Problem Set #2
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1 Problem 1

To see the effect of parameters on the steady state mRNA model, the original values were decreased and increased by 20%. Table One reports the "increasing" effects as the "decreasing" effect was more or less equal and opposite. Problem Set 1 includes all sources and rationales for original values. Note that k_A was assumed to be zero so changing this value wouldn't have an effect although increasing abortion would probably lower mRNA concentrations. Also note that increasing the level of change to a 50% difference didn't make the plots that didn't change start to change meaning they are not sensitive to these parameters. Finally, gene length L was not recorded because is has the predicted inverse effect of k_{Ej} due to the inverse proportion between them. Appendix A has every plot produced by CHEME5440HW2.m that motivates the descriptions in Table One.

Table One: Effect of Key Parameters on mRNA Profile

Parameter	*Symbol*	*Effect when Increased*
Elongation Rate	k_E	Increases mRNA concentration at all levels although more so once induced at high inducer concentration
Degradation rate	k_X^d	Decreases mRNA concentration at all levels although more so once induced at higher inducer concentration
Gene Concentration	G	No effect
Rate of Open Complex Formation	k_I	Increases mRNA concentration at all levels although almost all once induced at higher inducer concentrations
Rate of Closed Complex Formation	k_+	No effect
Rate of Closed Complex Dissociation	k_-	No effect
Saturation Constant	K_X	No effect
Specific Growth Rate	μ	Only decreases mRNA concentration once induced or higher inducer concentration
Cooperativity Constant	n	Only decreases mRNA at the regions not at kinetic limit or lower to middle inducer concentrations
RNA Polymerase Concentration	R_{XT}	Increases mRNA concentration at all levels although more so once induced at higher inducer concentration
Time Constant	τ	Decreases mRNA concentration at all levels although more so once induced at higher inducer concentrations
Weight Without Inducer	W_1	Only increases mRNA at the regions not induced or low inducer concentrations
Weight With Inducer	W_2	Only increases mRNA in the middle regions when the step is occurring but before kinetic limit
Affinity Constant	K	Only decreases mRNA in the middle regions when the step is occurring but before kinetic limit
Hill Function	f_I	Only decreases mRNA at the regions not at kinetic limit or lower to middle inducer concentrations

2 Problem 2 Part A

Starting with transcription, we can write an mRNA balance where the dilution term $B^{-1}B^{'}$ is already substituted as specific growth rate:

$$\frac{dm_1}{dt} = r_{X1}u_{X1} - k^d_{X1}m_1 - \mu m_1$$
$$\frac{dm_2}{dt} = r_{X2}u_{X2} - k^d_{X2}m_2 - \mu m_2$$
$$\frac{dm_3}{dt} = r_{X3}u_{X3} - k^d_{X3}m_3 - \mu m_3$$

Where

$$r_{Xj} = k_{EXj}R_{XT}\frac{G_j}{K_{Xj}\tau_{Xj} + (\tau_{Xj}+1)G_j}$$

And

$$u_{X1} = \frac{W_{RT1} + W_I f_I}{1 + W_{RT1} + W_I f_I}$$
$$u_{X2} = \frac{W_{RT2} + W_{12}f_{12} + W_{32}f_{32}}{1 + W_{RT2} + W_{12}f_{12} + W_{32}f_{32}}$$
$$u_{X3} = \frac{W_{RT3} + W_{13}f_{13} + W_{23}f_{23}}{1 + W_{RT3} + W_{13}f_{13} + W_{23}f_{23}}$$

Where each f_{ij} is described as:

$$f_I = \frac{I^n}{K^n + I^n}$$
$$f_{12} = \frac{p_1^n}{K^n + p_1^n}$$
$$f_{13} = \frac{p_1^n}{K^n + p_1^n}$$
$$f_{23} = \frac{p_2^n}{K^n + p_2^n}$$
$$f_{32} = \frac{p_3^n}{K^n + p_3^n}$$

Now translation of each gene can be described via analogy as:

$$\frac{dp_1}{dt} = r_{L1}u_{L1} - k^d_{L1}p_1 - \mu p_1$$
$$\frac{dp_2}{dt} = r_{L2}u_{L2} - k^d_{L2}p_2 - \mu p_2$$
$$\frac{dp_3}{dt} = r_{L3}u_{L3} - k^d_{L3}p_3 - \mu p_3$$

Where:

$$r_{Lj} = k_{ELj}R_{LT}\frac{m_j}{K_{Lj}\tau_{Lj} + (\tau_{Lj}+1)m_j}$$

And the control terms is at 1 because the system is assumed to be operating at the kinetic limit:

$$u_{Lj} = W_j = 1$$

Now in order to write this in matrix form we define $\mathbf{x}$ as the 6x1 mRNA/protein vector, A as the consumption matrix, S as the 6x6 stoichiometric matrix (here, read identity), and $\mathbf{r}$ as the 6x1 overall reaction rate vector:

$$\frac{d\mathbf{x}}{dt} = A\mathbf{x} + S\mathbf{r}$$

$$\frac{d}{dt}\begin{pmatrix} \mathrm{m}_1 \\ \mathrm{m}_2 \\ \mathrm{m}_3 \\ \mathrm{p}_1 \\ \mathrm{p}_2 \\ \mathrm{p}_3 \end{pmatrix} = \begin{pmatrix} -(\mu + k^d_{X1}) & 0 & 0 & 0 & 0 & 0 \\ 0 & -(\mu + k^d_{X2}) & 0 & 0 & 0 & 0 \\ 0 & 0 & -(\mu + k^d_{X3}) & 0 & 0 & 0 \\ 0 & 0 & 0 & -(\mu + k^d_{L1}) & 0 & 0 \\ 0 & 0 & 0 & 0 & -(\mu + k^d_{L2}) & 0 \\ 0 & 0 & 0 & 0 & 0 & -(\mu + k^d_{L3}) \end{pmatrix} \begin{pmatrix} \mathrm{m}_1 \\ \mathrm{m}_2 \\ \mathrm{m}_3 \\ \mathrm{p}_1 \\ \mathrm{p}_2 \\ \mathrm{p}_3 \end{pmatrix}$$

$$+ \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} \mathrm{r}_{X1} u_{X1} \\ \mathrm{r}_{X2} u_{X2} \\ \mathrm{r}_{X3} u_{X3} \\ \mathrm{r}_{L1} u_{L1} \\ \mathrm{r}_{L2} u_{L2} \\ \mathrm{r}_{L3} u_{L3} \end{pmatrix}$$

3 Problem 2 Part B

In order to solve this set of ODEs, we have to define all the parameters. Table Two defines them
Table Two: Relevant Parameters

