iGEM 2010 Presentation

University of St Andrews iGEM 2010 Team

November 6, 2010
Team

- 9 Undergraduates:
  - Biologists, Medics, Physicists, a Computer Scientist and a Chemist

- 5 Advisors:
  - 2 Postgraduates
  - 3 Faculty
Our Project: Cholera and Quorum Sensing
Outline

Cholera

Biology
  Introduction
  CqsA
  Bistable Switch

Modelling
  Introduction
  Models
  Parameter Testing

Characterisation

Human Practices
  Method
  Results
Cholera and Quorum Sensing

- **Vibrio cholerae**
  - Acute infection which is often fatal
  - Leads to dehydration and loss of electrolyte balance
  - Transmitted through contaminated food and water
  - Outbreaks often follow natural disasters striking vulnerable communities
Low Cell Density

Gut Lining

When V. cholerae populations are low the concentration of CAI-1 is also low. Genes are transcribed which promote attachment to the gut lining and the production of cholera toxin. Cholerae reproduce...
High Cell Density

Gut Lining

V. cholera population increases over a short period of time. Now, enough cholera toxin is being produced to overwhelm the host. Water loss occurs, increasing the gut flow.

CAI-1 is abundant and causes a change in gene transcription which favours detachment from the lining and escape from the dying host.
Hence...

If we can trick *V. cholerae* into thinking it is at high cell density it will become avirulent and safe
Expressing CqsA in *E. coli*

The Cholera Autoinducer: CAI-1
New Functionality From Existing Parts
Re-Engineering the Lux Operon

- Well understood quorum sensing system in *Vibrio fischeri*
- In the registry and commonly used by iGEM teams
Wild-Type Expression Pattern
Bistable Switch

Natural Operon

Bistable Operon

LuxR also now downstream of this promoter
Bistable Expression Pattern
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Model Design

- Based on ODEs
- Solved computationally using RK4
- Models written in C++
- Custom written solvers and models
Modelling Goals

- To create a mathematical representation of the *E.coli* Lux quorum sensing system
- To understand the bistable switch mechanism of our synthetic *E.coli*
- To predict how the CAI-1 production mechanism will be activated and deactivated under the control of the bistable switch
LuxR Quorum Sensing Circuit
Our Model

- Cell growth phase: doubling time of 20 minutes
- Cell death phase: halving time of 20 minutes
- Diffusion of HSL throughout the system is an instantaneous process
- HSL diffuses evenly across the entire system
GFP: High/Low Cell Density
Parameter Testing
What Parameters Affect Bistability?

- Association and dissociation
- HSL production and degradation
- LuxR degradation
- Promoter binding site strengths
Measuring Bistability

Bistability in the LuxR Quorum Sensing System

![Graph showing bistability with cell density on the x-axis and GFP concentration on the y-axis.](image)
Investigate $\Delta$ Cell density varying one parameter

Investigate $\Delta$ Cell density varying the other parameter
Tuning Our Switch

In the lab we can control:

- Promoter Strength (PoPs)
- Ribosome Binding Site Efficient

Use these parameters to control the operating point of our switch
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Ribosome Binding Site

- Sequence on mRNA
- Required in bacteria for translation
Determining RBS Efficiency

- Translation initiation determines rate of protein translation.
- Translation initiation rate depends on:
  - Downstream of RBS
  - Sequence of RBS
  - Upstream of RBS
Determining the Optimal RBS

The thermodynamic model quantifies the strength of the molecular interaction between an mRNA and the 30 S ribosome complex by Gibbs free energy\(^1\).

- RBS calculator uses this thermodynamic model ([http://voigtlab.ucsf.edu/software/](http://voigtlab.ucsf.edu/software/)).

RBS in vitro

- Ordered the sequence as primers
- Ligated them into I13401
- Measured the level of GFP expression by flow cytometry

![Graph showing average fluorescence per cell for different Biobrick variants]
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Human Practices

Introduction

Two views of human practices:

**Local** Data collected in sunny St Andrews
- Not everyone has an Internet connection
- Gets data from those not involved in the subject at hand
- Different groups of people than trend setters

**Global** Data collected from the World Wide Web
- Poll thousands of users easily
- Collect repeat data quickly
- Huge repository of data: Facebook, Twitter, Blogs, News etc
A new discovery in synthetic biology….
New field of synthetic biology requires regulation…..
Disaster ahead with synthetic biology …
Synthetic biology brings hope to millions….
Popularity of Synthetic Biology

Local

Synthetic changes should not be made to any organism / Organisms should not be genetically modified.
Popularity of Synthetic Biology

Global

Change of Opinion Over Time

- synthetic biology
- Genetic Engineering

Week
Opinion
1 2 3 4 5 6 7 8 9 10
0
10
20
30
40
50
60
70
80
Popularity of Sciences

Global

Change of Opinion Over Time
from web data

Week

Opinion

chemistry
biology
physics
computer science
mathematics
synthetic biology
Amount of Discussion of Sciences

Global

Amount of Web Content Over Time

- Chemistry
- Biology
- Physics
- Computer Science
- Mathematics
- Synthetic Biology

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iGEM 2010 Presentation
Popularity of Different Subjects
Conclusions

- Constructed a biobrick to generate the cholera autoinducer CAI-1
- Created a system of equations to describe the Lux quorum sensing network
- Predicted the RBS strength by the RBS calculator program and measured it in vitro
- Introduced two new RBS sequences with measured strength
- Introduced new approaches to human practices were introduced with the automated collection of large amounts of readily available web data pertaining to current opinion and trend
Sponsors

Takara
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Life Sciences
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Dr Elaine Campbell for helping us with the flow cytometry

The St Andrews Ice Cream Van which drives past the lab