sRNAs in Artificial Gene Circuits and Bioremediation Applications

University of Illinois Urbana-Champaign 2010 iGEM
The University of Illinois iGEM Bioware Team
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The 2010 UIUC iGEM project has two components:

Modular sRNA logic gates

Heavy metal bioremediation
Our goal is to develop a *sRNA-mediated artificial gene circuit* and to ultimately implement it into a *heavy metal bioremediation system*. 
Project Part I: Goal

• Our goal is to construct a bacterial decoder based on sRNA

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<th>D2</th>
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• A = INPUT

• D = OUTPUT
Approach: Small RNAs

• Small single stranded fragment of RNA
• Used as responders to cellular stress in bacteria
• Targets specific sequences on mRNA
• Can activate or repress target gene transcripts
Motivation: Small RNAs

• As engineered biological designs grow in complexity, multiple levels of genetic regulation will be required.

• Most applications in synthetic biology involve a system’s novel response to its external environment.

• The Parts Registry is still beginning to explore these areas.
**sRNA Activate Target Gene Expression**

**Biobricks**
- GadY

**sRNA Repress Target Gene Expression**

**Biobricks**
- MicA
- MicF

**GOAL**

**sRNA**

**BIOBRICKS**

**LOGIC GATES**

**DECODER**
**TARGET SEQUENCE::GFP FUSION**

- **ompF**
- **ompA**
- **gadX**

**FLUORESCENCE**

- 0 mM [IPTG] hfq+
- 1 mM [IPTG] hfq+

**OVERVIEW**
sRNAs can be used to make simple logic gates

**Statement**

A AND NOT B

atc AND NOT IPTG

<table>
<thead>
<tr>
<th>IPTG (B)</th>
<th>atc (A)</th>
<th>Expected Output</th>
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</table>

GOAL | sRNA | BIOBRICKS | LOGIC GATES | DECODER
<table>
<thead>
<tr>
<th>IPTG</th>
<th>atc</th>
<th>Expected Output</th>
<th>Observed Output</th>
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Fluorescence

0 mM [IPTG], 0 mM [atc]  1 mM [IPTG], 0 mM [atc]  0 mM [IPTG], 1 mM [atc]  1 mM [IPTG], 1 mM [atc]
sRNAs can be used in a Bacterial Decoder Function

Characterization of K021101 (lac-tet fusion promoter)

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<th>IPTG</th>
<th>atc</th>
<th>Expected Output</th>
</tr>
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<td>GFP 0 EYFP 0 ECFP 0 mCherry 0</td>
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<td>0</td>
<td>GFP 0 EYFP 1 ECFP 0 mCherry 0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>GFP 0 EYFP 0 ECFP 1 mCherry 0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>GFP 0 EYFP 0 ECFP 0 mCherry 1</td>
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</table>
### Performance of A Bacterial Decoder

<table>
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<tr>
<th>IPTG</th>
<th>atc</th>
<th>Expected Output</th>
<th>Observed Output</th>
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</thead>
<tbody>
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<td>1.00 0.01 0.00 0.01</td>
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<tr>
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<tr>
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<td>1</td>
<td>0 0 0 0 1</td>
<td>0.03 0.39 0.10 1.00</td>
</tr>
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Part I: Summary

• Submission of 7 basic sRNA Biobrick parts
• Successful construction of a bacterial decoder using sRNA regulation.
• Only 3 functional sRNAs
Our goal is to develop a sRNA mediated artificial gene circuit and to ultimately implement it into a heavy metal bioremediation system.

Project Part II: Gold and Arsenic Bio remediation System
Project Part II: Goal is to remove metals from water

Arsenic needs to be removed from drinking water.
Project Part II: Bacteria are inexpensive and effective solution
The Bioremediation system uses metal-binding proteins that attach to the cell’s outer surface and collect free-floating metal ions.
sRNA logic gates regulate efflux pumps. Arsenic and gold ions leave cell through efflux pumps, which keeps the cell alive.
Bioremediation Small RNA Construct

- Sensor
- Constitutive Promoter
- Inducible Promoter
- Efflux pump
- GadY
- GadX

