Paint By COLI

Abstract

Our project aims to introduce a triplet of NOT gates in *E. coli* to create a system which is finely controlled by exposure to light. This would be demonstrated using three pigments – cyan, magenta and yellow. Projecting an image onto a lawn of our modified bacteria would allow exact replication of the image in full colour, showcasing the versatility of utilising optical controls in a synthesis pathway. Our project is higly adaptable as it allows future iGEM teams to insert a gene of their choice into the system in place of the pigments, using the restriction enzymes *Bgl*II and *Bam*HI.

Light, pigments and our logic gates

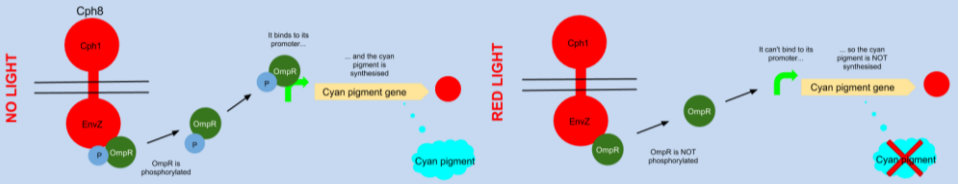
Exposing the *E. coli* to red, blue or green light at sufficient levels will halt the synthesis of cyan, yellow or magenta pigments respectively.

Constructs

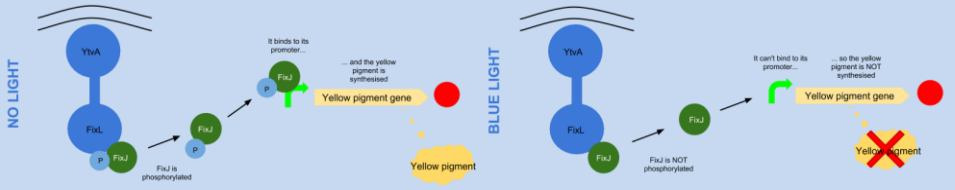
Red Construct

Blue Construct

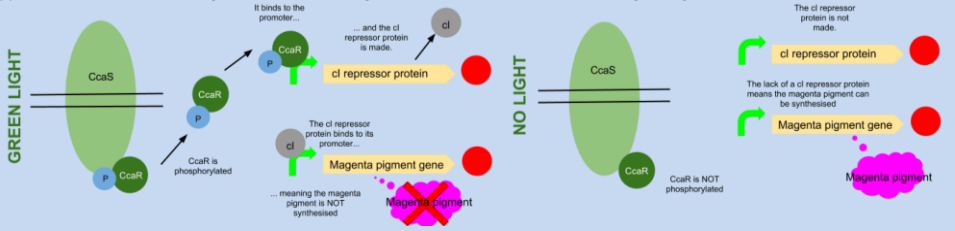
Green Construct



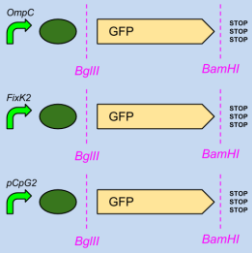
This diagram shows the proteins and pathways coded for by the Red Light Module. Absence of red light allows phosphorylation of an intermediate protein, which binds to a specific promoter, allowing transcription of the cyan pigment. Exposure to red light prevents the phosphorylation of the intermediate protein, so the cyan pigment gene is not transcribed. We built this Module using standard 3A Assembly



The Blue Light Module works in a very similar way, however the light sensing protein is an internal protein, rather than transmembrane. The Module was designed and ordered through DNA2.0. The Green Light Module required an inverter system, as exposure to green light allowed the phosphorylation of the intermediate protein; the opposite of the Red and Blue Light Modules. We designed the Module and ordered it through IDT gBlocks.



BglII and *BamHI*



The *Bgl*II and *Bam*HI cut sites within the output bricks allow "edits" of the bricks outside of the iGEM standard 3A Assembly.

