RiboTALes: A New Paradigm for Transcriptional Control
Synthetic Biology

Controlled by Transcription Factors
Current State of Affairs
Design Criteria for Ideal Transcription Factors

- Sequence Specific
- Easily Engineerable Target Specificity
- Tunable Output Levels
- Orthogonal
- Variable Input
- Usable in any Chassis
Transcription Factors

- Sequence Specific
- Easily Engineerable Target Specificity
- Tunable Output Levels
- Orthogonal
- Variable Input
- Usable in any Chassis
Designing Novel Transcription Factors

DNA

RNA

Protein
TALEs

As a Transcription Factor:
- Sequence Specific
- Easily Engineerable
- Target Specificity
- Tunable
- Usable in any Chassis

Designing Novel Transcription Factors

DNA → RNA → Protein
Riboswitches

Riboswitches Attached to Transcription Factors:
- Tunable Output Levels
- Orthogonal
- Variable Input

## Design Criteria for Ideal Transcription Factors

<table>
<thead>
<tr>
<th></th>
<th>Typical Transcription Factor</th>
<th>RiboTALes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence Specific</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Easily Engineerable Target Specificity</td>
<td>✗</td>
<td>✔</td>
</tr>
<tr>
<td>Tunable Output Levels</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Orthogonal</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Variable Input</td>
<td>✗</td>
<td>✔</td>
</tr>
<tr>
<td>Usable in any Chassis</td>
<td>✗</td>
<td>✔</td>
</tr>
</tbody>
</table>
Building Our Testing Constructs

1. Supplemental Tables

<table>
<thead>
<tr>
<th>Riboswitch Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>E</td>
</tr>
<tr>
<td>E*</td>
</tr>
</tbody>
</table>

Shen, Aaron W. Puri, Arash Komeili, Carolyn R. Bertozzi, June R. Scott and Justin P. Gallivan
Published Ahead of Print 8 October 2010.
Building Our Testing Constructs

Quantitative analysis of TALE-DNA interactions

1Genome Center and Department of Biochemistry and Molecular Medicine, University of California, Davis, CA 95616, USA, 2Department of Molecular and Cellular Biology, University of California, Davis, CA 95616, USA and 3Department of Biology, Institute of Genetics, Ludwig-Maximilians-University Munich, 82152 Martinsried, Germany

Received November 26, 2012; Revised January 18, 2013; Accepted January 23, 2013
Testing Construct

- **pBAD**
- **TALe**
- **pTET**
- **TBS**
- **GFP**
- **+ aTc**
Testing Construct

+ arabinose

pBAD

Riboswitch

+ aTc

pTET

TBS

TALe

GFP
Testing Construct

+ arabinose
pBAD

+ theophylline
Riboswitch

+ aTc
pTET

TALe
TALe
TALe
TALe
GFP

pTET
TBS
Testing Construct
Experiments

• **Host Organism:** E. coli MG1655Z1

• **Runs extended into cellular stationary phase**

• **Equipment:**
  o TECAN Infinite 200Pro Microplate Reader
  o 96 well plates
  o Awesome pipetting skills
Experiment 1:

- Subject RiboTALe to a theophylline gradient
Translation is modulated by theophylline
Experiment 2:

- Compare effect of TALe binding affinities on system response

![Diagram showing comparison of TALe binding affinities with pTET and GFP]

- pTET → TBS → TALe Kd = X → GFP
- pTET → TBS2 → TALe Kd = Y → GFP
TAL repressor binding affinities provide tunability

![Graph showing TAL repressor binding affinities](image)

**Baseline**
- 0% arabinose
- 0 mM theophylline
- Fluorescence/OD: 60000

**Experiment**
- 1% arabinose
- 0 mM theophylline
- Fluorescence/OD: 65000

**Repression**
- 1% arabinose
- 10 mM theophylline
- Fluorescence/OD: 30000

RiboTALes

RiboTALes, Kd = 240 nM
Experiment 3:

• Compare effect of riboswitch leakiness on system response
Riboswitch leakiness modulates activity
Targeting the Anderson Family of Promoters
Inducible Anderson Promoters

![Bar chart showing fluorescence/OD for parts K1212021, K1212022, K1212023, and K1212025 under conditions of no repression and repression with 10 mM theophylline and 1% arabinose.]

