Stockholm Modelling Report

By NUSGEM

Overview

We have completed modelling of the Tryptophan and Cumate Circuits with specific timings. Below you can find the related methodology, results and discussion. The modelling results should serve to guide your experiment as well provide you with an understanding of the characteristics of your genetic circuit.

Methodology

Given the genetic circuits provided from StockholmiGEM, we modelled Stockholm Cumate circuit, and Stockholm Tryptophan circuit. We first used the kinetic data provided, as well as some assumed kinetic values to generate a functional response model for both circuits. The functional response model is a model of how the circuit should ideally perform. From the functional model, we probed deeper and conducted a sensitivity analysis to measure the most sensitive parts in the circuit. From the list of most sensitive parts, we chose one part and conducted a combinatorial analysis of that part. The combinatorial analysis involved changing the kinetic values of one particular part and measuring the respective changes in response. We chose not to include the interaction between IM2 and E2 because toxin-antitoxin systems are known for high association dissociation rates and since this value is unknown, we chose not to include it. We hope that from the different response quantities, or in future models, we can include the rate at which these two molecules interact to show through modelling whether the host will die or not.

We also implemented timing of different states into both models. Both models run for a total simulation time of 4320s at time step 1s. The initial conditions of the inputs, Tryptophan and Cumate are 0 a.u., and at 2160s, the conditions of Tryptophan and Cumate are both 0.5 a.u. The timing state aims to simulate Tryptophan and Cumate being added at 36 minutes. We also assume that these values hold constant for the duration of the model.

To address the lack of data regarding the kinetics of interactions and parts used in both circuit, we assumed arbitrary values to replace the unknown kinetic values. In the Stockholm Tryptophan Circuit, we assumed the promoter strength of the StockholmTrp Operator to have Vmax of 1 RPU and hill-coefficient of 1. We varied the Km in the combinatorial analysis to illustrate the effect it has the on the response. In addition, we assumed the association and dissociation constant between Trp and Tryptophan, and between TrpTryptophan and Trp Operator, to be 1.5 and 0.7 respectively.

In the Stockholm Cumate Circuit, we assumed the promoter strength of StockholmT5Cumate to have Vmax of 1 RPU and hill-coefficient of 1. We then varied the Km of this promoter in the combinatorial analysis since it has the largest effect on the circuit. In addition , we assumed the association and dissociation constant between CymR and Cumate, and between CymRCumate and StockholmT5Cumate operator, were both 1.5 and 0.7 respectively.

The kinetic values of the other parts were characterised by AdvanceSyn using the PBbE8K vector with E. Coli MG1655 host. We can expect discrepancies in the kinetic values and interactions across different experimental conditions such as when testing in different strains and mediums.

