Softcoding of Genetic Programs
Genetic Program

Parameter
Genetic Program
Softcoding Platform

• Faster

• More affordable

• More predictable
Softcoding Platform

Genetic Circuits VS Electronic Circuits

introduction methodology application summary
RNA Controller Toolkit

Ligand responsive riboswitch/ribozyme

Ligand concentration $\leftrightarrow$ Translation strength
Softcoding Platform

introduction  methodology  application  summary
RBS calculator

RBS sequence \iff Translation strength
Softcoding Platform

AND gate

Bistable switch

Violacein synthetic pathway
RNA Controller Toolkit
RNA Controller Toolkit

Criteria
Appropriate range of regulation
Modularity
Orthogonality
Outline

✓ Harvest from existing RNA controllers

✓ Develop a rational and semi-rational design of ribozyme
Existing RNA Controllers


Theophylline Regulated Riboswitch

Result

RBS: AGGAGGU  dG = -5.9 kJ/mol

RBS: UGGAGUG  dG = -0.5 kJ/mol
TPP Regulated Ribozyme construction
TPP Regulated Ribozyme

Result

[Diagram showing regulation of GFP expression with different constructs: T7, eGFP, pBAD, and pBAD-Cl. A graph illustrates the inhibition ratio of GFP expression with varying concentrations of TPP (c(σPP/µM)).]
A General Method for RNA Controller Construction

Design

Various aptamers + Hammerhead ribozyme
A General Method for RNA Controller Construction

A General Method for RNA Controller Construction

Mock selection result

- Selected
- Control

on/off ratio vs dilution

- Selected
- Control

- 10^{-1}
- 10^{-2}
### A General Method for RNA Controller Construction

#### Table 1: Sequence (5'->3') and Activation Fold

<table>
<thead>
<tr>
<th></th>
<th>Sequence (5'-&gt;3')</th>
<th>Activation Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>TTCATA aptamer TATGAA</td>
<td>2.5</td>
</tr>
<tr>
<td>1.1</td>
<td>CCCATA aptamer TATATG</td>
<td>4</td>
</tr>
<tr>
<td>1.4</td>
<td>CAAATA aptamer TATCTT</td>
<td>2.5</td>
</tr>
<tr>
<td>2.1</td>
<td>CTGATA aptamer TATGTA</td>
<td>1.5</td>
</tr>
<tr>
<td>2.2</td>
<td>ATGATA aptamer TATTTA</td>
<td>1.5</td>
</tr>
</tbody>
</table>
RBS Calculator
Translation Strength

ΔG

Particular

Sequence

Ligand Concentration

Translation Strength

Particular

Sequence

Ligand Concentration
Derivation of $\Delta G$

A Thermodynamics-based Model

$$-\Delta G_{mRNA} + \Delta G_{mRNA:rRNA} - \Delta G_{\text{standby}}$$

Derivation of $\Delta G$

A Thermodynamics-based Model

\[ -\Delta G_{mRNA} + \Delta G_{mRNA:rRNA} - \Delta G_{\text{standby}} + \Delta G_{\text{spacing}} + \Delta G_{\text{start}} \]

The RBS Calculator

\[
\Delta G_{\text{tot}} = -\Delta G_{mRNA} + \Delta G_{mRNA:rRNA} - \Delta G_{\text{standby}} + \Delta G_{\text{spacing}} + \Delta G_{\text{start}}
\]

Translation Rate = \( Ke^{-\beta \Delta G_{\text{tot}}} \)

The RBS Calculator
Verification of Accuracy

Output Fluorescence Measurement
The RBS Calculator

Verification of Accuracy

\[ R^2 = 0.539 \]
\[ \beta = 0.24 \]
The RBS Calculator
→ Forward Engineering

Output Fluorescence
(Translation Rate)

RBS  AUG  Green Fluorescent Protein
The RBS Calculator

![Graph showing the relationship between ln(GFP/OD_{600}) and dG (kJ/mol). The line of best fit has an R^2 value of 0.842 and a beta value of 0.24.]

R^2 = 0.842
\beta = 0.24

RNA1995, 51nt
AND Gate Module
Architecture of AND gate

<table>
<thead>
<tr>
<th>Input1</th>
<th>0</th>
<th>1</th>
<th>0</th>
<th>1</th>
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<tbody>
<tr>
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<td>1</td>
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<tr>
<td>gfp</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Input 1

Input 2

supD

T7ptag

RBS

T7

GFP

introduction  methodology  AND gate  summary
Phase diagram
Step 1

TPP Hammerhead Ribozyme

Input 1

supD

RBS

T7ptag

GFP

Input 2

RBS

T7ptag

AUC TAG TAG

introduction methodology AND gate summary
Result of step 1

Ligand: TPP (M)

