

ChemE 5440/7770: Problem Set 2

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Problem 1

Sensitivity Analysis was performed using the Julia file (found in github repository). Based on graphed results during sensitivity analysis. The table below describes the behavior of the mRNA concentration as a function of $[I]$ at different values of $[I]$ due to an increase in the parameter of the first column. Explanations of the significance of changers are summarized below the graph.

Parameter Increased	Low $[I]$	Mid $[I]$	High $[I]$
L_j (length mRNA)	started higher	steeper slope	shift up
k_I	started higher	shift left/steeper slope	shift up
K_{ej}	no change	no change	no change
τ	shift down	shift right/less steep slope	shift down
k_{ej}	shift up	shift left/steeper slope	shift up
RNAP total	shift up	shift left/steeper slope	shift up
G_j	negligible change	negligible change	negligible change
K_m	negligible change	shifted right/same slope	negligible change
k_1	started higher	shifted left/same slope	negligible change
k_2	negligible effect	slope shifted left/same slope	negligible change
k_d	shift down	shift right/less steep slope	shift down
μ	negligible change	negligible change	shift up

L_j = length of mRNA

k_I = transcription initiation rate

K_{ej} = saturation transcription

τ = time constant

k_{ej} = transcription rate

RNAP total = total RNAP in the cell

G_j = copy in the cell

K_m = affinity of the inducer

k_1 = basal transcription level (leaky expression)

k_2 = weight of the inducer effect

k_d = mRNA degradation rate

μ = dilution rate

The Appendix contains the graphs used to make all of the conclusions. Half and double the original amount were used in all cases to look at the difference of changing the parameters.

The mRNA concentration at "low $[I]$ " starting higher means that the initial mRNA concentration before the inducer had significant effect increasing the basal level of mRNA production (leaky expression).

For "mid $[I]$ " -The slope refers to the critical range of $[I]$ needed for the drastic increases in mRNA concentration – meaning a steep slope indicates a narrower range for I that causes a dramatic increases (in others words, more sensitivity.) In the $[I]$ range around the slope, a steep slopes indicates that mRNA concentration is very sensitive to small changes in $[I]$.

For "mid $[I]$ " If the line is shifted left, it is good, because it means that a lower concentration of I is needed for a signification induction of mRNA transcription.

A higher final mRNA (shift up) at the range "high $[I]$ " means that the maximum equilibrium concentration of mRNA, and hence maximum velocity of mRNA production was higher due to an increase in the parameter in the first column. For "high $[I]$ " if there is little difference, it means that there is negligible effect of the increase of the parameter on the final maximum mRNA equilibrium concentration.

Problem 2

IMPORTANT NOTE:

Code contains values of all parameters and full equations. This is the high level summary.

Assumptions:

- Promoter control models Moon/Voigt formulation
- translation operates at the kinetic limit
- RNAP and Ribosome levels are constant

Knowns:

doubling time = 30 min

mass of cell water = 70 %

plasmid copies = 200

concentration basis = umol gDW-1

$L_X = 1000$ nt (characteristic length)

$L_T = 333$ AA (characteristic length)

$L_{X,1} = 1200$ nt

$L_{X,2} = 2400$ nt

$L_{X,3} = 600$ nt

$L_{L,i} = (1/3)L_{X,i}$

Equations for Mass Balances:

$$\frac{dm_i}{dt} = TX - kd_m * m_i - \mu_d \text{dilution} * m_i \quad (1)$$

$$\frac{dp_i}{dt} = TL - kd_p * p_i - \mu_d \text{dilution} * p_i \quad (2)$$

where m_i is the mRNA, and p_i is the protein, and $i = 1,2,3$ for both mRNAs and proteins.

Transcription rate is:

$$TX = k_{(e,j)} * \frac{G_j * RNAP_T}{\tau_x * K_{x,j} + (\tau_x + 1) * G_j} * u \quad (3)$$

and translations rate is:

$$TL = k_{e,L} * ribosomes_T * \frac{m_i}{\tau_L * K_L + (\tau_L + 1) * m_i} * u \quad (4)$$

Everything before u in both TX and TL is the maximum rate. u is the control function. For mRNA, the u function is not equal to one (see julia code for 2b "srkps2.jl"):

$$u! = 1 \quad (5)$$

For mRNA, the u function is equal to one, because it is at the kinetic limit (see julia code for 2b "srkps2.jl"):

$$u = 1 \quad (6)$$

FULL FORM OF EQUATIONS ARE IN THE CODES.