Parameter Name	*Symbol*	*Value*	*Units*	*Source*
Gene concentration	$G_{1,2,3}$	200	plasmids/cell	Given
Gene concentration	$G_{1,2,3}$	0.972	$\mu mol/gDW$	See Note 1 Below
Gene length	$L_{1,2,3}$	1200,2400,600	nucleotides	Given
E.coli TX Elongation Rate	e_X	42	nt/s	Gotta[1]
TX Rate of Elongation	$k_{EX1,2,3}$	0.0350,0.01750.07	s^-1	See Note 2 Below
RNAP Concentration	R_{XT}	8000	molecules/cell	Bremer[2]
RNAP Concentration	R_{XT}	38.88	$\mu mol/gDW$	See Note 1 Below
TX Rate of Open Complex Formation	k_I	1/42	s^-1	McClure[3]
TX Rate of Closed Complex Formation	k_+	5	$\mu M^{-1}s^{-1}$	See Note 3 Below
TX Rate of Close Complex Dissociation	k_-	0.1	s^{-1}	See Note 3 Below
TX Rate of Abortive Initiation	k_A	0	s^{-1}	$k_A << k_I or k_E$
Ribosome Concentration	R_{LT}	20,100	ribosomes/cell	Nilsson[4]
Ribosome Concentration	R_{LT}	97.68	$\mu mol/gDW$	See Note 1 Below
E. coli Translation Elongation Rate	e_L	14.5	aa/s	Dalbow[5]
TX Rate of Elongation	$k_{EL1,2,3}$	0.0362,0.0181,0.0725	s^-1	See Note 2 Below
TL Rate of Open Complex Formation	k_{LI}	1/15	s^-1	1/Time from Siwiak[6]
TL Rate of Closed Complex Formation	k_{L+}	5	$\mu M^{-1}s^{-1}$	See Note 3 Below
TL Rate of Close Complex Dissociation	k_{L-}	0.1	s^{-1}	See Note 3 Below
TL Rate of Abortive Initiation	k_{LA}	0	s^{-1}	$k_{LA} << k_{LI or} k_{EL}$
Bacterial Protein Half-Life	$t^p_{0.5}$	72000	s	Koch[7]
mRNA half-life	$t^m_{0.5}$	120	s	Li[8]
Doubling Time of *E.coli*	t_d	1800	s	Given
Weight of Inducer on 1	W_I	100	Unitless	See Note 4 Below
Weight of 1 on 2	W_{12}	100	Unitless	See Note 4 Below
Weight of 1 on 3	W_{13}	100	Unitless	See Note 4 Below
Weight of 2 on 3	W_{23}	0 or 10	Unitless	See Note 4 Below
Weight of 3 on 2	W_{32}	10	Unitless	See Note 4 Below
Basal Weight of 1	W_{RT1}	0.00001	Unitless	See Note 4 Below
Basal Weight of 2	W_{RT2}	0.00001	Unitless	See Note 4 Belwo
Basal Weight of 2	W_{RT3}	0.00001	Unitless	See Note 4 Below
Affinity Constant	K	0.3	mM	See Note 4 Below
Cooperativity Constant	n	2	Unitless	See Note 4 Below

Note 1
Molecules per cell were converted to $\mu mol/gDW$ Avogadro's Number, 30% dry weight given, the volume of a cell as 6.7e-10L/cell (Wang[9]), and the weight of the wet cell at $1.7g/L$ (Glazyrina[10]).
Note 2

$$k_{Ej} =< k_E > \frac{L}{L_j}$$

Where $< k_E >$ is the average elongation rate and L,L_j refer to the characteristic gene length (average here) and the current gene length (1200,2400,600 nt or 1/3 of that for protein). This simplifies to the elongation rate e_X (42 nt/s) divided by the gene length for example for transcription:

$$k_{EX1} = e_X/L_1 = 42/1200 = 0.035s^{-1}$$

This same method is used for protein k_{ELj} from e_L.
Note 3
The values of k_+ and k_- were derived from McClure's[3] formula and value for the slope of a tau plot in *E.Coli* D promoters:

$$McClureSlope = 1.04\mu M * s = \frac{k_- + k_I}{k_+ k_I}$$

$$1.04k_+(1/42) = k_- + 1/42$$

We can arbitrarily assign $k_+ = 5\mu M^{-1}s^{-1}$ which leads to $k_- = 0.1s^{-1}$. This is valid because when these values are used later in calculating K_{Xj}, the ratio of k_-/k_+ or some dissociation constant k_D is restored. This is best described below:

