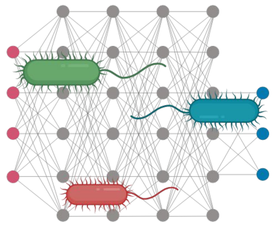
mini Imperial College London iGEM



motify

Meet the Team

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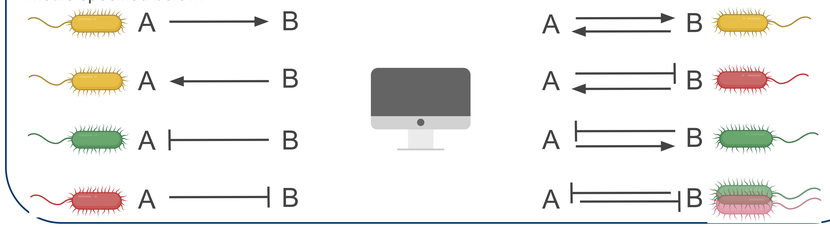
Abstract

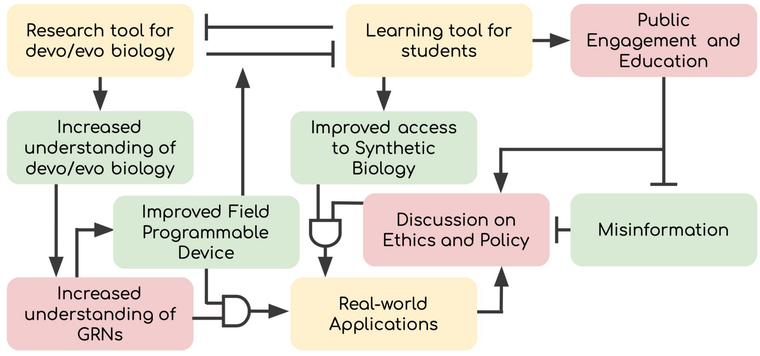
This project aims to develop a system for online computer-aided control of biological network motifs. The circuit proposed uses well-characterised optogenetic modules and synthetic dual activator-repressor systems based on the cI transcription factor. This enables switching between the 8 possible 2 node motifs specified below.



Motivation

Network motifs are the basic building blocks of gene regulatory networks (GRNs) that regulate cell behaviour. When these motifs are rewired through evolution, the resultant networks give rise to emergent properties which lead to complex biological phenomena such as complex body forms or neuronal development. Synthetic biology aims to extend, improve or design new cellular behaviours and in doing so better understand native systems. Control over interactions in a genetic network allows the creation of a biological field programmable array which not only gives better understand biology but also to improves customisation of synthetic biology devices.

RRI and Applications



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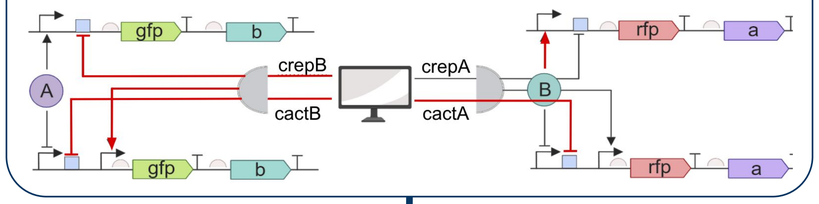
Design

Circuitry

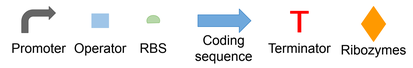


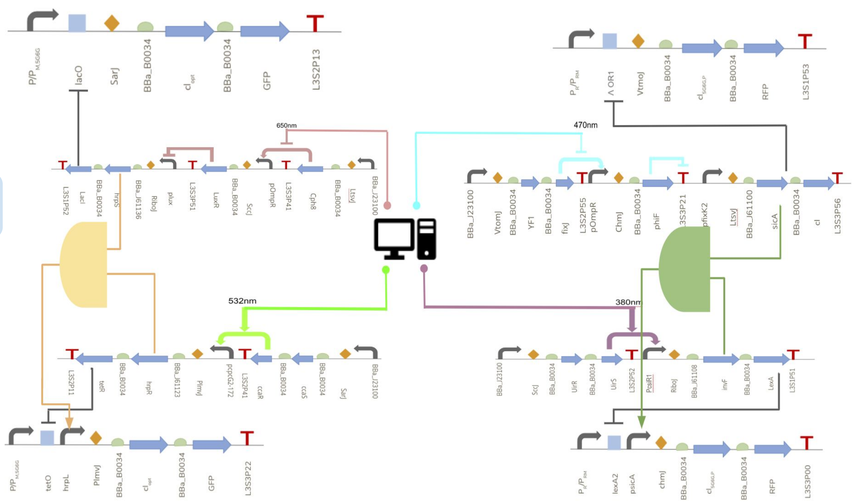
Logic Table

2 node Motifs (1-8)	Computer input by light			
	A1 [crepA]	A2 [cactA]	B1 [crepB]	B2 [cactB]
A → B	1	1	0	1
A ---| B	1	1	1	0
A ← B	0	1	1	1
A |--- B	1	0	1	1
A → B & A |--- B	1	0	0	1
A ---| B & A ← B	0	1	1	0
A → B & A ← B	0	1	0	1
A ---| B & A |--- B	1	0	1	0

Build

Biological Parts

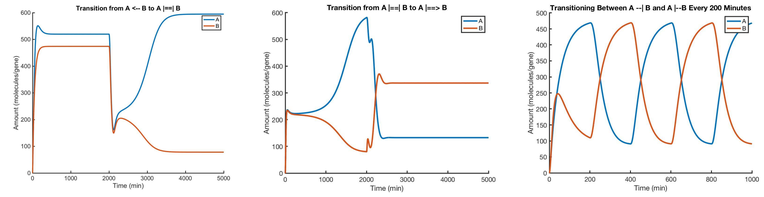




Test

State Transitions

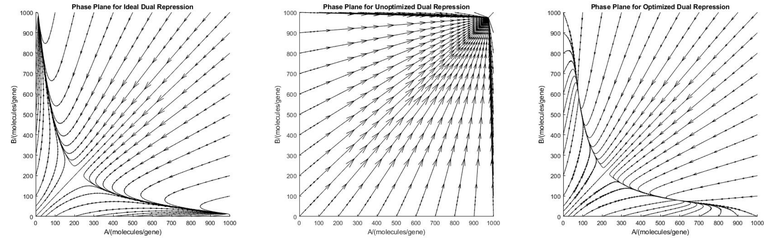
- State transitions do not affect steady-state behavior of each motif
- Transition time generally 300-600 min. Transition to A |==| B motif takes approx. 780+ min.
- Artificial oscillations can be made by continuously interrupting transitions before reaching steady-state.



Model optimisation

Initial simulations suggested that the system was not robust to transcriptional leakage of the optogenetic regulators. Three changes were made to improve system robustness:

1. The half-maximal repression concentrations of the optogenetic regulators were tuned to between the low and high steady state concentrations of the optogenetic regulators.
2. The half-maximal repression concentrations of the nodal transcription factors A and B were tuned to make them robust to a leaky level of constitutive expression.
3. The RBS strength of the AND gate components was decreased.



Learn

Biological Challenges

Our project is ambitious and the circuits we devised is complicated. For this reasons, we expect several challenges to arise in the wet-lab. Below listed are some feasibility issues:

- First time use of 4 orthogonal optogenetic systems. [1,3]
- Homologous Recombination.
- Unwanted crosstalk.
- AND gate leakage.
- Cell burden. [1]
- Plasmid size.