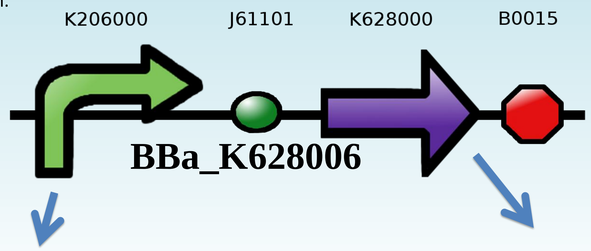
KILL SWITCH ENGAGE: INTRACELLULAR PROTEGRIN- 1 PRODUCTION AND ITS POTENTIAL APPLICATIONS

WHAT IS A KILL SWITCH ?

For the 2011 St Andrews iGEM Team project, we are creating an intracellular *Escherichia coli* "kill switch" that functions differently from any found in nature. Our biobrick synthesizes protegrin-1, an antimicrobial peptide which has microbidicidal activity against multiple species of unicellular and multicellular organisms.

Protegrin-1 functions by imbedding itself into the phospholipid bilayer and disrupting bacterial cell walls by creating pores within the membrane (Lam et al., 2006). Pore formation between the cytoplasm and the extracellular space inhibits the cell's ability to control ion movement, cytosol make-up, as well as its own structural integrity. The damage caused by this lack of control inevitably leads to cell death.



MODELLING

Aims:

- To create a working model based on the production of protegrin-1 in our biological system.
- To determine the optimum range for input arabinose concentration that would produce a sufficient concentration of protegrin-1 to kill the cells.

Method:

Through a series of ordinary differential equations, we used a model in MATLAB to investigate the parameters used in the system.

$$\frac{d[A_1]}{dt} = \Gamma_{diff}[A_0 - A_1] + \Gamma_{diss}[A_1AraC] - \Gamma_{ass}[A_1][AraC] - \gamma[A_1]$$

$$\frac{d[A_1AraC]}{dt} = \Gamma_{ass}[A_1][AraC] - \Gamma_{diss}[A_1AraC]$$

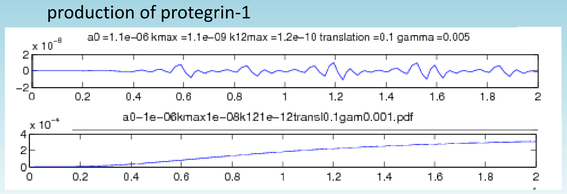
$$\frac{d[AraC]}{dt} = \Gamma_{diss}[A_1AraC] - \Gamma_{ass}[A_1][AraC] - \Gamma_{deg}[AraC]$$

$$\frac{d[PG-1mRNA]}{dt} = k_{maxprodRNA}\left(\frac{[A_1AraC]^n}{\left(k_{\frac{1}{2}maxprodRNA}\right)^n + [A_1AraC]^n}\right) + \Gamma_{deg}[PG-1mRNA]$$

$$\frac{d[PG-1]}{dt} = \Gamma_{trans}[PG-1mRNA] - \gamma[PG-1]$$

Results:

Two varieties of graphs were created with respect to the production of protegrin-1



Conclusion:

Instability was formed with respect to the last set of constraints when referring to the protegrin-1 production, meaning that our model was successful when using a certain selection of parameter ranges. As the translation rate of the protegrin-1 is altered we see how one type is incorrect as we have a negative concentration produced. Further work would involve more detailed study of the constant ranges.

LAB RESULTS

Aims:

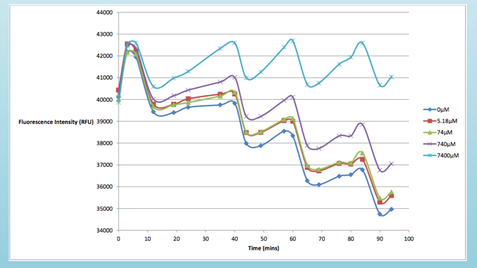
- To prove the functionality of BBa_K628006
- To test the efficacy of BBa_K628006 over a range of substrate concentrations

Method:

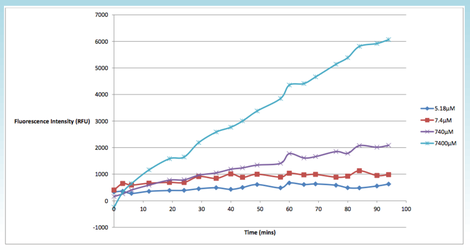
Using membrane-sensitive fluorescent dyes, we tested the efficiency of the K628006 biobrick to produce cell death over time.

Results:

We found that increasing levels of arabinose cause increasing levels of cell death.



