BENG 221 – Mathematical Methods in Bioengineering

Project Report

A Mathematical Model of a Synthetically Constructed Genetic Toggle Switch

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INTRODUCTION

Biological cells are extremely sophisticated systems capable of executing myriad complex functions. The impressive suite of tasks that cells are capable of -such as dynamically monitoring their environment and accurately performing tasks like cellular division in noisy environments with very low error rates- is a result of the workings of genetic circuits. A genetic circuit is a set of biomolecules that interact to perform a dynamical function[1]. Typical components of genetic circuits are genes, promoters and transcription factors.

A gene is defined as a region of DNA that is transcribed as a single unit and carries information for a discrete hereditary characteristic, usually corresponding to an end product of one or several proteins and in some cases one or several RNA's[2]. A promoter is a nucleotide sequence in DNA to which RNA polymerase binds to begin transcription, converting the DNA sequence of the gene into an RNA sequence[2].
A transcription factor is a protein that regulates the transcription rate of specific target genes. An activator is a transcription factor that increases the rate of transcription of a gene when it binds a specific site in the gene's promoter and a repressor is a transcription factor that decreases the rate of transcription when it binds a specific site in the promoter of a gene[1]. Figure 1 shows a diagram of a simple genetic circuit.

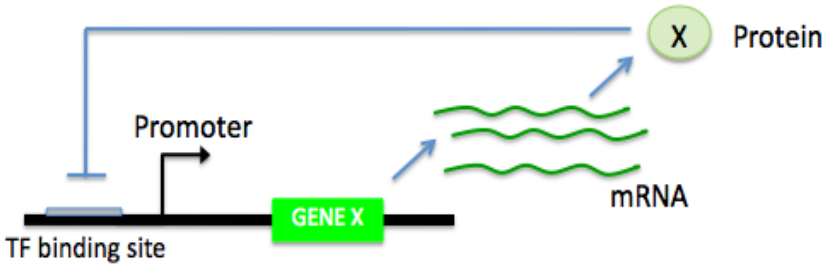

Figure 1: Diagram of a simple genetic circuit. Gene X produces protein X, which is a repressor and binds upstream of the promoter of gene X (indicated by the arrow) at the transcription factor (TF) binding site, thereby reducing the rate of its own transcription. This process is termed negative autoregulation[1].

In the past two decades, advances in genomics and genetic engineering have made it possible to design and construct *synthetic* gene circuits not found in nature, similar to the way an electrical engineer would construct an electrical circuit. Combined with computational approaches aimed at understanding the function of the synthetically constructed circuits, this practice is now known as synthetic biology. Synthetic biology was born with the broad goal of engineering or 'wiring' biological circuitry for manifesting logical forms of cellular control[3].

One of the first synthetic gene networks was the toggle switch[4], which was constructed in *Escherichia coli* by Tim Gardner, Charles Cantor and Jim Collins. The toggle switch emulates the ubiquitous and extremely useful Reset-set latch in electronics, and is also reminiscent of the naturally occurring bacteriophage λ switch. Figure 2 highlights the similarities among these switches.

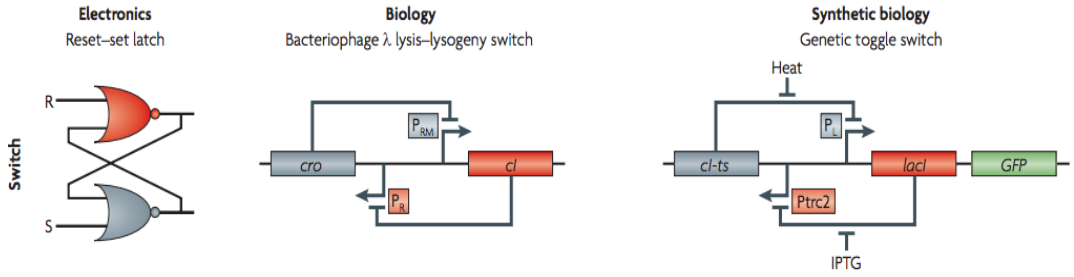

Figure 2: The reset-set latch, bacteriophage λ switch and a synthetically constructed genetic toggle switch[3].

The toggle switch is a useful construct because it exhibits bistability and as a result can be used as a form of memory storage. Although many different kinds of genetic toggle switches have been constructed, the overall design, shown in Figure 3, is similar.

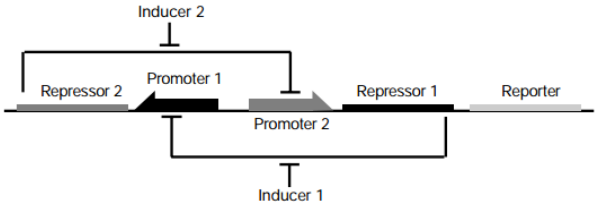

Figure 3: General overall design of a genetic toggle switch. Repressor 1 inhibit transcription from Promoter 1 and is induced by Inducer 1. Repressor 2 inhibits transcription from Promoter 2 and is induced by Inducer 2 [4].

Two promoters of equal strength drive expression of genes that are able to repress the other promoter (the one that did not result in their own expression). One of the promoters also drives expression of green fluorescent protein (or some other reporter gene), which is not able to repress any of the promoters, but rather serves as the 'high' output state of the circuit. Additionally, the repressor proteins are able to be inhibited by adding an inducer to the system; in the case of the first synthetic toggle switch the inducers used were heat the the small molecule IPTG. Thus by inhibiting repressors, inducers stimulate or induce gene expression from a promoter. Adding an inducer can flip the state of the system to the 'high' (GFP on) or 'low' state (GFP off) depending on which is added.

PROBLEM STATEMENT

The goal of this project was to create a mathematical model of the first synthetically constructed genetic toggle switch[4], in order to investigate the dynamic behavior of the circuit. This was accomplished by setting up a system of nonlinear ordinary differential equations based mass action kinetics. First, the system was simplified in order to arrive at an analytical solution and then the nonlinear system was solved using numerical techniques. The results of the two approaches were subsequently compared. Finally, results from both the analytical and numerical approaches were used to explore the stability of the system and how the output of the toggle switch is affected by various parameters and input combinations.

METHODS

Mass Action Kinetics

A common approach taken to model genetic circuits is based on mass action kinetics. Mass action kinetics state that the rate of a reaction is the product of a rate constant (k) times the mass(S). The mass in our case is concentration of the substrate (S) of the reaction[5]. In order to describe a genetic circuit in this way, the following assumptions were made:

1) Many complexities of the molecular interactions in a cell, such as polymerase binding, ribosome binding, etc. are ignored, as considering these factors would substantially increase the complexity of the model.
2) Since input signals usually change transcription factor activities on a sub-second timescale, binding of active transcription factors to their DNA sites often reaches equilibrium within seconds. Transcription and translation operate on minute time scales, and accumulation of protein can take many minutes to hours. Thus, the transcription factor activity levels can be considered to be at steady state within the equations that describe network dynamics on the slow timescale of changes in protein level[1].
3) Aspects of cellular heterogeneity are ignored, meaning spatial paramters, heat and diffusion gradients, transport times in space etc. are not considered, as these would give rise to partial differential equations.

