E. cerevisiae
Introduction

Diacetyl

E. coli

AHL

S. cerevisiae

Odr-10

Gpa1/chi

Ste4

Ste5

Ste11

Ste12

Ste18

Sst2

Fus3

Fus1-BFP

Fus1-HIS3

Cell-Cycle Arrest

BFP

SCUT
E. cerevisiae

Oscillating odorant
Outline

1. Oscillating odorant
2. Odorant sensing
3. Human practice
Oscillating odorant
The metabolism pathway of diacetyl in E.coli

- Transforming gene α-ALS
- Decarboxylating spontaneously

The metabolism pathway of diacetyl in E.coli

Oscillating odorant
Modeling

Central carbon pathway

Diacetyl production

Oscillation modeling

Oscillating odorant
Diacetyl production

The concentration of pyruvate is stable.

Simplify: model the diacetyl through the Michaelis-Menten equation.
Oscillation model

Oscillater across a microfluidic array simulate by “degrade-and-fire” model

The delay-differential equation:

\[
P' = \mu + \alpha_p X - \gamma_p P
\]

\[
\dot{X} = \frac{\alpha_0 (1 + \nu P_{\tau_2})}{\left(1 + \frac{X_{\tau_1}}{C_0}\right)^2} - \frac{\gamma_0 X}{k + X}
\]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meaning</th>
<th>Value (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha_0)</td>
<td>The corresponding rate of coupled positive-negative feedback</td>
<td>8.25</td>
</tr>
<tr>
<td>(\gamma_0)</td>
<td>The max enzymatic degradation rate of Luxl</td>
<td>5.75</td>
</tr>
<tr>
<td>(\nu)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>(\tau_1)</td>
<td>The delayed time of Luxl</td>
<td>10</td>
</tr>
<tr>
<td>(\tau_2)</td>
<td>The delayed time of (H_2O_2)</td>
<td>20</td>
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<tr>
<td>(C_0)</td>
<td>The maximum concentration of Luxl</td>
<td>6</td>
</tr>
<tr>
<td>(k)</td>
<td>Michaelis-Menten constant of Luxl</td>
<td>10</td>
</tr>
<tr>
<td>(\mu)</td>
<td>Production rate of (H_2O_2)</td>
<td>20</td>
</tr>
<tr>
<td>(\alpha_p)</td>
<td></td>
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</tr>
<tr>
<td>(\gamma_p)</td>
<td>Decay rate of free (H_2O_2)</td>
<td>10</td>
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</table>
Simulation result

“degrade-and-fire” model

Oscillating odorant
Experimental results

Oscillating odorant

SCUT
Experimental results

Oscillating odorant

SCUT
The promoter successfully induced by AHL-LuxR!

Oscillating odorant

SCUT
GFP under control of Lux pR

Green fluorescence from expression strain

Three plasmids have transformed into E.coli, expressing green fluorescence under confocal that prove GFP under control of luxpR
H$_2$O$_2$ emitting

Diagram showing interactions between various components, including oscillators,luxR, AHL, LuxR, ndh, H$_2$O$_2$, and coupling mechanisms.
H$_2$O$_2$ emitting

OD value of H$_2$O$_2$

- $\text{OD}_{240}$
- Detecting BBa_K1072003
- Concentration of H$_2$O$_2$ is 7.21*10$^{-2}$ mol/L;

- Then Detecting BBa_K1072022
- Concentration of H$_2$O$_2$ is 2.19*10$^{-1}$ mol/L;

Oscillating odorant

○ SCUT
Producer

Oscillating odorant

SCUT
Prove that α-ALS successfully expressed in E. coli!
• Produced diacetyl concentration is 21.56mg/L.