$$McClureSlope = \frac{k_- + k_I}{k_+ k_I}$$

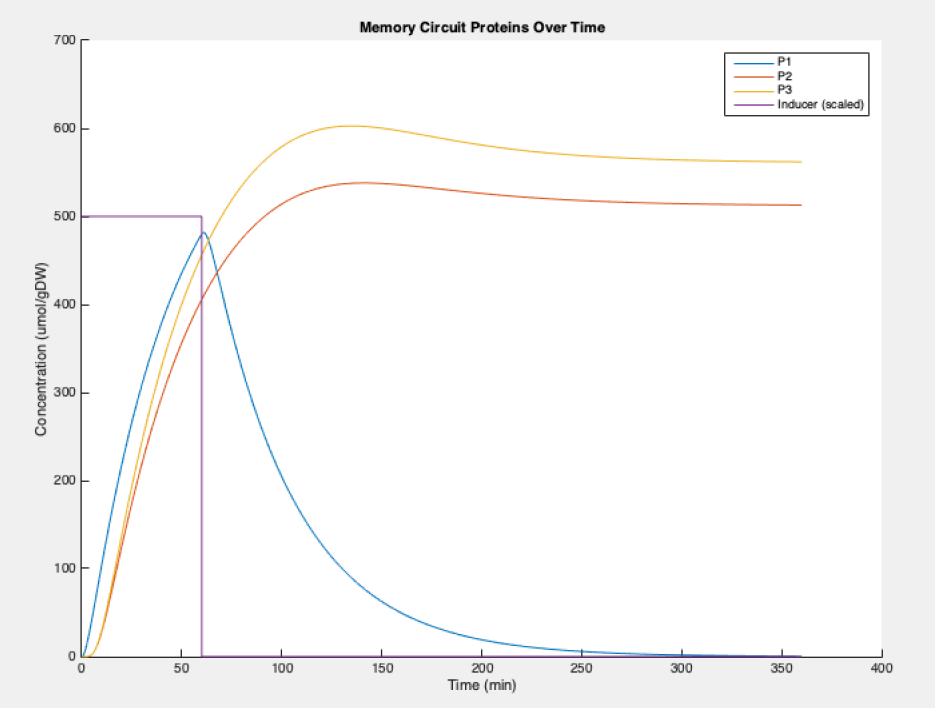
$$K_{Xj} = \frac{k_- + k_I}{k_+}$$

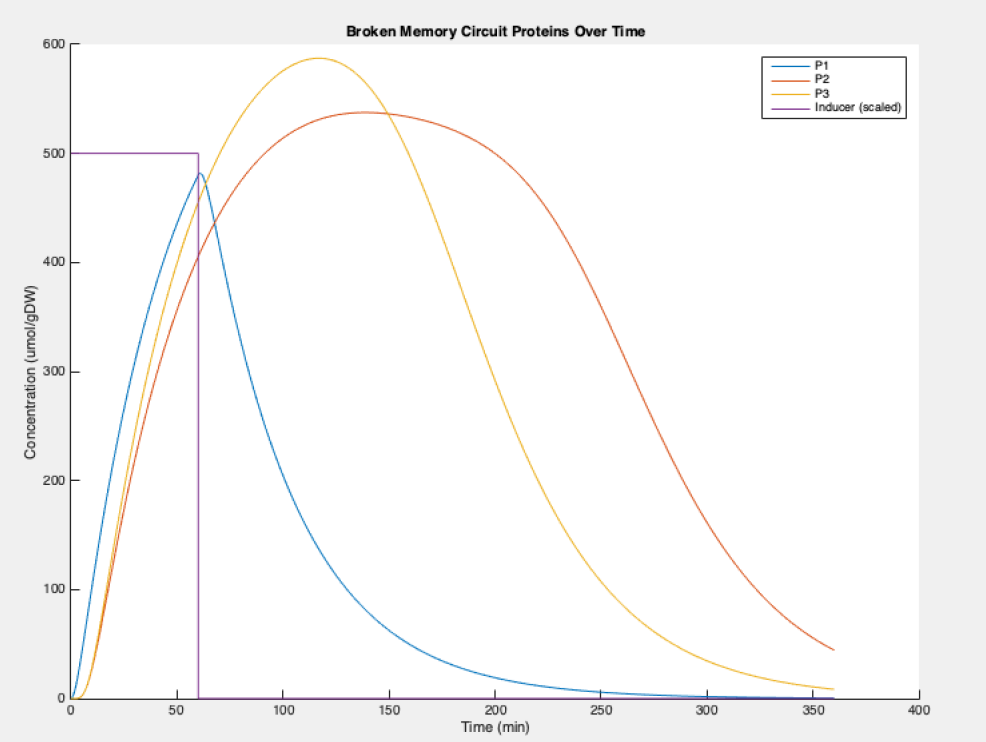
Thus:

$$K_{Xj} = McClureSlope * k_I$$

Plugging into either equation for K_{Xj} returns the same value. It also happens that K_D or k_-/k_+ for *E.Coli* with the lac promoter is $550nM$ (Bintu[11]) which is only one order of magnitude off our arbitrary assignment for transcription ($20nM$). The values for binding between a gene and RNAP and the values for a ribosome binding to mRNA were assumed to be the same. This is because the values are not easily measurable and have not been analyzed in McClure's fashion for translation. In other words, $k_- = k_{L-}$ and $k_+ = k_{L+}$. It is reasonable to assume they are on the same order of magnitude as some sort of enzyme is binding a large polymer substrate.
Note 4
The weights, cooperativity constants, and saturation constants mostly follow the trends or values given in Problem Set 1. They are on similar orders of magnitude. The basal rates were assumed to approach zero which is reasonable to say as the inducers should have a larger effect. n was raised to 2 to increase the effect of the Hill function step and produce more descriptive plots. The effect of proteins 2 and 3 on each other were decreased below protein 1's effect so that the readjustment on steady state could be observed as protein 1 dies off in a more timely manner (under 360 minutes). It should be noted that W_{23} is changed to be 0 when the circuit is broken.

The solutions for a memory circuit and broken circuit are plotted as follows. The broken circuit simply removes the weight or effect that protein 2 has on the translation of protein 3 ($W_{23} = 0$). Note that the inducer is scaled. HW2Run.m plots these values using ode45 calling function CHEME5440HW2B.m. The values for W_{23} were changed manually for each plot.