<table>
<thead>
<tr>
<th>Part</th>
<th>Fluorescence/OD</th>
<th>Parent Part</th>
<th>Relative Strength of Parent Part</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1212021</td>
<td>78,000</td>
<td>J23100</td>
<td>1.00</td>
</tr>
<tr>
<td>K1212022</td>
<td>67,000</td>
<td>J23101</td>
<td>0.70</td>
</tr>
<tr>
<td>K1212023</td>
<td>8,000</td>
<td>J23105</td>
<td>0.24</td>
</tr>
<tr>
<td>K1212025</td>
<td>1,000</td>
<td>J23109</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Inducible Anderson Promoters

The graph shows the fluorescence/OD levels in response to varying concentrations of theophylline (mM). Measurements were taken at 0.1% arabinose.

- **Parent Part**
  - J23100
  - J23101
  - J23105
  - J23109
Full Characterization of RiboTALe Device

+ arabinose

pBAD

+ theophylline

Riboswitch

J2310X TBS

TALe

GFP
3D RiboTALe Data Characterization
Modeling

pBAD  \rightarrow  TALe  \rightarrow  Riboswitch  \rightarrow  TALe  \rightarrow  GFP

J2310X  \rightarrow  TBS
Modeling

\[
\frac{d[RiboTALe\,mRNA]}{dt} = aP_{BAD} - \gamma_{mRNA} \cdot [RiboTALe\,mRNA]
\]

\[
\frac{d[RiboTALe\,mRNA\,\ast]}{dt} = k_{on}[RiboTALe\,mRNA][\text{theo}] - k_{off}[RiboTALe\,mRNA\,\ast]
\]

\[
\frac{d[TALe]}{dt} = b[RiboTALe\,mRNA\,\ast] - \gamma_{protein} \cdot [TALe]
\]

\[
\frac{d[GFP\,mRNA]}{dt} = a \ast P \ast P_{TBS} - \gamma_{mRNA} \cdot [GFP\,mRNA]
\]

\[
\frac{d[GFP\,int.]}{dt} = b[GFP\,mRNA] - \gamma_{protein} \cdot [GFP\,int.] - \beta[GFP\,int.]
\]

\[
\frac{d[GFP]}{dt} = \beta[GFP\,int.] - \gamma_{LVA tag} \cdot [GFP]
\]

\[a = \text{rate of transcription}\]

\[b = \text{rate of translation}\]

\[\gamma = \text{rate of degradation}\]

\[\beta = \text{rate of GFP maturation}\]

\[RiboTALe\,mRNA\,\ast = \text{active riboswitch}\]

\[P = \text{relative activity of Anderson promoter}\]
Modeling

\[
[GFP] = P \frac{\beta \cdot a_{GFP} \cdot b_{GFP} \cdot \gamma_{LVA} \cdot (\gamma_{prot} + \beta) \cdot \gamma_{mRNA} \cdot (1 + \frac{b_{RT} \cdot [\text{theo}] \cdot a_{RT} \cdot P_{BAD}}{K_{d_{TBS}} \cdot K_{d_{RS}} \cdot \gamma_{prot} \cdot \gamma_{mRNA}})}{\gamma_{mRNA}}
\]

- \(a\) = rate of transcription
- \(b\) = rate of translation
- \(\gamma\) = rate of degradation
- \(\beta\) = rate of GFP maturation
- \(\text{RiboTALe mRNA\textsuperscript{*}}\) = active riboswitch
Modeling