In the case of gene expression, the reactions of importance are those concerning the conversion of DNA to mRNA to protein, the central dogma of biology, which can be described in terms of kinetics as shown in Figure 3.

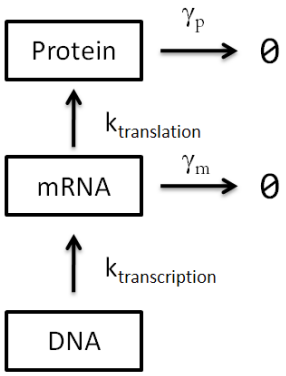

Figure 3: Information flow from a gene in terms of kinetics. The rate constant $k_{transcription}$ describes the rate at which DNA is converted to mRNA, $k_{translation}$ describes the rate at which mRNA is converted to protein and γ_m describes the rate at which mRNA is degraded. Finally γ_p describes the rate at which protein is degraded.

Using the rate constants and degradation rates shown above in Figure 3, it is possible to write out a set of chemical equations, shown below.

$$DNA \xrightarrow{k_{transcription}} DNA + mRNA$$

$$mRNA \xrightarrow{k_{translation}} mRNA + Protein$$

$$RNA \xrightarrow{\gamma_m} \emptyset$$

$$Protein \xrightarrow{\gamma_p} \emptyset$$

From these chemical equations it is then possible to develop a system of differential equations that describes the rate of change of mRNA and protein level over time. These ordinary differential equations are shown below.

$$\frac{d[mRNA]}{dt} = k_{transcription}[DNA] - \gamma_m[mRNA] \quad \textbf{(1)}$$

$$\frac{d[Protein]}{dt} = k_{translation}[mRNA] - \gamma_p[Protein] \quad \textbf{(2)}$$

A simple synthetic toggle switch can be modeled by expanding on these mass action kinetics.

The Input Function of a Gene for a Transcriptional Repressor

Before proceeding to use the above differential equations for modeling the synthetically constructed genetic toggle switch, it is necessary to formulate a more biologically accurate description of how a transcription factor regulates the expression of a gene. Two basic considerations need to be taken into account to create a more accurate mass action model:

1) Activators and Repressors must often to combine to form a complex of *n* proteins before they are functional. Therefore transcription factors cooperatively regulate expression of genes.

2) Inducers must bind to each activator or repressor in the complex.

When cooperativity is taken into account, it is possible to derive an 'input function' for a gene, known as the Hill Equation. Derivation of the Hill Equation for a repressor protein *S* is shown below.

Consider some free inducer *X** that binds to a multimer of *n* repressor proteins, giving rise to a complex *nSX*, and assume that there are no intermediate states were fewer than *n* molecules are bound. This can be summarized by the following chemical equation:

$$[nS] + [X^*] \leftrightarrow [nSX]$$

From the above equation, the following conservation equation, describing the total concentration of inducer can be obtained:

$$[nSX] + [X^*] = X_{TOTAL}$$

Furthermore, two rate equations, describing the formation and decomposition of the complex can be obtained:

$$\text{rate of complex formation} = k_{ON}[X^*][S^n]$$

$$\text{rate of dissociation} = k_{OFF}[nSX]$$

The rate of complex formation at steady state is therefore:

$$\frac{d[nSX]}{dt} = k_{ON}X^*S^n - k_{OFF}[nSX] = 0$$

The fraction of unbound repressor taking into account cooperativity of inducer binding and repressor dimerization is given by Equation 3. The rate of mRNA production is the given by Equation 4, where β is a constant equal to the rate of transcription. The constants $K^{n2}{}_X$ and $K^{n1}{}_d$ defined as k_{ON}/k_{OFF} .

$$\frac{X^*}{X_T} = \frac{1}{1 + \left(\frac{S}{K_X}\right)^{n_2}} \quad \textbf{(3)}$$

$$promoter\ activity = \frac{\beta}{1 + \left(\frac{X^*}{K_d}\right)^{n_1}} \quad \textbf{(4)}$$

Equations 3 and 4 can then be incorporated into 1 and 2 in order to yield Equations 5 -8. These will be the final form of the equations used to model the synthetically constructed genetic toggle switch. Note that $\beta = k_{transcription}$.

$$\frac{d[mRNA_1]}{dt} = \frac{k_{transcription}[DNA_1]}{1 + \left(\frac{[Repressor_1]}{\left(1 + \left(\frac{[Inducer_1]}{K_s}\right)^{n_2}\right)K_d}\right)^{n_1}} - \gamma_m[mRNA_1] \quad \textbf{(5)}$$

$$\frac{d[Repressor_2]}{dt} = k_{translation}[mRNA_1] - \gamma_p[Repressor_2] \quad \textbf{(6)}$$

$$\frac{d[mRNA_2]}{dt} = \frac{k_{transcription}[DNA_2]}{1 + \left(\frac{[Repressor_2]}{\left(1 + \left(\frac{[Inducer_2]}{K_s}\right)^{n_2}\right)K_d}\right)^{n_1}} - \gamma_m[mRNA_2] \quad \textbf{(7)}$$

$$\frac{d[Repressor_1]}{dt} = k_{translation}[mRNA_2] - \gamma_p[Repressor_1] \quad \textbf{(8)}$$

Analytical Solution for a Simplified System

In order to obtain a system of ODE's that could be solved analytically, the genetic toggle switch was 'cut in half', yielding a simple circuit consisting of a single repressible promoter, as shown in Figure 4. This system can be represented by one equation describing the rate of change of mRNA (such as Equations 5 or 7) and one equation describing the rate of change of protein (such as Equations 6 or 8).

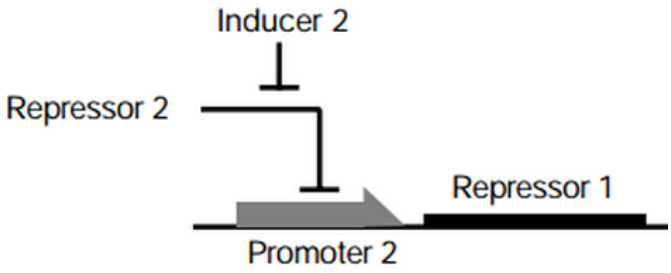

Figure 4: Simplified system used to obtain analytical solution.