Matrices - Answer to 2a

In matrix form the final equations are:

$$\frac{d\mathbf{x}}{dt} = \mathbf{A}\mathbf{x} + \mathbf{S}\mathbf{r} \quad (7)$$

where

$$\mathbf{A} = \begin{pmatrix} -\mu - kd_m & 0 & 0 & 0 & 0 & 0 \\ 0 & -\mu - kd_m & 0 & 0 & 0 & 0 \\ 0 & 0 & -\mu - kd_m & 0 & 0 & 0 \\ 0 & 0 & 0 & -\mu - kd_p & 0 & 0 \\ 0 & 0 & 0 & 0 & -\mu - kd_p & 0 \\ 0 & 0 & 0 & 0 & 0 & -\mu - kd_p \end{pmatrix}. \quad (8)$$

(kdm is degradation rate of mRNA, kdp is degradation rate of protein, mu is dilution rate.)

(m's are the mRNA, and p's are the proteins) and,

$$\mathbf{x} = \begin{pmatrix} m_1 \\ m_2 \\ m_3 \\ p_1 \\ p_2 \\ p_3 \end{pmatrix}. \quad (9)$$

and

$$\mathbf{S} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix}. \quad (10)$$

$$\mathbf{r} = \begin{pmatrix} TX_1 \\ TX_2 \\ TX_3 \\ TL_1 \\ TL_2 \\ TL_3 \end{pmatrix}. \quad (11)$$

where TX is the transcription rate, and TL is the translation rate.

Graphs - Answer to 2b

Full code is in both "srkps2.jl" which runs the odes encoded "srkps2balances.jl"

Images are uploaded and labeled.

p1 = black

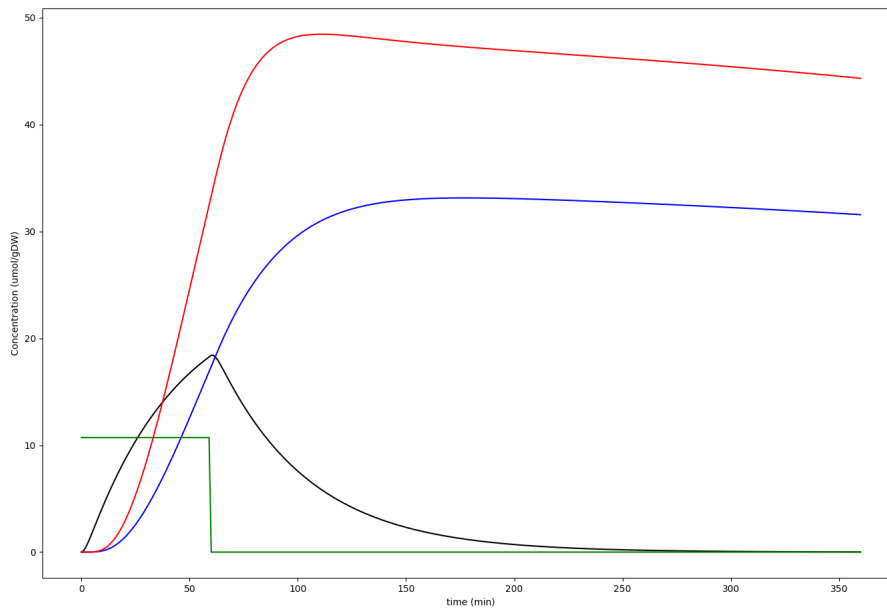
p2 = blue

p3 = red

I = green (scaled)

y-axis = umol/gDW ; x-axis = time (min)

i. not broken circuit

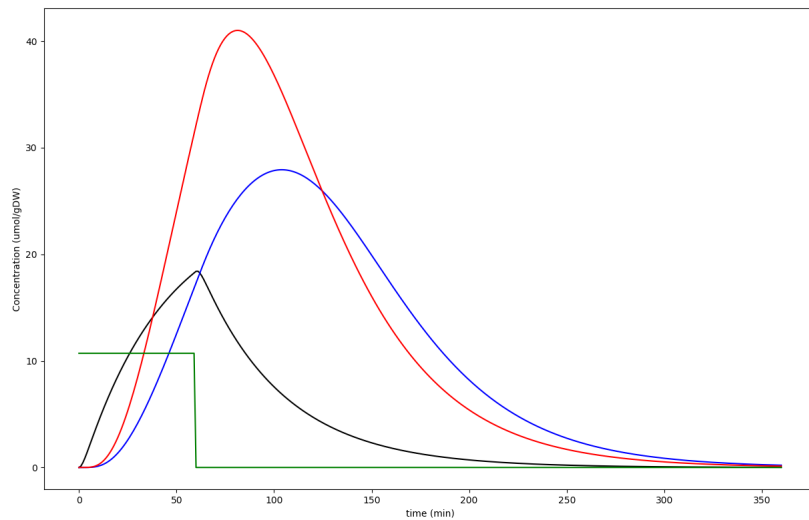


]

