CHEME5440: Take Home Prelim 1

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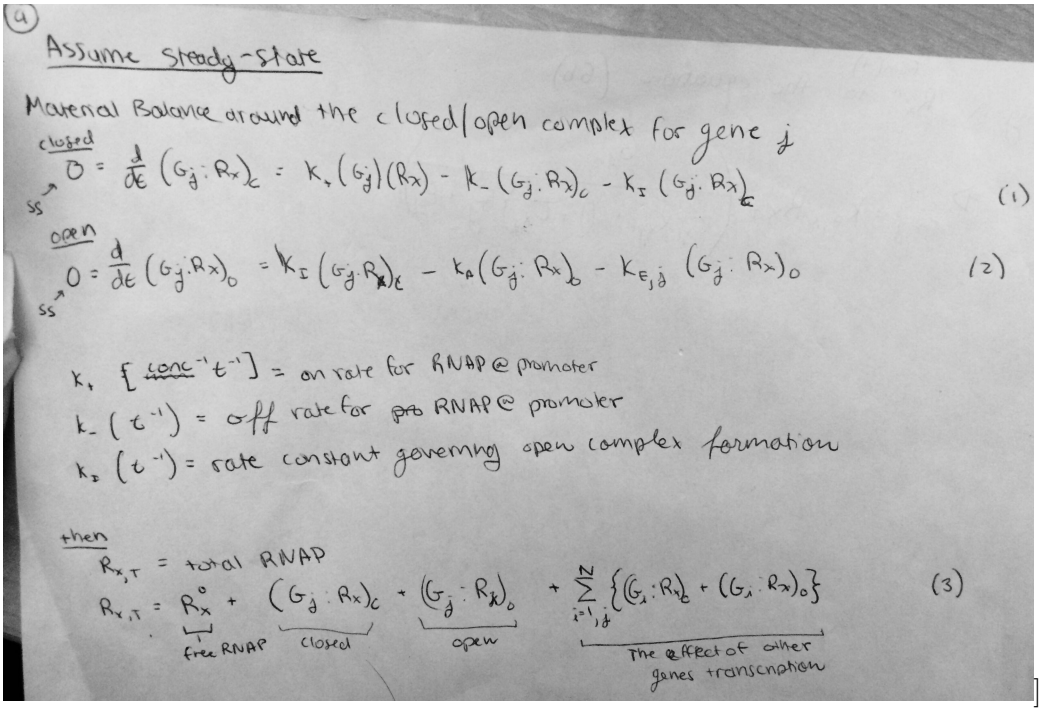
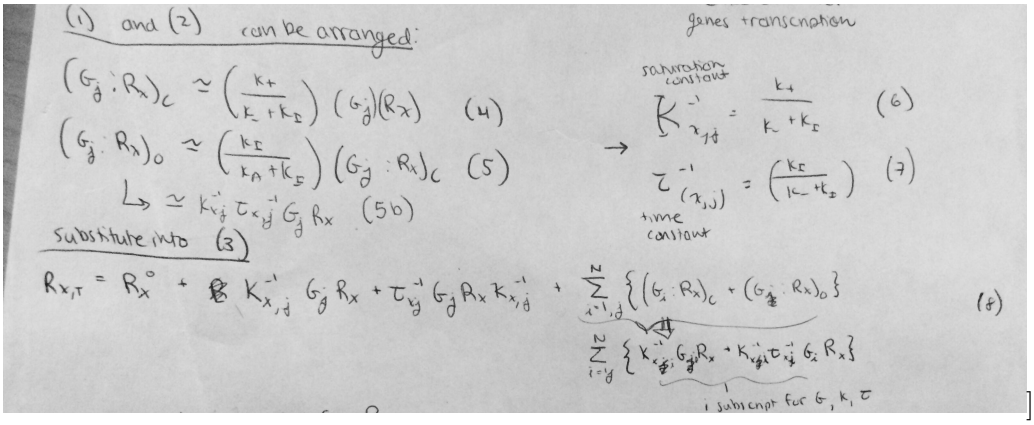
1 Problem 1

