Rainbofilm

A stratified biofilm expression system
Module extensible for bioreactor and biosensor

Overview

Our project is a novel expression system for synthetic biology. We used sensitive promoters to artificially induce differentiated expressions through the spatial distribution of biofilm. We proved that this system works by producing Rainbofilm, where different fluorescence proteins are expressed at different depth of the biofilm.

In application, biofilm forms spontaneously and has the natural resistance to high levels of toxin. These two properties render it a competitive platform of bioreactor and bio-sensor. The system can cater to different needs simply by changing its downstream genes, resulting in our highly extensible module X-film.

One possible application is Sugarfilm, in which multi-step reactions are carried out in different parts of the biofilm. The cellulose is degraded to monose from the bottom to the middle layer, and the butanol is produced in the surface layer to minimize the toxicity to inner cells. Our Rainbofilm system can also be transformed into Gluefilm to produce magic glue. We further utilized the behavior of biofilm in bio-detection to produce Sensorkin. The natural chemical gradient and high tolerance of biofilm can generate a new generation of biosensors that are more robust and quantitative in real practice.

Our project carries synthetic biology to a "tissue" level using a natural structure. We hope that biofilm can reduce stress to genetic engineered organisms by differentiated expression, protect them from toxicity by limiting contact with toxic substances, and increase efficiency by increasing protein concentration.

Moreover, we have done great work on human practice such as a funding guide, an iPhon, a novel, etc. The paper in part, we developed two helpful softwares.

Rainbofilm

So far synthetic biologists have made tremendous effort in and below the cell level. However, engineering work done in higher levels remains rare. This year, ZJU-China has been focusing on the tissue level; we want to make cells differentiated within a natural bacteria 'tissue'.

The left is the genetic circuit of our design. CFP is constitutively expressed when there is no tetR. YFP is expressed in micro-aerobic environment, and RFP is expressed in anaerobic environment. TetR is produced in both micro aerobic and anaerobic have enough oxygen, CFP is repressed.

For Rainbofilm modeling, we first built a model of the genetic circuit, then used results from parts characterization for parameter estimation. The right shows the relationship between three fluorescence proteins and partial pressure of oxygen. As oxygen level increases, RFP, YFP, and CFP express one another. We transform the different combination of three devices into E.coli.

Xfilm

Sugarfilm

Cellulose utilization is one of the hot approaches of bio-fuel production. Its big obstacle is the slow enzymatic break-down process from cellulose. We believe the employment of biofilm and its stratified feature would help to accelerate the degradation by the increased density of substrates and enzymes.

Sugarfilm, a three-layer biofilm-based system, is designed to directly attach to substrate. In this way, the distance between our "degradation engine" (E.coli cells) and the targeted substrate would be reduced greatly, leading to a more efficient system.

The MatLAB modeling represents the behavior of the sugarfilm system. We use a similar model with Team Edinburgh, but the degradation process in our model is separated into different layers. When the parameter from their modeling is adopted, the result of our sugarfilm system is similar with their scaffold system.

Gluefilm

With biofilm stratification, glue production can be limited to anaerobic layer. We can use Mps BioBrick made by the Berkeley 2009 iGEM team to produce glue.

In the microaerobic environment and oxygen rich environment no reaction occurs. As a result, the surface of gluefilm is not sticky. When a suitable surface is placed on the biofilm, oxygen penetration is limited and thus all the e.coli will produce glue and the two surface will be stuck together.

Sensorkin

Our biofilm system has great future prospects in the field of biosensing. The chemical gradient automatically formed throughout biofilm subjects each layer to different concentrations. At the same time, the biofilm can make cells more resistant to unfavorable growth conditions. These two properties give rise to the design of two biosensors, enabling quantification in lab and higher tolerance in field detection.

The first device is designed for quantification of Mercury/Lead in lab. A two layer stratification pattern is formed once detectable target chemical level occurs in the environment. By calculating the ratio of each layer's depth under confocal microscopy and fitting into a mathematical model, we could obtain the quantified concentration level. The second design uses quorum sensing to amplify and produce easily detectable signal across biofilm, making our biosensor more tolerant to unfavorable working conditions and of better performance in field practice.

Biofilm

Biofilm is a natural multi-cellular bacteria community best known for its resistance to environmental stress. We intend to utilize and modify it to develop a stratified protein expression system using the naturally formed oxygen gradient.

Our mathematical modeling calculates the biofilm growth. This helps us to simulate the oxygen gradient. We take in consideration of the oxygen diffusion and consumption. Although not exactly identical, these results are very similar to those of real lab results.

In many Gram-negative bacteria, quorum-sensing systems respond to N-acyl homoserine lactones (AHLs), which were proved to be essential for the architecture of biofilm. In addition, the universal quorum sensing signaling molecule autoinducer 2 (AI-2) can increase E.Coli’s biofilm biomass 30-folds. Thus we co-transformed our device along AI-2 producing devices under the control of pIac promoter, which could make biofilm grow thicker, which also could be controlled by the lac repressible promoter. A thicker biofilm make a more distinct oxygen gradient and further facilitate a more complicated stratification.

Tools

RainboSim

Our new software tool RainboSim helps to design and model and share all kinds of biofilms. Users can freely set their own promoter (sensing signal and coding sequence (function)) combinations, and then get a reasonable prediction of the biofilm behavior based on the model we built. The tool is highly flexible and promotes sharing of knowledge. Users can choose the current system we provide, update the system by changing parameter values or even add new design and data into our biofilm structure. Our RainboSim not only serves as an introduction and guide to engineering biofilm, but also exemplifies the extensibility of our Rainbofilm system.

Character

We also developed a MATLAB and Excel for parts characterization data analysis. One of the characterization methods is to use fluorescence proteins in the expression cassettes and measure culture’s optical density and fluorescence intensity in a time series. Our tool can automatically compute the transcription rate (PoPS) and produce four plots to visualize OD and fluorescence trends and also the relationship between OD and fluorescence.

We are hoping this could help to make characterization more standard and easier for people in a similar work in the future.