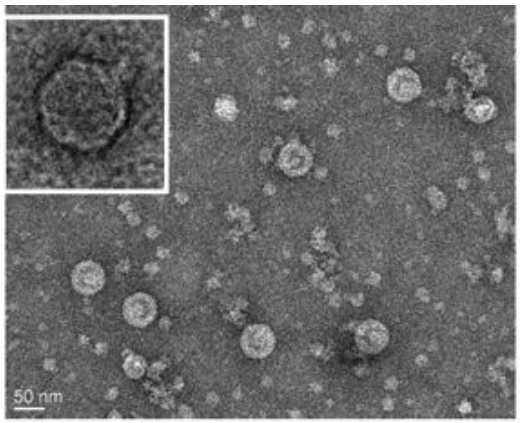


+ 10mM melibiose
in feeding

Genome replication, synthesis and self-assembly of the bacteriophage T7 in a single cell-free reaction

- lytic coliphage.
- 40 kbp, 60 genes (35 with known functions).
- almost host independent (2 host proteins required).
- has its own RNA polymerase.
- has its own DNA polymerase.



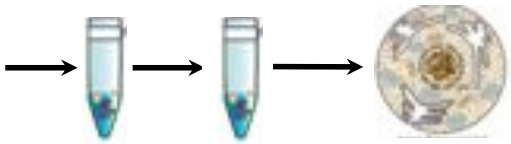


ACS Synthetic Biology, 2012.

Upcoming Technical Work and Goals

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

Filippo Caschera

Biochemical Wires

- Optimize assembly of origami wires w/ linkersTest signal carrying ability by hybridizing and dehybridizing fluorescent reportersDemo transcription of signal from genelets on microscope slides.

Open source information

- TX-TL protocols, data, tools: http://www.openwetware.org/wiki/breadboards
- TX-TL modeling library: http://www.sourceforge.net/projects/txtl
- TX-TL announcements mailing list: http://groups.google.com/d/forum/txtl-announce

Collaborations and Needs

TX-TL circuit testing

- Cornell (Lucks)
- Pivot Bio (Temme)
- MIT (Voigt)
- MIT (Del Vecchio)
- Hutchison (JCVI)
- Hoping to make all TXTL data available on internal LF website

Laboratory automation

- Stanford/Advanced Liquid Logic
- Visiting ALL on 8 Nov to work through details of TXTL based protocols
- Hoping to make use of Stanford protocols as they become available

Living Foundries web site

- Boyden, Lucks, Murray
- Draft site set up
- Would like to use for posting presentations (east/west coast LF meetings) + internal data
- Looking for volunteers to help test & maintain

What we need from others

- Help in trying out the protocols and identifying things that work and don't work
  - Protocols available on web: http://www.openwetware.org/wiki/breadboards
  - Workshops in Phase II, but happy to work with individuals at any time
- Larger collection of *in vitro* reporters (bulk + droplets); faster response times
- RNA scaffolds/origami for trying out biochemical wires in cells
- Sign up for web site: http://sites.google.com/site/livingfoundries
- TXTL announcements mailing list: http://groups.google.com/d/forum/txtl-announce