Assuming that the repressor and inducer concentration are held at a constant value allows the equation for the rate of change of mRNA over time to be greatly simplified. For the equation of *d[mRNA]/dt* for the simplified system in Figure 4, we defined a constant *K*, shown below:

$$K = \frac{k_{transcription}[DNA]}{1 + \left(\frac{[Repressor]}{\left(1 + \left(\frac{[Inducer]}{K_s}\right)^{n_2}\right) K_d} \right)^{n_1}}$$

Note that the value of *K* is small when *[Repressor]* is high *K* is large when *[Inducer]* is high. Plugging *K* into the rate equation for mRNA yields the following system of ordinary differential equations.

$$\frac{d[mRNA]}{dt} = K - \gamma_m[mRNA] \quad \textbf{(9)}$$

$$\frac{d[Protein]}{dt} = k_{translation}[mRNA] - \gamma_p[Protein] \quad \textbf{(10)}$$

Assuming complete repression at t<0 with repression completely removed at t=0 and initial conditions [mRNA](0)=0, [Protein](0)=0, gives the following result:

$$\frac{d[mRNA]}{dt} + \gamma_m[mRNA] = K$$

Solving for the homogeneous or steady state solution of the system gives the following result for the steady state concentration of mRNA:

$$\frac{d[mRNA]}{dt} + \gamma_m[mRNA] = 0$$
$$\lambda + \gamma_m = 0$$
$$\lambda = -\gamma_m$$
$$[mRNA]_h = C_1 e^{-\gamma_m t}$$

The particular solution was found using the method of undetermined coefficients, assuming the particular solution concentration of mRNA was equal to some constant *C*:

$$[mRNA]_p = C$$

Plugging this value into the ODE gives:

$$0 = K - \gamma_m C$$
$$C = \frac{K}{\gamma_m}$$
$$[mRNA]_p = \frac{K}{\gamma_m}$$

The solutions are then combined to give the final solution:

$$[mRNA] = [mRNA]_h + [mRNA]_p = C_1 e^{-\gamma_m t} + \frac{K}{\gamma_m}$$

The initial conditions were then plugged into the final solution in order to solve for the constants:

$$[mRNA] = 0 = C_1 e^0 + \frac{K}{\gamma_m}$$
$$C_1 = -\frac{K}{\gamma_m}$$
$$[mRNA] = \frac{K}{\gamma_m} - \frac{K}{\gamma_m} e^{-\gamma_m t} \quad \textbf{(11)}$$

The expression for [mRNA] was then plugged into Equation 10, the differential equation for the change of protein concentration over time to give:

$$\frac{d[Protein]}{dt} = k_{translation}[mRNA] - \gamma_p[Protein]$$
$$= k_{translation}\left(\frac{K}{\gamma_m} - \frac{K}{\gamma_m}e^{-\gamma_m t}\right) - \gamma_p[Protein]$$

Solving for the homogeneous or steady state solution of the system gives the following result for the steady state concentration of protein (i.e. Repressor 1):

$$\frac{d[Protein]}{dt} + \gamma_p[Protein] = 0$$
$$\lambda + \gamma_p = 0$$
$$\lambda = -\gamma_p$$
$$[Protein]_h = C_1 e^{-\gamma_p t}$$

The particular solution was found using the method of undetermined coefficients:

$$[Protein]_p = A + Be^{-\gamma_m t}$$

Plugging the over equation for the particular concentration of protein gives the following:

$$-\gamma_m Be^{-\gamma_m t} = k_{translation}\left(\frac{K}{\gamma_m} - \frac{K}{\gamma_m}e^{-\gamma_m t}\right) - \gamma_p(A + Be^{-\gamma_m t})$$

A and *B* were then solved for:

$$A = \frac{Kk_{translation}}{\gamma_p\gamma_m}$$

$$B = \frac{Kk_{translation}}{{\gamma_m}^2 - \gamma_p\gamma_m}$$

$$[Protein]_p = \frac{Kk_{translation}}{\gamma_p\gamma_m} + \frac{Kk_{translation}}{{\gamma_m}^2 - \gamma_p\gamma_m}e^{-\gamma_m t}$$

The solutions were then combined to give the full solution:

$$[Protein] = C_1 e^{-\gamma_p t} + \frac{Kk_{translation}}{\gamma_p\gamma_m} + \frac{Kk_{translation}}{{\gamma_m}^2 - \gamma_p\gamma_m}e^{-\gamma_m t}$$

Finally, the constant C_1 was solved for using the initial conditions:

$$[Protein] = 0 = C_1 e^0 + \frac{Kk_{translation}}{\gamma_p\gamma_m} + \frac{Kk_{translation}}{{\gamma_m}^2 - \gamma_p\gamma_m}e^0$$

$$C_1 = -\left(\frac{Kk_{translation}}{\gamma_p\gamma_m} + \frac{Kk_{translation}}{{\gamma_m}^2 - \gamma_p\gamma_m}\right)$$

This gives the final solution for the protein concentration:

$$[Protein] = \frac{Kk_{translation}}{\gamma_p\gamma_m} + \frac{Kk_{translation}}{{\gamma_m}^2 - \gamma_p\gamma_m}e^{-\gamma_m t} - \left(\frac{Kk_{translation}}{\gamma_p\gamma_m} + \frac{Kk_{translation}}{{\gamma_m}^2 - \gamma_p\gamma_m}\right)e^{-\gamma_p t} \quad \textbf{(12)}$$

In the previous model for the simplified system, it was assumed that there was no repression present at $t < 0$ and complete repression at $t=0$. Using the same general equations shown above, a more realistic solution was derived by changing the initial conditions so that at $t=0$ some repressor mRNA and protein is present in the system. The new initial conditions used were $[mRNA](0)=2.5\text{x}10^3$, $[Protein](0)=3.34\text{x}10^5$. When these initial conditions were used to solve Equations 9 and 10, the following solutions for mRNA and protein were obtained.

$$[mRNA] = \frac{K}{\gamma_m} + \left(2.5 \times 10^3 - \frac{K}{\gamma_m}\right)e^{-\gamma_m t} \quad \textbf{(13)}$$

$$[Protein] = \frac{Kk_{translation}}{\gamma_p\gamma_m} + \frac{Kk_{translation}}{{\gamma_m}^2 - \gamma_p\gamma_m}e^{-\gamma_m t} + \left(3.34 \times 10^5 - \left(\frac{Kk_{translation}}{\gamma_p\gamma_m} + \frac{2.5 \times 10^3 - \frac{K}{\gamma_m}}{\gamma_p - \gamma_m}\right)\right)e^{-\gamma_p t} \quad \textbf{(14)}$$

Parameters Values

Parameter values of typical genes and repressors were found in the literature[6] and are shown in Table 1 below. These values were used in all subsequent simulations and models.

Table 1: Parameter values used for the model.

Parameter	**Description**	**Value**
[DNA]	Concentration of DNA	150 copies/cell
$k_{translation}$	Rate of protein synthesis	2.85 min^{-1}
$k_{transcription}$	Rate of RNA synthesis	3.17 min^{-1}
γ_p	Protein degradation rate	2.13*10^-2 min^{-1}
γ_m	mRNA degradation rate	0.19 min^{-1}
K_S	Dissociation constant of inducer	1000 molecules/cell
K_d	Dissociation constant of repressor	0.05 molecules/cell
n_1	Cooperativity of repressor binding to promoter	2
n_2	Cooperativity of inducer binding to repressor	2

RESULTS and DISCUSSION

Analytical Solution

The plots of the analytical solutions for mRNA expression and protein expression over time under various degradation rates, when initial mRNA and protein levels were at zero (Equations 11 and 12) are shown in Figure 5 and 6, respectively.