Future Applications

- High resolution images
- Pigments don't necessarily have to be the output – *Bgl*II and *Bam*HI restriction sites allow insertion of any gene
- Applications in a vast array of fields – medical, industrial, experimental, even artistic
- Possibility of use in light targeted therapies
- Potential use of lasers for high spatial control
- Theoretically, light would be the only input (simple, cheap, effective)

Our model describes the expression of light controlled genes when the bacteria are exposed to various light spectra. It also calculates the change in the cell's absorption spectrum changes with time.
Initially the activation rate of each light sensor is calculated using MATLAB:

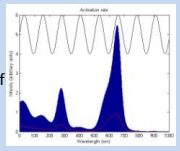
$$Ai(\lambda) = \int [I(\lambda) \cdot Ri(\lambda)] d\lambda$$

Where $A(I, R)$ is the activation rate of light a sensor species *i*

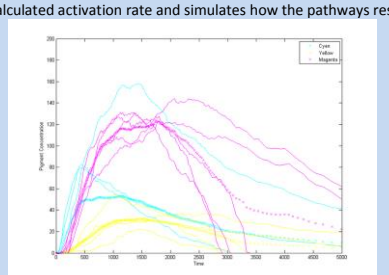
$I(\lambda)$ is the incident light spectrum

$Ri(\lambda)$ is the absorption spectrum of sensor species *i*

This method means that only light with a spectral overlap with a sensor can activate that sensor.
Furthermore it creates a saturation point, above which the sensors are all active; increasing the intensity of light will not change the number of activated sensors.



The primary component of our model is a rule-based stochastic simulation of the biological pathways above, written in Kappa and executed with KaSiM. It take the calculated activation rate and simulates how the pathways respond:

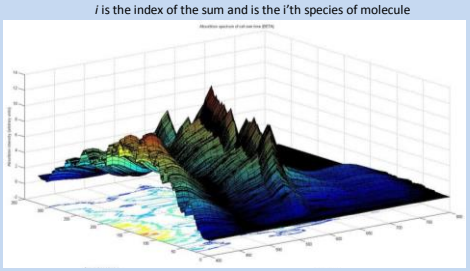


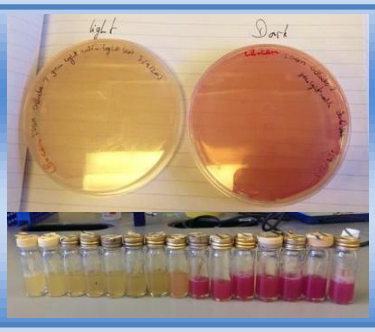
There was insufficient experimental data to test the model, however preliminary trials using ballpark figures for reaction rates suggest great instability and uneven pigment mixing. Increasing the number of light regulated DNA stabilised the system and can be used to correct the mix of colours.

Data from the stochastic simulation is combined with the absorption spectrum of the background cell, the light sensor and pigment proteins to produce a surface that describes how the absorption spectrum of the cell changes with time.

$$Spectrum(t, \lambda) = \sum Ni(t) \cdot Si(\lambda)$$

Where *Spectrum* is absorption spectrum over time
N is the number of molecules of species over time
S is the absorption spectrum
i is the index of the sum and is the i'th species of molecule





Our Green Light Module has been demonstrated to be functional; the synthesis of magenta pigment halts on exposure to white light.

Results

Green Light Module

Assembled using IDT gBlocks, the Green Light Module has been shown to function as expected on exposure to white light; the production of magenta pigment is inhibited.

*Bgl*II and *Bam*HI output brick

Also assembled using IDT gBlocks, this output brick responds to red light by producing GFP, clearly seen in the image to the right. Running the digested plasmid on a gel also verifies the presence of the *Bgl*II and *Bam*HI restriction sites. Replacing GFP with a gene of your choice would require use of simple standard primer based mutagenesis protocol.

Cyan pigment with specific promoter

ompC was successfully ligated to the cyan pigment, allowing synthesis of the pigment to be solely controlled by red light alone.

Green fluorescence indicates our Red Light Module output brick has been assembled correctly. Gels also indicate the *Bgl*II and *Bam*HI restriction sites are in place.

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