Since p1 was only induced by I, removing I causes p1 to drop significantly right away. Since p2 and p3 induce each other, they only decrease slightly after taking away p1, and reach a steady equilibrium dropping where they are just being lowered in concentration due to degradation/dilution.

Drops are due to degradation and dilution of the proteins.

ii. broken circuit



Since p1 was only induced by I, removing I causes p1 to drop significantly right away. p2 no longer induces p3, so p3 decreases next when p1 is gone.

Since p3 does induce p2, p3 needs to decrease a significant amount before p2 concentration is affected. That is why p2 decreases *after* p3 decreases. p2 eventually reachings almost zero as well (only basal transcription.)

Drops are due to degradation and dilution of the proteins.

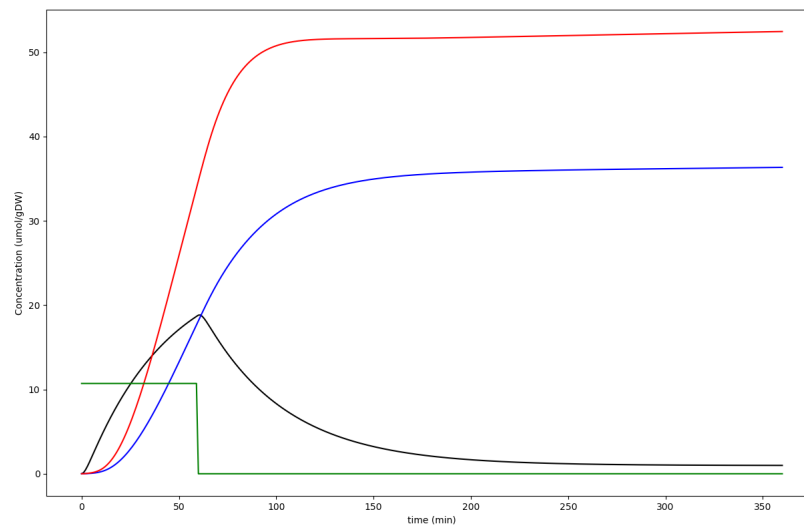
iii. Why does it go to zero?

Basal transcription level (not induced) was set really low (weight = .008), which is why the protein 1 concentration drops to nearly 0 and is not readable on the graph.

Essentially, you assume there is negligible basal transcription.

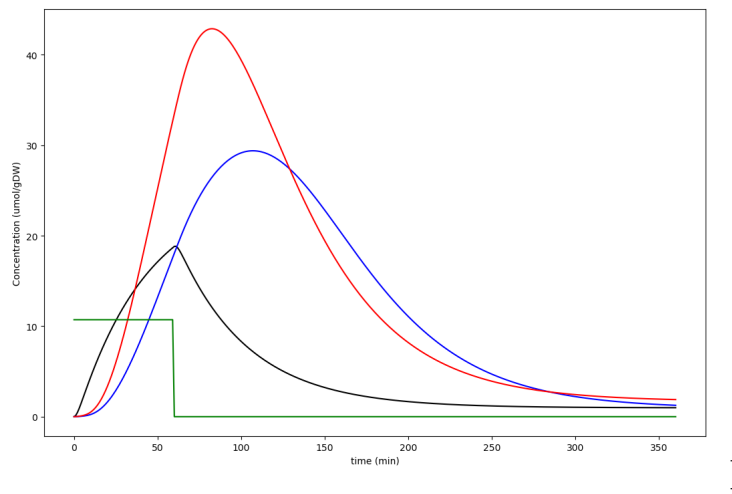
If basal transcription weight were set higher, protein concentrations would have reached equilibrium as some amount a bit more greater than 0.

– i, basal weight = 0.01, not broken circuit



basal.PNG

– ii, basal weight = 0.01, broken circuit



Graphs - Answer to 2c

Full code is in both "srkps2-2c.jl" which runs and graphs the "supercool" math. "srkps2.jl" is used to overlay with the answers to 2b.