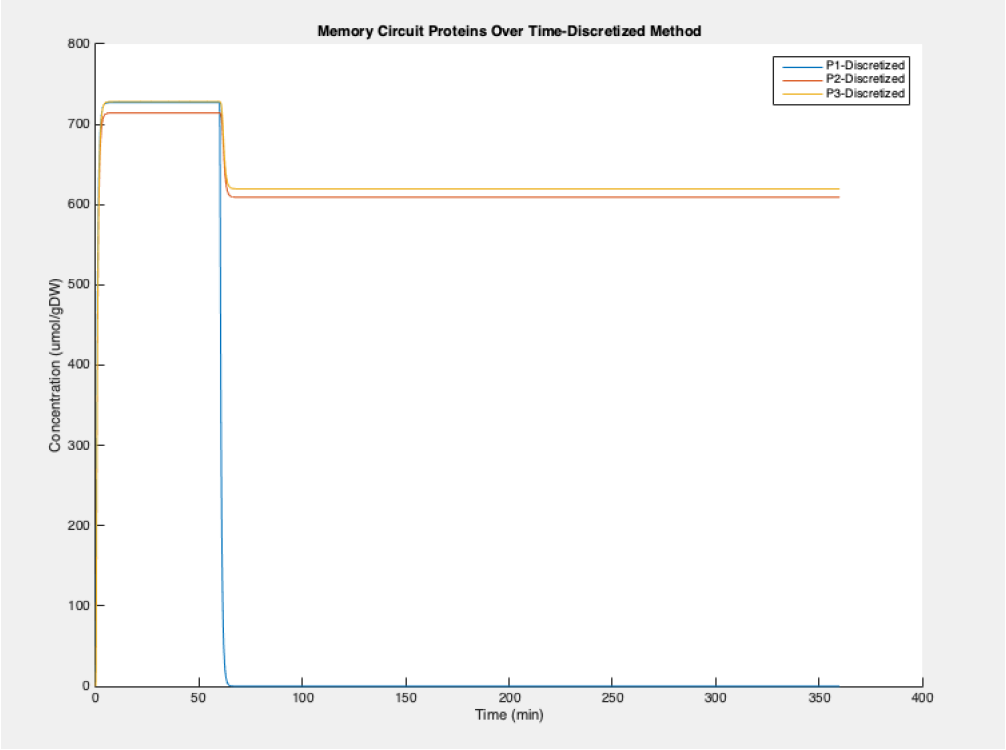


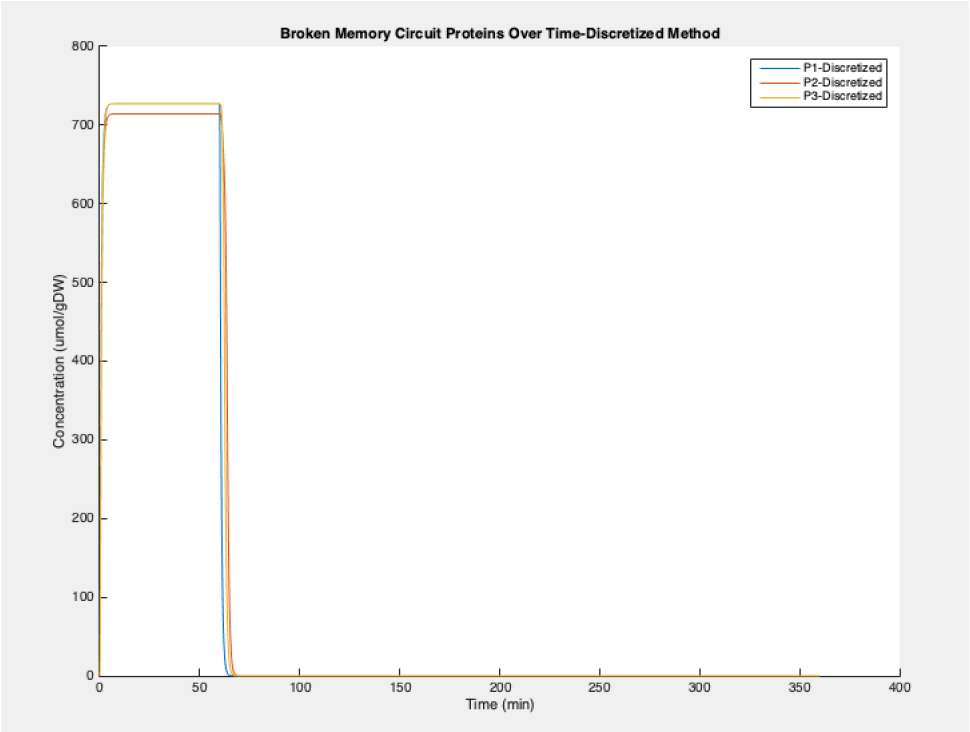


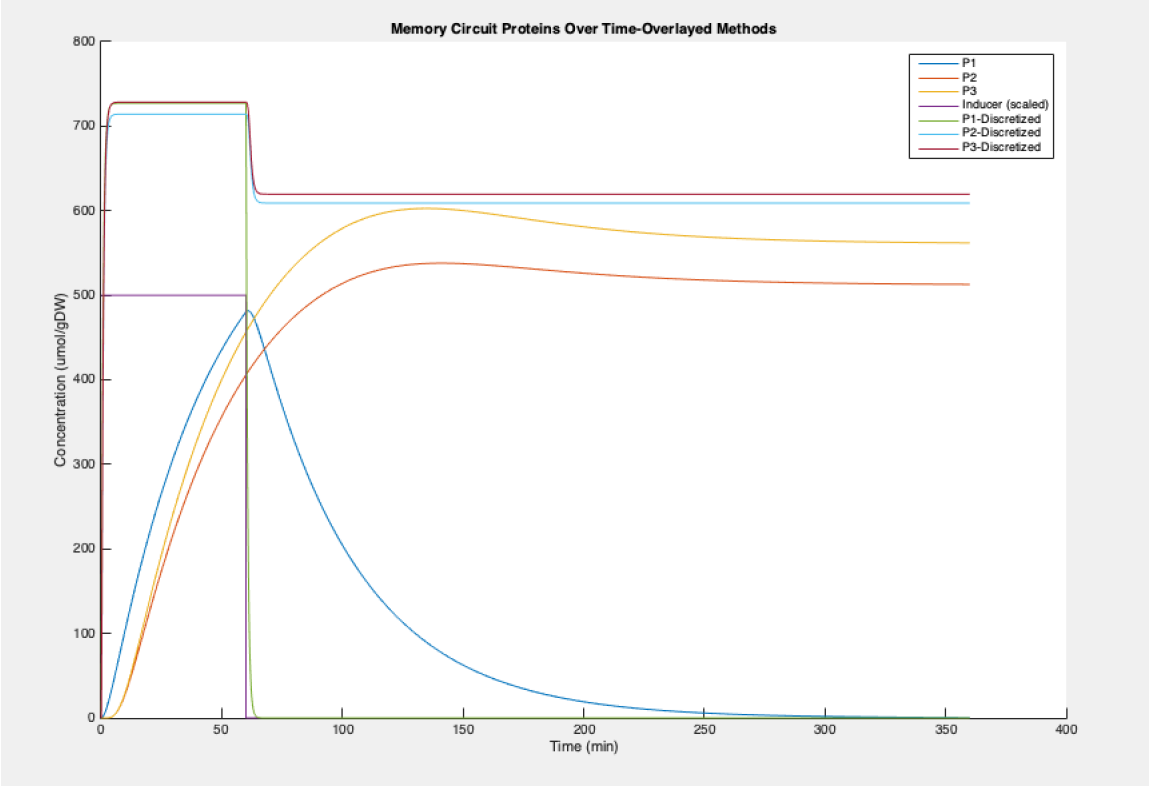
4 Problem 2 Part C

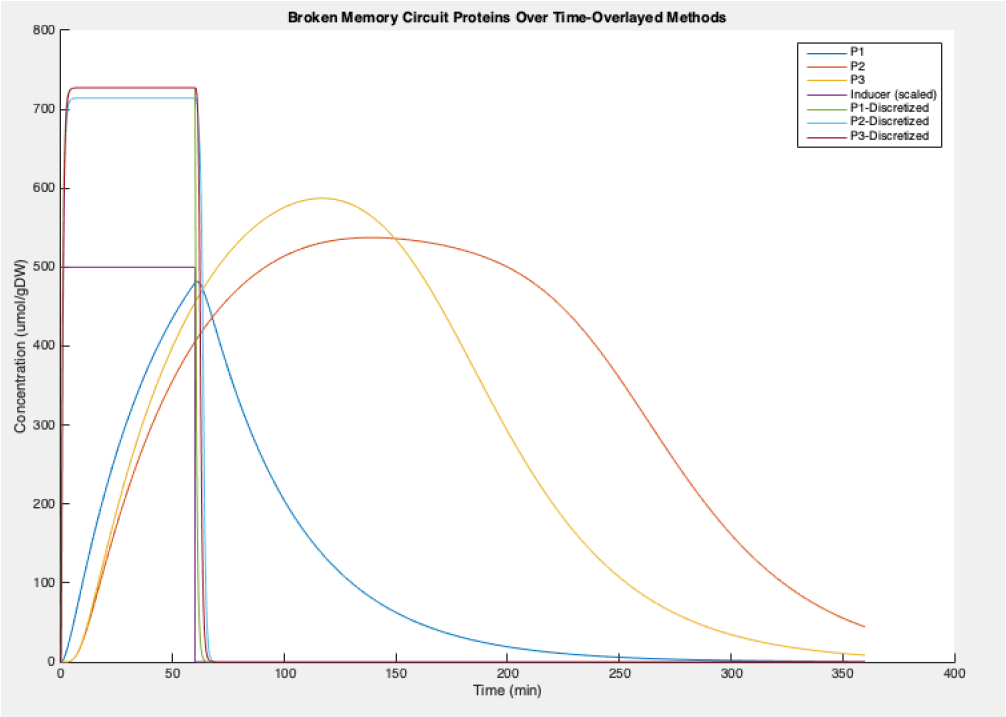
Using the given discretization, CHEME5440HW2C.m plots the protein profile over time. It calls the function f.m that simply calculates the Hill Functions (f_I). The plots for the discretization method, discretization method overlayed with the numerical solution, and discretization method with decreased time step (0.01 min) are given below. It is notable that the discretization gives much less resolution in the kinetics as the protein concentrations

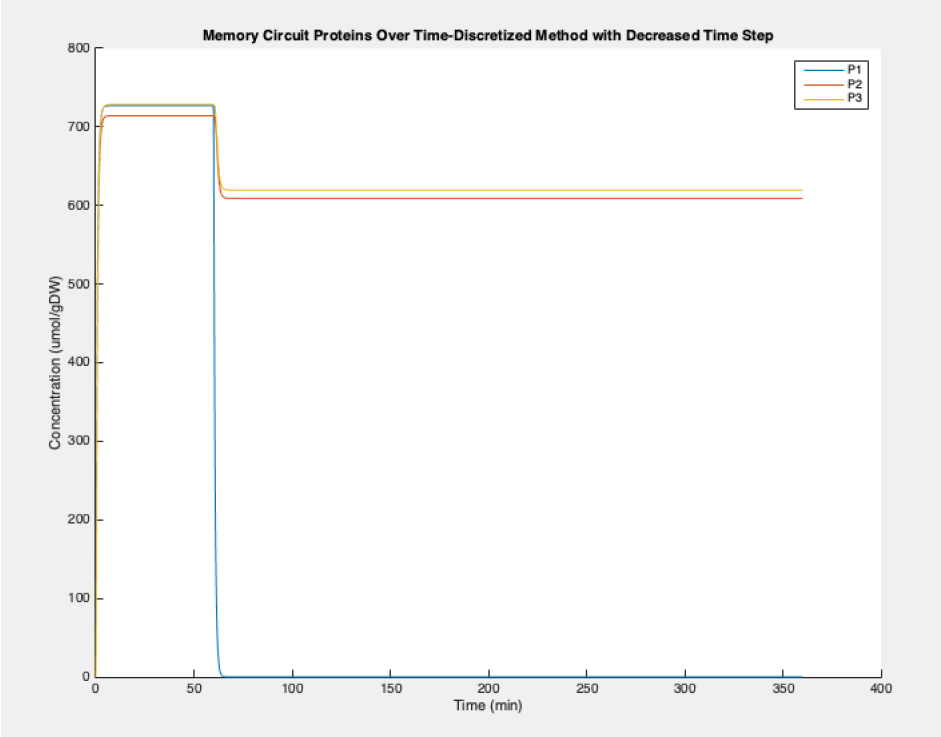