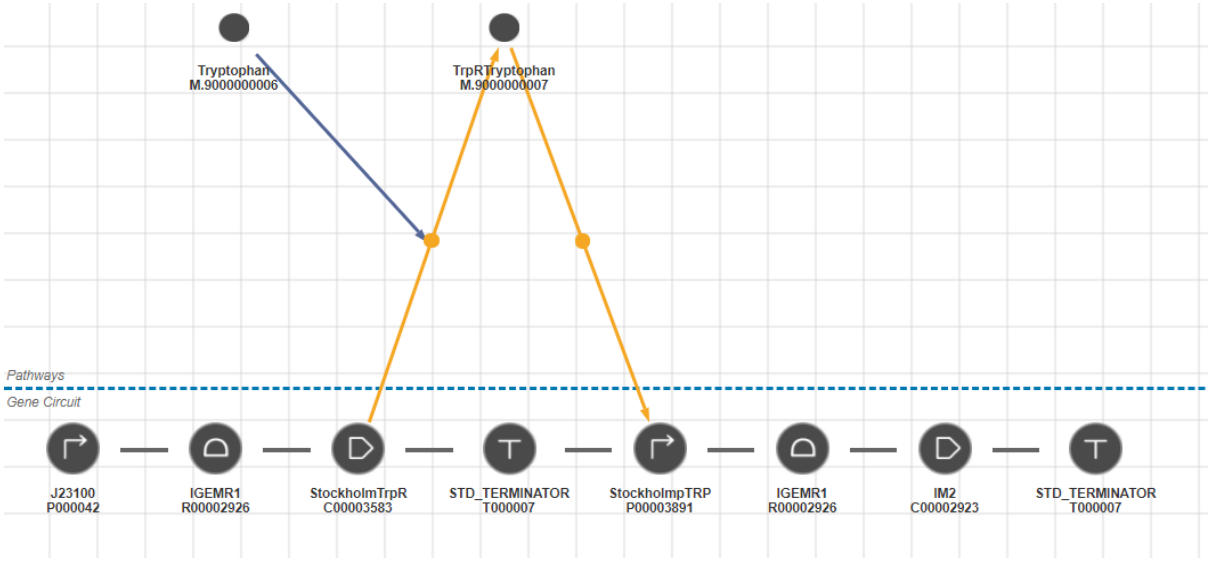


Figure 1 Gene Circuit of the Stockholm Tryptophan System

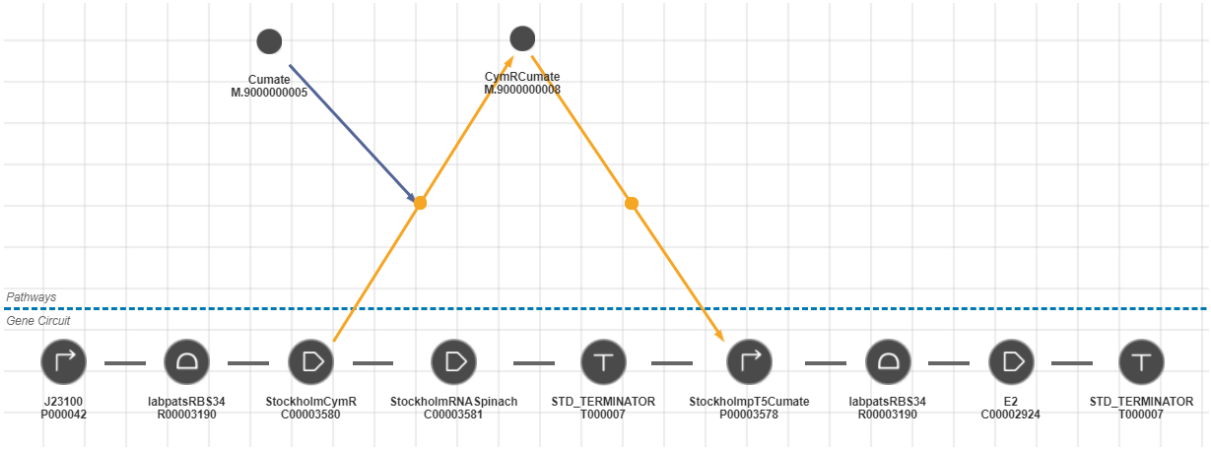