(a) see hand derivation below

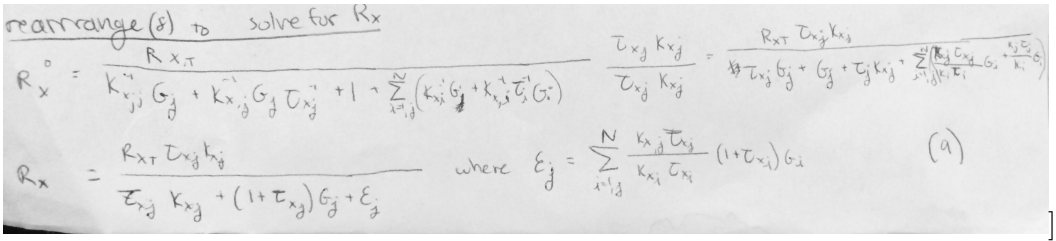


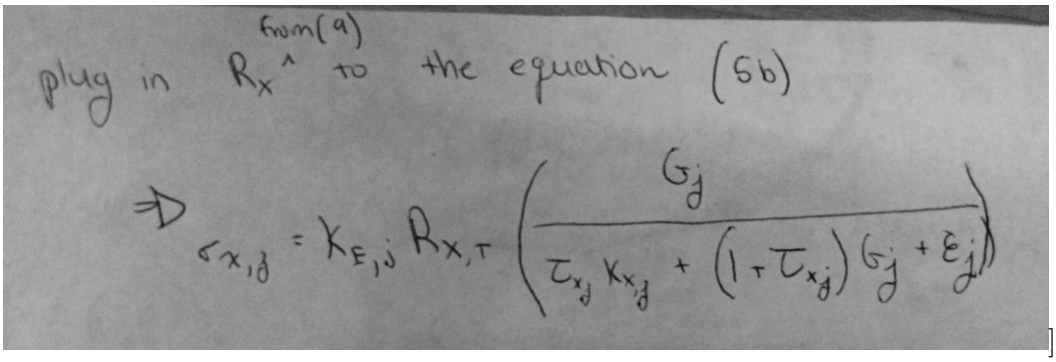
$k_A(t^{-1})$ = the rate constant governing abortive initiation.
Also, the first term in equation (2) is $k_I(G_j : R_x)_C$



The definitions of τ and K are incorrect in the above picture. Correctly,

The saturation and time constants are defined as $K_{X,j}^{-1} \equiv k_{+,j}/\left(k_{-,j}+k_{I,j}\right)$ and $\tau_{X,j}^{-1} \equiv k_{I,j}/\left(k_{A,j}+k_{E,j}\right)$, respectively.





In the above, (9) is plugged into (5b) to give:

$$(G_j : R_x)_O = R_{X,T}\frac{G_j}{\tau_{X,j}K_{x,j} + (1+\tau_{X,j})G_j + \epsilon_j} \tag{1}$$

which in turn was plugged into

$$r_{X,j} = k_{E,j}(G_j : R_x)_O \tag{2}$$

So therefore, this is the derivation of

$$r_{X,j} = k_{E,j}R_{X,T}\frac{G_j}{\tau_{X,j}K_{x,j} + (1+\tau_{X,j})G_j + \epsilon_j} \tag{3}$$

where

$$\epsilon_j = \sum_{i=1,j}^{N}\frac{K_{X,j}\tau_{X,j}}{K_{X,i}\tau_{X,i}}(1+\tau_{X,i})G_i \tag{4}$$

(b) The equation for when there is only one gene being considered is

$$r_{X,j} = k_{E,j} R_{X,T} \frac{G_j}{\tau_{X,j} K_{x,j} + (1 + \tau_{X,j}) G_j} \tag{5}$$

This is only missing the ϵ in the denominator. This would happen if:

$$\tau_{X,j} K_{x,j} + (1 + \tau_{X,j}) G_j >> \epsilon \tag{6}$$

which would in turn occur if

$$\epsilon \approx 0 \rightarrow K_{X,j} \tau_{X,j} << K_{X,i} \tau_{X,i} \tag{7}$$

So, in what circumstances would this happen? Roughly, the saturation constant $K_{X,i}$ is dissociation over association, so a $K_{X,i} > K_{X,j}$ would happen in the system where there less affinity of the RNAP for the other genes i in the system compared to affinity for gene j which we are focusing on. Also roughly, the time constant $\tau_{X,i}$ is RNAP open complex elongation/abortion over RNAP open complex forming, so $\tau_{X,i} > \tau_{X,j}$ would happen if the initiation happens slower and elongation/abortion happens faster for the other genes i in the system compared to gene j.(A way to think about this is that the RNAP will be more focused for transcribing gene j compared to genes i.)

If all of these conditions/circumstances happen, then $K_{X,j} \tau_{X,j} << K_{X,i} \tau_{X,i}$, and hence an $N >> 1$ system can be simplified to a $N = 1$ system.

