Building Adaptable Photosynthetic Consortia through Programmable Interactions

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Hope, Hype, & Hard Reality

Fundamental biology & practical engineering

+ Innovative biodesign strategies

INDUSTRIAL SUCCESS

Challenges in algal bioengineering:

- Yield improvements come at the expense of fitness
- High productivity requires well-controlled environments
- Is the metabolic and regulatory space of single species adequate?

Learning from nature:

- Natural photosynthetic communities are diverse systems, which display high productivities and remarkable stability
Photoautotrophic communities are built around division of labor

**Primary producers:**
- Fix CO$_2$
- Partitioned it to biomass, storage compounds, and DOC.
- Provide energy for heterotrophs

**Heterotrophs:**
- Couple oxidation of C$_{org}$ to aerobic anaerobic metabolisms
- Facilitate biogeochemical cycling of other key elements (critical to primary producers)

Evolutionary selection leads to synergism and interdependencies
Implementing Plug-and-Play Design

- Microbial “sabermetrics” facilitates modular assembly via inter-dependencies
- Modular design enables:
  - Parallel optimization of multiple functions
  - Metabolic versatility & ability to use various carbon sources
- Control over consortial structure and function:
  - Feedback/forward control
  - Generalizable communication

Photoautotroph (primary producer)

Excreted sugar

O₂ and excreted organic C (DOC, EPS)

CO₂

hv

C₀rg wastewater, biogas

CO₂

Heterotroph (consumer)

Macro/micro-nutrients

N, S, P, Fe, Mn

Actuator function (selection for function or viability)

Signal output (functional or compositional integrity)

CONTROL MODULE
Metabolite Sensor Devices

- DNA-based sensors use transcriptional factor (TF) regulation
  - Well characterized devices
  - Need a priori knowledge of TF
  - Need to be “insulated” to avoid cross-talk

- RNA-based regulatory devices operate at transcriptional and translational level
  - Context independent
  - Modular structure
  - Can use combinatorial methods for design
In Vivo Platform for Riboswitch Engineering

- Sensor development using *tetA* as readout
- Bacteriostatic action of Tet has fitness effect on the host (proxy for aptamer-ligand *K*<sub>d</sub>)

**In vivo selection using riboswitch libraries**

- **Absence of ligand**
  - TetA translation
  - No TetA translation

- **Presence of ligand**
  - TetA translation
  - No TetA translation

**Tet selection**

- **Add ligand**
  - Ligand
  - No ligand

High-throughput sequencing of the switch sequences
### In Vivo Identification of Sensor Devices

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Number of Aligned Reads</th>
<th>Number of unique riboswitches with &gt; 5 reads</th>
<th>Number of unique riboswitches in common between + and - cultures</th>
<th>Number of riboswitches with &gt;2-fold change in abundance (comparing + vs. - cultures)</th>
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</thead>
<tbody>
<tr>
<td>+ THP</td>
<td>2,286,512</td>
<td>1,964</td>
<td>1,703</td>
<td>103</td>
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<tr>
<td>- THP</td>
<td>2,097,399</td>
<td>2,242</td>
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<tr>
<td>+ pAF</td>
<td>4,010,381</td>
<td>3,211</td>
<td>2,817</td>
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<tr>
<td>- pAF</td>
<td>3,730,613</td>
<td>3,593</td>
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<td>146</td>
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</table>

#### Positive Riboswitches

![Graph of positive riboswitches responding to Theophylline (THP)](image1)

#### Negative Riboswitches

![Graph of negative riboswitches responding to pAF](image2)
## Multiple Riboswitches Enriched Repeatedly

### THP-Responsive Positive Riboswitch

<table>
<thead>
<tr>
<th>Sequence</th>
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<tbody>
<tr>
<td>CAGGATAGGGTCGCCC</td>
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<tr>
<td>CAGGATGGGCTGCCC</td>
</tr>
<tr>
<td>CAGGATGGGGGCTCCC</td>
</tr>
<tr>
<td>CAGGATGGGGGCACC</td>
</tr>
<tr>
<td>CAGGATGGGGTCGCC</td>
</tr>
<tr>
<td>CAGGATGGGTTGCC</td>
</tr>
<tr>
<td>CAGGATGGGCTGCCC</td>
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<tr>
<td>CCACGTGGGCAGGCC</td>
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</table>

### pAF-Responsive Positive Riboswitch

<table>
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<tbody>
<tr>
<td>GGTTGTCCTTAAGAT</td>
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<td>GGTTGTCCTTAAGAT</td>
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<tr>
<td>GGTTGTCGGCAAGAT</td>
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<tr>
<td>GGTTGCTTTAAGAT</td>
</tr>
<tr>
<td>GGTTTTGCCTAAGAT</td>
</tr>
</tbody>
</table>
Riboswitch Modularity Allows Grafting

THP Aptamer and Switch

12 nucleotides of lacZ

tetA

THP-Riboswitch_N12_tetA

+THP

-O.D. 600

0.9

0.8

0.7

0.6

0.5

0.4

0.3

0.2

0.1

0

1 2 3 4 5 6

Time (hr)

+THP

-THP

Graph showing O.D. 600 over time for THP-Riboswitch_N12_tetA with and without THP.
Riboswitch grafting onto devices with different readout genes and selection can be facilitated using \textit{in vivo} selection approach.

Development of novel riboswitch sensors for “encoded” cell-cell communication and their integration with available circuitry.
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