RiboTALs:
A New Paradigm for Transcriptional Control
Synthetic Biology

Controlled by Transcription Factors
Need for Input Diversity

- Controlled by Transcription Factors
Design Criteria for Ideal Transcription Factors

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

- ✔ Sequence Specific
- ◯ Easily Engineerable Target Specificity
- ✔ Tunable Output Levels
- ✔ Ability to Multiplex
- ◯ 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
## Design Criteria for IdealTranscription Factors

<table>
<thead>
<tr>
<th></th>
<th>Typical Transcription Factor</th>
<th>RiboTALes</th>
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<tbody>
<tr>
<td>Sequence Specific</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Easily Engineerable</td>
<td>☒</td>
<td>✓</td>
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<tr>
<td>Target Specificity</td>
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<tr>
<td>Tunable Output Levels</td>
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<tr>
<td>Ability to Multiplex</td>
<td>✓</td>
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<tr>
<td>Variable Input</td>
<td>☒</td>
<td>✓</td>
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<tr>
<td>Usable in any Chassis</td>
<td>☒</td>
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### Supplemental Tables

#### Riboswitch Sequence

<table>
<thead>
<tr>
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<th>Riboswitch Sequence</th>
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<tr>
<td>A</td>
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<td>B</td>
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<td>C</td>
<td><code>GGUACCGUGAUACCAGCAUCGUCUUGAUGCCCUUGGCCAGACCUCUGAAGGGCAACAAGAUG</code></td>
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<tr>
<td>D</td>
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<tr>
<td>E</td>
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Building Our Testing Constructs

Quantitative analysis of TALE-DNA interactions

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<tr>
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<td>N</td>
<td>I</td>
<td>A</td>
<td>C</td>
<td>N</td>
<td>G</td>
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<tr>
<td>II-NNp II-G</td>
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<td>N</td>
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<td>G</td>
<td>T</td>
<td>A</td>
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</tr>
</tbody>
</table>

Apparent $K_{a}$ (nM)

- I-Nlp I-A: 240 ± 40
- II-NNp II-G: 1.3 ± 0.3

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Testing Construct

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

- **+ arabinose**
  - pBAD
    - Riboswitch
    - TALe

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

- **+ arabinose**
  - pBAD
  - TALe

- **+ theophylline**
  - Riboswitch
  - TALe

- **+ aTc**
  - pTET
  - TBS
  - GFP
Testing Construct
Experiments

- Host Organism: E. coli MG1655Z1

- Runs extended into cellular stationary phase

- Equipment:
  - TECAN Infinite 200Pro Microplate Reader
  - 96 well plates
  - 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 two systems with different TALe Kd values and corresponding GFP expression. One system with TALe Kd = X and the other with TALe Kd = Y.]
TAL repressor binding affinities provide tunability

**Graph:**
- **Baseline:**
  - 0% arabinose, 0 mM theophylline
  - 1% arabinose, 0 mM theophylline

- **Experiment:**
  - 1% arabinose, 10 mM theophylline

- **Repression:**
  - RiboTALe1, Kd = 240 nM
Experiment 3:

- Compare effect of riboswitch leakiness on system response
Riboswitch leakiness modulates activity

- Baseline:
  - 0% arabinose
  - 0 mM theophylline

- Experiment:
  - NO REPRESSION:
    - 1% arabinose
    - 0 mM theophylline
  - REPRESSION:
    - 1% arabinose
    - 10 mM theophylline

Fluorescence/OD
Experiment 4:

- Full characterization of RiboTALe device
3D RiboTALe Data Characterization

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

- Mathematical support for device functionality.
- Can be developed into a predictive tool for RiboTALe design
Raw Data for RiboTALs

- Complex system with multiple inducers
- How can we best share?
Scaling to All Parts
Initial Population

- UCSF
- Stanford-Brown
- Bielefeld-Germany
- ZJU-China
A Walkthrough: Uploading

Upload An Experiment

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

Title: 
Part Type: Generator
Upload Raw Data: Choose File No file chosen
Upload Standardized Plate Layout: Choose File No file chosen
Upload Standardized Measurement Reads: Choose File No file chosen
Upload Metadata File: Choose File No file chosen

Submit
A Walkthrough: Searching

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--
# A Walkthrough: Results

<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>
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Output from Data Depot

- After Downloading Data:
  - Able to Visualize

![Graph showing data visualization](image-url)
Other Efforts in Human Outreach

- Provided lab materials for a biotechnology class at Davis Senior High School.
- Worked with Nicholas Armstrong to create a Synthetic Biology Club at UC Davis
  - Platform for new student research
Accomplishments and Future Directions

• Introduced a novel set of transcription factors known as RiboTALs.
  • New riboswitch and TALe combinations
  • Use modeling to predict and tune device behavior

• Developed the Depot for raw data characterization of Biobricks.
  • Further develop the Depot
  • Expand the amount of data in the Depot
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References


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