ecolibrium

Imperial College London

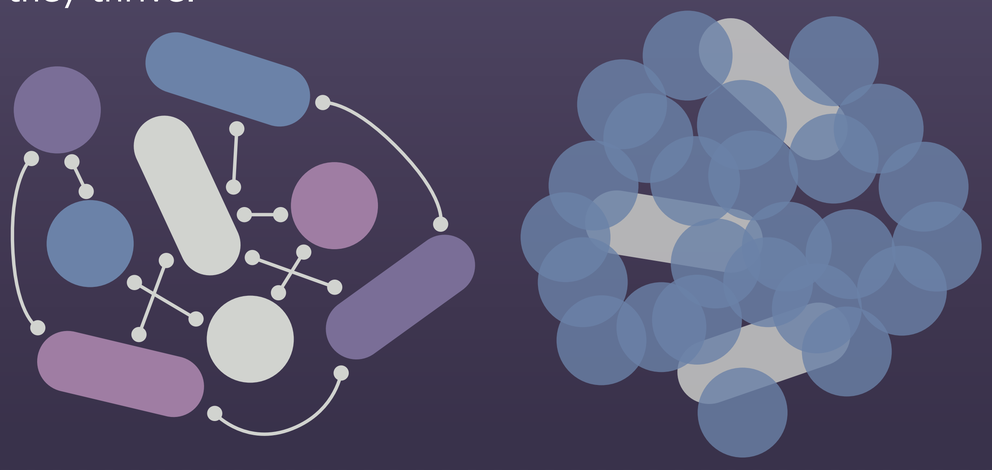
iGEM

Developing a framework for engineering co-culture

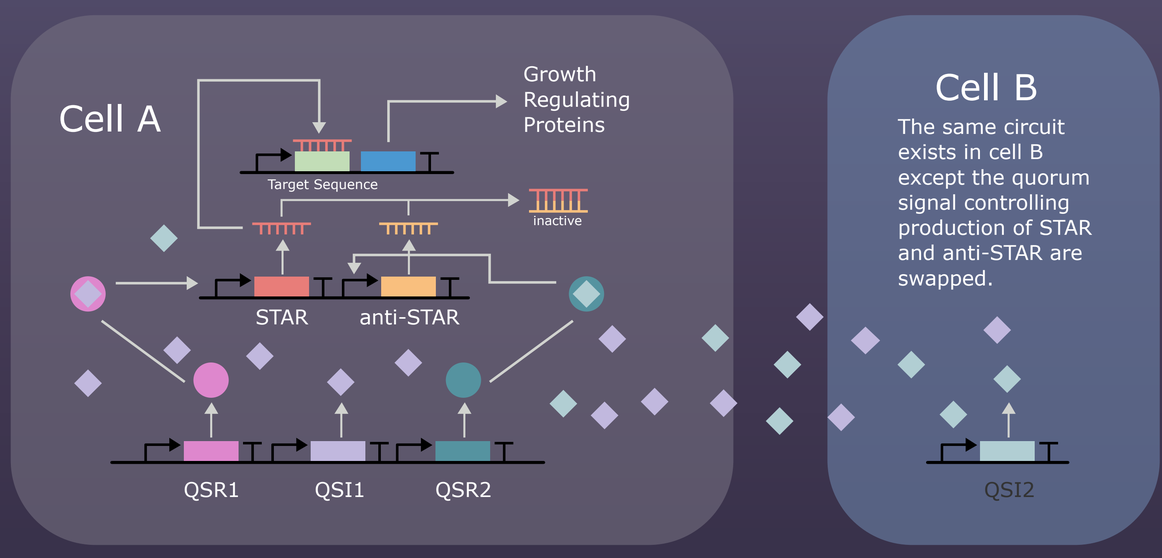
In nature microorganisms do not exist in isolation but interact within complex ecosystems - a phenomenon which synthetic biology has yet to harness.

But why?

We found that researchers struggle to determine the ideal conditions for their co-cultures to thrive. Different species have varied growth rates and optimal conditions in which they thrive.



We set out to develop a genetic circuit dubbed Genetically Engineered Artificial Ratio (G.E.A.R.) to allow ratiometric control of populations in a co-culture. Our genetic circuit utilises three distinctive modules; communication, comparison and growth regulation. In addition to the circuit, we have produced a software tool, the Advanced Logging Interface for Culture Experiments (A.L.I.C.E). A.L.I.C.E is a repository for culture data and aids synthetic biologists in the design of their own co-cultures.



Advance Logging Interface for Culture Experiments

We have created a repository, the Advanced Logging Interface for Culture Experiment (A.L.I.C.E.), for synthetic biologists to store information about optimal growth conditions and growth characterisation for their cultures. We hope that they will then be able to use this information to design their own co-cultures.

