Oceanfloat and Oceanfeel

OUC-China iGEM Team
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Qingdao
Negative Effect of Harmful Algal Bloom
Fig. 1 Variations in surface inorganic N and inorganic P concentrations in relation to red tide occurrences in inner Tolo Harbour for the years 1984–1989. (Original data courtesy of the Director, Environmental Protection Department, Government of Hong Kong.)
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Overview

Sensor Device
2. Modify of the two promoters.

Floating Device
1. Make *Escherichia coli* float using natural *gvp* cluster.
2. Make *E.coli* float using synthetic *gvp* cluster only containing *gvpA* and *gvpC* genes.

Decision-making device
1. Construct a platform for replaceable sRNA
2. Construct model-driven comparator
3. Construct model-driven ratio sensor
Sensor Device

2. Modify of the two promoters.
Decision-making device

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3. Construct model-driven ratio sensor
1. Make *Escherichia coli* float using natural *gvp* cluster.

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Input: Sensor Device

--- Sensors that can respond to the environmental phosphate and nitrate and we transform them into ‘readable input’ for decision-making device.
Input: Sensor Devices

2. Modification and fine-tuning of both promoters.
Mechanism
Design

Testing platform

Phosphate-sensitive promoter

Nitrate-sensitive promoter

PphoB

Pugp

PyeaR (obtained from 2009 Edinburgh)
Testing platform

Phosphate-sensitive promoter

PphoB

Nitrate-sensitive promoter

Pugp

PyeaR (obtained from 2009 Edingburgh)
Characterization  Problem-directed modeling
Nitrate-sensitive Promoter

Fig. 2 Ultrasensitive response of nitrate-sensitive promoter (Pyear) is shown above with Top 10 control. A strong promoter that facilitate our fine-tuning.
Fig.3 Low-phosphate induction of two Pi-sensitive promoter, Pugp and PphoB. The downtrend can be seen but keep in quite a low level. Why?
Population heterogeneity simulate by Gillespie algorithm simulation in typical TCS

Fig. 4 The Gillespie algorithm simulation of a typical two component system which HK expression is low. The molecules number of GFP changes with time is shown.
Population heterogeneity simulate by Gillespie algorithm simulation in increased HK expression

Fig. 5 Initiation transcription rate of the promoter upstream of the HK is enhanced to 0.80s⁻¹ from 0.31s⁻¹. The method is introduced by Andrzej M. Kierzek.

Solution to the problem
To verify the feasibility of this method, we firstly constructed an amplifier composed of constitutive promoter J23106 and HK phoR. If our amplifier could increase the fluorescence value, we could confirm that it works as expected.
Further Design

modeling
Fine-tuning to be compatible with our decision-making device!
CPU: Decision-making Device
How to deal with PoPs input promoted by nitrate/phosphate sensor?

Fig. 6 Nitrate/Phosphate sensor promotes PoPs1 input to decision-making device.

*PoPs stands for Polymerase Per Second, transcription rate.
Fig. 7 Comparator recognize the difference between PoPs1 and PoPs2 quantitatively.
Fig. 8 The expected response curve for Comparator.
*We adopt GFP as substitute output for trial device. The expected response curve for Comparator.

PoPs1 > PoPs2, Fluorescence High;
PoPs1 < PoPs2, Fluorescence Low.
Design

Trial device:
Ternary system for information processing.

Fig. 9 An overview of our trial device. 
“[ ]” stands for their concentration and “k” stands for reaction constant.
Trial device: Ternary system for information processing.

Fig.9 An overview for our trial device. "[ ]" stands for their concentration and "k" stands for reaction constant.
Small RNA pairs RBS region, blocking translation, initiating degradation
The molecular mechanisms of the comparator

Small RNA

the 3′BS region blocking translation, initiating decay
Buffer RNA: higher affinity in competition establishes tunable threshold that stands for warning limit.
Buffer RNA: higher affinity in competition establishes tunable threshold that stands for warning limit
Is it feasible?

Fig. 10 Three ODE for ternary system

\[
\begin{align*}
\frac{d[s_1]}{dt} &= \alpha_{s1} - \beta_{s1}[s_1] - ks[s_1][s_2] - km[s_1][m] \\
\frac{d[s_2]}{dt} &= \alpha_{s2} - \beta_{s2}[s_2] - ks[s_1][s_2] \\
\frac{d[m]}{dt} &= \alpha_m - \beta_m[m] - km[s_1][m]
\end{align*}
\]
Yes, it’s feasible.

Table 1: Investigated parameter set

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Dimension</th>
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<tbody>
<tr>
<td>$\alpha_s$</td>
<td>0–40</td>
<td>nM/min</td>
</tr>
<tr>
<td>$\alpha_m$</td>
<td>18</td>
<td>nM/min</td>
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<tr>
<td>$\beta_s$</td>
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<td>1/min</td>
</tr>
<tr>
<td>$\beta_m$</td>
<td>0.1</td>
<td>1/min</td>
</tr>
<tr>
<td>$k_s$</td>
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<td>1/nM*min</td>
</tr>
<tr>
<td>$k_m$</td>
<td>0.4</td>
<td>1/nM*min</td>
</tr>
</tbody>
</table>

Fig. 11 ODE analysis of comparator
And, The Output is robust in noise.

Fig.12 Stochastic analysis
Are there more directions from theoretical simulation?

Fig. 13 Illustration on ‘slope
It indicates ultrasensitivity.

Fig. 14 Parameter sweep results
Repression efficiency of small RNA must be low; mRNA synthesis rate should be medium.