shoot to steady state values and remain there until parameters change. The numerical ode solver gives much smoother transitions and better resolution. However, both solvers give the general idea and magnitude of the protein levels when being produced at certain levels of inducer and inducing protein values. The discretization magnitudes are slightly higher, however, which probably has to do with some effect being less strong when concentration is rising in the discretization (such as dilution or degradation). Note that decreasing the time step does not increase discretization resolution.



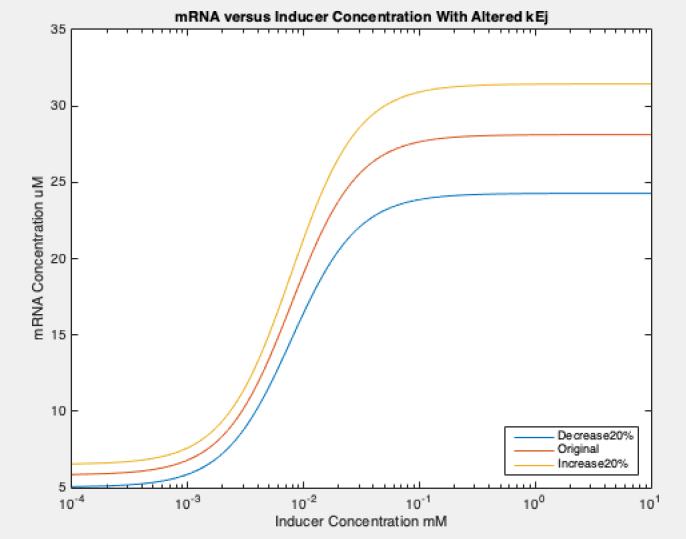


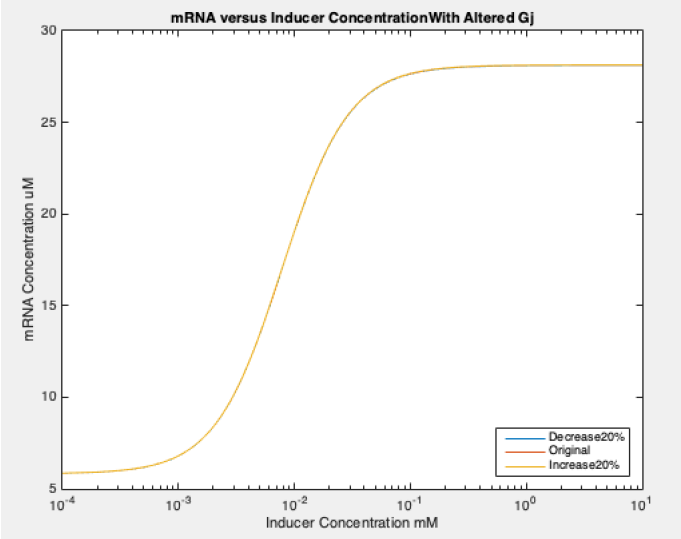

Memory Circuit Proteins Over Time-Overlayed Methods
P1
P2
P3
Inducer (scaled)
P1-Discretized
P2-Discretized
P3-Discretized
Concentration (umol/gDW)
Time (min)
0
100
200
300
400
500
600
700
800
0
50
100
150
200
250
300
350
400