Images are uploaded and labeled.

p1 = purple

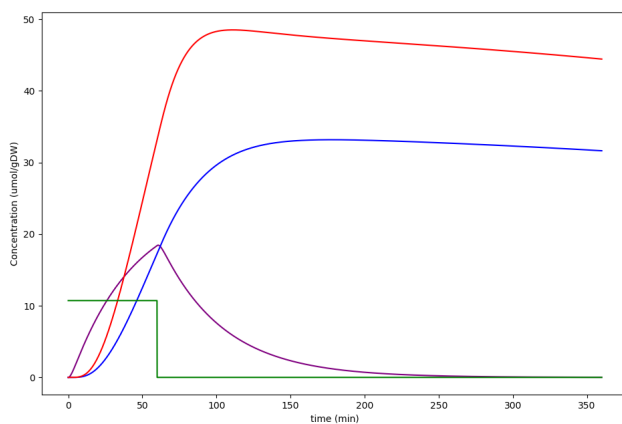
p2 = blue

p3 = red

I = green (scaled)

y-axis = $\mu\text{mol/gDW}$; x-axis = time (min)

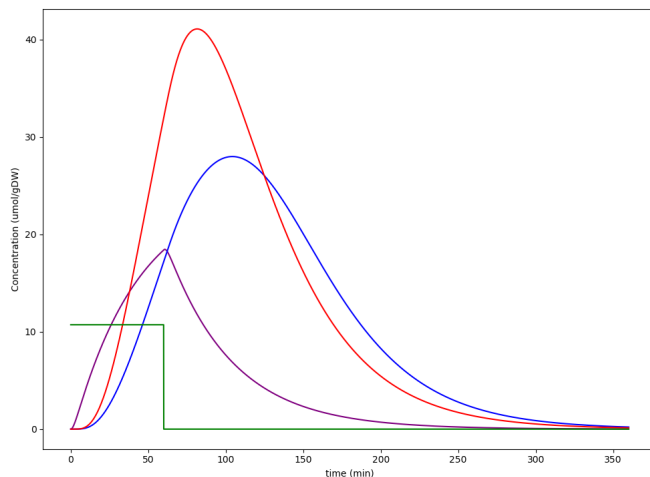
i. not broken circuit



Since p1 was only induced by I, removing I causes p1 to drop significantly right away. Since p2 and p3 induce each other, they only decrease slightly after taking away p1, and reach a steady equilibrium dropping where they are just being lowered in concentration due to degradation/dilution.

Drops are due to degradation and dilution of the proteins.

ii. broken circuit



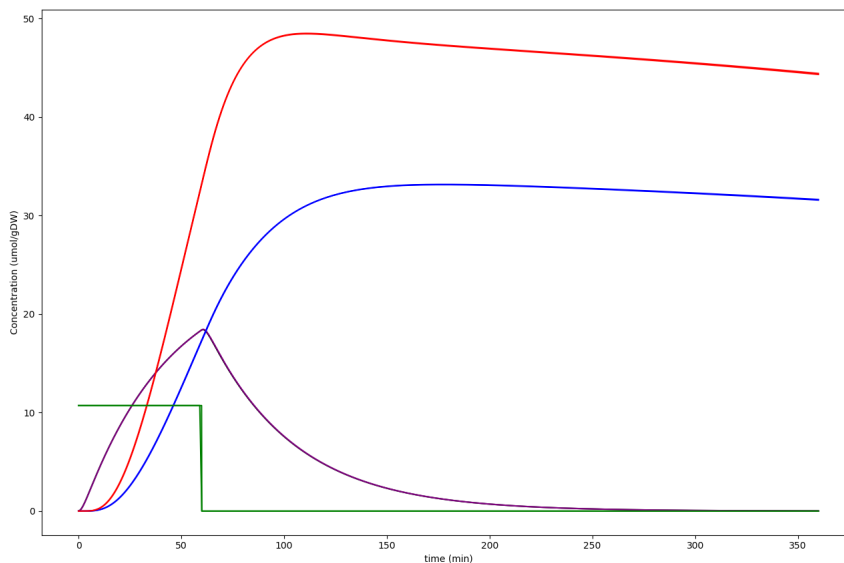
Since p1 was only induced by I, removing I causes p1 to drop significantly right away. p2 no longer induces p3, so p3 decreases next when p1 is gone.

Since p3 does induce p2, p3 needs to decrease a significant amount before p2 concentration is affected. That is why p2 decreases *after* p3 decreases. p2 eventually reachings almost zero as well (only basal transcription.)