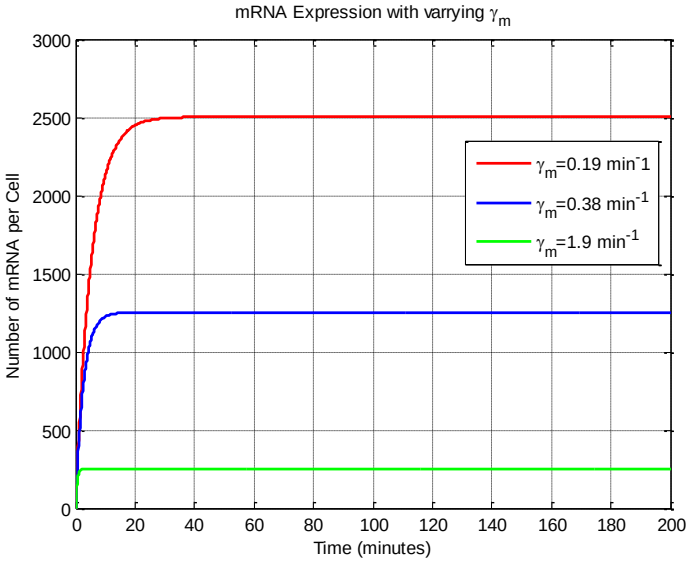

Figure 5: mRNA expression levels over time with various degradation levels γ_m.

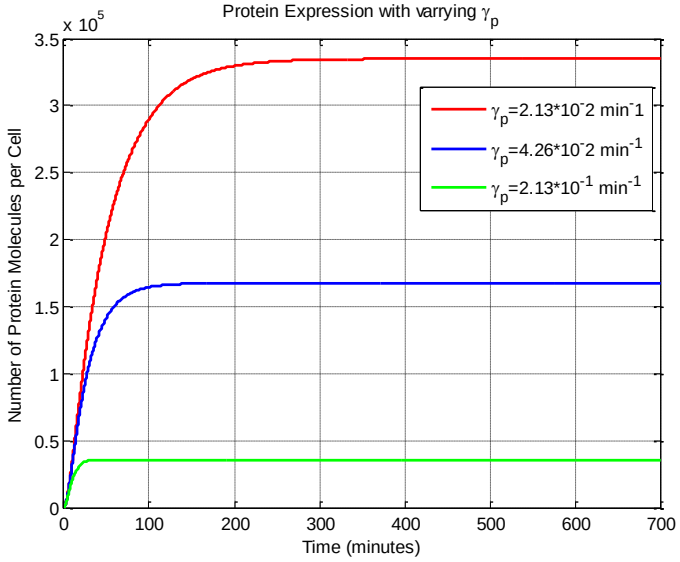

Figure 6: Protein expression levels over time with various degradation levels γ_p.

The system is stable because the negative root of the characteristic equation. Repressor and Inducer concentrations were assumed to be constant and initially not present at t=0. In the absence of any initial repressor, the promoter drives expression of protein until it reaches so steady state constant value, which is the maximal rate of production. However as shown by the plots, the degradation rate of the protein can affect the mRNA and protein steady state values. It can also be seen in Figure 5 and 6 that the degradation rate does not appear to affect the time the system takes to reach steady state, only the maximal value which it can reach.

The initial conditions of the system were changed to $[mRNA](0)=2.5\text{x}10^3$, $[Protein](0)=3.34\text{x}10^5$. The results for steady state mRNA and Protein levels at these initial conditions are shown in Figure 7 and 8 respectively.

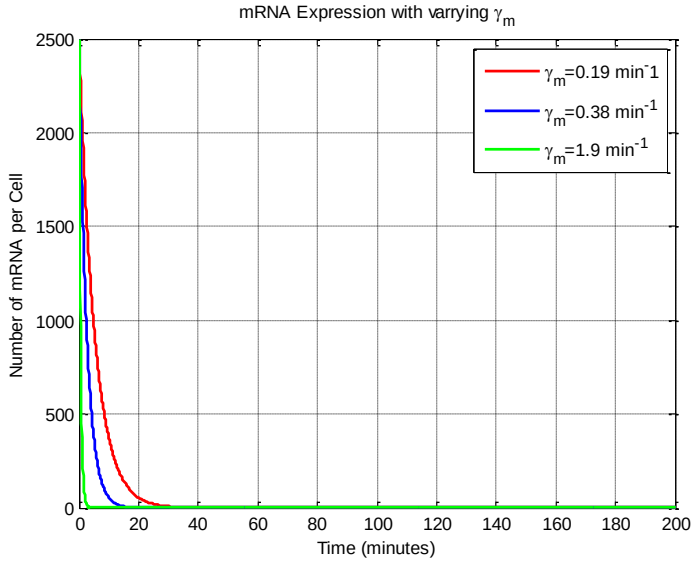

Figure 7: mRNA expression levels over time with various degradation levels γ_m.

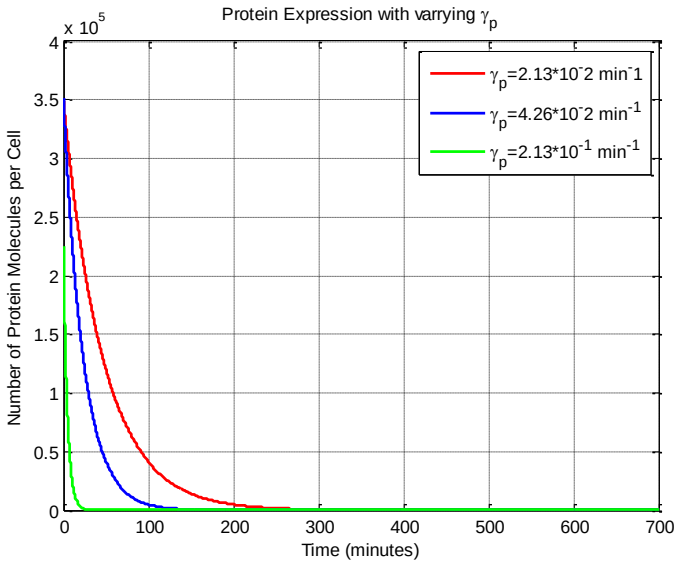

Figure 8: Protein expression levels over time with various degradation levels γ_p.

