WELCOME ALL
OUR TEAM.

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Content

- Project idea
- The Model
  - Defining the Model
  - Simulating the Model
- Characterisation of the system
- Implementing the Project Objective
- Purpose of Project
- Acknowledgement and References
Project idea

Engineering E.coli strain to be responsive to multiple wavelength.
The Initial system

Red light - change state of phytochrome

Sensing

Photoreceptor

EnvZ-OmpR 2 component regulatory system

Autophosphorylation

Transcription and translation of OmpC promoter gene and LacZ reporter gene, which promotes the activity of LacZ

OUTPUT

LESS BLACK PRECIPITATE
Characterising the system

Firstly, varying wavelengths:

- Dark
- Blue
- Green
- Red
- Control
- Control
- Control
- Control

Wavelength (nm)
Findings from varying the wavelength

- Sample in the dark produced most precipitate.
- Gradual decrease of precipitate production as input wavelength increases.
- All control strain has similar output.
- Gene expression under red light is most inhibited.

<table>
<thead>
<tr>
<th>Time</th>
<th>0</th>
<th>3hr</th>
<th>6hr</th>
<th>9hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td><img src="image1.png" alt="Images" /></td>
<td><img src="image2.png" alt="Images" /></td>
<td><img src="image3.png" alt="Images" /></td>
<td><img src="image4.png" alt="Images" /></td>
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<tr>
<td>Black Control</td>
<td><img src="image5.png" alt="Images" /></td>
<td><img src="image6.png" alt="Images" /></td>
<td><img src="image7.png" alt="Images" /></td>
<td><img src="image8.png" alt="Images" /></td>
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<tr>
<td>Blue</td>
<td><img src="image9.png" alt="Images" /></td>
<td><img src="image10.png" alt="Images" /></td>
<td><img src="image11.png" alt="Images" /></td>
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<td>Blue Control</td>
<td><img src="image13.png" alt="Images" /></td>
<td><img src="image14.png" alt="Images" /></td>
<td><img src="image15.png" alt="Images" /></td>
<td><img src="image16.png" alt="Images" /></td>
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<td>Green</td>
<td><img src="image17.png" alt="Images" /></td>
<td><img src="image18.png" alt="Images" /></td>
<td><img src="image19.png" alt="Images" /></td>
<td><img src="image20.png" alt="Images" /></td>
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<tr>
<td>Green Control</td>
<td><img src="image21.png" alt="Images" /></td>
<td><img src="image22.png" alt="Images" /></td>
<td><img src="image23.png" alt="Images" /></td>
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<tr>
<td>Red</td>
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<td><img src="image26.png" alt="Images" /></td>
<td><img src="image27.png" alt="Images" /></td>
<td><img src="image28.png" alt="Images" /></td>
</tr>
<tr>
<td>Red Control</td>
<td><img src="image29.png" alt="Images" /></td>
<td><img src="image30.png" alt="Images" /></td>
<td><img src="image31.png" alt="Images" /></td>
<td><img src="image32.png" alt="Images" /></td>
</tr>
</tbody>
</table>
Secondly, varying light intensity

- The samples and controls are stored in LB Broth.
- At different intensities, measured by a light intensity probe.
- Exposed in red light and incubated for 12hrs.
- Perform Miller Assay – to quantify the activity of beta-galactocidase enzyme.
- The photospectrometer used to measure the optical density of each sample.
- Parameters collected.
THE MODEL
A QUICK FLASH BACK

The Initial system

Photoreceptor

EnvZ-OmpR 2 component regulatory system

Light

Sensing

Control autophosphorylation

Transcription and translation of OmpC promoter gene and LacZ reporter gene, which promotes the activity of LacZ.

LESS BLACK PRECIPITATE

OUTPUT
TRANSFORMING THE FLOW DIAGRAM TO A CONTROL BLOCK GIVES

Output

Light

Regulator

EnvZ - OmpR system

Enzymatic reaction (Transcription and Translation)

Black precipitate (Activity of LacZ)

Sensor

Photoreceptor

External parameter
INDIVIDUAL INSIGHTS TO EACH BLOCKS THE 2 COMPONENT REGULATORY

EnvZ – OmpR

OmpR
The 3 main processes can be simplified as:

1. \( \text{EnvZ} + \text{ATP} \rightarrow \text{EnvZ} - \text{P} + \text{ADP} \) - autophosphorylation
2. \( \text{EnvZ} + \text{OmpR} - \text{P} \rightarrow \text{EnvZ} + \text{OmpR} + \text{Pi} \) - 3-phosphatase
3. \( \text{EnvZ} - \text{P} + \text{OmpR} \rightarrow \text{EnvZ} + \text{OmpR} - \text{P} \) - phosphotransfer

Assumptions behind the model:

- ATP concentration is constant and absorbed into the rate constant \( k_k \).
- Concentration of \( \text{EnvZ} \) is divided into: \( \text{EnvZ} \), \( \text{EnvZ} - \text{P} \), \( \text{EnvZ} - \text{P}/\text{OmpR} \), and \( \text{EnvZ} - \text{P}/\text{OmpR} \).
- Concentration of \( \text{OmpR} \) is divided into: \( \text{OmpR} \), \( \text{OmpR} - \text{P} \), \( \text{EnvZ} - \text{P}/\text{OmpR} \), and \( \text{EnvZ} - \text{P}/\text{OmpR} \).
- Total concentration of \( \text{OmpR} \) and \( \text{EnvZ} \) is constant; therefore the cycle goes on and on.