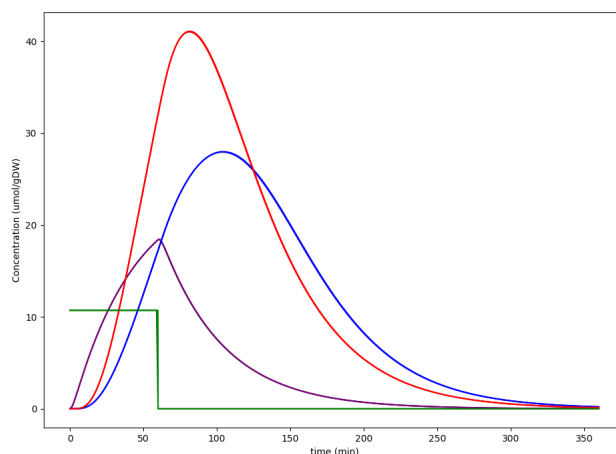
Drops are due to degradation and dilution of the proteins.

Graphs with the method from 2c overlaid almost perfectly with those from 2a. This shows that the supercool math trick works well.

i. not broken circuit overlay



ii. broken circuit overlay



iii. Why does it go to zero?

As with 2b, in 2c the basal weight was set really low in order to really observe the effect of the inducer and p1 (weight = 0.008). Essentially, you assume there is negligible basal transcription.

Appendix: Graphs for Problem 1

For all graphs:

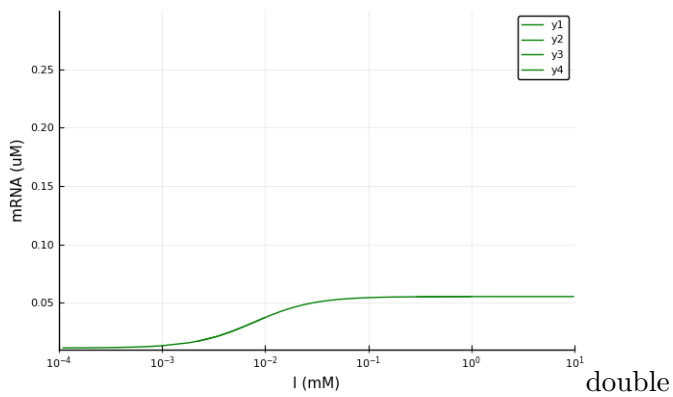
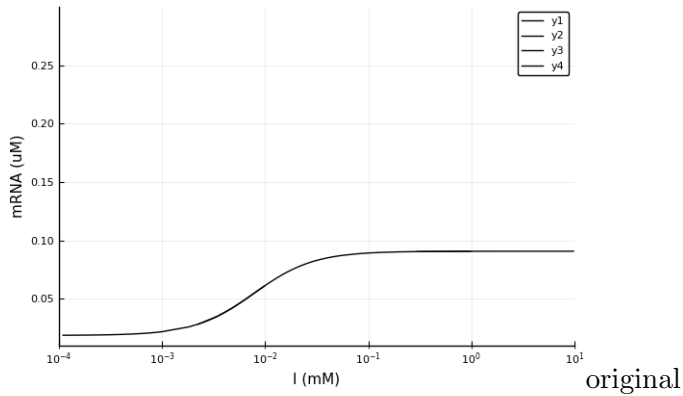
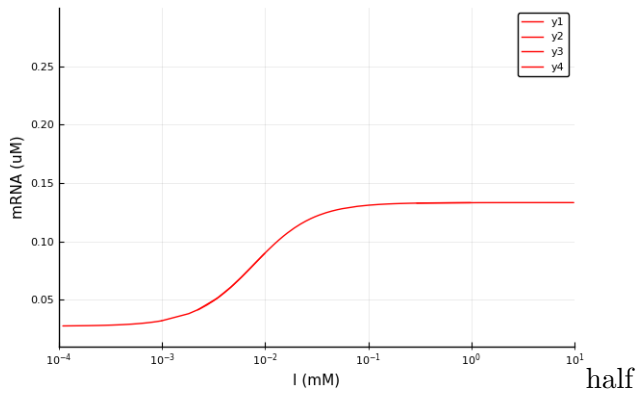
Green means parameter was doubled.

Red means parameter was halved.

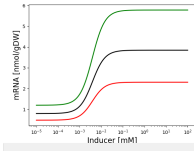
Black means the original parameter values.