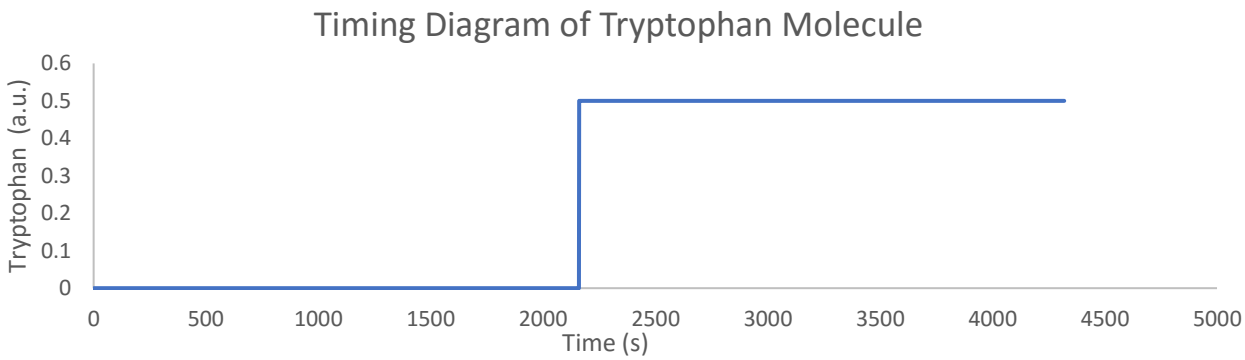
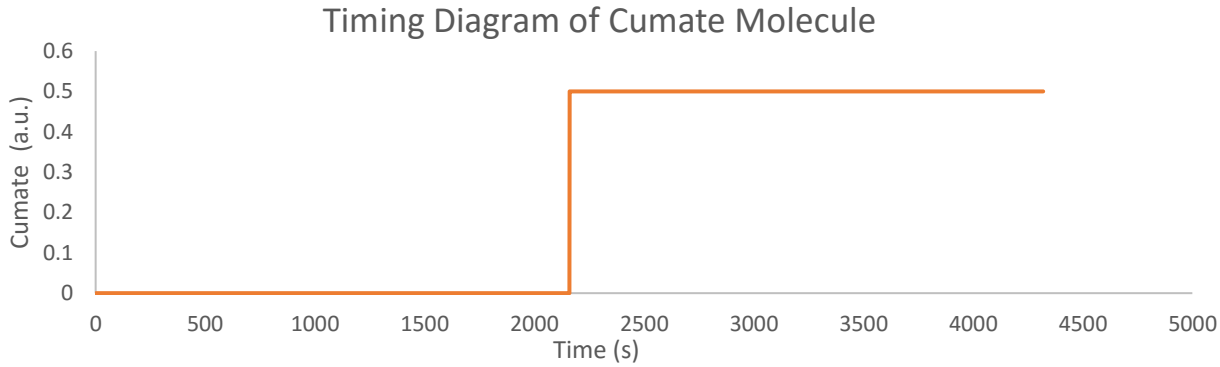
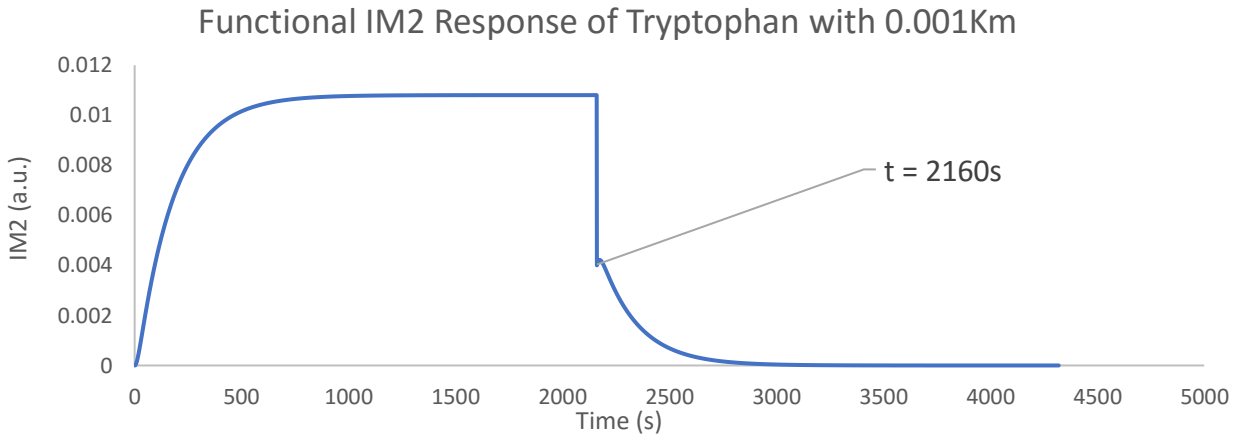