Modeled at 0.01% arabinose

Effect of theophylline induction levels on relative GFP fluorescence
Modeling

Modeled at 1.0% arabinose

Effect of theophylline induction levels on relative GFP fluorescence

- Blue line: $P = 1$
- Green line: $P = 0.7$
- Red line: $P = 0.47$
- Cyan line: $P = 0.24$
- Purple line: $P = 0.04$

X-axis: [theophylline] (uM)
Y-axis: Relative GFP Fluorescence
Modeling
3D RiboTALe Data Characterization

With lots of data, what is the best way to share it?
Seeking Advice

• How can we best share?
The BioBrick Data Depot
Uploading Data

Upload An Experiment

Please adhere to the standards outlined at the UC-Davis iGEM 2013 page linked here.

Title: ____________
Part Type: ____________
Upload Raw Data: ____________
Upload Standardized Plate Layout: ____________
Upload Standardized Measurement Reads: ____________
Upload Metadata File: ____________

Submit
Retrieving Data

Search Experiments

Searching by part name yields a downloaded .csv file containing all the specified part name’s data.

Searching by experiment title yields a .zip download of all the files that went into uploading the data (raw data file, standardized plate layout, standardized measurements, standardized metadata).

Some queries make take up to twenty seconds to complete.

Search by
Part Name:     OR Experiment Title:  --Select--
## Retrieving by Part Name

<table>
<thead>
<tr>
<th>Part Name</th>
<th>Time, seconds</th>
<th>Temperature, Celsius</th>
<th>Fluorescence, AU</th>
<th>Cell Density, Optical Density at 700nm</th>
<th>Experiment Title</th>
<th>Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBa_J23101</td>
<td>0</td>
<td>37.3</td>
<td>206.0</td>
<td>0.36</td>
<td>Initial Population: The Anderson Promoter Collection</td>
<td>ZJU-China</td>
</tr>
<tr>
<td>BBa_J23101</td>
<td>934.1</td>
<td>37</td>
<td>242.0</td>
<td>0.39666666666667</td>
<td>Initial Population: The Anderson Promoter Collection</td>
<td>ZJU-China</td>
</tr>
<tr>
<td>BBa_J23101</td>
<td>1868.2</td>
<td>37.1</td>
<td>242.3333333333</td>
<td>0.41666666666667</td>
<td>Initial Population: The Anderson Promoter Collection</td>
<td>ZJU-China</td>
</tr>
<tr>
<td>BBa_J23101</td>
<td>2802.3</td>
<td>37</td>
<td>241.0</td>
<td>0.44</td>
<td>Initial Population: The Anderson Promoter Collection</td>
<td>ZJU-China</td>
</tr>
<tr>
<td>BBa_J23101</td>
<td>3736.4</td>
<td>37.1</td>
<td>241.0</td>
<td>0.47333333333333</td>
<td>Initial Population: The Anderson Promoter Collection</td>
<td>ZJU-China</td>
</tr>
<tr>
<td>BBa_J23101</td>
<td>4670.5</td>
<td>37.1</td>
<td>243.0</td>
<td>0.5</td>
<td>Initial Population: The Anderson Promoter Collection</td>
<td>ZJU-China</td>
</tr>
<tr>
<td>BBa_J23101</td>
<td>5604.6</td>
<td>36.8</td>
<td>245.6666666667</td>
<td>0.53</td>
<td>Initial Population: The Anderson Promoter Collection</td>
<td>ZJU-China</td>
</tr>
<tr>
<td>BBa_J23101</td>
<td>6538.6</td>
<td>36.7</td>
<td>253.0</td>
<td>0.57333333333333</td>
<td>Initial Population: The Anderson Promoter Collection</td>
<td>ZJU-China</td>
</tr>
<tr>
<td>BBa_J23101</td>
<td>7472.7</td>
<td>37</td>
<td>251.6666666667</td>
<td>0.61666666666667</td>
<td>Initial Population: The Anderson Promoter Collection</td>
<td>ZJU-China</td>
</tr>
<tr>
<td>BBa_J23101</td>
<td>8406.8</td>
<td>37.1</td>
<td>256.3333333333</td>
<td>0.66666666666667</td>
<td>Initial Population: The Anderson Promoter Collection</td>
<td>ZJU-China</td>
</tr>
<tr>
<td>BBa_J23101</td>
<td>9340.9</td>
<td>36.8</td>
<td>265.6666666667</td>
<td>0.72</td>
<td>Initial Population: The Anderson Promoter Collection</td>
<td>ZJU-China</td>
</tr>
</tbody>
</table>
Experience With Community Data
Experience With Community Data