All parameter values are shown in the code "jv-ps1-solution.jl"

L_j = length of mRNA

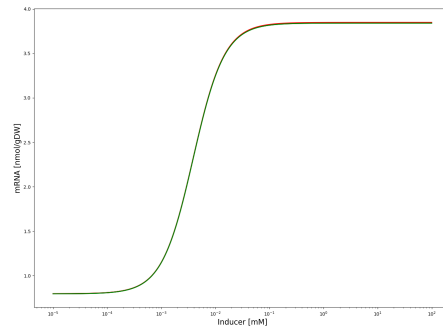


k_I = transcription initiation rate



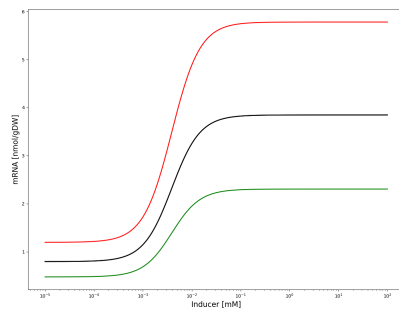
change.PNG

K_{ej} = saturation transcription



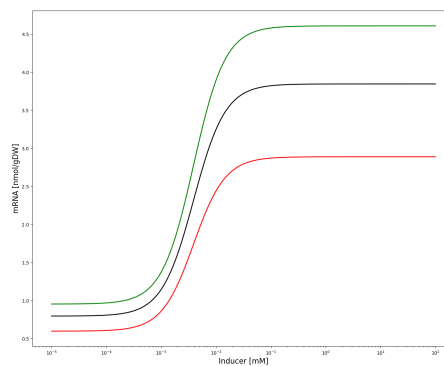
big change.PNG

τ = time constant



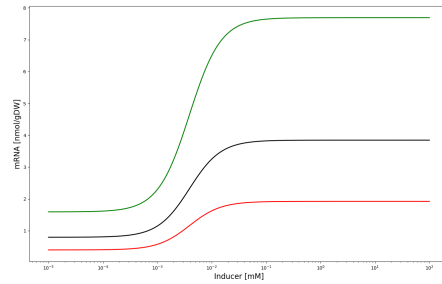
tau.PNG

k_{ej} = transcription rate



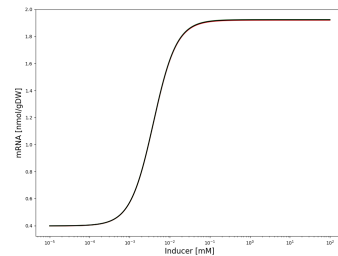
transcription rate change.PNG

RNAP total = total RNAP in the cell



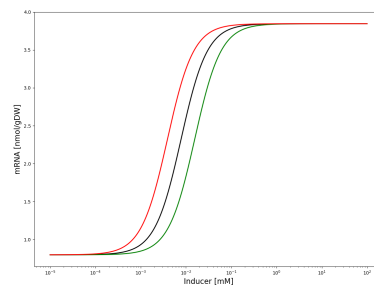
total change.PNG

G_j = copy in the cell



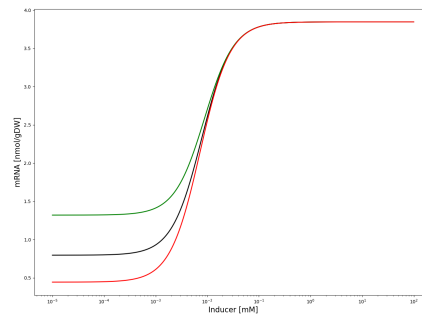
number change.PNG

K_m = affinity of the inducer



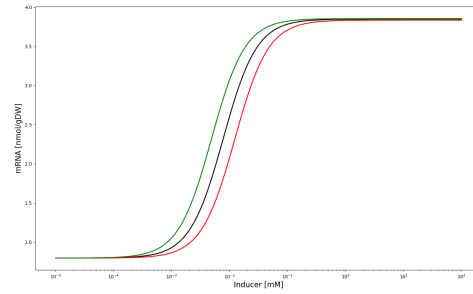
K_m - binding affinity constant.PNG

k_1 = basal transcription level (leaky expression)



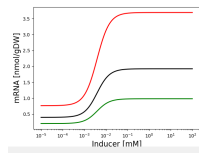
k_1 - basal transcription rate.PNG

k_2 = weight of the inducer effect



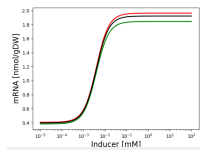
k2 - weight of inducer.PNG

kd = mRNA degradation rate



change.PNG

mu = dilution rate



dilution rate change.PNG