By subtracting the value of the control (0 μM) from each fluorescence reading, we can see the relationship between the varying substrate concentrations.



Conclusions:

- Proved BBa_K628006 functions as expected
- Explored how BBa_K628006 functions across a range of substrate concentrations

WHAT IS PROTEGRIN -1?

Protegrin-1 is an antimicrobial peptide which was first identified in porcine leukocytes. It has a length of 18 amino acids, and can fold *in vivo* without the uses of chaperone proteins.

Protegrin-1 has high microbicidal activity against *E. coli* (gram-negative), *N. gonorrheae* (gram-positive), and HIV-1 (lipid-coated virus), amongst several other bacterial and virion species.

Protegrin-1's secondary structure is a β-sheet conformation including a β-hairpin turn, as displayed on the left. This β-hairpin turn is what allows protegrin-1 to be microbidicidal, as it is this specific folding confirmation that allows for integration into prokaryotic membranes.

POTENTIAL APPLICATIONS

BIO-SAFETY

The risk of synthetic organisms escaping the laboratory and damaging the environment or other species is a serious concern for both scientists the world over, as well as the public at large. In order to decrease the chance of unwanted GEM colonization, our kill switch could be inserted into cells and then designed to activate upon contact with non-laboratory conditions. This could even be a precursor to **site-based testing** with synthetic organisms, which is current safety issue amongst synthetic biologists. Thus the possibility of a GEM damaging its environment is greatly reduced.

DRUG DELIVERY

The natural specificity of bacteria to colonize their preferred ecological niche is an untapped resource within synthetic biology. Our kill switch functions by forming pores within the membrane of prokaryotic bacteria, causing intracellular cytosol to leak into the extracellular matrix. If a "drug" of some design was also present within the cytosol, it too would be exported from the cell.

Combined with the bacteria's natural affinity to colonize certain areas of the body (i.e. E. coli → the gut), we believe that our kill switch, once inserted into bacteria, could be used to form a **bacterial-vector drug delivery system** that would allow drugs to be delivered to the specific site of absorbance. This would also mean that future drugs could be redesigned to be site-specific, potentially increasing their efficacy and improving treatment as a result.

PANEL DEBATE

The University of St Andrews iGEM Team 2011 collaborated with the University of Dundee iGEM Team 2011 and held a panel debate at the World Schools Debate Championships, where we gave a presentation about synthetic biology and answered questions from the audience.

iGEM REVIEW

Aims:

To investigate the equality of iGEM by reviewing the team structure and success from 2008-2010

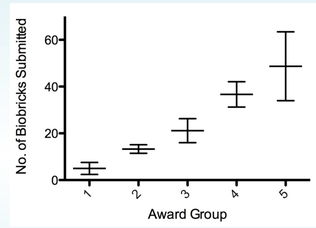
Method:

We collated relevant data from various sources and applied statistical methods, using PSAW and R programming, to discover any correlating factors in the figures. We considered the p-value, which relates to the existence of an effect from a variable on the data but not the size of the effect, to determine the importance of the significant variables in a statistical model. The measurement of team success was based on the following scale:

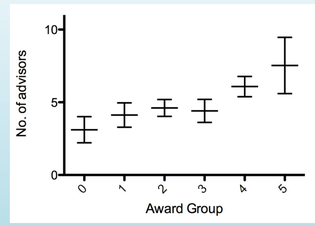
0	Team Withdrew
1	None
2	Bronze
3	Silver
4	Gold
5	Finalist
6	Grand Prize

Results:

We found that the outcome, number of biobricks produced, was an indicator of the success of a team:



Of a greater significance, as it incorporates an input into the model rather than a secondary output from the competition, is the total number of advisors:



The p-values that were calculated for each variable was compared against a reference team (2008 South Africa) and output as such. The p-value for the number of biobricks submitted is $p < 0.0001$ and similarly the p-value for the total number of advisors is $p < 0.0001$.

Conclusions:

We found two significant conclusions from these statistical tests

- there is a highly significant effect of the number of advisors on the success of the team
- there is a highly significant effect of the number of biobricks submitted on the success of the team

REFLECTIVE ESSAYS

In addition to the statistical analysis, our iGEM team also wrote reflective essays which provide information about our iGEM experiences and our development from them. Prospective iGEM applicants could use our insights to educate themselves on the basis of iGEM.

CONCLUSIONS

- **Successfully created a biobrick responsible for prokaryotic cell death** via intracellular protegrin-1 production
- **Discussed several potential future uses** of our kill switch
- **Created a model** measuring the rate of protegrin-1 production
- **Completed a review of 2008-2010 iGEM team data**, resulting in extraordinary findings about what determines a team's medal

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