Biofilm
Natural aggregation of bacteria

Graded substances

High resistance

High cell density
Modeling Results

• Biofilm Formation Modeling
  – Biofilm Growth
  – Biofilm Thickness
  – Oxygen Gradient
  – Meaning to wet-lab experiments
DIY Biofilm Reactor

- Cell Culture Plate Method
- Silicone Tube Method
- Bubbling Method
Comparison of Methods

Silicone Tube
- Frozen section
- Thickness 100µm
- However, it is unstable.

Cell Culture Plate Method
- Confocal observation
- Best maintained structure
- Very stable.
Introduction

Natural gradients within biofilm

Fig. Oxygen concentration profiles for P. aeruginosa colony biofilms. Triplicate data sets are shown for each strain. Depth zero on the x axis corresponds to the air-colony interface.
Introduction

stratified Biofilm formation

cell attachment  0~12h

Liquid  →  Cell monolayer
Substratum

active biofilm  12~48h

Liquid  Biofilm
Substratum

depth of biofilm

concentration of oxygen

mature biofilm  48h~

Liquid  Biofilm
Substratum

depth of biofilm

concentration of oxygen

Rainbofilm
Ptet’s activity under aerobic conditions
Anaerobic
Results

Fluorescence Intensity

Frequency
Rainbofilm

Fig. 4 D Merged fluorescent biofilm picture of CFP (aerobic condition induced) YFP (micro-aerobic condition induced) and RFP (anaerobic condition induced) under Zeiss LSM510 Meta confocal laser scanning microscope, using a 63X oil-immersion objective.
Results
Fig.1 Vgb promoter. Revised from *ArcA works with Fnr as a positive regulator of Vitreoscilla (bacterial) hemoglobin gene expression in Escherichia coli* (2005) J. Yang et al.
Mercury and lead promoters

**PmerTPCAD**

TTGAC TCCTCGT ACATGACTAC GCAAGTAAGG TTACGCTATC

**PpbrA**

TTGAC TCTCTAT AGTAAC TAGAAG GTGTTAAA TCGGCAACGC

-35

**Conservative Region**

-10

**Mutation Region**

-1
X film
Intriguing features of Rainbofilm?

A stratified system

Robustness
Into real-world, into practice

How can we expand it?
A stratified system

Adherence

Robustness

Bio-reactor Design

- Better for producing unfavorable products
- Multi-step synthesizing pathway
Upper Layer:

```
ptetR  alxS  adh2  kivd
```

Middle Layer:

```
pvgb  pglD  tetR
K561001  B0032  K132026  B0034  C0040  B0010  B0012
```

Bottom Layer:

```
pfdhF  cex  cenA  tetR
K387003  B0034  K118022  B0034  K118023  B0034  C0040  B0010  B0012
```

Note:  
- **Cex** - *Cellulomonas fimi* exo-glucanase  
- **CenA** - *Cellulomonas fimi* endo-glucanase  
- **BglX** - *Cytophaga hutchinsonii* beta-glucosidase  
- **AlxS** - coding sequence of desired enzyme(s) in the following pathway  
- **Adh2** - *S.cerevisiae* alcohol dehydrogenases  
- **Kivd** - *L.lactis* 2-keto-acid decarboxylases
Modeling

Overall Stimulation

Bottom Layer

Middle Layer

Time (hours)

Relative value of mass

Amount of (g/kg)

Time (hours)
Over Sugarfilm and More

Concentrated enzyme distribution

Neat

Porous vessel bioreactor with immobilized biocatalyst

\[
\text{influent} \quad \rightarrow \quad \text{nutrient} \quad \rightarrow \quad \text{biofilm} \quad \rightarrow \quad \text{effluent} \quad \rightarrow \quad \text{final product} \quad \rightarrow \quad \text{by-product}
\]
Gluefilm

FdhF mgfp-5 Glue

Aerobic and microaerobic

FdhF mgfp-5 Glue

anaerobic
When two surfaces fit

FdhF  Glue
When two surfaces don’t fit

FdhF Glue
X film Sensorfilm
Shortcomings of traditional biosensors

- Confined upper detection limit
- Qualitative but not quantitative

Advantages of sensorfilm

- Higher metal concentration sensitivity
- Quantitative metal concentration gradient
Sensorfilm I

Sensorfilm II
Sensorfilm System II

- arabinose
- pBAD
- RBS
- LuxR
- RBS
- MerR
- terminator
- SAM
- AHL
- pMerT
- RBS
- LuxI
- terminator
- pLuxR
- RBS
- LuxI
- RBS
- Luciferase
- terminator
Rainbosim

- Design
- Model
- Share
- Web based
Template

Device A
- P(tetR)
- Coding Sequence A

Device B
- Promoter B
- Coding Sequence B
- tetR

Device C
- Promoter C
- Coding Sequence C
- tetR

You Design
## Config Parameters

### Devices

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<th>Part</th>
<th>Description</th>
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<td>BBa_K561000</td>
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<td>BBa_K561002</td>
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### Properties

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<th>Name</th>
<th>Value</th>
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<tbody>
<tr>
<td>Dissociation constant for P(TetR) to TetR</td>
<td>5e-08</td>
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<td>Hill coefficient for promoter TetR</td>
<td>3</td>
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<td>mRNA degradation rates</td>
<td>0.00288 s^-1</td>
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<td>Degradation rates protein TetR</td>
<td>0.00289 s^-1</td>
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<td>Degradation rates protein ecfp</td>
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<td>Degradation rates for proteins eyfp and mRFP1</td>
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<td>Translation rates</td>
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<tr>
<td>Max. transcription rate for promoter TetR</td>
<td>6.55e-08 s^-1</td>
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### Modeling

<table>
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<th>Transcription rates of promoter vgb</th>
<th>M/s</th>
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[Start modeling]
Expression Level of C, Y, R. Varying with time, $\alpha = 4 \times 10^{-9}$, $\beta = 6 \times 10^{-9}$, 1000s

Database
Table:

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<th>Time (h)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>0.2</td>
<td>0.35</td>
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<td>0.95</td>
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<td>Fluorescence</td>
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<td>2500</td>
<td>4100</td>
<td>6800</td>
<td>12500</td>
<td>19300</td>
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</table>

Graphs:
- Expression Rate
- Growth Rate

Tools:
- Matlab
- Excel
Human practice
HUMAN PRACTICE

STUDENT ORGANIZATION:
THE SYNTHETIC BIOLOGY CLUB
AN ORIGINAL NOVEL

In the Name of God
• Play all-around!
• Build a path, and fulfill a task!
• Gain Knowledge by tips!
• Stop by our Poster and go ahead to enjoy!
How do funding problems affect iGEM?

Funding Manual

Through the survey analysis, we've summarized the following funding experiences for sharing:

1. Applying for funding as early as possible
   Through our survey, most of the successful funding applications are early (from Feb. to June). So don't wait until summer vacation!

2. Look around yourself first
   Before applying for funding, consider alternative sources and alternative strategies to secure funding.
Realized standard biofilm formation in lab easier, cheaper and quicker.

Cheap Biofilm!!

Constructed an oxygen dependent stratified expression system and extend the input signal of it.

Our Design Works!!

Carried out a new approach for bioreactor design.

Created a new system to produce glue.

Updated traditional biosensor to stronger and quantitative biofilm sensor.

Extensive Application!!
Developed a tool for biofilm reactor design.

Designed a tool for better and easier characterization.

Founded a synthetic biology club and wrote a splendid novel.

Developed an iPhone APP to promote synthetic biology.

Wrote Funding Guide to help future iGEM teams.

Long Live iGEM!!!
Thank you!
Questions?