Stabilize!
## Parts involved in bioremediation

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Gold</th>
<th>Arsenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensor</td>
<td>GolS</td>
<td>ArsR</td>
</tr>
<tr>
<td>Efflux</td>
<td>GesABC</td>
<td>ArsB</td>
</tr>
<tr>
<td>Binder</td>
<td>GolB-LamB</td>
<td>ArsR-LamB</td>
</tr>
<tr>
<td>Promoter</td>
<td>PGolS</td>
<td>PArsR</td>
</tr>
</tbody>
</table>
Final Construct

- **Efflux pump GadX**
- **GadY**
- **Sensor**
- **Binder**
- **Efflux**
- **Constitutive Promoter**
- **Inducible Promoter**

Stabilize!
Strong Arsenic Removal Based on Cell Concentration and Affinity for Arsenic

The projected Arsenic collection for our system is well over 50ppb, particularly at cell concentrations over 7mg/mL.
Biobricks in Au-As Bioremediation System

<table>
<thead>
<tr>
<th>Biobricks</th>
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<tbody>
<tr>
<td><strong>PGolS</strong></td>
<td>Promoter</td>
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<tr>
<td><strong>GolS</strong></td>
<td>Sensor</td>
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<tr>
<td><strong>GolB-LamB</strong></td>
<td>Binder</td>
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<tr>
<td><strong>Ges-GadX</strong></td>
<td>Efflux</td>
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<tr>
<td><strong>GadY (sRNA)</strong></td>
<td>Ges stabilizer</td>
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<table>
<thead>
<tr>
<th>Biobricks</th>
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<tbody>
<tr>
<td><strong>P_{ArsR}</strong></td>
<td>Promoter</td>
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<tr>
<td><strong>ArsR</strong></td>
<td>Sensor</td>
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<td><strong>ArsR-LamB</strong></td>
<td>Binder</td>
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<td><strong>ArsB-GadX</strong></td>
<td>Efflux</td>
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<tr>
<td><strong>GadY (sRNA)</strong></td>
<td>ArsB stabilizer</td>
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Overview

Goal: better connect with our community to spread the word about synthetic biology.

Customize presentation: survey
Meet in a variety of settings: public presentations
The Survey

- Short Survey on public conceptions and beliefs about synthetic biology
- Distribution Method: online
## Survey Population Characteristics

<table>
<thead>
<tr>
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<th>Number of Responses</th>
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<td>Indiana</td>
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<td>Taiwan</td>
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<tr>
<td>Vietnam</td>
<td>1</td>
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</table>
Survey Questions/Answers:

Q: What are the first 3 words that come to mind when you hear the words “Synthetic Biology?”

- Man-Made
- Controversial
- Biology
- Crops
- Genetics
- Bacteria
- Fake
- Confusing
- Engineering
- Medicine
- Complicated
- Genetically Modified Organisms
Survey Questions/Answers

Q: What kind of science and/or synthetic biology do you see in pop culture (music, movies, tv etc.)?
Public Outreach

- Engineering Open House
- Biology Open House
- Science Olympiad
- National Tournament
- G.A.M.E.S. Camp
- Quad Day
- Surrounded By Science
Continuing Work

• Continue human practices
• Integrate small RNA logic gates with bioremediation system
• Test the metal system
• Compare our collection system to existing systems
• Prepare for 2011 UIUC iGEM team!
Medal Criteria

Bronze:

- Successfully complete and submit a Project Summary form. Create and share a Description of the team's project via the iGEM wiki
- Present a Poster and Talk at the iGEM Jamboree
- Enter information detailing at least one new standard BioBrick Part or Device in the Registry of Parts
- Submit DNA for at least one new BioBrick Part or Device to the Registry of Parts
Medal Criteria

Silver:

- Demonstrate that at least one new BioBrick Part or Device of your own design and construction works as expected.

- Characterize the operation of at least one new BioBrick Part or Device and enter this information on the Parts or Device page via the Registry of Parts.
Medal Criteria

Gold:

• Help another iGEM team
We collaborated on various levels with a number of teams

- UIUC Software
- Project Advice:
  - Peking
  - Purdue
- Surveys:
  - Warsaw
  - METU Turkey Software
  - Edinburgh

Software Team
Medal Criteria

Gold:

- Help another iGEM team
- Characterize or improve an existing BioBrick Part or Device and enter this information back on the Registry.
- Completed a Human Practices project
Acknowledgements