Promoter Characterization using Fluorogen-Activated Biosensors

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The Problem

Car Engine

- Racing
- Environmental
- Small Car

Unlabeled Black Boxes?
- Trial and Error
- Suboptimal Fit

Promoter

- Production
- Small Constructs
- Single Molecule Localization

Unlabeled Black Boxes?
- Trial and Error
- Suboptimal Fit
Traditional methods (qPCR, blotting)

- Invasive – lyse cells
- Labor/Time-Intensive

Current synthetic biology approach

- Fuse promoters of interest with green fluorescent proteins
- **Indirect** measurement of promoter activities
Fluorogen-Activated Biosensor

Advantages

- Translation Efficiency
- Transcription Rate
- Real-time
- Non-invasive
- Modular
- Spatial localization
- Short maturation time
Spinach and DFHBI

Paige et al., Science 2011.
http://mfold.rna.albany.edu/?q=mfold
Ben and MG

Emission spectra of Ben is well-separated from Spinach

http://zhanglab.ccmb.med.umich.edu/I-TASSER/

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Assumptions for the Model

- Spinach and FAP are limiting reactants and will produce signal proportional to the concentration of the protein or RNA that is bound (1:1 ratio)
- Every Spinach and FAP is in the correct conformation to bind to their dye
- Malachite green and DFHBI are both cell permeable
- DFHBI ($pK_a=5.5$) is fully deprotonated at cytosolic pH (6.5-7)
Dosage Curve - Spinach

-Dosage curve experiments to determine binding affinities of our constructs *in vivo*.

<table>
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<tr>
<th>Method</th>
<th>Comp.</th>
<th>Society</th>
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<tr>
<th>K_d In vitro (literature)</th>
<th>K_d In vivo (our results)</th>
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<td>437nM</td>
<td>~1nM</td>
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K_d

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Dosage curve experiments to determine binding affinities of our constructs *in vivo*.

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**Dosage Curve - FAP**

- **K\textsubscript{D} in vitro (literature)**
  - 1.2nM

- **K\textsubscript{D} in vivo (our results)**
  - ~50nM

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T7 Promoters and the Lac Operator

Traditional inducible promoter

- T7 promoters derive from the T7 bacteriophage and require a specific RNA polymerase in order to begin transcription.
- Lac operator (LacO) binds the LacI repressor, which prevents transcription. The LacI repressor dissociates when lactose is bound. IPTG is a lactose analog that is not consumed.
- These promoters are widely used but are not widely represented in the Registry of Standard Biological Parts. (Only 4 catalogued)
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Rationally designed T7/lac promoters

- T7 promoters have 3 sections: Recognition site, melting box and the initiation site.
- We made point mutations to develop mutants that we transformed into cells analyzed with our biosensors.
- Once we developed a model of the transcription/translation process, we could determine parameters specific to each promoter.
Measurements of Real-Time Fluorescence

1. Our expression strain is BL21(DE3), a strain that contains the gene for T7 RNAP, which we transformed with a high-copy plasmid (pIVEX).
2. We filled a 96 well plate with 100µL of our transformed cells and added 200µM DFHBI into half of the wells and 10µM MG into the other half.
3. We added IPTG and took time course measurements for 3.5 hours.
Rationally Mutated T7 Promoters

**Intro**

**Method**

**Comp.**

**Society**

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<th>Melting</th>
<th>Initiation</th>
<th>Lac operator</th>
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<tr>
<td>&gt;BBa_K921000</td>
<td>TAATGC GACTCACT</td>
<td>TATAAGAG---ACAATTGTGGGCGCGACAACAAATTCCAA</td>
<td>AATTCG AATTCG AATTCG</td>
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| >BBa_K921001 | TAATAC GACTCACT | TACAGGGC---GGAAATTGTGAGCGGATAACAATTCCAA | CAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA...
The Model – Big Picture

DNA promoter type

transcription

mRNA

transcriptional strength

translational efficiency

degradation

FAP-MG

MG

degradation

mRNA-DMHBI

DMHBI

translation

protein

Intro Method Comp. Society

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The Model - Inputs

**Intro**

**Method**

**Comp.**

**Society**

**RNA fluorescence measurements** ($t, R(t)$)

**Protein fluorescence measurements** ($t, P(t)$)

**Outputs**

Promoter characterization model
The Model – \( Ts & Tl \)

**Transcriptional strength**

\[
\frac{d[R]}{dt} = Ts \cdot [D] - \alpha \cdot [R]
\]

\[
Ts = \frac{[R] \cdot \alpha}{[D] \cdot (1 - e^{-\alpha t})}
\]

