Developing designable genetic control systems for applied synthetic biology

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Control functions balance the supplies and demands for cellular resources

Control functions balance the supplies and demands for cellular resources

Approach: Develop RNA-based systems to solve regulatory control problems and enable high levels of chemical production from engineered cells

Goal: Designable RNA-based genetic control systems that dramatically increase the sizes and complexities of systems that can be engineered.
E. coli platform for \( p \)-aminostyrene (\( p \)-AS) production

14 enzymatic transformations

Goal: Engineer RNA-based genetic systems to solve pathway control problems and enable production of \( p \)-AS for advanced polymer composites

E. coli platform for $p$-aminostyrene production

Goal: Engineer RNA-based genetic systems to solve pathway control problems and enable production of $p$-AS for advanced polymer composites
Functional RNAs regulate gene expression and control cellular activities

**S. mansoni** hammerhead ribozyme

Phosphodiester bond cleavage

**B. subtilis** purine riboswitch aptamer domain

Dynamic, ligand-responsive
Functional RNAs can be evolved in the laboratory with *in vitro* selection.

**Aptamers**
- GTP aptamer

**Ribozymes**
- hammerhead rbz

**Aptazymes**
- Aptamer-controlled ribozyme

### Kinetic Constants
- $k_d$: range: mM to nM
- $k_{cat}$: up to $\sim 10$ min$^{-1}$
- $k_{cat}$ (±): up to 1000

*Carothers, Goler et al. & Keasling. Nucl. Acids Res. 2010.*
Formalized framework for designing static and dynamic genetic devices with quantitatively-predictable outputs

- Genetic device design goals
- Functional design
- Physical implementation
- Assembly & verification

- Static RNA devices
- Dynamic RNA devices

- Systems-level models
- Refine Systems-level models
- Systems-level functions

1. Targeted device outputs
2. Biochemical models for identifying component specifications
3. Biophysical models for designing transcripts
4. Biochemical models for predicting device outputs
5. Devices generating targeted outputs

- Engineer components
- Kinetic folding models
- Mechanistic models
- Assembly & verification

- Ribozyme (rREDs)
- Aptazyme (rREDs)
Excellent correspondence between predictions and observations validates underlying models and overall approach.
Design of FadR-based dynamic sensor regulator system to improve fatty acid ethyl ester (FAEE) biodiesel production in *E. coli*

**Approach:** Interrogate system designs by performing design variable global sensitivity analysis with biochemical parameter uncertainty.

Design of FadR-based dynamic sensor regulator system to improve fatty acid ethyl ester (FAEE) biodiesel production in *E. coli*

Modeled parameter ranges where dynamic sensor regulator system increases FAEE production

Experimentally measured 3 fold greater yields with FadR-dynamic sensor regulator system (28% theoretical maximum from glucose)


Broad range of dynamic regulatory topologies gave improved production compared to pathway gene expression from constitutive promoters
E. coli platform for \( p \)-aminostyrene production

Last 6 of 14 steps shown

Goal: Engineer RNA-based genetic systems to solve pathway control problems and enable production of \( p \)-AS for advanced polymer composites
**E. coli** platform for \( p \)-aminostyrene production

Potential genetic control mechanisms

![Diagram showing the pathway of \( p \)-aminostyrene production with key enzymes, metabolites, and regulatory mechanisms.]

**Approach:** Generate 728 unique pathway models to map space of dynamic control topology inputs to production outputs.
E. coli platform for \(p\)-aminostyrene production

Predicted production outputs for 728 core regulatory topologies

\[ \text{Output Production Ratio} \]

\[ p\text{-ACA Output Ratio } (\alpha_{\text{rel}}) = \text{dynamic / static production} \]

9-fold production increases (20% theoretical maximum) readily achievable with dynamic multi-device, RNA-based genetic control systems
E. coli platform for \( p \)-aminostyrene production

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*E. coli* platform for *p*-aminostyrene production

Predicted production outputs for 728 core regulatory topologies

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### p-ACA Output Ratio ($\alpha_{rel}$)

$$p\text{-ACA Output Ratio (}\alpha_{rel}\) = dynamic / static production$$

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<thead>
<tr>
<th>Core Topology Number</th>
<th>Output Production Ratio</th>
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<tbody>
<tr>
<td></td>
<td>aRED only + sRNA + TF</td>
</tr>
<tr>
<td>1</td>
<td>1e10 1e-5 1 1e5</td>
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<tr>
<td>10</td>
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<tr>
<td>26</td>
<td>1e10 1e-5 1 1e5</td>
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</tbody>
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1. RNA devices with quantitatively-programmable genetic outputs can be assembled from component parts generated and characterized in vitro, in vivo, and in silico.

2. Simulation analysis can inform the design of Dynamic Sensor-Regulator Systems that increase production of FAEEs.

3. Multi-device, RNA-based genetic systems can be designed to solve control problems and potentially enable high levels of production.
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