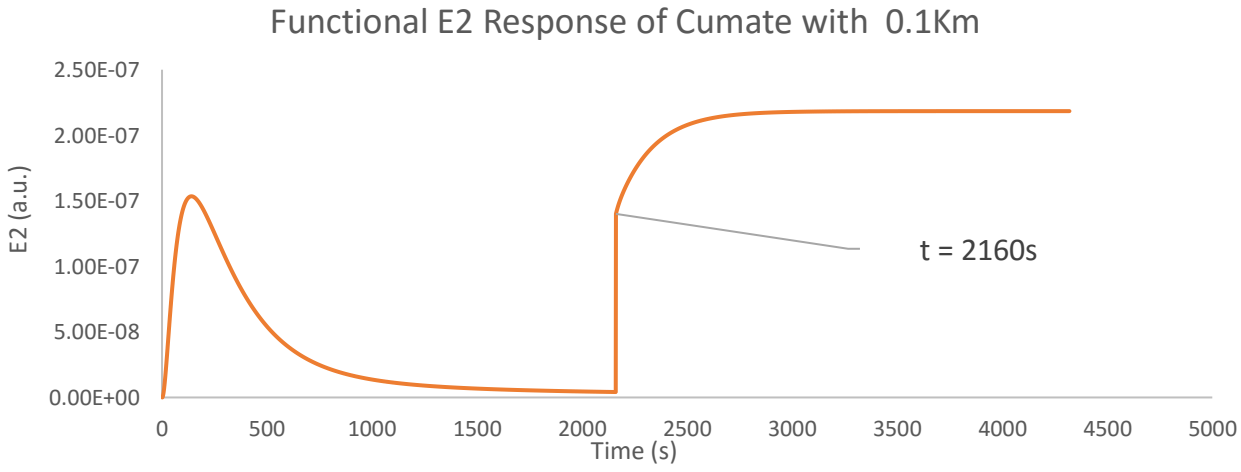
Figure 2 Gene Circuit of Stockholm Cumate System

Results


Timing Diagram of Tryptophan Molecule
Tryptophan (a.u.)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
Time (s)


Timing Diagram of Cumate Molecule
Cumate (a.u.)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
Time (s)


Functional IM2 Response of Tryptophan with 0.001Km
IM2 (a.u.)
0.012
0.01
0.008
0.006
0.004
0.002
0
t = 2160s
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
Time (s)


Functional E2 Response of Cumate with 0.1Km
E2 (a.u.)
2.50E-07
2.00E-07
1.50E-07
1.00E-07
5.00E-08
0.00E+00
t = 2160s
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
Time (s)

