Luminesensor
Luminescence

Peking University
Luminesensor

Activation

DNA

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Luminesensor

Rebinding

DNA

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OPTOGENETICS
In Eukaryotes >100 Fold Turn-up

Application in Prokaryotes

>100 Fold Turn-up

<100 Fold Turn-up

ON/OFF Ratio

Sensitivity

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Light Sensing Domain

ONLY in Eukaryotes

Function Domain

Application in Prokaryotes

Optimization

ON/OFF Ratio

Sensitivity

Programming Cells through Light

Luminesensor
Luminesensor

Function Domain

Optimization

Sensing Natural Light

Illuminance Scales

- Full Moon
- Firefly Luminescence
- Candle at 10cm
- Indoor Lighting
- Sunny Day

- LED Array at 10cm
- Laser Beam

(w/m²)

Laser Beam

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A practical function domain should \textit{not interfere} with the intrinsic genetic context of the cell.
Building up our sensor

Light sensing domain
Vivid protein (VVD)

Function domain
LexA protein

LOV
N cap

LOV
N cap

LOV
N cap

‘Luminesensor’

+ dimerization

DNA binding
Function mechanism

- reporter gene

In the dark

In the light

target sequence

NTD

LOV

NTD

LOV

NTD

LOV

NTD

LOV

NTD
Testing sensitivity

Luminesensor

- Sensitivity
- Orthogonality
- ON/OFF Ratio
Testing sensitivity

Fluorescence Intensity of GFP (A.U.)

Light Intensity of Different Attenuators

14.5  1.46  0.159  0.0574  Dark
(W/m²)

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Bacterial luciferase from *Vibrio fischeri*

$10^{-4} \sim 10^{-3}$ W/m$^2$

Hardly detectable!
Testing sensitivity

Fluorescence intensity of GFP (A.U.)

The Very First Time!
Problem of orthogonality

Sensitivity

Orthogonality

ON/OFF Ratio
Problem of orthogonality

LOV  LOV

No binding

native LexA

Mutagenesis sequence

GFP

under light

in the dark

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Testing orthogonality

Fluorescence intensity of GFP (A.U.)

- precA408-GFP + luminesensor
  - light: 350,000
  - dark: 0

- precA408-GFP + luminesensor
  - light: 350,000
  - dark: 0

- psulA408-GFP + luminesensor
  - light: 350,000
  - dark: 0
mutations in LOV domain to enhance VVD dimerization:

<table>
<thead>
<tr>
<th>mutation</th>
<th>structural effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>C71V</td>
<td>occupies C71’s position in light state</td>
</tr>
<tr>
<td>Q56K</td>
<td>form a salt bridge with Asp68</td>
</tr>
<tr>
<td>M135I</td>
<td>changes FAD molecule’s electronic environment</td>
</tr>
</tbody>
</table>

all mutations suggested to improve dimerization equilibrium constant

Brian D Zoltowski, Brian Vaccaro & Brian R Crane Nature Chemical Biology Vol.5 No.11, 827-834 (2009)
Almost 400 fold!
High sensitivity

Light communication

High on-off ratio

Bio-Printing
Light communication

Illuminance Scales

Without physical contact

Easy to reset

Interkingdom communication

Luciferase Luminescence

A Single LED at 10cm

LED Array at 10cm

Laser Beam

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Sender cell

Bacterial Luciferase
(Luxbrick—Cambridge 2010)
Sender cell

Spectrum of bacterial luciferase

Intensity vs. Wavelength (nm)

Intensity:
- 0
- 100
- 200
- 300
- 400

Wavelength (nm):
- 300
- 350
- 400
- 450
- 500
- 550
- 600

Luminesenser absorption scale
Setup

Receiver Cells

Sender Cells

$O_2$
Light communication

Experiment result

- Bio-luminescence
- Non-luminescence
- Blue LED
- Dark

Fluorescence intensity of GFP (A.U.)
Different dilution ratio of bacterial luciferase.
Light Communication

Sender  Receiver  Control
Light Communication

17:20

The Very First Time!

