Circadian Release of Propionate for Antihypertensive Therapy

HUST-CHINA
BACKGROUND

**Main complications of persistent high blood pressure**

- Brain: Cerebrovascular accident (strokes) - Hypertensive encephalopathy - Confusion - Headache - Convulsion

- Heart: Myocardial infarction (heart attack) - Hypertensive heart disease - Heart failure

- Kidney: Hypertensive nephropathy - Chronic kidney disease

- Blood: Elevated sugar levels

**High blood pressure, a major risk**

Almost 1 billion people worldwide have high blood pressure.

**SOURCE:** High Blood Pressure and Health Policy, 2006

**24-hour BP profile in untreated hypertensive patients (n=20)**

Key: BP = blood pressure
A Novel Method

Engineered Bacteria

Antihypertensive Drug

24-hour BP profile in untreated hypertensive patients (n=20)
Propionate

- Interacts with Olfr78, a GPCR expressed in smooth muscle cells of small blood vessels
- Induce vasodilatation and hypotension

A Novel Method

Propionate

Engineered Machine

Oscillator
Our Progresses

Oscillator
- Plasmids
- Modeling
- Results

Propionate
- Methods
- Results

Other
- Future plan
- Human Practice
Background

Wood-Werkman Reaction

\[
\begin{align*}
\text{Sbm} & \quad \text{Methylmalonyl-CoA epimerase} \\
\text{YgfG} & \quad \text{Decarboxylation from methylmalonyl-CoA to propionyl-CoA} \\
\text{YgfH} & \quad \text{Catalyzes a COA-transfer reaction} \\
\text{YgfD} & \quad \text{Indispensable in the pathway and have two possible functions}
\end{align*}
\]

Plasmids Construction

- Insert four genes into pET-28a(+) plasmids
- Transform them into BL21 E.coli strains
Protein Expression
Successfully validate the expression of sbm, ygfD, ygfG, and ygfH.

<table>
<thead>
<tr>
<th>(KDa)</th>
<th>Marker</th>
<th>sbm</th>
<th>ygfD</th>
<th>ygfG</th>
<th>ygfH</th>
<th>Control</th>
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<tbody>
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Production Increaseement

Propionate's output can be increased over **10%** with ygfD

HPLC analysis of wild-type BL21 and recombinant BL21 with four genes (4hrs after IPTG induction)

<table>
<thead>
<tr>
<th>Concentration of propionate (mg/L)</th>
<th>Control</th>
<th>ygfH</th>
<th>ygfG</th>
<th>sbm</th>
<th>ygfD</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>22787.7</td>
<td>23810.4</td>
<td>24051.3</td>
<td>24986.3</td>
<td>25765.1</td>
</tr>
<tr>
<td>Increased (%)</td>
<td>n.s.</td>
<td>4.49%</td>
<td>9.41%</td>
<td>9.65%</td>
<td>13.07%</td>
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**P<0.01**
Question: How can we make ygfD expressed periodically?
Our **Progresses**

### Oscillator
- Plasmids
- Modeling
- Results

### Propionate
- Methods
- Results

### Other
- Future plan
- Human Practice
Hybrid Promoter

Activation from araBAD promoter

Repression from lacZYA promoter

+Arabinose
+AraC

Hybrid Promoter

-IPTG
+LacI
Oscillator

+Arabinose  +IPTG

Hybrid Promoter  araC

Hybrid Promoter  lacI
Issues

Can the oscillator be applicable?

- The single-cell level
- The multi-cell level

How can we adjust its period?

- Arabinose, IPTG, and delay($\tau$)
- Determine the key parameter
The Single-Cell Level

Delay Differential Equations (DDEs)

AraC’s oscillation, $\tau = 30\text{min}$, $T = 11\text{ hours}$

$\tau \sim N(30.0, 1.0^2)\text{min}$

$T_1 = 644.8\text{min}$, $T_2 = 639.2\text{min}$, $T_3 = 654.1\text{min}$, $T_4 = 642.6\text{min}$, $T_5 = 645.1\text{min}$

Random Period
The Multi-Cell Level

A group of genetic oscillating cells can oscillate as well as a single one does.
Adjusting Period

The period range is limited to 44min – 50min when altering Arabinose and IPTG, with $\tau = 2$ min.

Not Satisfying
Adjusted Period

Period increases linearly and unlimitedly when delay $\tau$ increases.
Achieve rapid protein degradation

Oscillator Construction

+Arabinose +IPTG

Hybrid Promoter

araC

LAA

mRFP

LAA

Reporter

pACYCDuet-1

Hybrid Promoter

lacI

LAA

pET-28a(+)

pET-28a(+)

LAA
Validation of Oscillator

Validation of constructed pET-28a(+) Immobilized cell with oscillator expresses RFP periodically
Validation of Oscillator

Fluorescence density of multi-cells changes in an oscillating way.
Wet-Lab & Modeling

Wet-lab work and modeling results consistent with each other well.
Our Progresses

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Future Plan

Circadian release of high-level propionate

Replace mRFP with ygfD
Future Plan

- Regulating the period of propionate release under the direction of modeling
- Mathematically simulate the “in vivo” environment
Future Plan

Transfer the engineered machine into Bifidobacterium
Human Practice

A Lecture In The High School

Collaborations

Crosswords Game
Human Practice

A Talk to The Public

Questionnaire
Accomplishments

I. Subcloned sbm, ygfG, ygfH and ygfD, constructed and documented the expression vectors of the four genes

II. Constructed the oscillator with mRFP and LAA tags

III. Found the gene that can realize the highest production of propionate

IV. Achieved the periodical fluorescence intensity simulated working process of the oscillator

V. Analyzed the sensitivity and the robustness of the oscillator, providing ways to adjust for a wanted period

VI. Verified that oscillators in multi cells can oscillate as well as in a single one

VII. A new application in hypertension treatment

VIII. Shared information with WHU & HZAU, and help HZAU complete a biobrick
Acknowledgement

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Jeff Hasty  Roya Mahmoudi
We are HUST-China
Circadian Release of Propionate for Antihypertensive Therapy

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