

Biomolecular Breadboards for Prototyping and Debugging

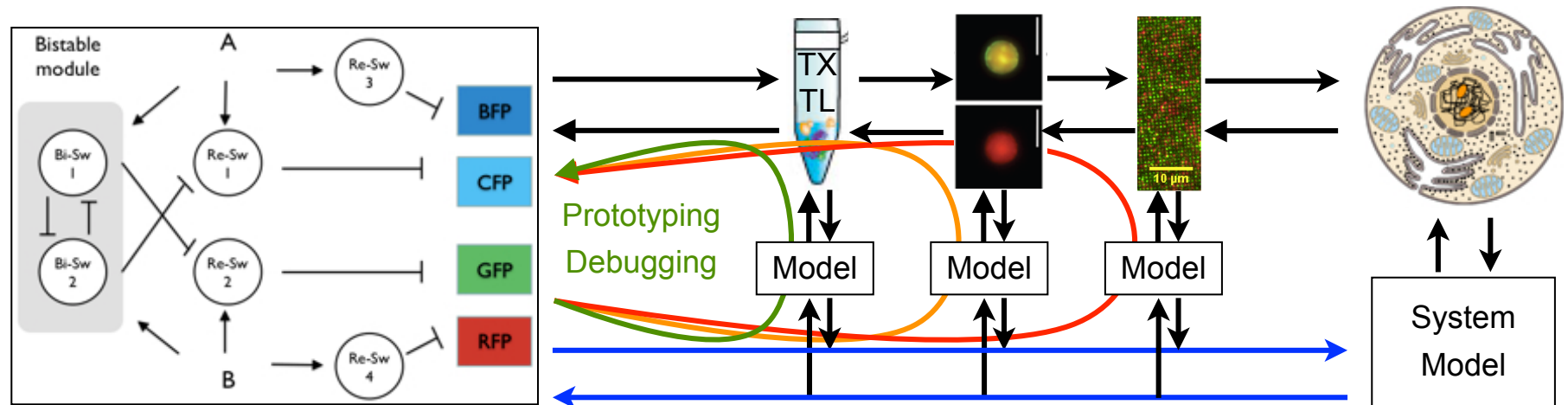
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Goal: >10X reduction in design cycle time

- Breadboard-based prototyping
- Parallel testing (plate reader + droplets)
- MATLAB-based TX-TL modeling toolbox

Cell-free extract (TX-TL)

- *E. coli*-based extract, with processing
- Allows linear DNA (PCR products)
- Cheap: \$0.03/ul Fast: 1 day cycle time

Artificial cell (phospholipid vesicles)

- Spatial constrained reactions
- 1-1000 fL volumes via vesicles

Spatial localization (DNA origami)

- Spatially localized reactions
- 10 µm ribbons [+ 50 x 50 arrays]

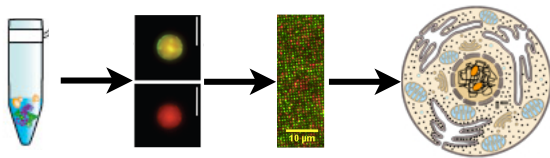
Circuit testing

- Testing LF partner circuits in TX-TL

Accomplishments to Date (Q3 highlighted)

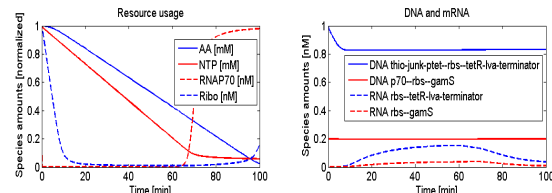
TX-TL cell-free toolbox

- \$0.03/ul, 1 day cycle time
- Linear DNA (w/ protect'n)
- Protein degradation (via YbaQ and ssrA tags)
- Detailed protocols (JoVE)
- Circuits: switch, IFFL, toxin-antitoxin + partners
- Next: design 3-6 gene circuit, *in vitro* → *in vivo*



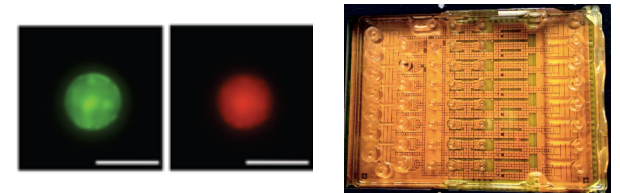
TX-TL modeling toolbox

- MATLAB (Simbiology) based toolbox with 10 line circuit specs
- Validated models for gene expression, regulation, w/ resource lims
- Full source code and user documentation available on web



TX-TL vesicles & droplets

- Inducer-based expression in droplets
- Time course measurements of reporters in 0.3 µl droplets on ALL R110
- Next: protocols for mixing, merging, splitting plus improved imaging



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, Impact and Needs

TX-TL circuit testing (LF and [non-LF])

- Cornell (Lucks) - RNA sensing TFs
- Pivot Bio (Temme) - orthogonal RNAPs
- MIT (Voigt) - 11 gene logic circuit
- MIT (Del Vecchio) - Resource limits
- Hutchison (JCVI) - DNA replication
- Silver (Harvard) - RNA scaffolds
- [Tabor (Rice)] - light inducible TFs
- [Kortemme (UCSF)] - small molecule sensors
- [Seigal-Gaskins (Caltech)] - toxin-antitoxin (TA)

Lessons learned so far

- Many mechanisms do not work directly in TX-TL on first try
 - Reporters often too weak
 - Resource limits get in the way
- Fast design/test cycles are possible
 - Eg: daily test cycles for toxin-antitoxin systems
- Models give good insights
 - Eg: effects of resource limits

Droplet-based automation (with ALL) - appears very powerful; need to exploit!

Phase II plans and ideas

- Move toward integrated set of testbeds (TX-TL bulk, droplet, vesicle, origami)
- Increase role and use of models as primary mechanism in design cycles
 - Allows mapping between breadboards and cells, including normalizing for limits
- Increase role and available of debugging tools in TX-TL (especially for composition)
 - Need a library of mRNA and protein-based reporters that can be added in
- Increase use of laboratory automation (ALL droplet system, nano-spotter, LH robot)