Light communication
Bio-Printing

Higher resolution

Higher sensitivity
Mask Printing
Can simple biological systems be built from standard, interchangeable parts and operated in living cells? Or is biology so complicated that every case is unique?

The Claimed Ultimate Goal of iGEM Competition
Summary
Summary

- **Ultra-sensitive**: sensing natural light and even bioluminescence without exogenous substrates;

- **Bio-orthogonality**: working independently of host genetic context;

- **On-off ratio enhanced**: enhancing the on-off ratio of our luminesensor;

- **Widespread application**: implementing cell-cell communication and bio-printing;

- ……
Perspective
Inter-species/Inter-kingdom communication

Mammalian cell

Yeast

Bacteria
A new signaling channel
Human Practice
Sowing Tomorrow’s Synthetic Biologists
Pre-investigation

Had an discussion with high school teachers.

Visited their labs for Molecular Biology.
Lectures to High School students

Life as machines

Rational design for artificial biological systems

Peking iGEM 2012
Beijing Teenager S&T Club
Lectures to High School students

XIONG Hongyu is giving his lecture.

ZHOU Wenyuan is answering students’ questions.
Lectures to High School students

Over 100 students and teachers from 24 high schools came to the lecture.
Funding Application & Guidance

Question 9: If you’d like to participate in the iGEM(HS) from which aspects of support do you need
Director of NO.4 High School came to discuss with us.
Funding Application & Guidance

Brochures on the competition for the students.
High school students are doing mini-prep with our guidance
Human Practice: Historical iGEM Project Review

Video of Historical iGEM Project Review (available on our wiki)
Extension of “Sowing”

Visiting Team ZJU-China

Visiting Team OUC-China

Collaboration on Propagate SB
Sowing Tomorrow’s Synthetic Biologists is our responsibility!
Acknowledgement

Prof. YANG Yi

Prof. OUYANG Qi

ZHANG Haoqian

CHEN Shuobing
Acknowledgement

Team members: ZHAO Zhilei, XIONG Hongyu, ZHOU Wenyuan, ZHANG Hong, LIU Jintan, YU Zhou, XING Wenmin, LI Dayi, LI Hanxi, LU Tian, QIU Zhen, SUN Sibai, Tina Chen, YAN Jiawei, YANG Lu, ZHANG Zidong, YU Ye
Acknowledgement

Bmaffitt, Muviag, Strid3r21 for video
Programming
Cells through
Light

Thank you
Ensuring orthogonality

Specific binding

PrecA408

Mutated target sequence

P40A, N41S, A42S

PrecA408-GFP

Andrew T. Thliveris and David W. Mount. P.N.A.S. Vol.89, pp.4500-4504
Ensuring orthogonality

LexA

specific binding

CTGT
ACAG

precA408

LOV LOV

specific binding

CCGT ACGG

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precA408-GFP+ luminesensor in the dark
precA408-GFP+Luminesensor under light
Testing orthogonality

Fluorescence intensity of GFP (A.U.)

- psulA408-GFP
- psulA408-GFP + luminesensor light
- psulA408-GFP + luminesensor dark
Testing enhancement

Signal-background ratio

Wild type luminesensor
Optimized luminesensor

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sensitive parameter analysis

[Diagram with mathematical expressions and symbols related to design, orthogonality, and dynamic performance]
reversibility test

using mCherry as reporter gene

under light

throw into darkness

Experiment performed in ΔLexA strain
testing orthogonality

- CTGT
- ACAG
- pSulA
- CCGT
- ACGG
- pSulA_{408}

ΔLexA

Point mutation

LOV
- N
- cap
- cap
- NTD
- NTD

Wild type

LOV
- N
- cap
- cap
- 408
- 408

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result
LOV

transcription activator

histidine kinase

expansion

red light

‘AND gate’
5-1. Big Pocket!
5-2. Conjugation group of FAD: riboflavin
5-3. Red-shifted riboflavin analogue
5-4. Where to mutate?
5-5. Molecular Docking