**Translational efficiency**

\[
\frac{d[P]}{dt} = [R] \cdot Tl - \beta \cdot [P]
\]

\[
Tl = \frac{Ts \cdot [D]}{(\alpha \cdot \beta)} \cdot \frac{[P] \cdot e^{\beta t}}{(1 - e^{-\beta t}) - \frac{Ts \cdot [D]}{\alpha \cdot (-\alpha + \beta) \cdot (e^{-\alpha t} - e^{-\beta t})}}
\]

**RNA fluorescence measurements \( (t, R(t)) \)**

**Protein fluorescence measurements \( (t, P(t)) \)**
The Model

Promoter characterization model

\[ TS = \frac{[R] \cdot \alpha}{[D] \cdot (1 - e^{-\alpha t})} \]

\[ TL = \frac{[P] \cdot e^{\beta t}}{TS \cdot [D] \cdot (1 - e^{-\beta t}) - \frac{TS \cdot [D]}{\alpha \cdot (\alpha + \beta)} \cdot (e^{-\alpha t} - e^{-\beta t})} \]
Another Option: Code

**RNA fluorescence measurements** ($t, R(t)$)

```
function [ PoPSans ] = Fluoro2( matrix, matrix2, DNA, modeldata )
%Fluoro 2: This function takes in a matrix of titrations and determines
%both the possible percentage for bound mRNA as well as the actual
%fluorescent mRNA concentration from fluorescent input values.
```

```
controlc = matrix(end,:); %concentration and fluorescence of the control
controlconc = controlc(1); %concentration of dye for the control
```
Human Practices

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We Want Our Human Practices to be Widely Adopted

High School Teachers

Increase SAT Score

Teach Synthetic Biology

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Human Practices - Goals

Sharing and Outreach

Interactive
Relatable
Easily shared and improved
Electronic analog of our BioBrick design

Dye-Complex

 ↑
Light Emitting Diode
(LED)

DFHBI Dye

Malachite Green Dye

Promoter X

tRNA stabilizer

Spinach RNA-fluorophore

RBS

FAP

Fluorimeter

↓

Photoresistor

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Circuit Kit - Details
‘Mini-game’ to find the best promoter
Taught students about potential problems in synthetic biology
- Mutations
- Circuit failure

Discussed bioterrorism and the uncontrolled release of synthetic bacteria
Circuit Kit - Relatable

- Physical, interactive
- Brings experiment/lab to students
- Graphical User Interface plots realistic graphs (uses modeling function)
- Comprehensive teaching presentation to introduce concepts
Circuit Kit –
Easily Shared and Improved

Pennsylvania Academic Standards for Science and Technology and Engineering Education

- 3.2.12.D: Analyze and use the technological design process to solve problems.
- 3.2.10.B: Apply process knowledge and organize scientific and technological phenomena in varied ways.
- Try to incorporate this lesson/s after:
  - 3.2.10.D: Identify and apply the technological design process to solve problems.

Your Design!

- Portable, cheap and versatile circuit.
- Lower barrier for adoption
- Open source
Submitted BioBricks

- Temporal Protein data
- Temporal RNA data
- Leaky RNA levels
- Leaky Protein levels
- Estimated Parameters

Previously...

No characterization data

Now!

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What We Accomplished

Devised new system of characterizing promoters
Introduced 3 novel well-characterized T7Lac promoters
What We Accomplished

Devised new system of characterizing promoters
Introduced 3 novel well-characterized T7Lac promoters

Created a model to analyze the data
Devised new system of characterizing promoters
Introduced 3 novel well-characterized T7Lac promoters

Created a model to analyze the data

Created a circuit kit to act as a teaching tool
Future Work

- What can be built upon our work

- Correlate actual concentration of protein/fluorescence (verification)

- Characterize more promoters – potential collaborations!

- Test the same promoters in different cell strains (chassis standardization)

- Choose other approaches for modeling
Instructors:
• Dr. Cheemeng Tan
• Dr. Natasa Miskov-Zivanov

Advisors:
• Dr. Catalina Achim
• Dr. Diana Marculescu
• Dr. Aaron Mitchell
• Dr. Ge Yang
Thank you!

Synthetic biological systems

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We were unsatisfied with how our data fit our model.

MATLAB simulation takes into account IPTG induction and can simulate experimental errors (i.e. too little dye, not enough IPTG, etc.)

Provides results consistent with experimental data.
Control Experiments

Spinach-DFHBI Functionality

FAP-MG Functionality