Figures 7 and 8 show that if an initial amount of repressor protein is present in the system at time zero then this will lead to a steady state in which no protein or mRNA is present. The figures also show that for both mRNA and protein, the system decays toward the steady state value of zero faster for higher degredation rates. This result makes sense because the initial condition of a finite amount of repressor protein present will quickly block transcription from the promoter (which at t=0 is on) and therefore the only protein or mRNA molecules present are those that initially made before transcription was fully blocked. Thus the number of protein and mRNA molecules in the cell is heavily affected by the their respective degradation rates. These results also indicate that the initial concentration of repressor (3.34×10^5) at t=0 is enough to result in full repression of the system (steady state value of 0).

Solving for the steady state of the output can also provide useful information about the appropriate level of inducer to use. Figure 9 shows a plot of the protein concentration for various combinations of repressor and inducer levels.

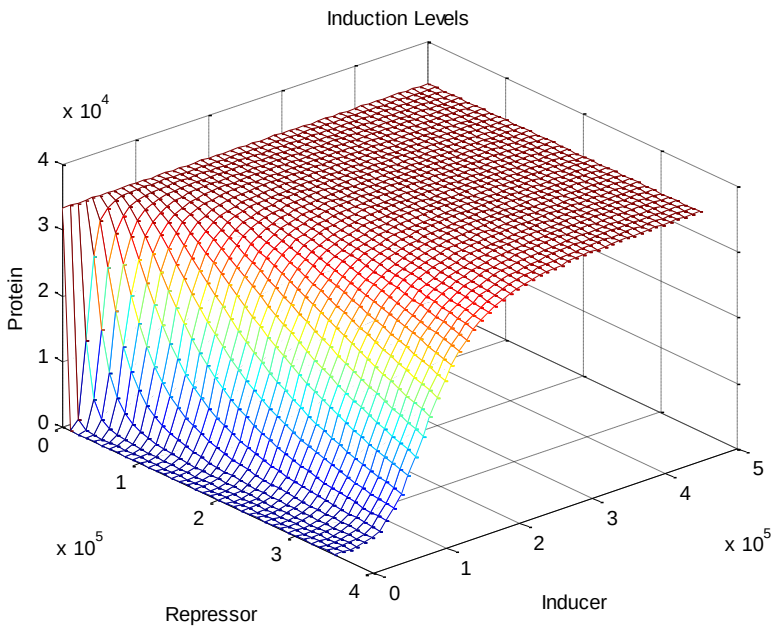

Figure 9: Surface plot describing protein concentration (the output of the system) as a function of the Repressor and inducer levels (inputs of the system).

Numerical Solution

The numerical solution of the system of nonlinear ordinary differential equations (Equations 5-8) for the full genetic toggle switch was obtained using ode45. The numerical and analytical results for the concentrations of the repressor proteins for a various inducer levels are shown below in Figures 10 to 13.

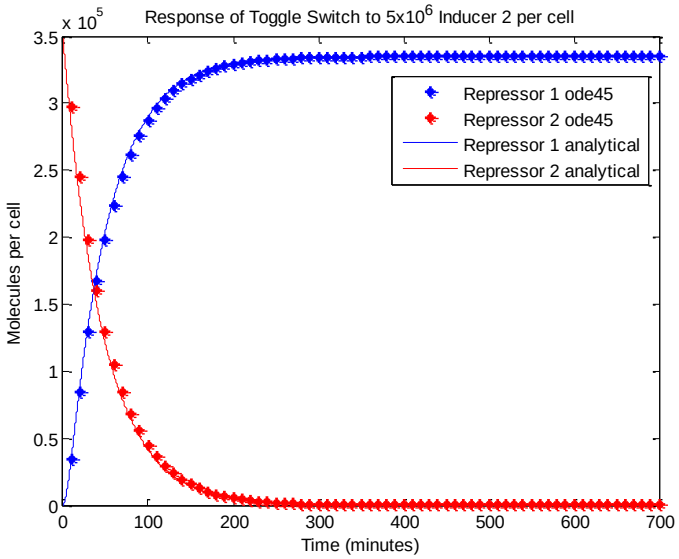

Figure 10: Numerical vs. Analytical solution of the genetic toggle switch with 5×10^6 Inducer 2.

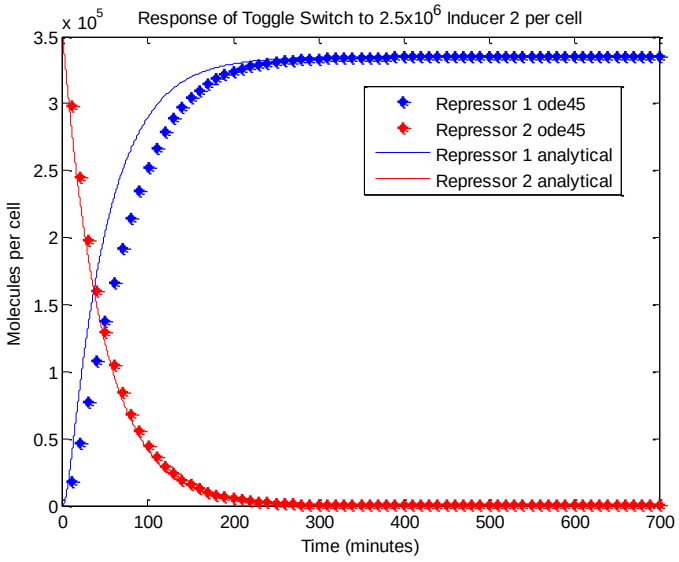

Figure 11: Numerical vs. Analytical solution of the genetic toggle switch with 2.5×10^6 Inducer 2.

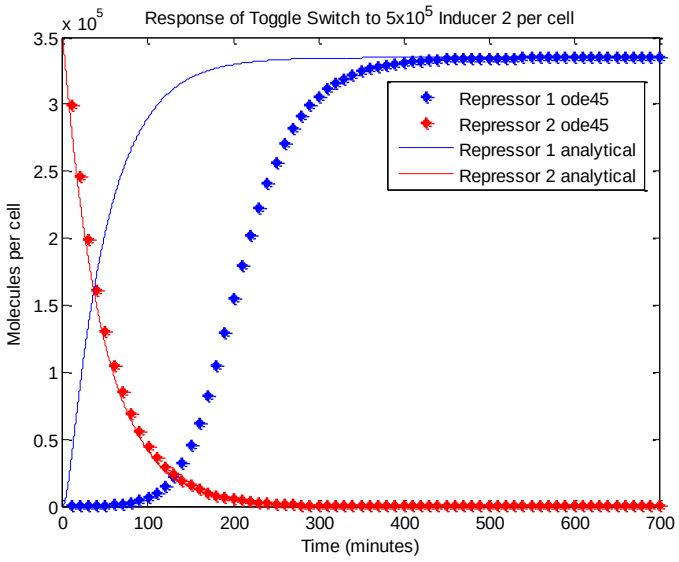

Figure 12: Numerical vs. Analytical solution of the genetic toggle switch with 5×10^5 Inducer 2.

