BioBots
Developing the Next Generation of Biosensors
Team Georgia Tech
What is a BioBot?
A biological robot that can be programmed to sense and interact with its environment.
What would make a good BioBot?
Multiple Configurations
What are the naturally occurring cell sensors that interact with the human body?
Integrins

• Previous attempts to express integrins on the surface of bacterial cells were unsuccessful
• Differences in the intercellular organizations of mammalian and bacterial cells
• We propose to redesign the integrin extracellular sequence and use bacterial
What are the synthetic biology tools that we need to achieve this goal?
Our Project
Our Project

- **Transcribe** (1)
- **Transport** (2)
- **Dimerize** (3)
- **Bind ligand** (4)

**Steps:**
1. **RBS Primer**
2. **PET-mCherry Z-Domain**
3. **Split GFP**
4. **KQAGDV-GFP**
Z-domain
KQAGDV-GFP
Split GFP
PET-mCherry
RBS Primer
HybB Promoter
Small parts, such as a Promoter, Ribosomal Binding Site, or Terminator, are hard to quickly and efficiently place in front of a BioBrick.

How can small parts be combined efficiently?

Here is our solution/new technical standard.

Transcribe
Complete RBS Primer
RBS Primer

iGEM Kit

ATG Parts from iGEM Well Plates

PCR reaction
RBS Primer

- Amplification indicates presence of site
- Amplification of 21/24 BioBricks from plate
**RBS Primer**

<table>
<thead>
<tr>
<th>New RBS Primer</th>
<th>3A Assembly</th>
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<tbody>
<tr>
<td><strong>Time</strong></td>
<td></td>
</tr>
<tr>
<td>15 min active</td>
<td>48 hr process</td>
</tr>
<tr>
<td>2 hr PCR</td>
<td></td>
</tr>
<tr>
<td><strong>Labor Intensive?</strong></td>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>Success Rate</strong></td>
<td>&lt;10%</td>
</tr>
<tr>
<td>87.5%</td>
<td></td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td></td>
</tr>
<tr>
<td>$</td>
<td>$$$$</td>
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**Antisense Primer:** Standard Sequencing Primer

GTATTACCGCCTTTGAGTGAGA
How do we move sensors to the surface of cells?
Autodisplay
PET-mCherry

- Optimized for *E. coli*
- Removal of Standard 10 restriction sites
- Addition of T7, lacI, and RBS
- Packaged for Biobrick Standard 10

Prefix

mCherry

Suffix

T7 + LacI + RBS

SP

passenger

linker

β-barrel

<table>
<thead>
<tr>
<th>SS</th>
<th>Passenger domain</th>
<th>AC</th>
<th>α-β-barrel</th>
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<tr>
<td>(BB) SS</td>
<td>HP</td>
<td>AC</td>
<td>α-β-barrel</td>
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<tr>
<td>(BP) SS</td>
<td>HP</td>
<td>AC</td>
<td>α-β-barrel</td>
</tr>
<tr>
<td>SS</td>
<td>Ag85B</td>
<td>AC</td>
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</tr>
<tr>
<td>SS</td>
<td>Pertactin</td>
<td>AC</td>
<td>α-β-barrel</td>
</tr>
</tbody>
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PET-mCherry gBlock
PET-mCherry

PET_mCherry Expression through IPTG induction!
PET-mCherry

- Characterization using:
  - Flow cytometry
  - Immunostaining
    - Monoclonal mouse and polyclonal rabbit Ab
    - Secondary fluorescently tagged anti-mouse or anti-

PET-mCherry

Pet-mCherry

Pet-mCherry IPTG

Control

Control + IPTG
Does LacI work?

YES!
Is PET-mCherry extracellularly expressed?

Most Likely...
What is an alternative to autotransporters?

<table>
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<td>(BP) SS</td>
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<tr>
<td>SS</td>
<td>Ag85B</td>
<td>ESAT-6</td>
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<tr>
<td>SS</td>
<td>Pertactin</td>
<td>AC</td>
<td>β-barrel</td>
</tr>
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</table>
• IgG binding domain that naturally occurs in *staphylococcus aureus*

• Redesigned for *E. coli*

• Double binding site Z domain
Z Domain

E. Coli Cell
How do we characterize dimerization?

Split GFP

Transcribe
Transport
Dimerize

(1)
(2)
(3)
• Sequence on Registry is correct

• However restriction digest found *EcoRI* site

• Site directed mutagenesis used to correct error
How do we determine active binding after dimerization?
KQAGDV-GFP

- Binding region of fibrinogen fused with fluorescent protein
- Fibrinogen is the natural ligand for $\alpha_{2b}\beta_3$ integrins
Summary of Work

1. Transcribe
2. Transport
3. Dimerize
4. Bind ligand

Prefix: BioBot
BioBrick
Is HybB a cold-shock promoter?
HybB Promoter

HybB → YFP

Control Promoter

fdrA → CFP
There is no difference!
Recap

Although we did not end up expressing the integrin, major steps have been made towards our goal:

• The development of the standard RBS primer
• Indications that PET mCherry (large autotransporter) is expressed on cell surface
• Developed alternative autotransporter with Z-Domain
• Fixed Split GFP from previous years, which can indicate presence of expressed integrins
• Helped Lambert iGEM through experimentation with hybB promoter
Future Work

- Fuse $\alpha 2b \beta 3$ integrin subunits to an autotransporter or IgG binding domain
- Characterization of our BioBot interaction
- Outside-in activation
### GeorgiaTech 2013 iGEM Team Parts Sandbox

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Description</th>
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<tbody>
<tr>
<td>BBa K1156000</td>
<td>Composite</td>
<td>RBS_T7_Pet(mCherry)</td>
</tr>
<tr>
<td>BBa K1156001</td>
<td>Protein_Domain</td>
<td>Z domain</td>
</tr>
<tr>
<td>BBa K1156002</td>
<td>Composite</td>
<td>GFP-KQAGDV</td>
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<tr>
<td>BBa K1156003</td>
<td>Composite</td>
<td>T7 lacI</td>
</tr>
<tr>
<td>BBa K1156004</td>
<td>Composite</td>
<td>RBS_NGFP</td>
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<tr>
<td>BBa K1156005</td>
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<td>RBS_CGFP</td>
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<td>BBa K1156006</td>
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<td>T7 lacI_RBS_NGFP</td>
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<td>T7 lacI_RBS_KQAGDV</td>
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<tr>
<td>BBa K1156008</td>
<td>Composite</td>
<td>T7 lacI_RBS_CGFP</td>
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Resources

By Integrin.png:Juergen Bode at de.wikipedia derivative work: Odysseus1479 (Integrin.png) [CC-BY-SA-3.0 (http://creativecommons.org/licenses/by-sa/3.0)], from Wikimedia Commons

Sevastsyanovich et al. Microbial Cell Factories 2012, 11:69
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96 well plate [Web Photo]. Retrieved from http://www.4ti.co.uk/files/cache/e7199a9f456dacab058c6be0b54e9235.jpg

The Team

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- Jessica Siemer

Facilitators:
- Dr. Anton Bryksin
- Haylee Bachman
- Vince Fiore
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- Dr. Kirill Lobachev
- Dr. Gang Bao
- Dr. Eric Goucher
- Edward Burdette
- Jesus Mata-Acosta
Thank you!
Questions?