ODE's derived:

\[
\begin{align*}
\frac{d[(\text{EnvZP})\text{OmpR}]}{dt} &= k_1[\text{EnvZP}][\text{OmpR}] - (k_{-1} + k_t)[(\text{EnvZP})\text{OmpR}] \quad [1] \\
\frac{d[(\text{EnvZ})\text{OmpRP}]}{dt} &= -(k_p + k_{-2})[(\text{EnvZ})\text{OmpRP}] + k_2[\text{EnvZ}][\text{OmpRP}] \quad [2] \\
\frac{d[\text{EnvZ}]}{dt} &= k_{-k}[\text{EnvZ}] \quad [3] \\
\frac{d[\text{EnvZP}]}{dt} &= k_k[\text{EnvZ}] - k_{-k}[\text{EnvZP}] + k_{-1}[(\text{EnvZP})\text{OmpR}] - k_1[\text{EnvZP}][\text{OmpR}] \quad [4] \\
\frac{d[\text{OmpR}]}{dt} &= k_{-1}[(\text{EnvZP})\text{OmpR}] - k_1[\text{EnvZP}][\text{OmpR}] + k_p[(\text{EnvZ})\text{OmpRP}] \quad [5] \\
\frac{d[\text{OmpRP}]}{dt} &= k_t[(\text{EnvZP})\text{OmpR}] + k_{-2}[(\text{EnvZ})\text{OmpRP}] - k_2[\text{EnvZ}][\text{OmpRP}] \quad [6]
\end{align*}
\]

Eric Batchelor and Mark Goulian. Robustness and the cycle of phosphorylation and dephosphorylation in a two-component regulatory system. PNAS January 21, 2003 vol. 100 no. 2 691-696.
Transcription state;

\[ \frac{d[mRNA]}{dt} = t_1[\text{OmpR-P}] - d_1[mRNA] \]

- \( t_1 \) – transcription coefficient
- \( d_1 \) – transcription degradation / decay rate

Translation state;

\[ \frac{d[\text{LacZ}]}{dt} = t_2[mRNA] - d_2[\text{LacZ}] \]

- \( t_2 \) – translation coefficient
- \( d_2 \) – translation degradation / decay rate

\( d_1 << d_2 \), Because protein is much more stable than Lac Z
What’s the role of the photoreceptor?

• It is known and verified from the wet labs that red light inhibits the process of autophosphorylation.

It is known and verified from the wet labs that red light inhibits the process of autophosphorylation.
SIMULATING THE MODEL
Constant and parameters used;

\[
\begin{align*}
k1 &= 0.01, \\
k-1 &= 0.01, \\
k2 &= 0.01, \\
k-2 &= 0.01, \\
kp &= 0.01, \\
kt &= 0.01, \\
t1 &= 0.1, \\
t2 &= 0.1, \\
d1 &= 0.01, \\
d2 &= 1.0 \\
\end{align*}
\]

\[
\begin{align*}
\text{EnvZ} &= 1\text{M}, \\
\text{EnvZP} &= 1\text{M}, \\
(\text{EnvZP})\text{OmpR} &= 1\text{M}, \\
\text{OmpRP} &= 1\text{M}, \\
\text{OmpR} &= 1\text{M}, \\
\text{EnvZ(}\text{OmpRP}\text{)} &= 1\text{M}. \\
\end{align*}
\]

• What do we do with constants Kk and K-k? Which are varied by the intensity of red light shined on it.
We decided to vary the constants with intensity as shown below;

- all ODE’s tends to a steady state after some time
- Without the presence of red light, the concentration of OmpRP should increase. Therefore the constant Kk which affects the rate of reaction should decrease with intensity. Whereas K-k should increase as intensity increases.
From the graphs above it is clear that at high intensity OmpRP concentration is less, therefore activity of LacZ should be less as well.
Characterisation of the initial system

- **3hrs**: The system is very unstable, the beginning of translation and transcription of gene, LacZ activity is erratic, does not form a trend proportional to intensity over the first 3 hours.
- **6hrs**: The system is still unstable, but relatively calmer.
- **9hrs**: The system is more similar to expected trend.
- **12hrs**: The system behavior as expected in the paper, LacZ activity decreases as light intensity increase.

- The control strain has produced a similar trend to the experimental values.
• Using system identification procedures, a prediction for a design process could be made; either intensity or time dependent.
Implementing the project objective

Initial system

LacZ activity
**INTENTION**: taking this as an example

<table>
<thead>
<tr>
<th>Colour of fluorescent protein</th>
<th>mRFP1</th>
<th>EGFP</th>
<th>ECFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation wavelength</td>
<td>584 nm</td>
<td>488 nm</td>
<td>434 nm</td>
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</table>

protein to LacZ
<table>
<thead>
<tr>
<th>INITIAL SYSTEM</th>
<th>FP</th>
<th>OUTPUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Final System

The initial phytochrome

BioBrick representation

ho1 BBa_I15008

pcyA BBa_I15009

Cph8 BBa_I15010

LacZ

FP
Barriers we have to overcome to achieve our main objective:

1. Choice of FP.

2. How to fuse the chosen FP to LacZ.
Purpose of the project

These are the works of the authors of ‘Engineering E.coli to see light’

1. We hope that our system can produce multi-colour images.

2. Biosensor for multiple wavelengths
REFERENCES:

1. Levskaya et al. Engineering Escherichia coli to see light Nature 24 November 2005 DOI:10.1038/nature04405 A.

2. Eric Batchelor and Mark Goulian. Robustness and the cycle of phosphorylation and dephosphorylation in a two-component regulatory system PNAS January 21, 2003 vol. 100 no. 2 691-696.
Acknowledgement

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