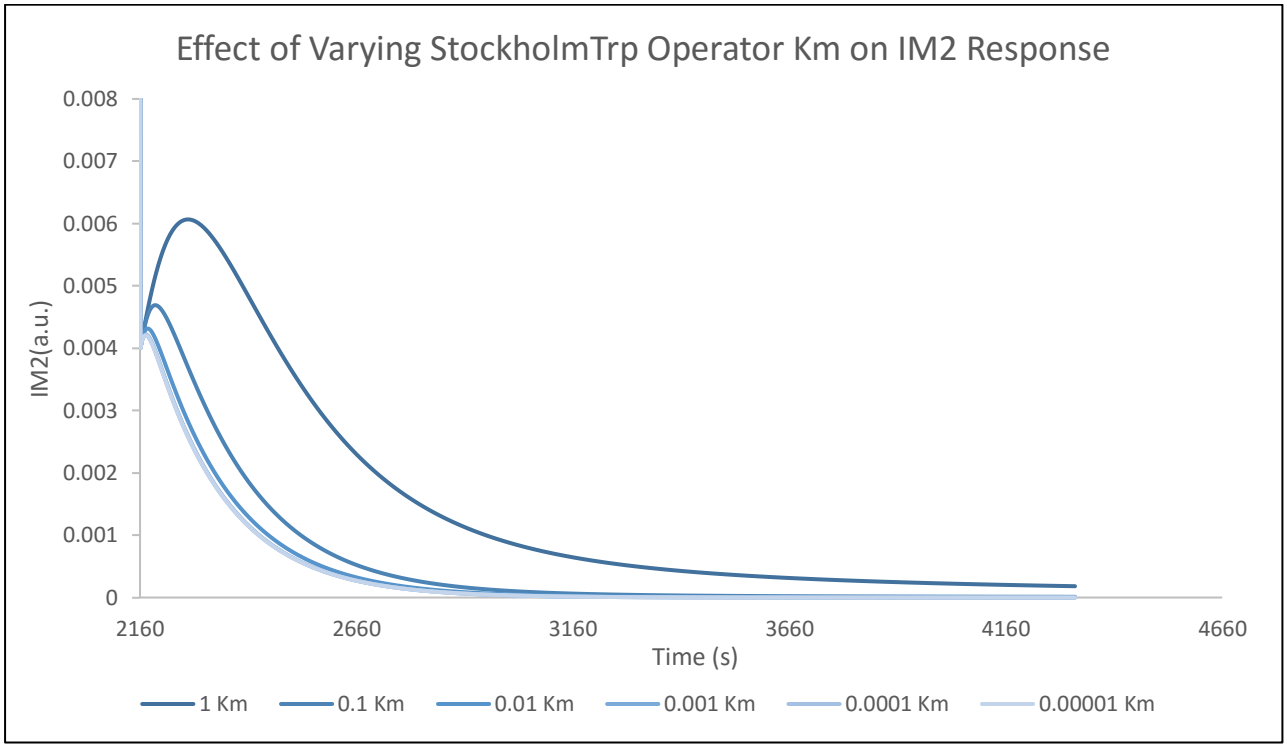
The above models show the timing diagrams of Tryptophan and Cumate, and functional models from both circuits. The functional models can be generated by using Stockholm Trp with 0.001Km, and StockholmT5 Cumate with 0.1Km.