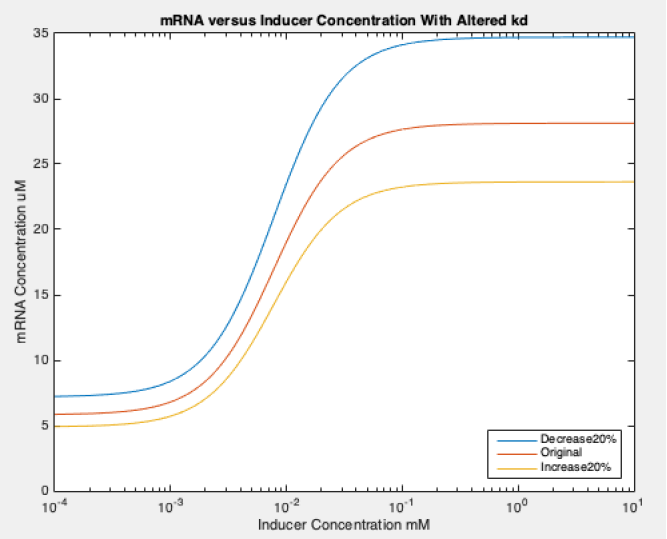

Broken Memory Circuit Proteins Over Time-Overlayed Methods
P1
P2
P3
Inducer (scaled)
P1-Discretized
P2-Discretized
P3-Discretized
Concentration (umol/gDW)
Time (min)
0
100
200
300
400
500
600
700
800
0
50
100
150
200
250
300
350
400

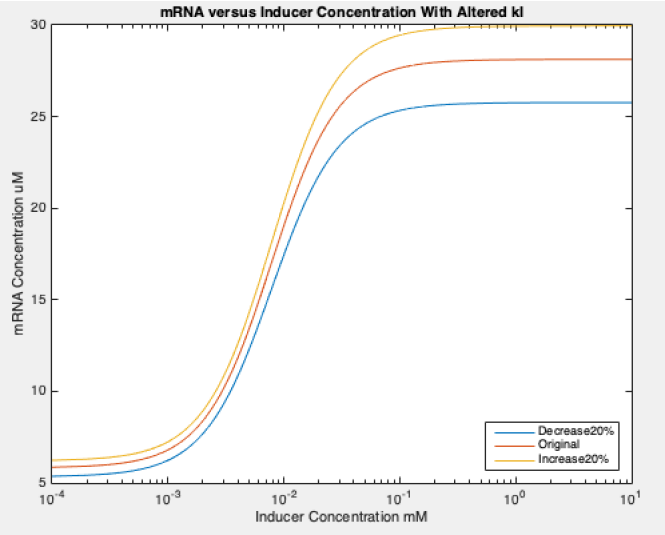

Memory Circuit Proteins Over Time-Discretized Method with Decreased Time Step
P1
P2
P3
Concentration (umol/gDW)
Time (min)
0
100
200
300
400
500
600
700
800
0
50
100
150
200
250
300
350
400

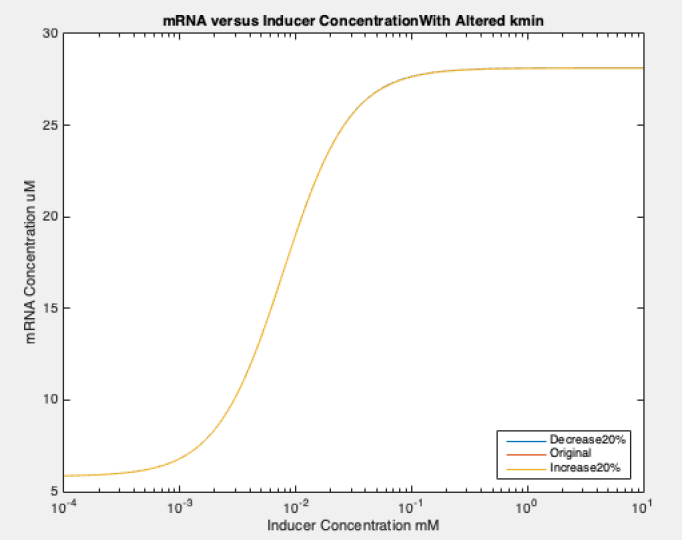
5 Appendix A

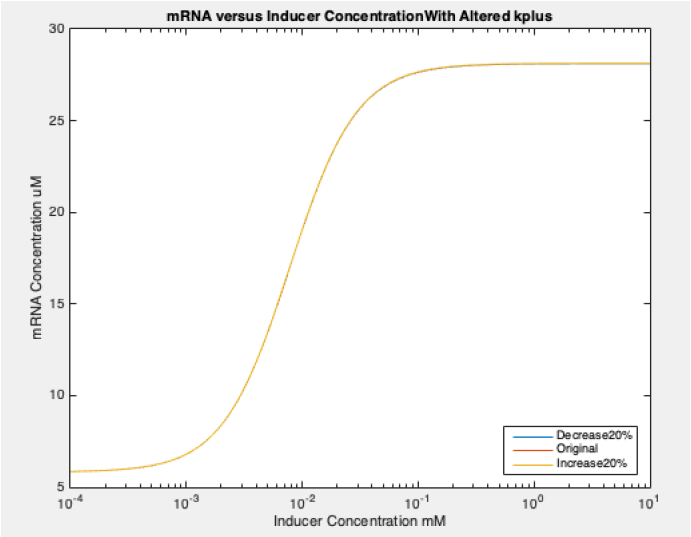

mRNA versus Inducer Concentration With Altered kEj
mRNA Concentration uM
Inducer Concentration mM
Decrease20%
Original
Increase20%

