University of Exeter

iGEM

A synthetic riboswitch with the potential to detect any given RNA sequence.

RIBONOSTICS

TOEHOLD DESIGN

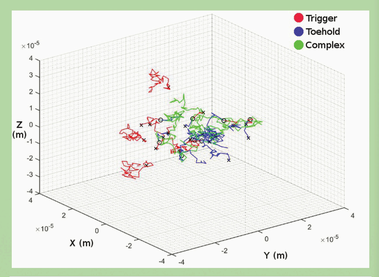
The toehold switch structure was originally designed by Green *et al.* (2014) [1]. To design our toehold switch, we used this general structure and NUPACK to generate an optimal sequence, which was then edited to ensure functionality. In addition to this, we kept to the following required constraints:

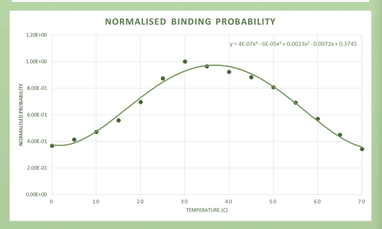
- RBS must be within the loop region.
- The first 15 nucleotides of the main linker must be complementary to the end of the switch region.
- The AUG start codon must be positioned shortly after the RBS, within the stem.
- The main linker must be a multiple of three in length and must not contain a stop codon.

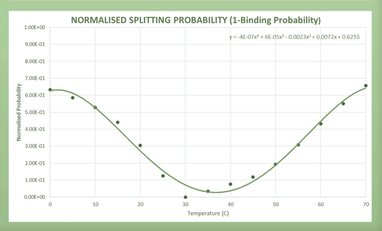
SIMULATION

We set out to produce a 3D simulation of the interactions of particles in our cell free system, providing a visual and numerical output. We hope this can be used by future teams to help their understanding of their own systems.

The graphical output is designed to perform several roles. It enables a visual interface to monitor the workings of our code and to ensure correct matlab functionality, whilst also being a tool to aid in our explanation of the system. Construction of this graphical output has been instrumental in influencing the experimental choices, such as in the use of Low-Binding affinity consumables. Using NUPACK to provide *in silico* data, enabled a robust estimation of binding and splitting probability functions across a range of temperatures.

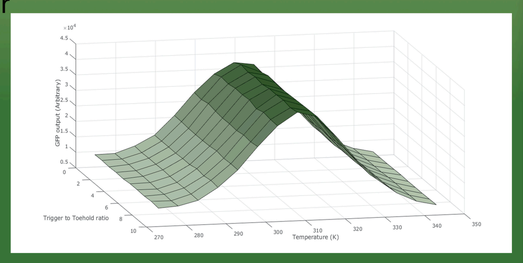






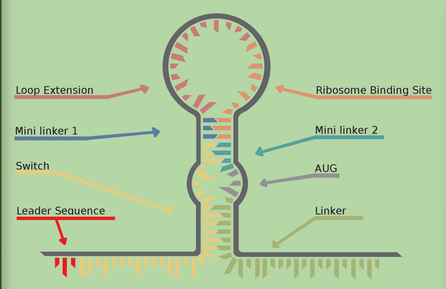
PARAMETER SCANNING

The parameter scanning element of the simulation was intended to perform multidimensional comparative scanning and to isolate peak islands from which we could infer the optima for each parameter. Foremost of these was temperature, as it affects the stability of the system's components, including the trigger RNAs, the toeholds themselves, and the translational machinery. Trigger/Toehold ratio was also scanned, to investigate the optimal concentrations necessary for a response; Viscosity was considered for the design of a functional prototype of our system. However, since we were unable to validate our simulation using lab generated data, the parameter scanning function worked as a tool for validating our simulation's operation against the *in silico* data from NUPACK.



INTRODUCTION

Toehold switches are a type of riboregulator which bind specific trigger sequences. Synthetic toeholds have been engineered to work in higher dynamic ranges and lower cross talk [1] [2]. We aim to show proof for an easy, adaptable, and specific diagnostic test using synthetic toeholds. The immediate application for our test is the detection of *Mycobacterium bovis*, the cause of tuberculosis in cattle.

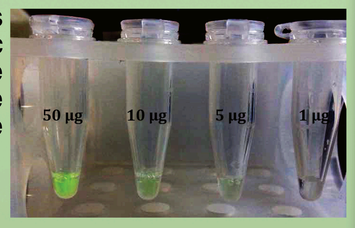


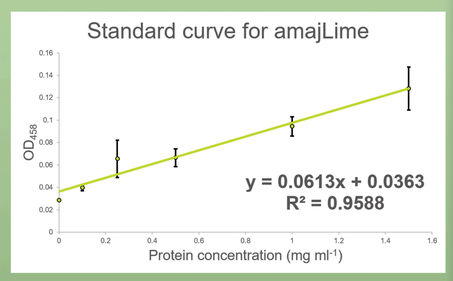
Chromoprotein Characterisation

Chromoproteins could be used as potential indicators for our diagnostic toehold, as they are clear and easy to see in the field. We further characterised the following three chromoproteins to make their measurement and detection easier:

- eforRed
- aeBlue
- amajLime

In order to determine the minimal amount of chromoprotein required to see a colour change, we obtained a visual limit for these chromoproteins by diluting known amounts of protein extract until it was no longer visible. Total volumes were 50µl, the same as the cell-free reaction.