Key achievements:

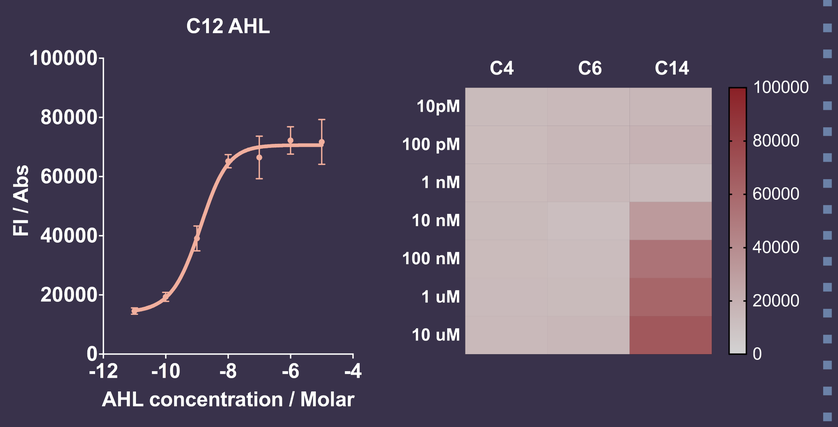
Characterised the growth of 7 different chassis under various temperature and media conditions in monoculture.
Characterised the growth of 3 different co-cultures under various temperature and media conditions.
Collaborated with 7 iGEM teams around the world to generate data for 12 different chassis.

Communication

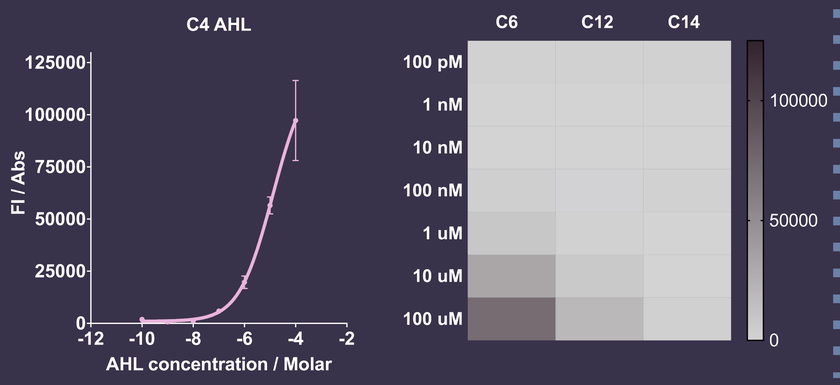
The quorum module provides a way for the cells to detect the density of each population.

Key Achievements:

Characterisation of the Las quorum sensing system, including crosstalk characterisation.

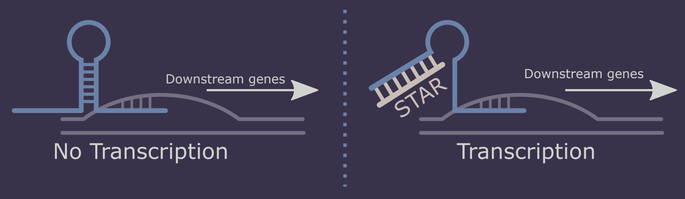


Characterisation of the Rhl quorum sensing system, including crosstalk characterisation.

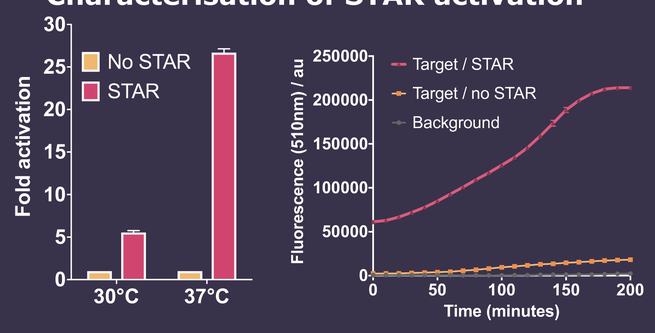


Comparator

STAR (Small Transcription Activating RNA), a novel RNA-logic technology making its debut in iGEM with our project, allows for rapid and robust regulation of gene transcription (Chappell et al, 2015). The two different quorum signals from the communication module control the respective transcription of STAR and anti-STAR, a novel RNA designed by us to deactivate STAR. This allows each cell to compare its own population density to the other cell's population density.



Characterisation of STAR activation



Key Achievements:

Characterisation of STAR-mediated gene activation in various conditions.