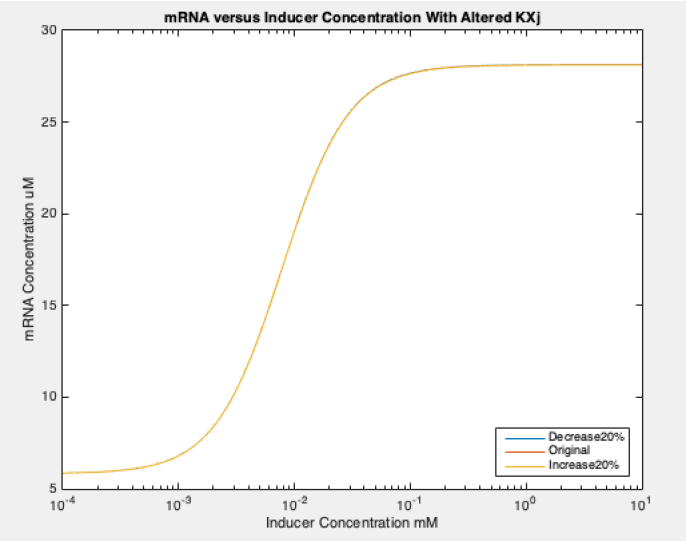

mRNA versus Inducer ConcentrationWith Altered Gj
mRNA Concentration uM
Inducer Concentration mM
Decrease20%
Original
Increase20%

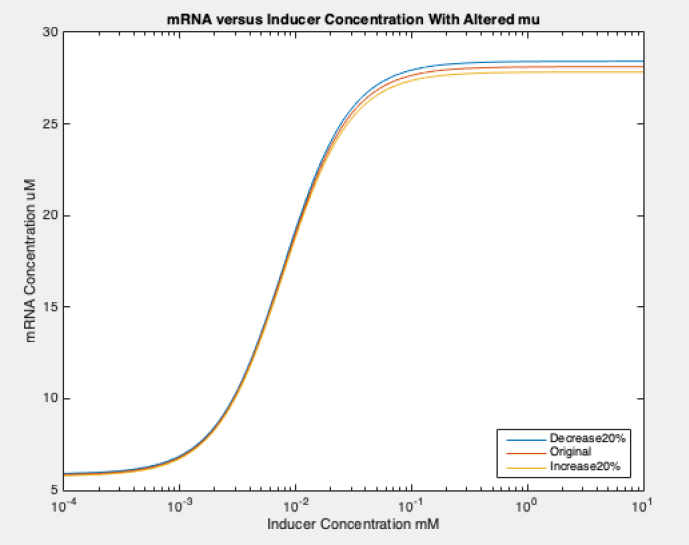

mRNA versus Inducer Concentration With Altered kd
mRNA Concentration uM
Inducer Concentration mM
Decrease20%
Original
Increase20%

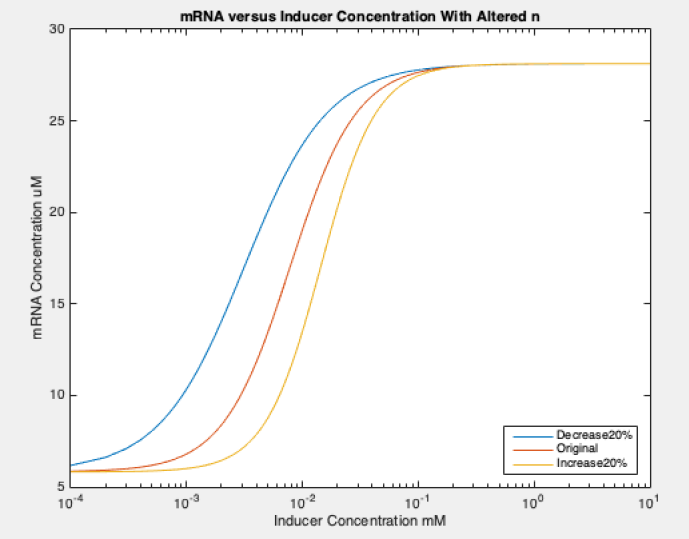

mRNA versus Inducer Concentration With Altered kl
mRNA Concentration uM
Inducer Concentration mM
Decrease20%
Original
Increase20%

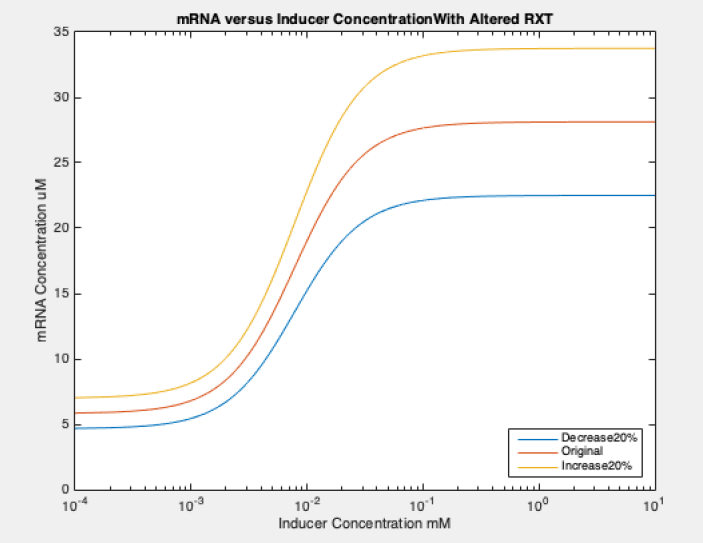

mRNA versus Inducer ConcentrationWith Altered kmin
mRNA Concentration uM
Inducer Concentration mM
Decrease20%
Original
Increase20%

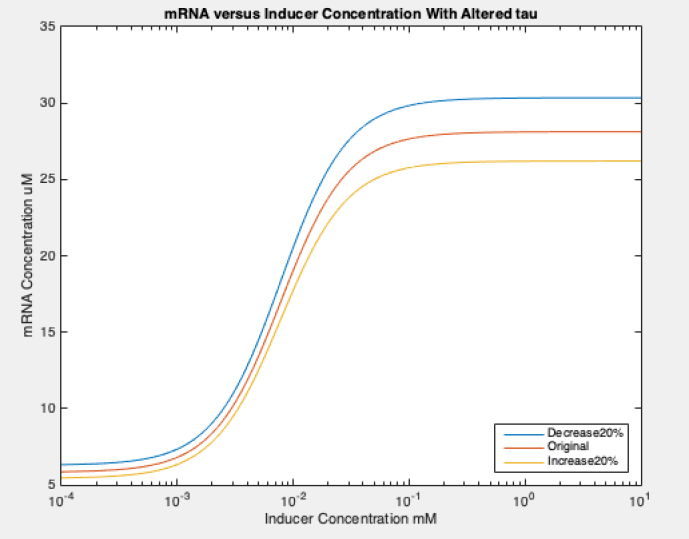

mRNA versus Inducer ConcentrationWith Altered kplus
mRNA Concentration uM
Inducer Concentration mM
Decrease20%
Original
Increase20%