BBa_K1072005

Prove that diacetyl indeed produce in E.coli!
Diacetyl producing

a: green fluorescence from *E. coli*

b: blue fluorescence from *S. cerevisiae*

**Oscillating odorant**
<table>
<thead>
<tr>
<th>W</th>
<th>BBa_K1072000</th>
<th>Lux pL+RBS+LuxR+2TM+Lux pR+RBS+LuxI+2TM+Lux pR+RBS+GFP+2TM</th>
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<tr>
<td>W</td>
<td>BBa_K1072001</td>
<td>Ndh</td>
</tr>
<tr>
<td>W</td>
<td>BBa_K1072002</td>
<td>Lux pR+RBS+Ndh+2TM</td>
</tr>
<tr>
<td>W</td>
<td>BBa_K1072003</td>
<td>Lux pL+RBS+LuxR+2TM+Lux pR+RBS+LuxI+2TM+LuxpR+RBS+Ndh+2TM</td>
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<tr>
<td>W</td>
<td>BBa_K1072004</td>
<td>alpha-ALS</td>
</tr>
<tr>
<td>W</td>
<td>BBa_K1072005</td>
<td>PT7+RBS+alpha-ALS+T7TM</td>
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<tr>
<td>W</td>
<td>BBa_K1072006</td>
<td>Lux pR+RBS+alpha-ALS+2TM</td>
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<td></td>
<td>BBa_K1072007</td>
<td>Lux pL+RBS+LuxR+2TM+Lux pR+RBS+LuxI+2TM+Lux pR+RBS+GFP+2TM+Lux pR+RBS+alpha-ALS+2TM</td>
</tr>
</tbody>
</table>
Outline

1. Oscillating odorant
2. Odorant sensing
3. Human practice
Odorant sensing
**Design**

- Odr-10 is a heterogenous receptor for *S.cerevisiae*, belonging to GPCRs.
- Diacetyl as the ligand to odr-10 produced by *E.coli*.
- Fus1 as the promoter of reporter system.

**Odorant sensing**

- SCUT
Receptor and Reporter

Odr-10 vector construction

BBa_K1072017

BBa_K1072019

Odorant sensing
Optimization-Gpa1/chi

Replace the Gpa1 with Gpa1/odr-3 chimera

Diacetyl

Cell-Cycle Arrest

Fus1-BFP
Fus1-HIS3
Fus1-LacZ
Five amino acids of Gpa1 are replaced by corresponding residues from odr-3 subunit.

Depend on two discoveries:

1. the specificity
2. the crystal structure
Optimization - Knock out redundancy gene

- Knock out Far1 gene disabling the cell-cycle arrest

- Sst2 knockout?

Later you will know
Gene Far1 knockout

Left arm → Far1 → Right arm

zeocin

Final fragment

Odorant sensing
Modeling

- ODE analysis
- Sensitivity analysis
- Parameter sweep
- Noise analysis
ODE analysis

Is the pathway feasible?

\[ v_1 = k_{odr-10a}[odr-10][P] \]
\[ v_2 = k_{odr-10a}[odr-10^*] \]
\[ v_3 = k_{gpa}[odr-10^*][G\alpha\beta\gamma] \]
\[ v_4 = k_{gpi\alpha}[G\alpha GTP] + k_{gpi\alpha Sst2}[G\alpha GTP][Sst2^*] \]
\[ v_5 = k_{pGformation}[G\alpha GDP][G\beta\gamma] \]
\[ v_6 = k_{Dsyn}[C][G\beta\gamma] \]
\[ v_7 = k_{Ddg}[D] + k_{DdgSst2a}[D][Sst2^*] \]
\[ v_8 = k_{fus3a}[D][Fus3] \]
\[ v_9 = k_{fus3a}[Fus3^*] + k_{fus3aSst2}[Sst2][Fus3^*] \]
\[ v_{10} = k_{Sst2a}[X^*][Sst2] \]
\[ v_{11} = k_{Sst2aSst2}[Sst2^*] \]
\[ v_{12} = k_{odr-10d}[odr-10^*] \]

“[ ]” stands for concentration; “k” stands for the reaction constant
Feasibility of odr-10 pathway