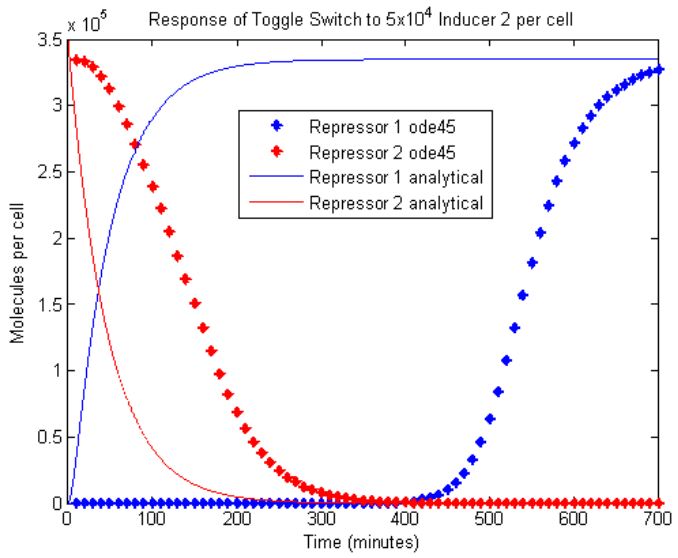

Figure 13: Numerical vs. Analytical solution of the genetic toggle switch with 5×10^4 Inducer 2.

It can be seen from Figures 10 to 13 that there are discrepancies between numerical and analytical solutions as the level of inducer 2 decreases. The analytical response is increasingly faster than the numerical solution as the amount of inducer is increased. The reason for this has to do with the assumptions used to create the analytical model; in the simplified system used to obtain the analytical solution, it is assumed that repressor is instantaneously present or not present. In reality it takes a finite amount of time for repressor levels to build up enough to cause a noticeable effect. This effect is not seen

much when inducer levels are high because enough inducer is present to free up almost all of the promoters driving Repressor 1 expression. The results in a fast transition time between zero and full repression of promoter 1. However as the level of inducer is decreased, the time it takes for Repressor 1 to accumulate is increased. Thus a longer transition time exists between the no repression and full repression states of promoter 1. The numerical solution accounts for this repressor feedback and the analytical does not, therefore this explains the discrepancies between the two solutions.

Using ode45, the toggle switch was simulated over time, as shown in Figure 14.

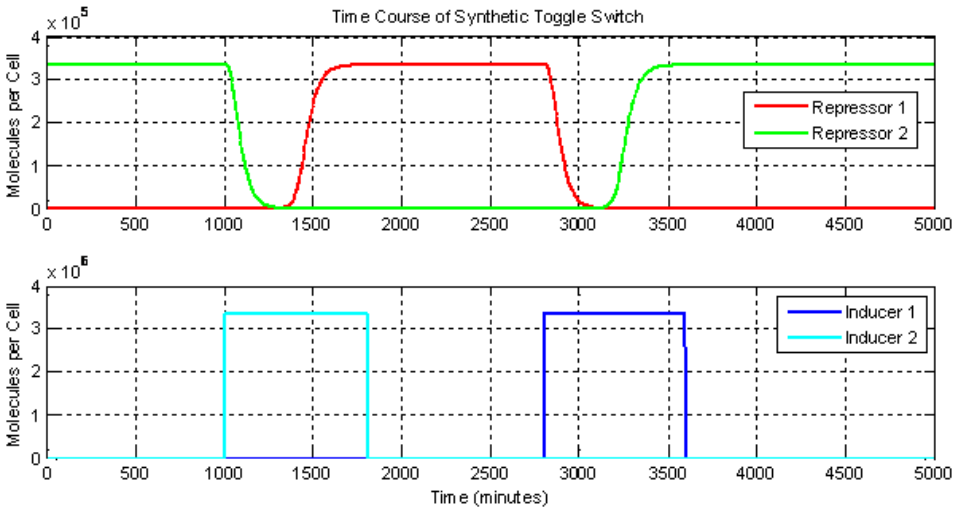

Figure 14:The dynamic behavior of the synthetically constructed genetic toggle switch as inducers are pulsed on and off.

Figure 14 highlights the bistability of the system and show that once the system reaches one of the steady states (Repressor 1 off, Repressor 2 on or Repressor 1 on, Repressor 2 off) it can remain there even after the inducer is taken away.

Conclusion

In this project, we presented a mathematical model of the genetic toggle switch constructed by Tim Gardner, Charles Cantor and Jim Collins. We formulated a system of ordinary differential equations based on mass action kinetics, including the effects of cooperatively in repressor and inducer binding. We then set up a simplified version of the circuit to arrive at an analytical solution and then compared this analytical solution to a numerical solution for the full system and compared the two results. Furthermore, we explore the stability of the system and how the output of the toggle switch is affected by various parameters and input combinations.

Our results show that a simplified analytical solution can accurately describe certain aspects of the system and that more complex models can capture more intricacies and details of the system. It was shown that the simplified analytical solution accurately describes the system under certain conditions and that under the assumed conditions and parameters, the system will reach a stable state.

In reality, finding parts in biology that perfectly fit these parameters is difficult. However progress in synthetic biology over the past decade has been rapid, and will eventually lead to more real-world applications of biological technologies in medicine. Mathematical modeling is a critical component of effective circuit design and will continue to be as the field of synthetic biology advances. Figure 15 gives an example of the power of mathematical modeling and how it can be used to investigate circuit design and function, even if currently those circuits are too difficult to experimentally build.

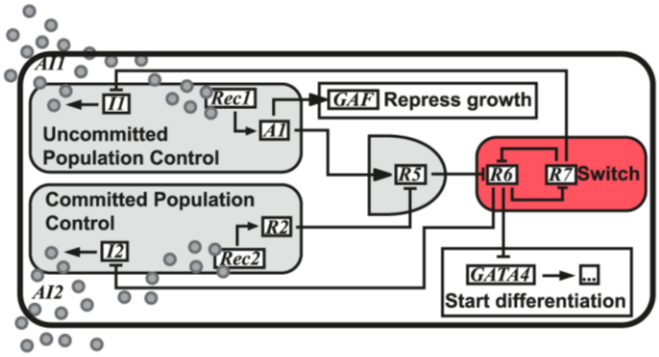

Figure 15: A synthetic gene circuit for artificial tissue homeostasis. This paper presents the computational design and analysis of a diabetes therapy based on synthetic biology where genetically programmed stem cells maintain a steady population of β-cells despite continuous turnover. A toggle switch (highlighted in red) is a critical component of this system.[7]

REFERENCES

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3) Khalil et al., "Synthetic Biology: Applications Come of Age." *Nature Reviews Genetics* (2010)

4) Gardner et al., "Construction of a Genetic Toggle Switch in *Escherichia Coli*." *Nature* (2000)

5) http://www.ccam.uchc.edu/ccb/Kinetics/RateLaws/MassAction.htm

6) Ceroni et al., "A computational model of gene expression in an inducible synthetic circuit." *Pacific Symposium on Biocomputing* (2010)