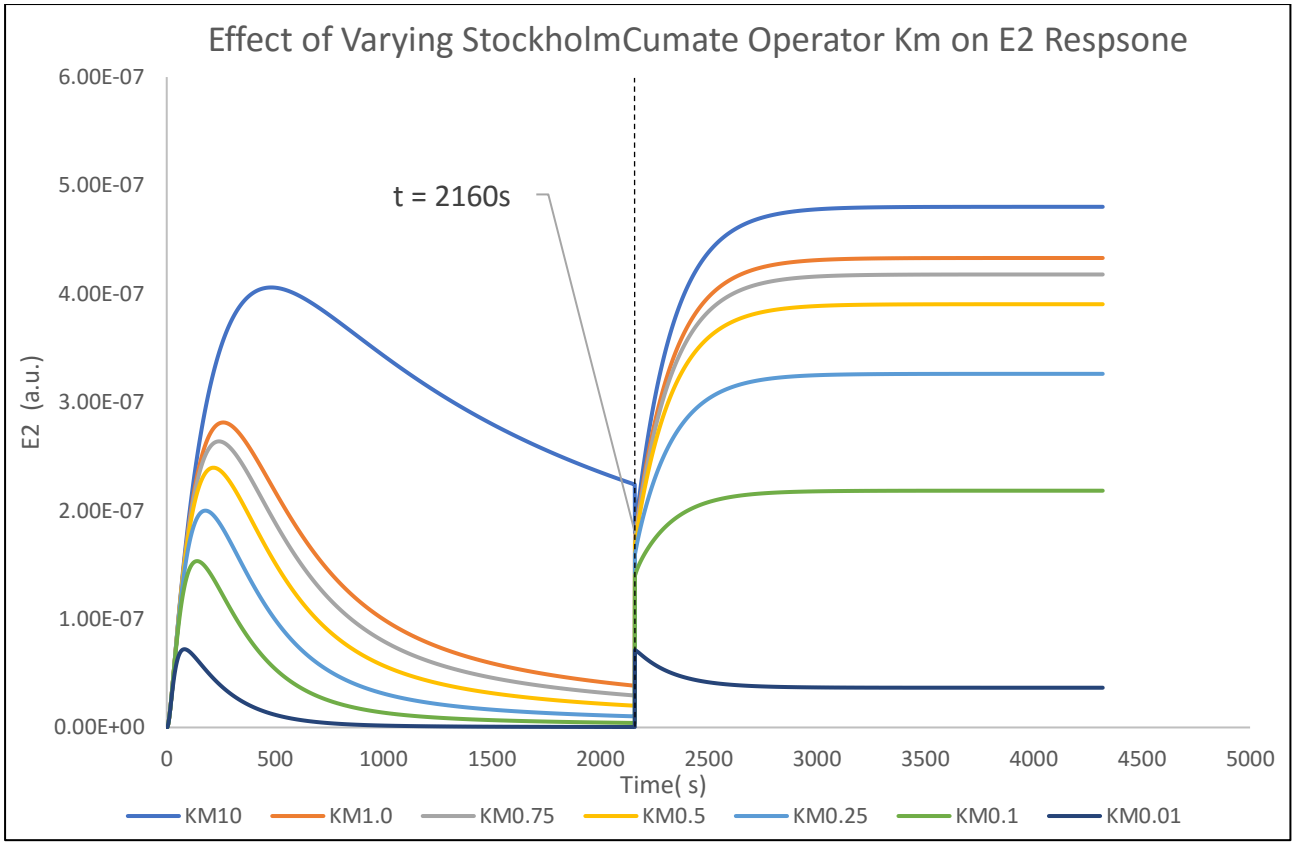
Sensitivity Analysis

Most Sensitive Parts in Descending Order	
Tryptophan Circuit	Cumate System
Vmax StockholmTrp Operator	Initial Condition of CymR
RBS connected to Constitutive Promoter	Km of StockholmCumate Operator
Vmax of Constitutive Promoter	Vmax of StockholmCumate Operator
Degradation rate of TrpRTryptophan Molecule	
Km of StockholmTrp Operator	

Based off the sensitivity analysis, we decided to examine how varying Km of the Trp and T5 Cumate operators impact the response of the circuit and how insights of these analysis can be used to help understand the circuit.

Combinatorial Analysis of Varying Km





Discussion

In the functional model of Tryptophan Circuit at t=2160s, we can observe a bulge in the response. This bulge is an undesired response where IM2 is being produced in a stage it where IM2 should not be produced. Likewise, in the initial stages of the functional model of the Cumate Circuit, we can observe E2 being undesirably produced and then diminishing away. These two undesired responses are caused by propagation delays between the repressor molecules being produced, and subsequently binding to the promoters.

In the Tryptophan Circuit, there is an abundance of TrpR produced in the first half of the model. In the second half of the model where Tryptophan is introduced to the circuit, we can observe a sudden drop in IM2 production as all the accumulated TrpR at steady state, readily binds to IM2 to repress the Trp operator. This sudden drop in IM2 production is caused by this rapid repression of the Trp Operator. However, TrpR must still be produced to maintain repression of the Trp Operator and because of the delay it takes to produce TrpR, we observe a small bulge that is indicative of time taken for TrpR to be produced, bind to TrpRTryptophan and reach the necessary quantity to repress the Trp Operator.

Likewise, in the Cumate Circuit, the repressor molecule CymR is not in high enough quantities to repress T5 Cumate and the downward expression of E2. As such, the undesired response in the model is representative of the time taken for CymR to reach the quantity needed to fully repress expression of E2.