Fig. 15 Relationship between mRNA binding rate($K_M$), mRNA synthesis rate($a_m$) and slope.
With first direction, we chose Spot42 and galK::mRNA finally.

**Fig. 16** Circuit for Spot42 characterization

**Fig. 17** Repression efficiency of Spot42, 2 fold as expected.
Spot42 is a multitarget sRNA whose targets have varied affinity.

Fig. 18 Secondary structure of Spot42 and galK complex.

Fig. 19 Secondary structure of Spot42
Buffer RNA: fuse other target leading sequences to Spot42 scaffold.

Fig. 20 Modular architecture of buffer RNA.
Fig. 21 Full construct and mechanism of comparator. The rose like protein is RNA chaperone.
Datapage:
An apparent behavior of comparing

Fig. 22 YtfJ_Comparator The datapoints are collected by 96-well plate reader, and processed by MATLAB surface tool.
We have other versions of comparator with varied ultrasensitivity.

Fig. 23 SrlA_Comparator, the buffer RNA seems less sensitive than small RNA when response to inducers.
Fig. 24 Jge means constitute galk::GFP generator, L1 means IPTG controlled Spot42 generator with galk::GFP generator, L3 means constitute Spot42 generator with galk::GFP generator.
My section is over, let’s move on to novel gas vesicle next!
The natural gene cluster
The recombined gene cluster can be control the expression as the decision-making output.
Design

Structure

Realize
Fig. 25 The buoyancy of cultures of the Pla 9402 of *Planktothrix rubescens* (taken by OUC-China)

Fig. 26 Pla 9402 of *P. rubescens* cell in microscope (taken by OUC-China)
Fig. 27 The arrangement of gas vesicle genes gvpA and gvpC in *P. rubescens*. The genome of this species has a compact cluster of gas vesicle gene with repeats of alternating gvpA and gvpC (Reference: S.J. Beard et al./ FEMS Microbio letters 215 2002 189-195)
Fig.28 Structure of gas vesicle. And the two proteins *gvpA*, which forms the ribs of the cylindrical structure, and the larger, hydrophilic *gvpC*, which attaches to the outer surface.
BBa_k737010

promoter  RBS  gvpA  RBS  gvpC  Terminator

BBa_k737010
The structure of the recombined gvpA/C cluster and can be controlled its expression.
Experiment & Result
Fig.29 The floating *E.coli*.
Fig. 30 The Trace stratified take sample device invent by OUC-China.
Fig. 31 the result of Flow Cytometer
Fig. 32 The result of stratified density distribution. The result shows the stratified population distribution is obvious in test tube. And by statistical analysis with control group we can prove that it has the significant difference of distribution due to the expression of gas vesicle.
Fig. 33 The result shows the gas vesicle in the cells after expressed in the overall trend count the cell number by using the Flow cytometry.
Fig. 34: The gas vesicle can be seen in the protoplast by using the electron microscope on the left. The right is the control of *E. coli* JM109 strain.
Fig. 35 The *gvp* gene cluster in 1% agarose gel.
Fig. 36 The result of gvp SDS-PAGE.
We can see that, the RFU of Gvp and GFP Generator is about 2000, and the RFU of Gvp and GFP Generator is about 4000, while top 10 is about 0 as control.
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Summary
Recombined *gvpA/C* cluster
Fig. 37 The floating bacteria. We have succeeded in transforming the two \textit{gvp} structure parts in two plasmids and got the same effect with nature gene cluster.
The diagram illustrates two promoters:

- **Nitrogen sensitive promoter**
- **Phosphorus sensitive promoter**

The arrows indicate the direction of gene expression in response to the respective nutrients.
Achievement

Sensor

✓ Characterized the nitrate-sensitive promoter

✓ Characterized the phosphate-sensitive promoter

Gas vesicle

Get floating E. coli by transforming gas vesicle gene cluster, two plasmids containing gene gvpA and gvpC separately, either an artificial gene cluster constructed by ourselves.

RNA Comparator

✓ Construct platform for testing small RNA
✓ Construct comparator with theoretical
**Sensor**

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Get floating *E.coli* by transforming gas vesicle gene cluster, two plasmids containing gene *gvpA* and *gvpC* separately, either an artificial gene cluster constructed by ourselves.

Get the result of SDS-PAGE and electron microscope
Achievement

**Sensor**
- Characterized the nitrate-sensitive promoter

**Gas vesicle**
Get floating E. coli by transforming gas vesicle gene cluster, two plasmids containing gene gvpA and gvpC separately, either an artificial gene cluster constructed by ourselves.

**RNA comparator**
- Construct platform for testing small RNA
- Construct comparator with theoretical

Get the result of SDS-PAGE and electron microscope
Transforming the biological signal into electrical signal by detecting the turbidity of water surface.
Constructing a series of biosensors able to detect the content of heavy metal in seawater.
A whole set of design ideas for TCS modifications

RNA calculator and information process

A platform used to work in the water surface
Future Work

- Transforming the biological signal into electrical signal by detecting the turbidity of water surface.

- Constructing a series of biosensors able to detect the content of heavy metal in seawater.

- A whole set of design ideas for TCS modifications

- RNA calculator and information process

- A platform used to work in the water surface

- SENSOR

- SCREEN-MAKER

- SUB VEHCLE
Teams

- iGEM
  - Peking University

- South University of Science and Technology of China

- Universitas Bostoniensis

Organization

- ETH
  - Eidgenössische Technische Hochschule Zürich
    - Swiss Federal Institute of Technology Zurich

- Functional Genomics Group
Professors
Instructors

Advisors
Departments

Cooperators
TEAM: OUC-China

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