Operation Echo

Brought to you by Tsinghua-A

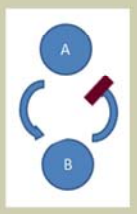
Everything you always wanted to know about Operation ECHO

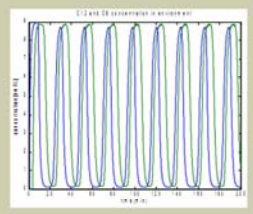
Summary

Our goal is to construct a biological oscillator, which we call 'ECHO', the abbreviation of "The E.Coli Homochronous Oscillator".

To manage this, we apply the Predator-Prey Model.

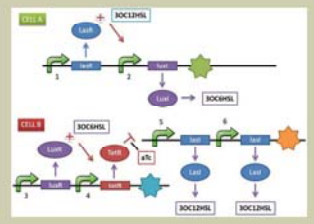
With two Escherichia coli populations expressing gene one after another, they give red and green fluorescent light alternately. E.coli populations communicate bi-directionally by a class of signaling molecules involves in bacteria quorum sensing, that is, N-Acyl homoserine lactones (AHL), to regulate the gene expression of each other.





More Details

A induces B, B represses B2, and B2 induces A, forming a feedback network with a delay part. Corresponding to the network is the three lines of gene, based on the interactions of C6/Lux, C12/Las and TetR/aTc.

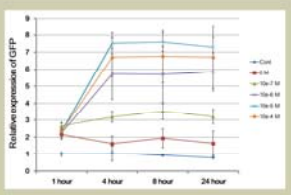


BioBrick Mechanism

Two E.coli populations (CELL-A & B) regulate each other to realize oscillation under the QS modules, LuxI/LuxR together with LasI/LasR. The constitutive promoter p1 in CELL-A produces LasR, a transcriptional regulator, who maintains its high concentration within the cell.

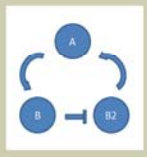
Then, an acyl-homoserine latone (AHL), 3OC12HSL from LasI in CELL-B will bind with LasR, lighting the green fluorescence and inducing the expression of LuxI, who synthesize 3OC6HSL. C6 accumulates in the culture and permeates into CELL-B, where it binds with LuxR. This process activates the expression of TetR and CFP, and therefore, represses LasI to produce C12.

GFP Results



Improved

In our expectation, ECHO is not just a single oscillator. Three parameters that determine the property of an oscillation is the amplitude, the period and the phase, and we hope to maneuver each of the three independently.



Modelling

Simplified DDE Model

First we describeb the system thoroughly without leaving out any seemingly unimportant actions and factors. We listed all 19 ODEs in Our Team Wiki.(contains every possible mass actions as hill kinetics, Henri-Michaelis-Menten kinetics and etc.)

The follow DDEs are essential enough for the simplified version of our Model.

$$\frac{dAL_1(t)}{dt} = k_0 \cdot AL_{1,0} + \gamma \cdot (AL_2 - AL_{1,0})$$

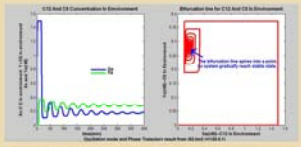
$$\frac{dAL_2(t)}{dt} = k_{1,0} \cdot \frac{AL_1(t-\tau_1)^{n_1}}{\left(\frac{K_{M1}}{A_{1,0}}\right)^{n_1} + AL_1(t-\tau_1)^{n_1}} + \gamma \cdot (AL_1 - AL_{2,0}) - k_2 \cdot AL_{2,0}$$

$$\frac{dAL_3(t)}{dt} = k_{2,0} \cdot \frac{\left(\frac{K_{M2}}{A_{2,0}}\right)^{n_2}}{\left(\frac{K_{M2}}{A_{2,0}}\right)^{n_2} + AL_2(t-\tau_2)^{n_2}} + \gamma \cdot (AL_2 - AL_{3,0}) - k_3 \cdot AL_{3,0} \quad \text{with } (t > \tau_1) \cap (t < \tau_2)$$

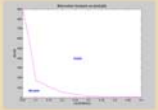
$$\frac{dAL_4(t)}{dt} = -k_4 \cdot AL_{4,0} + \gamma \cdot (AL_3 - AL_{4,0})$$

$$\frac{dAL_1}{dt} = -\alpha AL_1 - \gamma \cdot \frac{P \cdot n_{1,0}}{1 - p \cdot (1 + n_{1,0})} \cdot (AL_1 - AL_{4,\tau}) + \gamma \cdot \frac{P}{1 - p \cdot (1 + n_{1,0})} \cdot (AL_1 - AL_{2,0})$$

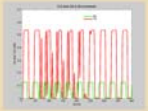
$$\frac{dAL_2}{dt} = -\alpha AL_2 - \gamma \cdot \frac{P \cdot n_{2,0}}{1 - p \cdot (1 + n_{2,0})} \cdot (AL_2 - AL_{1,\tau}) + \gamma \cdot \frac{P}{1 - p \cdot (1 + n_{2,0})} \cdot (AL_2 - AL_{3,0})$$



We did bifurcation analysis on the Hill parameters. What we had to do was find the critical points where the system can nearly oscillate but a little disruption may lead to a steady state.



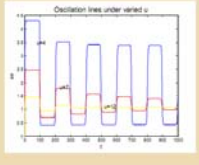
Depicting all those critical points, as shown in the figure, the system could oscillate when cellB's Hill parameters were located in the area named 'Bistable'.



The oscillation phase was adjusted by adding araC, which could induce the pBad promoter, in cell type B. After adding araC to our system at certain time, the oscillation was interrupted and the phase turned out to be changed.

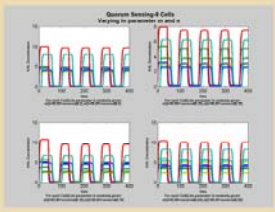
Dimensionless

In order to make a further analysis on stability of the system, and sensitivity of parameters, we further simplified the model to make them dimensionless. In addition, we tried to introduce feedback to our system and made a brief analysis on different types of feedback we introduced.



Quorum Sensing

Quorum-sensing oscillator is not simply a matter of expansion in magnitude, but a matter of robustness in allowing difference of each individual cell. Moreover, we test the adjustment of phase and period of oscillation in this part.



Human Practice

Background

The ideas of electronic circuit based design have long been a part of the fundamental research since the very beginning of synthetic biology. However, as the scale of designs becomes larger and larger, researchers start to find it difficult to adapt those ideas of circuits to the biological system, in which two main properties bottleneck the realization of these ideas.

Bottom-up Method

In modern electronics, people rarely focus on the detail of a particular component when designing systems, for these single components have already been well-characterized and standardized. All that requires is innovating rather than just repeating. When it comes to biology, we are surprised to find that some basic parts, such as AND gate, register and toggle switch, have been applied to genetic machines. While, there is still a far way to go.

Applications

CALCULATOR

TAKING MEDICINE

INSOMNIA CURING

Team

Advisors

Michael Q. Zhang

Instructors

Xiaowo Wang
Guoqiang Chen
Yang Chen
Xianshuang Yang

Students

Junyu Chen
Qi Sang
Silu Yang
Lei Wei
Weixi Liao
Pei Xie
Jian Wen
Zhirong Wu

Contact us:

Lei Wei

Dept. of Automation,Tsinghua Univ.

Beijing,PRChina. 100084

cubicstonewei@gmail.com