Circadian Release of Propionate for Antihypertensive Therapy
Background

Main complications of persistent High blood pressure

- Brain:
  - Cerebrovascular accident (strokes)
  - Hypertensive encephalopathy:
    - confusion
    - headache
    - convulsion

- Blood:
  - Elevated sugar levels

- Kidneys:
  - Hypertensive nephropathy
  - Chronic renal failure

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

- Retina of eye:
  - Hypertensive retinopathy

- 2010

WHO, Geneva 1996

Hypertension

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

- Systolic BP
- Diastolic BP

Blood pressure (mm/Hg)

- 18:00
- 22:00
- 02:00
- 06:00
- 10:00
- 14:00
- 18:00

Key: BP = blood pressure

High blood pressure, a major risk factor globally

Almost 1 billion people worldwide have high blood pressure, a recent report found.

Source: High Blood Pressure and Health Policy, 2008
Traditional Treatment


eg: Reserpine

Losartan

Morning surge

Novel Method!

Propionate

Engineered Bacteria
Propionate

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

A Novel Method!

Propionate

Oscillator

Engineered Machine
Our Progresses

- Propionate
  - Methods
  - Results

- Oscillator
  - Plasmids
  - Modeling
  - Results

- Others
  - Future Plan
  - Human Practice
Background

Wood-Werkman Reaction

Sbm
Methylmalonyl-CoA epimerase

YgfG
Decarboxylation from methylmalonyl-CoA to propionyl-CoA

YgfH
Catalyzes a CoA-transfer reaction

YgfD
Indispensable in the pathway and have two possible functions

Plasmids Construction

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

HPLC Analysis of Wild-type BL21 and Recombination BL21 With Four Genes (4h After IPTG Induction)

Propionate's output can be increased over 10% with ygfD
How can we make ygfD expressed periodically?
Our Progresses

- **Propionate**
  - Methods
  - Results

- **Oscillator**
  - Plasmids
  - Modeling
  - Results

- **Others**
  - Future Plan
  - Human Practice
Hybrid Promoter

- +Arabinose
- +AraC

Hybrid Promoter

+ Arabinose
+ AraC

Activation from araBAD promoter

Repression from lacZYA promoter

-IPTG
+ LacI
Oscillator

+Arabinose  +IPTG

Hybrid Promoter  araC

Hybrid Promoter  lacI

-IPTG
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, $\tau = 30\text{min}$, $T = 11\text{ hours}$

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

$T_1 = 644.8\text{min}, T_2 = 639.15\text{min}, T_3 = 654.05\text{min}, T_4 = 642.6\text{min}, T = 645.05\text{min}$

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 $\tau = 2\text{ min}$. 

Not Satisfying
Period increases linearly and unlimitedly when delay $\tau$ increases.
Oscillator Construction

+Arabinose  +IPTG

Hybrid Promoter

aroC

lact

mRFP

LAA

pACYCDuet-1

pET-28a(+)

Achieve rapid protein degradation
Genetic Oscillator

Immobilized cell with oscillator expresses RFP periodically
Current Progresses

- Propionate
  - Methods
  - Results

- Oscillator
  - Plasmids
  - Modeling
  - Results

- Others
  - Future Plan
  - Human Practice
Future Plan

Replace mRFP with ygfD

Circadian release of high-level propionate
Future Plan

- Regulating the cycle period of propionate
- Mathematically simulate the “in vivo” environment
Future Plan

➢ *Transfer the engineered machine into Bifidobacterium*
Human Practice

Public Awareness

Corporation and Share

Crosswords Quiz
Accomplishments

- Constructed and documented the expression vectors of sbm, ygfG, ygfH and ygfD
- Constructed the oscillator with mRFP and LAA tags
- Found the gene that can realize the highest production of propionate
- Achieved the periodical fluorescence intensity simulated working process of the oscillator
- Analyzed the sensitivity and robustness of the oscillator, providing ways to adjust for a wanted period
- Verified that oscillators in multi cells can oscillate as well as in a single one
- A new application in hypertension treatment
- Shared information with WHU & HZAU, and help HZAU complete a biobrick
Acknowledgement

尚广东    Jingnan Lu
Jeff Hasty  Roya Mahmoudi
We are HUST-China
Q & A