Implementing Non-Native Quorum Sensing in *E. coli*

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The Impact of Diabetes

• According to The American Diabetes Association, in 2012, over 29 million Americans were affected by diabetes.
• Total cost of diagnosed diabetes in the United States in 2012: $245 billion
• Arises as a consequence of the body’s inability to regulate blood glucose levels.
• There are 2 forms of diabetes:
  Type I: Inability to produce insulin
  Type II: Cells not responsive to insulin
Diabetes

**Beta Cell**
- Proinsulin
- Endopeptidases
- Export via exocytosis

**Muscle Cell**
- Tyrosine kinase receptor
- Intermediary signaling molecules
- Glucose uptake via endocytosis & glycogen production

Insulin

Export via exocytosis
A Synthetic Cell-Cell Communicator

Cell 1

- pConstitutive
- aTc
- pTet
- Ligand-exporting genes
- Export complex
- TetR

Cell 2

- Histidine kinase receptor
- Response Regulator
- Reporter (GFP)
- pResponse

Post-translationally modified signaling peptide
Quorum Sensing

- A gene regulation system correlated with bacterial population size/density
- Used to coordinate gene expression among large populations of bacteria
- Bacteria secrete signaling molecules detected by receptors on other bacteria, affecting gene expression.

• Response mechanism
  – Organisms sense and respond to changes
• Histidine Kinase
  – Membrane-bound
  – Sensor
  – Auto-phosphorylates
• Response Regulator
  – Mediator
  – Expression of target genes

3 Quorum Sensing Systems

- **agrBDCA**
- **lamBDCA**
- **fsrABC**
3 Quorum Sensing Systems

- agrBDCA
- lamBDCA
- fsrABC

GBAP (Mature Peptide)
Ligand Export Testing

Caltech iGEM

11/2/2014

Ligand-exporting genes

Export complex

Response Regulator

Histidine kinase

Reporter (GFP)

Cell 1

Cell 2

Post-translationally modified signaling peptide

 Reporter

Response

Regulator

TetR

pConstitutive

pTet

aTc

11/2/2014

Caltech iGEM

Ligand Export Testing
Western Blot - Background

**Western Blot Protocol:**

1. **Sample Preparation:**
   - **lamB** and **lamD** proteins.
   - FLAG-tagged prepeptide.
   - **Amp. Resist.**

2. **Antibody Staining:**
   - **Anti-FLAG Antibody** binds to FLAG-tagged proteins.
   - **Secondary Antibody** binds to the primary antibody.
   - **Horseradish Peroxidase** labeled secondary antibody.
   - **Chemiluminescent substrate** for detection.

3. **Imaging:**
   - **Flagged ligand** and **Flagged prepeptide visible.**

**Diagram:**

- **lamB** and **lamD** in a circular plasmid with **Amp. Resist.**
- **Flagged prepeptide** and **Flagged ligand** in the reaction.
- **Chemiluminescent substrate** for visualization.

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Western Blot – Potential Fragments

**FsrABC System**

- **30.3 kDa**
  - fsrB Membrane Component
  - 3xFLAG
  - GBAP
  - fsrB

- **29 kDa**
  - fsrB Membrane Component
  - 3xFLAG
  - GBAP

- **25 kDa**
  - fsrB Membrane Component
  - 3xFLAG

- **5.4 kDa**
  - 3xFLAG
  - GBAP
  - fsrB

- **4 kDa**
  - 3xFLAG
  - GBAP
Western Blot – Cell Lysate

Fsr lanes

0 nM  50 nM  150 nM  250 nM  350 nM  450 nM

30 kDa  25 kDa  5 kDa

fsrB Membrane Component  3xFLAG  GBAP  fsrB

fsrB Membrane Component  3xFLAG

3xFLAG  GBAP  fsrB  or  3xFLAG  GBAP
Western Blot – Supernatant

Fsr lanes

<table>
<thead>
<tr>
<th>Concentration (nM)</th>
<th>0 nM</th>
<th>50 nM</th>
<th>150 nM</th>
<th>250 nM</th>
<th>350 nM</th>
<th>450 nM</th>
</tr>
</thead>
</table>