It is feasible

Parameter set:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meaning</th>
<th>Value (Range)</th>
<th>Dimension</th>
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<tbody>
<tr>
<td>$k_{odr,10a}$</td>
<td>Receptor activation</td>
<td>0.071</td>
<td>1/(nM * min)</td>
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<tr>
<td>$k_{odr,10ia}$</td>
<td>Receptor inactivation</td>
<td>2.457</td>
<td>1/min</td>
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<tr>
<td>$k_{gpa}$</td>
<td>G-Protein activation</td>
<td>0.724</td>
<td>1/(nM * min)</td>
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<tr>
<td>$k_{odr,10ag}$</td>
<td>Receptor-ligand(odr10-diacyl) degradation</td>
<td>1.199</td>
<td>1/min</td>
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<tr>
<td>$k_{gpaSst,2}$</td>
<td>G-Protein inactivation under phosphorylated Sst2</td>
<td>1.124</td>
<td>1/(nM * min)</td>
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<tr>
<td>$k_{gpaia}$</td>
<td>G-Protein inactivation</td>
<td>1.259</td>
<td>1/min</td>
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<tr>
<td>$k_{pgformation}$</td>
<td>Heterotrimeric G-protein formation</td>
<td>0.002</td>
<td>1/(nM * min)</td>
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<tr>
<td>$k_{Dsyn}$</td>
<td>Complex D synthesis</td>
<td>0.021</td>
<td>1/(nM * min)</td>
</tr>
<tr>
<td>$k_{Ddig}$</td>
<td>Complex D degradation</td>
<td>0.004</td>
<td>1/min</td>
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<tr>
<td>$k_{DdigSst,2a}$</td>
<td>Complex D degradation under phosphorylated Sst2</td>
<td>0.261</td>
<td>1/(nM * min)</td>
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<tr>
<td>$k_{Fus,3a}$</td>
<td>Fus3 activation</td>
<td>0.818</td>
<td>1/(nM * min)</td>
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<tr>
<td>$k_{Fus,3ia}$</td>
<td>Fus3 inactivation</td>
<td>0.0</td>
<td>1/min</td>
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<tr>
<td>$k_{Fus,3iaSst,2}$</td>
<td>Fus3 inactivation by Sst2</td>
<td>0.017</td>
<td>1/(nM * min)</td>
</tr>
<tr>
<td>$k_{Sst,2a}$</td>
<td>Activation of Sst2</td>
<td>0.342</td>
<td>1/(nM * min)</td>
</tr>
<tr>
<td>$k_{Sst,2ia}$</td>
<td>Inactivation of Sst2</td>
<td>0.643</td>
<td>1/min</td>
</tr>
</tbody>
</table>
Knock out Sst2 gene or not...

Gene sst2 knockout would result in pathway being extremely sensitive

Fig. wild type

Fig. Comparator (knock out the Sst2 gene)
Through the noise analysis, the system is stable.
Parameter sweep

Discover the optimized value of important parameters and the best results

\[ S_i = \frac{\partial y_i}{\partial p} \approx \frac{y_i(p + \Delta p) - y_i(p)}{\Delta p} \]
Conclusion: the repression efficiency of fus3a should be small
Validation of promoter of odr-10

galactose

Odorant sensing
Validation of promoter of odr-10

The promoter Works and not leaks

a : induced by galactose

b : not induced by galactose
Localization of odr-10 at membrane

Odorant sensing

S. cerevisiae

SCUT
Localization of odr-10 at membrane

Our odr-10 was localized at the plasma membrane **successfully**!
Stimulation of the pathway by diacetyl

Diacetyl

S. cerevisiae

Odorant sensing

SCUT
Stimulation of the pathway by diacetyl

The whole pathway reconstructed was stimulated by diacetyl!