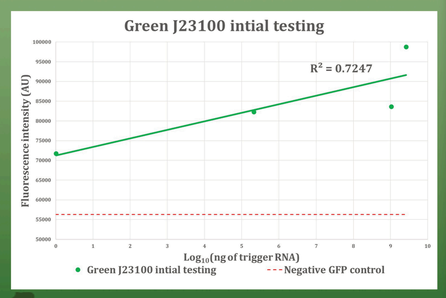




To allow easy quantification, varying protein extract concentrations of each of the chromoproteins were used to make a standard curve.

TESTING TOEHOLDS

In order to demonstrate functionality of a toehold switch in a cell-free system, we measured the response of GreenFET1J to increasing amounts of correct and incorrect trigger RNA. As can be seen from the graph, as amount of trigger increases, so does expression of GFP. Further testing has also shown that the toehold is not efficiently activated by incorrect trigger RNA.



Why we chose bovine TB

The current Tuberculin skin test for TB is unable to differentiate between vaccinated and infected cattle [3], meaning that it is illegal to vaccinate cattle in the UK against TB. The UK loses a dairy herd every other day due to culling of TB infected cattle. DEFRA plans to invest £1 billion over the next decade in order to control Bovine TB [4]. These are just some of the facts that accompany the grim reality of Bovine TB in the UK, a reality that affects farmers, vets and politicians.

If successful, our test would allow the reintroduction of the BCG vaccination and offer a multipurpose test that could provide a smart solution to bTB in the UK.

INTO THE FIELD

Bovine TB affects the livelihoods of a range of people. For the Human Practices aspect of our project we wanted to work out exactly what challenges the UK faces to eradicate Bovine TB. To do this we met with a number of relevant individuals, with differing priorities and opinions. Combining what we learned from these relationships allowed us to integrate the various needs of each party into the design and application of our toehold test.

Richard Sibley helped us understand the current limitations of the Tuberculin skin test and explained situations where our test would be most useful. He stressed the importance of a higher degree of sensitivity.

Phil Leighton helped us with the design of our test for use 'in the field'. It was important our test could fit with the current methods of collecting a blood sample.

Michael Ross explained what his company (Saved & Safe) does to tackle bovine TB and how a new test like ours could offer an incredibly useful animal health management tool. The simplicity of our test would allow it to be used by any untrained individual, including farmers.

George Eustice, MP and Minister of State for Farming, Food and the Marine Environment, helped us as a team to establish the position and approach of DEFRA with controlling and eradicating bTB. Development of a DIVA (differentiate between infected and vaccinated animals) test is of high priority for DEFRA and our RNA based diagnostic tool fits this criterion.

FUTURE

The Ribonostics project has ample potential for further development; proposed improvements include:

- Optimisation of chromoprotein production, toehold switch efficiency and the cell free system
- New ways to visualise the chromoprotein in a blood sample - eg. the use of metal strips, a paper-based system [2], or an enzyme indicator
- Providing a toolkit for simulating a cell free system
- Developing a prototype which we could use in the field.

The flexibility of our system allows a wide range of potential applications; ranging from adaptation towards the diagnosis of many diseases right through to use as a valuable tool in academic research.

Our prototype

References

[1] Green, A.A. *et al.* 2014. 'Toehold Switches: De-Novo-Designed Regulators of Gene Expression'. Cell. 159, pp. 925-939.
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[4] Department for Environment, Food and Rural Affairs, 2014. A strategy for achieving Officially Bovine TB Free status for England. [Online] [Accessed on 18/08/2015] Available from: http://tinyurl.com/ksmcy9x
[5] Saved and Safe Ltd., *Saved and Safe Ltd. - UK*. [Online] [Accessed 20th August 2015]. Available from: http://savedandsafe.org/en/uk
[6] Dirks, R.M. *et al.*, *NUPACK: Nucleic Acid Package* [Online] [Accessed 15th September 2015]. Available from: www.nupack.org

Team

Jasmine Bird, Biological Science
Bradley Brown, Biochemistry
Todd Burlington, Physics
Rachel Cooley, Biochemistry
Yemi Fenuyi, Medical Science
Daniel Herring, Natural Science
David Markham, Biological Science
Amy Perrelet, Physics
Joseph Slack, Biological Science
Georgina Westwood, Medical Science
Emilia Wojcik, Biological Science

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Our Wiki