- **30 kDa**: fsrB Membrane Component 3xFLAG
- **25 kDa**: fsrB Membrane Component 3xFLAG
- **5 kDa**: 3xFLAG GBAP fsrB

**or**

- **3xFLAG GBAP**

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LC/MS - Background

Example Read-out

\[ \text{Counts} \quad m/z = 200 \quad \text{Time} \]

\[ \text{Counts} \quad m/z = 300 \quad \text{Time} \]
LC/MS – lamBDCA System

m/z = 577.3

LamBDCA QS System

m/z = 373.2

250nM aTc Induction

m/z = 345.2

m/z = 260.1
LC/MS – fsrABC System

fsrABC
QS System
500nM aTc
Induction

All m/z channels

9.14
387.193

9.00 9.25 9.50
Conclusions – Ligand Export

Post-translationally modified signaling peptide

Export complex
- agrBDCA
- lamBDCA
- fsrABC

Ligand-exporting genes
- agrBDCA
- lamBDCA
- fsrABC

Response Regulator

Reporter (GFP)

Cell 1
- pConstitutive
- aTc
- pTet

Cell 2
- TetR
- pResponse
Ligand Reception Testing

Post-translationally modified signaling peptide

Cell 1
- pConstitutive
- aTc
- pTet
- Ligand-exporting genes
- Export complex

Cell 2
- Histidine kinase receptor
- Response Regulator
- pResponse
- Reporter (GFP)
Scaffolds in Two Component Systems

Histidine kinase

Response regulator

GFP
Testing Scaffold Protein

GFP Fluorescence for p1521 & p1523

GFP Fluorescence (AU)

Concentration of Arabinose (%)

p1521 (no scaffold)  p1523 (scaffold)
Combinatorial Promoter Testing

Cell 1
- pConstitutive
- aTc
- pTet
- TetR
- Ligand-exporting genes

Cell 2
- Histidine kinase receptor
- Response Regulator
- Reporter (GFP)
- pResponse

Export complex
- Post-translationally modified signaling peptide

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Combinatorial Promoters

pTet/pLac promoter acts as AND gate
pLac/pTet acts as asym-AND gate

![Graph showing GFP Fluorescence (au) vs. [IPTG] in μM and [aTc] in ng/ml. The graph illustrates the interaction between IPTG, RNAP, aTc, LacI, TetR, and GFP.]
pBAD/pTet acts as a single-input gate.
Conclusions

**Positive Control Scaffold System successfully tested**

**Export complex**
- agrBDCA
- lamBDCA
- fsrABC

**Ligand-exporting genes**
- agrBDCA
- lamBDCA
- fsrABC

**Post-translationally modified signaling peptide**

**Response Regulator**

**Reporter (GFP)**
- pTet/pLac AND pLac/pTet asym-AND pBAD/pTet single-input

**TetR**

**aTc**

**pTet**

**pConstitutive**
Additional Contributions

• TXTL Characterization of Existing Biobrick Promoters
• Novel Safety Considerations
• Participation in Collaborative Survey with UVA iGEM Team
What is TXTL?

TXTL is a cell-free transcription translation expression system that allows for rapid prototyping and debugging of biological circuits.

Hong SH, Kwon YC, Jewett MC. Non-standard amino acid incorporation into proteins using Escherichia coli cell-free protein synthesis. Front Chem. 2014 Jun 10; 2:34
Promoter TXTL Characterization

Measured expression levels of RFP under regulation by Anderson family of constitutive promoters (Berkeley iGEM 2006) in TX-TL

*in vivo values as reported in Biobrick registry
How feasible would it be to test and distribute a drug that can treat diabetes in animals and humans?
• Interviewed Kathleen Gilbert from Caltech Safety Office
• IACUC Animal Testing Guidelines
  – Proper housing facilities
  – Standard protocol for care and management
  – Euthanasia of sick and dying animals
• IRB Human Testing Guidelines
  – Protocol Application
  – Informed Consent Document
• FDA Guidelines
  – Good Clinical Practices (GCPs)
  – Human Subject Protection (HSP)
  – Bioresearch Monitoring Program
We contributed to the Synthetic Biology Global Awareness and Acceptance Survey Map spearheaded by the UVA iGEM Team.
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