Odorant sensing
## Results

### Biobricks

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<tr>
<th></th>
<th>Biobrick ID</th>
<th>Type</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>W</td>
<td>BBa_K1072008</td>
<td>Coding</td>
<td>Flag tag</td>
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<tr>
<td>W</td>
<td>BBa_K1072009</td>
<td>Coding</td>
<td>Bovini Rhodopsin signal peptide (Brho) with optimized codon</td>
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<tr>
<td>W</td>
<td>BBa_K1072010</td>
<td>Coding</td>
<td>Odr-10 (diacetyl) GPCR with optimized codon</td>
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<tr>
<td>W</td>
<td>BBa_K1072011</td>
<td>Coding</td>
<td>pGpa1+Chimeric Gpa1 (Gα subunit)</td>
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<tr>
<td>W</td>
<td>BBa_K1072012</td>
<td>Composite</td>
<td>pGpa1+Chimeric Gpa1 (Gα subunit)+Rfp+ADH1</td>
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<td>W</td>
<td>BBa_K1072013</td>
<td>Composite</td>
<td>pGAL1+Flag+Odr-10+GFP+ADH1</td>
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<td>W</td>
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<td>Composite</td>
<td>pGAL1+Brho+Flag+Odr-10+GFP+ADH1</td>
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<td>BBa_K1072015</td>
<td>Composite</td>
<td>pGAL1+Flag+Brho+Odr-10+GFP+ADH1</td>
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<td>W</td>
<td>BBa_K1072016</td>
<td>Composite</td>
<td>pGAL1+Brho+EGFP+ADH1</td>
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<tr>
<td>W</td>
<td>BBa_K1072017</td>
<td>Composite</td>
<td>pGAL1+Flag+Odr-10+GFP+ADH1+Fus1+BFP reporter+ADH1</td>
</tr>
<tr>
<td>W</td>
<td>BBa_K1072018</td>
<td>Composite</td>
<td>pGAL1+Flag+Brho+Odr-10+GFP+ADH1+Fus1+BFP reporter+ADH1</td>
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<tr>
<td>W</td>
<td>BBa_K1072019</td>
<td>Composite</td>
<td>pGAL1+Brho+Flag+Odr-10+GFP+ADH1+Fus1+BFP reporter+ADH1</td>
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<td>W</td>
<td>BBa_K1072020</td>
<td>Reporter</td>
<td>FUS1+BFP+ADH1</td>
</tr>
<tr>
<td>W</td>
<td>BBa_K1072023</td>
<td>Promoter</td>
<td>FUS1</td>
</tr>
</tbody>
</table>
Co-culture of E.coli and yeast
Co-culture of E.coli and yeast

Yeast Control

Yeast(s) + E.coli(L)

E.Coli(s)

Yeast(s) + diacetyl

Co-culture
More ideas

1. Microfluidic device.
2. Supposing the same Concentration of $\text{H}_2\text{O}_2$ across the two devices.
Two way communication
Outline

1. Oscillating odorant
2. Odorant sensing
3. Human practice
Human Practice
Overview

Number of IGEM Teams

- Around the World
- In Asian
- In China

Year
0 5 14 41 59 88 113 128 159 191 223

Human practice

SCUT
iGEM Everywhere
IGEM Teach-in
Cultivating--Bio-model competition

Novel approach!
Cultivating—IGEM workshop
Harvest

Laboratory open day for undergraduate and ready to Asian jamboree

Human practice
Harvest

And this year, SCUT also help us to characterize the part, by comparing its growth-inhibiting effect with the original hbax that we improve, we can see from the picture that the inhibition level of hbax mutant (label as hbax) is significantly higher than the hbax already exist. And they can both inhibit the growth comparing to the control. However, since 2% galactose is a very high induction level, it turns out that the highest expression level already reach at this point, so there are almost no difference between 2% and 5%. For more accurate gradient data, we need to set the gradient between 1% and 2%.
Achievements

✔ Team registration and Complete Judging form.
✔ Team Wiki, Present a poster and a talk at the iGEM Jamboree.
✔ Document at least one new standard BioBrick Part or Device.

✔ Experimentally validate that at least one new BioBrick Part or device works as expected, BBa_K1072008, BBa_K1072010 and so on.
✔ Document the characterization of this part in the “Main Page” section.
✔ Submit this new part to the iGEM Parts Registry.
✔ Describe one or more ways in which these or other broader implications have been taken into consideration in the design and execution.

✔ Improve the function of an existing BioBrick Part or Device, BBa_K1072022,BBa_K1072017 and so on.
✔ Help SYSU iGEM team characterizing a part, BBa_K1061006.
Acknowledgement

Prof. Pan Li

Prof. Lin Ying

Prof. Wang Jufang

Zhang Junjie

Dr. Zhang Li

Gong Jianhui

Ou Min
Acknowledgement
Thank you
Any questions
Lane1-4 amplified the zeocin

Lane1-4 didn’t amplified the fragment in far1