BROWN
Projects

Light-Pattern Controlled Circuit (LPC)

Subproject: E. cargo
Issues with Induction

Toxicity

Efficiency

Cost

Complexity
Light-Pattern Controlled Circuit

Light Input

Binary input -> 4 outputs

Automatic control via induction device
CIRCUIT DESIGN
Previously...

Step 1: OFF

Step 2: ON

Step 3: OFF

Step 4: ON
Our Goal

Step 1
OFF

Step 2
ON

Step 3
OFF

Step 4
ON

state 1

state 2

state 3

state 4
Circuit Design

Conversion Module
Circuit Design

Memory Module

[Diagram showing a bistable switch with components labeled RFP, CI, PRM, PR, CI434, and GFP over time]
Circuit Design

Logic Module

IF&NOT gate:

AND gate:
The image contains a biological circuit diagram. The diagram includes several labeled regions:

- **Logic Modules**: Components such as LacI + IPTG, Mnt, pLac/Mnt, S3, and others.
- **Conversion Module**: J23119, RBS, tetR, ter, LovTAP, and LIGHT.
- **Memory Module**: S1, S2, Cl, Mnt, GAL4, PRM, PR, and others.

The diagram also includes notations for OFF and ON states, indicating the activation status of various components.

References:

- 2010 Brown iGEM
2010 Brown iGEM
Experimental Setup

- Composing Conversion Module
  - Modification of Double Repressor
  - Addition of TFs

- Assembly with Memory Module

- Testing of State 1 and 2

- Assembly of Logic Modules and testing!
MODELING
Ordinary Differential Equations

\[
\frac{d[S1]}{dt} = \beta_5 + \alpha_1 \left( \frac{K_{d3}}{[CI]^{n3} + K_{d3}} \right) - \mu_{13}[S1]
\]

- Change in concentration
- Basal expression
- Maximal expression
- Hill equation
- Degradation
Parameters

- 58 values
Results
State 1

Light Pattern Controlled Circuit Simulation

- **Ci434**, **Ci AraC**, **Met**, **LacI**, **GAL4**, **S1**, **S2**, **S3**, **S4**
- **Conversion Module**
- **Memory Module**
- **Logic Modules**

**Graph**: Cellular concentration (nM) vs. time elapsed (min)
State 2
State 3
State 4
After *in-silico* mutation...
Oscillation between states 3-4
TESTING THE LIGHT CIRCUIT: E.CARGO

A quick and easy protein delivery system
Testing a genetic circuit

1. Split circuit into small pieces
2. Develop constructs for each transcription factor
3. Use various promoters to adjust TF production
4. Insert TF plasmids
5. Select multi transformants and collect data
E. cargo method

- Produce E. cargo transcription factors in a separate cell culture
- Purify and apply transcription factors to the part of interest
Advantages of E. cargo

- Easily variable concentration
- Fewer plasmids per cell
- Transient activation/repression
The key: Tat-PTD

- 11-amino acid domain (YGRKKRRQRRR)
  - Allows protein to traverse membranes
  - Mechanism is not fully known

E. cargo Workflow

1. Tat-Linker Construction
2. Tat-Transcription Factors
3. Reporter Constructs
4. Testing Approach
1. Construction of modular Tat-Linker

- Tat amino acid sequence attached to polyglycine chain
- Attach RFC25 (protein fusion) assembly sites

Compatible with RFC10, RFC25
2. Tat-Transcription Factors: Constructs

- Two bacterial TFs from Registry:
  - AraC (BBa_C0080)
  - LacI (BBa_I732100)

- Modifications:
  - Add His tag to C-terminus for purification purposes
  - Remove 3-aa rapid degradation tag
  - Add RFC25 sites to entire construct

```
Constitutive promoter

RBS  Tat—glycine linker  AraC  ter
```

```
Constitutive promoter

RBS  Tat—glycine linker  LacI  ter
```
3. Tat Reporter Constructs

- LacI reporter construct from Registry (BBa_J04421)

- AraC reporter construct assembled from parts:

- Create cell lines
4. Testing

- Purify Tat-TF with Ni-NTA column
- Apply purification product to cells externally
- Compare fluorescence to controls

Expected results:

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacl</td>
<td><img src="image" alt="LacI fluorescence before" /></td>
<td><img src="image" alt="LacI repression after" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LacI represses fluorescence</td>
</tr>
<tr>
<td>AraC</td>
<td><img src="image" alt="AraC fluorescence before" /></td>
<td><img src="image" alt="AraC induction after" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AraC induces fluorescence</td>
</tr>
</tbody>
</table>
Applications of E. cargo

- Tat-transcription factors
  - Inducing systems remotely
  - Control of cell cycle, differentiation

- Tat-antibody
  - In vivo tagging of cell structures
  - Competitive binding to knock out function

- Others?
Project Summary

- Problem with manufacturing:
  - Induction methods
  - Management of different signals

- Solution: a light-pattern controlled cell circuit
  - Clean induction
  - Simple input

- Modeling our circuit in silico through four states
- Devised E. cargo method to test individual parts
The Future

- From molecular synthesis to complex biological manufacturing
- Ex. The *Encapsulator* from Imperial College London 2009

![Diagram showing steps of production](http://2009.igem.org/Team:Imperial_College_London)

- Step 1: IPTG induction
- Step 2: glucose levels
- Step 3: 42°C temperature

- Streamlined production of whole-cell devices
Thanks!

Sponsors of Brown iGEM:

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Convergence Module

Logic Modules

Memory Module

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