It should be noted that in practice, the delay of E2 repression is significantly more detrimental than the delay of IM2 repression. This is because E2 is a toxin and its undesired expression can kill the host bacteria at an unsuitable stage, for example in a stage before the bacteria has completed its primary function.

Realising this issue, we conducted a sensitivity analysis and then varied the Km of Trp Operator and Cumate operator to possible solutions that can be useful in rectifying this issue. Increasing the Km of the operators increases the concentration of molecule needed to repress the operator. In the combinatorial analysis of Trp operator Km, changing the Km only had an effect after t=2160s. In this situation, we identified that lower Km reduced the undesired expression levels of IM2. However, we also identified that at lower Km, the effect of repressing undesired expression of IM2 became less significant which is suggestive that there is a limit when Km decreases. For example, the difference in expression of IM2 between 0.0001Km and 0.00001Km is extremely small yet, the difference in expression of IM2 between 1Km and 0.1K m is large. We believe this limit to be associated with minimum time it takes for a molecule of TrpR to be produced, converted to TrpRTryptophan and then bind to the Trp Operator. Therefore, we suggest that the TrpR Operator of Km 0.0001 or lower is the most suitable choice for this circuit because it will repress undesired IM2 production to a level where the quantities are extremely low and potentially insignificant. As Km was not the most sensitive part in the circuit, it may be insightful to vary the strength of the Trp Operator and RBS connected to J23100 and examine the impact changing these parts has on the output response.

Likewise, we also varied the Km of the T5 Cumate operator and measured the respective E2 response. We note that decreasing the Km is successful in reducing the undesired expression of E2 to very low levels that may prevent avoid accidental killing. However, decreasing the Km of the T5 Cumate operator also means that the promoter is more sensitive to CymR and therefore, overall expression of E2 is also decreased. Since biological systems are never completely “tight”, small quantities of CymR unbounded to Cumate - that at higher Kms would regarded as insignificant - begin to repress the T5 Cumate operator and expression of E2. Therefore, at lower Km, we will see a decrease in undesired expression of E2 as well as overall expression of E2 which may compromise the killing mechanism. As we do not know the kinetics of toxin-antitoxin interactions, we propose that at the T5 Cumate operator at 0.1Km is suitable because we assume that only low concentrations of antitoxin are needed for the killing mechanism. At 0.1Km, we believe a balance is achieved between overall expression of E2 and undesired expression of E2. In addition, since IM2 will also be produced in the initial stages, we propose that with the two circuits coupled, the effect that undesired E2 has on the circuit will not be as significant. If experimenters are concerned that the undesired E2 expression is still too high, one can modify the initial concentration of CymR or modify the strength of promoters to rectify this issue.

Limitations of the Model

Models of the Tryptophan and Cumate circuit represent a general understanding of the system; however, they are not without their limitations. As stated previously, the models are limited due to assumptions in regarding kinetics of the promoter, interactions between the repressors and the molecules, interactions between the repressor and the promoters, and the interaction between E2 and IM2. To rectify these issues, further characterisation of the kinetics involved is needed. Within the model, we have tried to assume reasonable arbitrary values of unknown kinetics as well as provide possible solutions that deal with unwanted behaviour of the model that can perhaps be caused by different kinetic values in practice.

Finally, modelling results are not true representations of results but rather only general representations; models only offer a guide for experimenters. Parameters such as humidity, pH, medium, strain type and temperature are only a few of the many parameters known to affect results. However, as current modelling systems cannot capture all possible parameters, they cannot match experimental results in terms value. Therefore, the purpose of modelling is to guide the experimenter by presenting them with insight and understanding of their system. This advantage, saves the experimenter time and resources during the experiment phase.

We hope you find these models helpful. Do let us know if you have any questions. For collaboration purposes, if you can display the graphs on your website, or acknowledge our contribution in any other way that would fantastic. We have listed StockholmiGEM's contributions to our project with the delivery of E2 embedded in the genome.

All the best and see you in Boston!

NUS-GEM