<table>
<thead>
<tr>
<th>Ara Sal</th>
<th>0</th>
<th>1*10^{-8}</th>
<th>3*10^{-8}</th>
<th>1*10^{-7}</th>
<th>3*10^{-7}</th>
<th>1*10^{-6}</th>
<th>3*10^{-6}</th>
<th>1*10^{-5}</th>
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</thead>
<tbody>
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</tr>
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</table>

ON/OFF ratio

-6

-4

dG(k)

inhibition ratio

T7-GFP
T7-eGFP
pBAD-eGFP
pBAD-Ci-eGFP
Result of step 2

$\Delta G = 1.33 \text{ kJ/mol}$

$\Delta G = -0.4873 \text{ kJ/mol}$

RBS: ACACAGGAC

RBS: GGAGUGG
Bistable Switch Module
Construction of Bistable Switch

[Diagram of bistable switch with mRFP, CI, PRM, CI 434, and eGFP]
Softcoding of Bistable Switch

TPP hammerhead ribozyme

No TPP  \( dG=4.6 \text{kJ/mol} \)  High TPP conc.  \( dG=7.0 \text{kJ/mol} \)

Translation strength of CI434
Stochastic Model of Bistable Switch

Introduction

Methodology

Bistable Switch

Summary
Stochastic Model of Bistable Switch

Fit with Softcoding Result
Prediction of a RBS sequence

No TPP: $dG=4.6\text{kJ/mol}$
High TPP conc.: $dG=7.0\text{kJ/mol}$

$dG=5.3\text{kJ/mol}$

RBS: CGAGACC

RBS calculator

RBS calculator
Fine-tuning biosynthetic pathway
Violacein pathway

L-tryptophan → VioA → indole-3-pyruvic acid imine (IPA imine) → VioB → IPA imine dimer → VioE → prodeoxyviolacein

deoxychromoviridans → VioD → proviolacein
Violacein pathway

- **L-tryptophan** → **VioA** → **indole-3-pyruvic acid imine (IPA imine)**
- **VioB** → **IPA imine dimer**
- **VioE** → **prodeoxyviolacein** → **deoxychromoviridans**
- **VioD** → **proviolacein**
Design

IPA imine dimer

VioE

Prodeoxyviolacein

VioD

Proviolacein

Deoxy-chromoviridans
Fine-tuning pathway

Translation Strength

control

violacein
Fine-tuning pathway

- Solvent
- TPP
- TPP 10nM
- TPP 100nM
- TPP 1µM
- TPP 10µM
Fine-tuning pathway

Area Ratio
(providacein/dexoychromovirdins)

TPP concentration (nM)

3 4 5 6 7 8 9 10 11

3 10 100 1000
Summary
Idea Summary

Soft-coding

Ligand Conc. Translation Strength RBS Sequence
Achievements

• RNA controllers & RBS calculator
  – New ribozymes with different aptamers
  – Improved existing ribozymes
  – Improved Reverse translation strength prediction

• Three applications
  – AND gate
  – Bistable switch
  – Violacein bio-synthesis
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RNA Controllers
Achievements

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RBS Calculator

Green Fluorescent Protein

\[ \ln(\text{GFP/OD}_{800}) \]

\[ R^2 = 0.842 \]
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Achievements

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Applications
Prospect & future plans

• New generation
  – Quantitative
  – Predictable
  – Fast
• Improvement
  – Precise
  – General
Parts

• **Favorite Parts**

  BBa_K598000
  *TPP Down-regulated Hammerhead Ribozyme 2.5 with Native RBS+E0040+B0015*

  BBa_K598001
  *Theophylline Responsive Riboswitch 1G1 with Engineered RBS+GFP generator*

  BBa_K598002
  *Bistable Switch Mutant 68*

• **Parts Sandbox**

  BBa_K598003--BBa_K598027
Human Practice
Collaboration

Project From TEAM

PEKING SIGNALING
Dear Luo Zheng and Helen,
Haoqian said you two would be helping me with my members. I suppose you two are the team leaders for members. I include you two are the team leaders for members. I include 

interview all the iGEM members (including yourselves) forward this email to the teams so that each member that's most suitable for him/her.

INVITATION FROM EDINBURGH
the Online Video Talk with Dr. Hu

IGEM 2011 CHINA MEETUP
Peking R in USTC

THE GUEST FROM SLOVENIA
Professor Miha Mraz's visit in Peking
Special thanks

- Prof. Qi Ouyang
- Prof. Ming Tian
- Prof. Chongren Xu
- Prof. Hongwei Guo
- Prof. Wensheng Wei
- Prof. Xinxiang Zhang
- Prof. Luhua Lai
- Prof. Lei Liu
- Prof. Peng Chen
THANK YOU FOR YOUR ATTENTION