- This process gets to the core of human practices
  - Time
  - Not so simple
The Depot
Sharing BioBrick Characterization Data
Other Efforts in Human Outreach

• Organized NorCal iGEM Meetup

• Worked with Nicholas Armstrong to create a Synthetic Biology Club at UC Davis
  - Platform for new student research
Accomplishments

• Introduced a novel set of transcription factors known as RiboTALs.
• Developed a database for raw data characterization of Biobricks.

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Description</th>
<th>Designer</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBa_K1212011</td>
<td>Composite</td>
<td>pBAD+Riboswitch 2+TAL1</td>
<td>Amy Soon</td>
<td>2612</td>
</tr>
<tr>
<td>BBa_K1212012</td>
<td>Composite</td>
<td>pBAD+Riboswitch 2+TAL8</td>
<td>Amy Soon</td>
<td>2616</td>
</tr>
<tr>
<td>BBa_K1212013</td>
<td>Composite</td>
<td>pTet+TBS 1+RBS+GFP+T</td>
<td>Aura Ferreiro</td>
<td>1003</td>
</tr>
<tr>
<td>BBa_K1212014</td>
<td>Composite</td>
<td>pBAD+Riboswitch 1+TAL 1</td>
<td>Amy Soon</td>
<td>2616</td>
</tr>
<tr>
<td>BBa_K1212015</td>
<td>Composite</td>
<td>pBAD+Riboswitch 1+TAL 8</td>
<td>Amy Soon</td>
<td>2620</td>
</tr>
<tr>
<td>BBa_K1212016</td>
<td>Composite</td>
<td>pTet+TBS 2+RBS+GFP</td>
<td>Amy Soon</td>
<td>1003</td>
</tr>
<tr>
<td>BBa_K1212021</td>
<td>Composite</td>
<td>J23100+TBS 2+RBS+GFP</td>
<td>Amy Soon</td>
<td>988</td>
</tr>
<tr>
<td>BBa_K1212022</td>
<td>Composite</td>
<td>J23101+TBS 2+RBS+GFP</td>
<td>Amy Soon</td>
<td>974</td>
</tr>
<tr>
<td>BBa_K1212023</td>
<td>Composite</td>
<td>J23105+TBS 2+RBS+GFP</td>
<td>Amy Soon</td>
<td>974</td>
</tr>
<tr>
<td>BBa_K1212024</td>
<td>Composite</td>
<td>J23106+TBS 2+RBS+GFP</td>
<td>Amy Soon</td>
<td>988</td>
</tr>
<tr>
<td>BBa_K1212025</td>
<td>Composite</td>
<td>J23109+TBS 2+RBS+GFP</td>
<td>Amy Soon</td>
<td>974</td>
</tr>
</tbody>
</table>
Acknowledgements

Our Advisers:
• Dr. Marc Facciotti
• Dr. Ilias Tagkopoulos
• Dr. Justin B. Siegel

Technical Guidance:
• Andrew Yao
• Dr. Navneet Rai
• Linh Nguyen

Special Thanks to:
• Nick Csicsery
• Dr. Justin B. Gallivan
• Dr. Yohei Yokobayashi
• Dr. Bertram Ludaescher
• Sven Koehler
• Anandarup Sarkar
• Hector Garcia Martin
• Keegan Owsley
References


Thank You
Theophylline Toxicity Assay