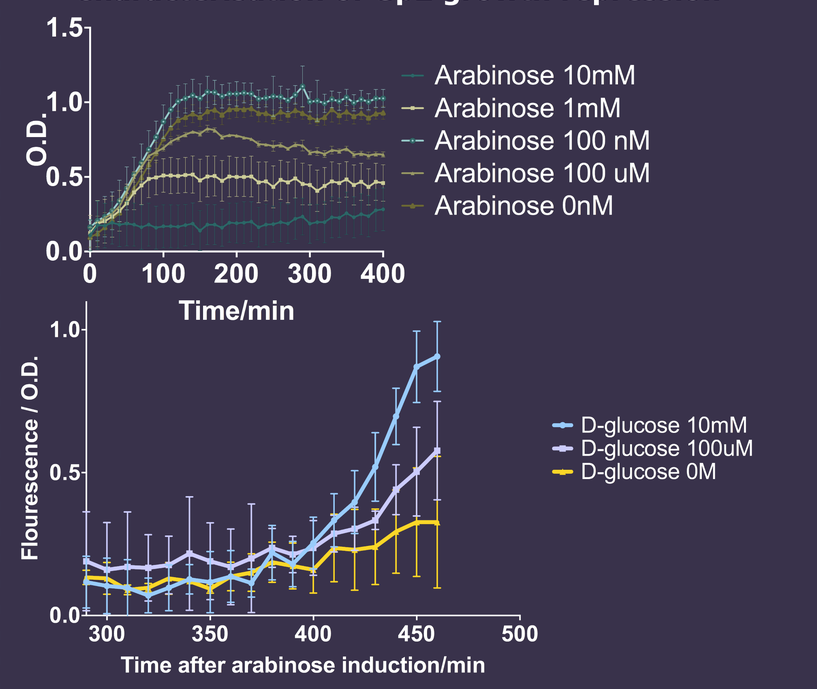
Design of a new anti-STAR to deactivate STAR-mediated gene activation.

Expansion of the BioBrick Registry library by addition of new STAR and anti-STAR technology.

Growth Regulation

If the comparator module calculates a population imbalance, it activates the transcription of a Gp2 phage protein, a novel growth regulatory system, that binds reversibly to RNAP to achieve rapid growth regulation.

Characterisation of Gp2 growth repression



Key Achievements:

Improved the pBad-AraC construct by adding a reverse terminator.

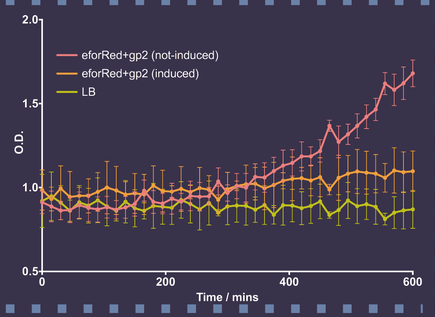
Characterised our improved pBAD-AraC construct.

Characterised a novel, effective and reversible growth control method for E. coli, the phage gene Gp2, using the improved pBAD-AraC construct.

Biotone

BIOTONE, our eco-friendly library of colours, is an example of how our circuit standardizes the behaviour of a co-culture: our mixed colours are only possible if set population ratios are maintained.

Key achievements:
77 different colours were made from 7 base chromoproteins.
Demonstrated need for maintaining fixed ratios as each chromoprotein slows the growth of its host by differing amounts.

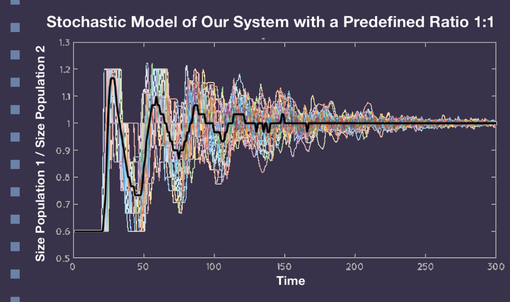


Modelling

Our single cell model was constructed in the Matlab Simbiology Toolbox. We modelled the 4 quorum systems, STAR and anti-STAR behaviour and 4 different growth regulator systems in silico. We used RNAstruct developed by Matthews Lab to help aid the development of the anti-STAR.

Human Practices

We adapted the Socio-Technical Integration Research protocol (S.T.I.R.) protocol, a formalised approach to "reflexivity", to enable iGEM teams to structure their discussions about the impact of decisions made inside and outside the lab.
This process allowed us to create our "Visual Strategies Experiential Guidebook". The handbook goes over visual strategies for communicating research that impacted how we presented our project.



Population models of GP2 growth regulation in two population and a simplified version of our circuit with GP0.4 growth regulation into two populations of *E. coli* were constructed using Simbiology and Gro programming language, developed by the University of Washington, respectively.

Conclusion

We hope that our contributions to the Biobrick registry provide synthetic biologists with the tools to design and build their co-cultures.
Let us take synthetic biology to a higher level !

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Our supervisors and advisors : Polizzi K., Stan G.B., Freemont P., Kitney R., Jensen K., Kylilis N. and Smith R.

We would like to acknowledge the help of the synthetic biology community at Imperial College London with their help with our projects.

References: Chappell, J., Takahashi, M. & Lucks, J. (2015) Creating small transcription activating RNAs. Nature Chemical Biology. [Online] 11 (3), 214-220.