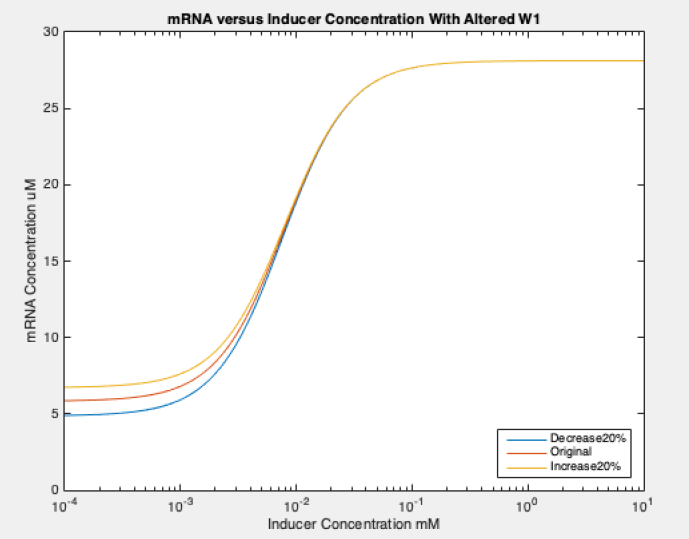

mRNA versus Inducer Concentration With Altered KXj
mRNA Concentration uM
Inducer Concentration mM
Decrease20%
Original
Increase20%

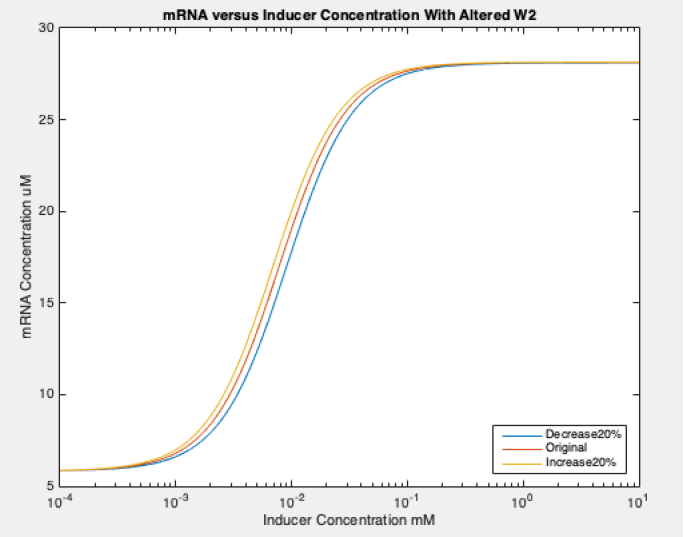

mRNA versus Inducer Concentration With Altered mu
mRNA Concentration uM
Inducer Concentration mM
Decrease20%
Original
Increase20%

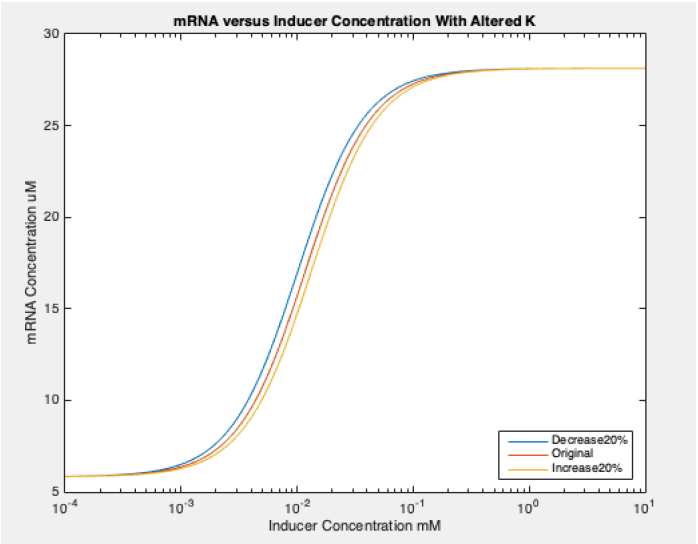

mRNA versus Inducer Concentration With Altered n
mRNA Concentration uM
Inducer Concentration mM
Decrease20%
Original
Increase20%

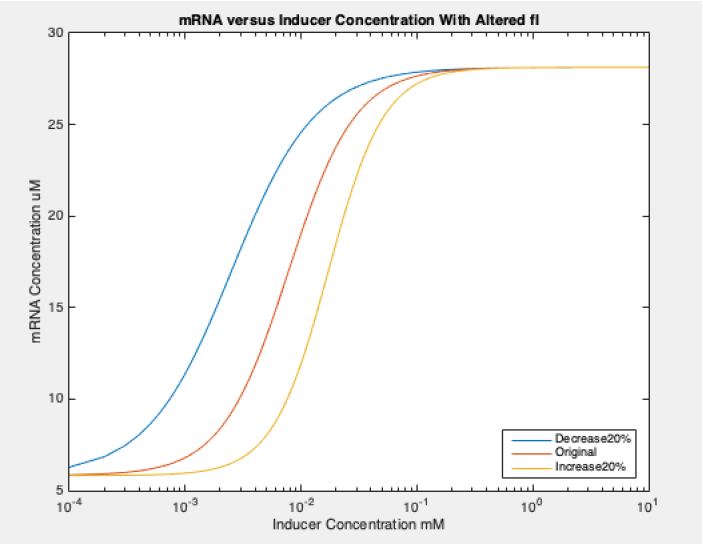

mRNA versus Inducer ConcentrationWith Altered RXT
mRNA Concentration uM
Inducer Concentration mM
Decrease20%
Original
Increase20%


mRNA versus Inducer Concentration With Altered tau
mRNA Concentration uM
Inducer Concentration mM
Decrease20%
Original
Increase20%


mRNA versus Inducer Concentration With Altered W1
mRNA Concentration uM
Inducer Concentration mM
Decrease20%
Original
Increase20%







References

[1] S. L. Gotta, O. Miller, and S. L. French, "rrna transcription rate in escherichia coli.," *Journal of bacteriology*, vol. 173, no. 20, pp. 6647–6649, 1991.

[2] H. Bremer, P. P. Dennis, *et al.*, "Modulation of chemical composition and other parameters of the cell by growth rate," *Escherichia coli and Salmonella: cellular and molecular biology*, vol. 2, no. 2, pp. 1553–69, 1996.

[3] W. R. McClure, "Rate-limiting steps in rna chain initiation," *Proceedings of the National Academy of Sciences*, vol. 77, no. 10, pp. 5634–5638, 1980.

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