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MATLAB CODE

```
%Nicholas Csicsery and Ricky O'Laughlin
clear
clc

%Set Parameters
ktr=3.17; %mRNA synthesis rate per minute (rate of transcription)
ktrans=2.85; %Protein synthesis rate per minute (rate of translation)
yp=2.13*10^-2; %Per minute
ym=0.19; %Per minute
Ks=1000; %Dissociation constant for inducer
Kd=0.05; %Dissociation constant for repressor
DNA=150; %Number of plasmids per cell
n1=2; %Cooperativity of repressor
n2=2; %Cooperativity of inducer

%Assume the the repressor is removed at t=0
Repressor=0;
Inducer=0;

t=0:0.1:700; %Set time span

%Calculate mRNA and Protein values
K=ktr*DNA/(1+(Repressor/((1+(Inducer/Ks)^n2)*Kd))^n1);
mRNA=K/ym-K/ym*exp(-ym*t);
Protein=K*ktrans/(yp*ym)+K*ktrans/(ym^2-yp*ym)*exp(-ym*t)-
(K*ktrans/(yp*ym)+K*ktrans/(ym^2-yp*ym))*exp(-yp*t);
disp('Steady State RNA Concentration:')
disp(mRNA(end)) %Steady state mRNA value
disp('Steady State Protein Concentration:')
disp(Protein(end)) %Steady state Protein value

%Plot protein concentrations over time
clf
figure(1)
plot(t,Protein,'r', 'LineWidth',2)
grid on
hold on
yp=4.26*10^-2; %Change protein degradation rate
Protein=K*ktrans/(yp*ym)+K*ktrans/(ym^2-yp*ym)*exp(-ym*t)-
(K*ktrans/(yp*ym)+K*ktrans/(ym^2-yp*ym))*exp(-yp*t);
plot(t,Protein,'b', 'LineWidth',2)
yp=2*10^-1; %Change protein degradation rate
Protein=K*ktrans/(yp*ym)+K*ktrans/(ym^2-yp*ym)*exp(-ym*t)-
(K*ktrans/(yp*ym)+K*ktrans/(ym^2-yp*ym))*exp(-yp*t);
plot(t,Protein,'g', 'LineWidth',2)
xlabel('Time (minutes)')
ylabel('Number of Protein Molecules per Cell')
title('Protein Expression with varrying \gamma_p')
legend('\gamma_p=2.13*10^-2 min^-1','\gamma_p=4.26*10^-2 min^-
^1','\gamma_p=2.13*10^-^1 min^-^1')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
clear mRNA Protein
```

```
%Assume Repressor is added at t=0
Repressor=3.5*10^5;
yp=2.13*10^-2; %Set a new protein degradation rate

%Calculate mRNA and Protein values
K=ktr*DNA/(1+(Repressor/((1+(Inducer/Ks)^n2)*Kd))^n1);
mRNA=K/ym+(2.5*10^3-K/ym)*exp(-ym*t);
Protein=K*ktrans/(yp*ym)+K*ktrans/(ym^2-yp*ym)*exp(-ym*t)+(3.34*10^5-
(K*ktrans/(yp*ym)+(2.5*10^3-K/ym)/(yp-ym)))*exp(-yp*t);

%Plot protein concentrations over time
figure(2)
plot(t,Protein,'r', 'LineWidth',2)
grid on
hold on
yp=4.26*10^-2; %Change protein degradation rate
Protein=K*ktrans/(yp*ym)+K*ktrans/(ym^2-yp*ym)*exp(-ym*t)+(3.34*10^5-
(K*ktrans/(yp*ym)+(2.5*10^3-K/ym)/(yp-ym)))*exp(-yp*t);
plot(t,Protein,'b', 'LineWidth',2)
yp=2.13*10^-1; %Change protein degradation rate
Protein=K*ktrans/(yp*ym)+K*ktrans/(ym^2-yp*ym)*exp(-ym*t)+(3.34*10^5-
(K*ktrans/(yp*ym)+(2.5*10^3-K/ym)/(yp-ym)))*exp(-yp*t);
plot(t,Protein,'g', 'LineWidth',2)
xlabel('Time (minutes)')
ylabel('Number of Protein Molecules per Cell')
title('Protein Expression with varrying \gamma_p')
legend('\gamma_p=2.13*10^-2 min^-1','\gamma_p=4.26*10^-2 min^-
^1','\gamma_p=2.13*10^-^1 min^-^1')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Create 3D plot of Steady State Protein expression
R=0:10000:3.5*10^5; %Set range of repressor values
I=0:10000:5*10^5; %Set range of inducer values
[Repressor,Inducer]=meshgrid(R,I);
K=ktr*DNA./(1+(Repressor./(1+(Inducer/Ks).^n2)*Kd).^n1); %Calculate
Proteinss=ktrans*(K./ym)./yp;
figure(3)
mesh(R,I,Proteinss); %Plot mesh
xlabel('Repressor')
ylabel('Inducer')
zlabel('Protein')
title('Induction Levels')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Solve the toggle switch problem numerically
[t2,P]=ode45(@syntoggle,0:10:700,[2.5*10^3, 3.34*10^5, 0, 0]);
%Repressor 2 starts high

%Plot the solution to the numerical approximation
figure(4)
plot(t2,P(:,4),'*b','LineWidth',2)
hold on
plot(t2,P(:,2),'*r','LineWidth',2)

%Recreate the analytical case where Repressor is removed at t=0
clear mRNA Protein K Repressor Inducer
```

```
Repressor=0;
Inducer=0;
yp=2.13*10^-2; %Per minute
K=ktr*DNA/(1+(Repressor/((1+(Inducer/Ks)^n2)*Kd))^n1);
Protein=K*ktrans/(yp*ym)+K*ktrans/(ym^2-yp*ym)*exp(-ym*t)-
(K*ktrans/(yp*ym)+K*ktrans/(ym^2-yp*ym))*exp(-yp*t);
plot(t,Protein,'b') %Plot with numerical solution

%Recreate the analytical case where Repressor is added at t=0
clear mRNA Protein K Repressor Inducer
Repressor=3.5*10^5;
Inducer=0;
yp=2.13*10^-2; %Per minute
K=ktr*DNA/(1+(Repressor/((1+(Inducer/Ks)^n2)*Kd))^n1);
Protein=K*ktrans/(yp*ym)+K*ktrans/(ym^2-yp*ym)*exp(-ym*t)+(3.34*10^5-
(K*ktrans/(yp*ym)+(2.5*10^3-K/ym)/(yp-ym)))*exp(-yp*t);
plot(t,Protein,'r') %Plot with the numerical solution
xlabel('Time (minutes)')
ylabel('Molecules per cell')
title('Response of Toggle Switch to 5x10^6 Inducer 2 per cell') %Change
depending on inducer concentration
legend('Repressor 1 ode45','Repressor 2 ode45','Repressor 1
analytical','Repressor 2 analytical')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Observe Time Course for Toggle Switch
clear

%Same as previous numerical solution, but inducer values pulsed
[t,P]=ode45(@syntoggle2,[0,5000],[2.5*10^3, 3.34*10^5, 0, 0]);
Inducer1=3.34*10^6*(t>2800&t<3600); %Pulse each inducer
Inducer2=3.34*10^6*(t>1000&t<1800);

%Plot over time
figure(5)
subplot(2,1,1) %Plot of protein levels
plot(t,P(:,4),'r','LineWidth',2)
hold on
plot(t,P(:,2),'g','LineWidth',2)
grid on
ylabel('Molecules per Cell')
legend('Repressor 1','Repressor 2')
title('Time Course of Synthetic Toggle Switch')
axis([0,5000,0,4*10^5]);

subplot(2,1,2)%Plot of inducer levels
plot(t,Inducer1,'b','LineWidth',2)
hold on
plot(t,Inducer2,'c','LineWidth',2)
grid on
xlabel('Time (minutes)')
ylabel('Molecules per Cell')
legend('Inducer 1','Inducer 2')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Observe RNA dynamics
```

```
clear

Repressor=0; %Case where Repressor is removed at t=0

ktr=3.17; %mRNA synthesis rate per minute (rate of transcription)
ktrans=2.85; %Protein synthesis rate per minute (rate of translation)
yp=2.13*10^-2; %Per minute
ym=0.19; %Per minute
Ks=1000; %Dissociation constant for inducer
Kd=0.05; %Dissociation constant for repressor
DNA=150; %Number of plasmids per cell
n1=2; %Cooperativity of repressor
n2=2; %Cooperativity of inducer
Inducer=0;
t=0:0.1:200; %Time span

%Calculate mRNA concentration
K=ktr*DNA/(1+(Repressor/((1+(Inducer/Ks)^n2)*Kd))^n1);
mRNA=K/ym-K/ym*exp(-ym*t);

%Plot
figure(6)
plot(t,mRNA,'r','LineWidth',2)
hold on
ym=0.38; %Change mRNA degradation rate
mRNA=K/ym-K/ym*exp(-ym*t);
plot(t,mRNA,'b','LineWidth',2)
ym=1.9; %Change mRNA degradation rate
mRNA=K/ym-K/ym*exp(-ym*t);
plot(t,mRNA,'g','LineWidth',2)
grid on
xlabel('Time (minutes)')
ylabel('Number of mRNA per Cell')
title('mRNA Expression with varrying \gamma_m')
legend('\gamma_m=0.19 min^-1','\gamma_m=0.38 min^-^1','\gamma_m=1.9
min^-^1')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
clear

Repressor=3.5*10^5; %Case where Repressor is added at t=0

ktr=3.17; %mRNA synthesis rate per minute (rate of transcription)
ktrans=2.85; %Protein synthesis rate per minute (rate of translation)
yp=2.13*10^-2; %Per minute
ym=0.19; %Per minute
Ks=1000; %Dissociation constant for inducer
Kd=0.05; %Dissociation constant for repressor
DNA=150; %Number of plasmids per cell
n1=2; %Cooperativity of repressor
n2=2; %Cooperativity of inducer
Inducer=0;
t=0:0.1:200; %Time span

%Calculate mRNA concentration
```

```
K=ktr*DNA/(1+(Repressor/((1+(Inducer/Ks)^n2)*Kd))^n1);
mRNA=K/ym+(2.5*10^3-K/ym)*exp(-ym*t);

%Plot
figure(7)
plot(t,mRNA,'r','LineWidth',2)
hold on
ym=0.38; %Change mRNA degradation rate
mRNA=K/ym+(2.5*10^3-K/ym)*exp(-ym*t);
plot(t,mRNA,'b','LineWidth',2)
ym=1.9; %Change mRNA degradation rate
mRNA=K/ym+(2.5*10^3-K/ym)*exp(-ym*t);
plot(t,mRNA,'g','LineWidth',2)
grid on
xlabel('Time (minutes)')
ylabel('Number of mRNA per Cell')
title('mRNA Expression with varrying \gamma_m')
legend('\gamma_m=0.19 min^-1','\gamma_m=0.38 min^-^1','\gamma_m=1.9
min^-^1')
```

Functions

```
function dP=syntoggle(t,P);

%Parameters
ktr=3.17; %mRNA synthesis rate per minute (rate of transcription)
ktrans=2.85; %Protein synthesis rate per minute (rate of translation)
yp=2.13*10^-2; %Per minute
ym=0.19; %Per minute
Ks=1000; %Dissociation constant for inducer
Kd=0.05; %Dissociation constant for repressor
DNA=150; %Number of plasmids per cell
Inducer1=0; %Specify inducer values
Inducer2=5*10^6; %This value is changed for the different plots
n1=2; %Cooperativity of repressor
n2=2; %Cooperativity of inducer

%Define components of ODEs
mRNA1=P(1);
Repressor2=P(2);
mRNA2=P(3);
Repressor1=P(4);
K1=ktr*DNA/(1+(Repressor1/((1+(Inducer1/Ks)^n2)*Kd))^n1);
K2=ktr*DNA/(1+(Repressor2/((1+(Inducer2/Ks)^n2)*Kd))^n1);

%Write ODEs
dP(1)=K1-ym*mRNA1;
dP(2)=ktrans*mRNA1-yp*Repressor2;
dP(3)=K2-ym*mRNA2;
dP(4)=ktrans*mRNA2-yp*Repressor1;

dP=dP'; %Transpose matrix

end
```

```
function dP=syntoggle2(t,P);

%Parameters
ktr=3.17; %mRNA synthesis rate per minute (rate of transcription)
ktrans=2.85; %Protein synthesis rate per minute (rate of translation)
yp=2.13*10^-2; %Per minute
ym=0.19; %Per minute
Ks=1000; %Dissociation constant for inducer
Kd=0.05; %Dissociation constant for repressor
DNA=150; %Number of plasmids per cell
Inducer1=3.5*10^6*(t>2800&t<3800); %Give pulses of each inducer
Inducer2=3.5*10^6*(t>1000&t<2000);
n1=2; %Cooperativity of repressor
n2=2; %Cooperativity of inducer

%Define components of ODEs
mRNA1=P(1);
Repressor2=P(2);
mRNA2=P(3);
Repressor1=P(4);
K1=ktr*DNA/(1+(Repressor1/((1+(Inducer1/Ks)^n2)*Kd))^n1);
K2=ktr*DNA/(1+(Repressor2/((1+(Inducer2/Ks)^n2)*Kd))^n1);

%Write ODEs
dP(1)=K1-ym*mRNA1;
dP(2)=ktrans*mRNA1-yp*Repressor2;
dP(3)=K2-ym*mRNA2;
dP(4)=ktrans*mRNA2-yp*Repressor1;

dP=dP'; %Transpose matrix

end
```