2 Problem 2

(a) **ps2a_run.jl** = run file for the graphs

ps2_parameters.jl = includes calculations for all variables and input parameters based on given information

Control Functions

For this problem, the control functions u_X and u_L which have the range [0,1] are used to modulate the rate of transcription and translation respectively. The form of the u_X equation is similar for each mRNA: the numerator contains the terms that represent when the corresponding protein is activated; the denominator contains all possible states of the protein.

$$u_{X,1} = \frac{W_{1,1} + W_{I,1} f_{I,1}}{1 + W_{1,1} + W_{I,1} f_{I,1}} \tag{8}$$

$$u_{X,2} = \frac{W_{2,2} + W_{1,2} f_{1,2}}{1 + W_{2,2} + W_{1,2} f_{1,2}} \tag{9}$$

$$u_{X,3} = \frac{W_{3,3} + W_{1,3} f_{1,3}}{1 + W_{3,3} + W_{1,3} f_{1,3} + W_{2,3} f_{2,3}} \tag{10}$$

where the subscripts $1, 2, 3$ denote the mRNA u_X functions for P1, P2, and P3 respectively. The weights are from Table 1, following the same subscript notation. To note, $W_{i,i}$ where i is a protein #, denotes basal transcription in this case.

Each of these terms above contain the term f. Within the code, the f is determined by the given effector and target. Assuming that you use the correct k and n parameters for the given system (Table 2) and the concentration of protein i, the following is the formula for f:

$$f_{i,j}(p_i, k_{i,j}, n_{i,j}) = \frac{p_i^{n_{i,j}}}{k_{i,j}^{n_{i,j}} + p_i^{n_{i,j}}} \tag{11}$$

The f defined as $f_{i,j}$ is where p_i is the effector protein i concentration, the target protein is j, $k_{i,j}$ is the binding parameter, and $n_{i,j}$ is the binding coefficient. More cleanly written:

$$f_{i,j}(p_i, k_{i,j}, n_{i,j}) = \frac{p_i^{n}}{k_{i,j}^{n} + p_i^{n}} \tag{12}$$

Within the julia file code, the inputs for each of the $f(p_i, k_{i,j}, n_{i,j})$ can be found in the u_X control function equations for transcription. We assume that translation happens at the kinetic limit, so $u_L = 1$ for all proteins.

kinetic equations

For mRNA, the kinetic limit of transcription rate is:

$$r_{X,j} = v_{X,j} R_{X,T} \left(\frac{G_j}{\tau_X K_X + (\tau_X + 1) G_j}\right) \tag{13}$$

where $v_{X,j}$ is the transcription elongation rate for the given protein gene (calculated within the julia code based on gene length), $R_{X,T}$ is the total RNAP concentration, G_j is the gene concentration (same for all proteins), K_X is the RNAP saturation constant, and τ_X is the transcription time constant. All values are defined in the julia code.

For protein, the kinetic limit of translation rate is:

$$r_{L,i} = v_{L,i} R_{L,T} \left(\frac{m_i}{\tau_L K_L + (\tau_L + 1) m_i}\right) \tag{14}$$

where $v_{L,i}$ is the translation elongation rate for the given protein i amino acid length (calculated within the julia code based on number of amino acids), $R_{L,T}$ is the total ribosome concentration, m_i is the corresponding mRNA concentration, K_L is the ribosome saturation constant, and τ_L is the translation time constant. All values are defined in the julia code.

From there the transcription rates for j mRNA were:

$$TX_j = r_{X,j} u_{X,j} \tag{15}$$

and the translation rates for j protein were:

$$TL_j = r_{L,j} u_{L,j} \tag{16}$$

mRNA and protein balances

Assumptions in the balances include: (1) Well-mixed assumption, (2) abundance of mRNA and P in the continuous limit, (3) ignore intermediates and conformations, and (4) ignore resource limitations (ATP/GTP, dXTP, AAs)

The balance for mRNA j is:

$$\frac{dm_j}{dt} = TX_j - k_{X,j}^d m_j - m_j \beta^{-1} \beta \tag{17}$$

where the terms denote the transcription rate, degradation rate, and dilution from left to right. In this $k^d_{X,j} = \frac{0.693}{halflife}$ is the mRNA degradation rate (assume 1st order).
The balance for protein j is:

$$\frac{dp_j}{dt} = TL_j - k^d_{L,j}p_j - p_j\beta^{-1}\beta \tag{18}$$

where the terms denote the translation rate, degradation rate, and dilution from left to right.In this $k^d_{L,j} = \frac{0.693}{halflife}$ is the protein degradation rate (assume 1st order)
In both these cases the dilution term μ_{dil} is:

$$\mu_{dil} = \beta^{-1}\beta = 0.693/doublingtime \tag{19}$$

As before, all values are defined and calculated within the julia code.

Matrix Form

In matrix form the final mass balances are defined as:

$$\frac{d\mathbf{x}}{dt} = \mathbf{A}\mathbf{x} + \mathbf{S}\mathbf{r} \tag{20}$$

where

$$\mathbf{A} = \begin{pmatrix} -\mu_{dil} - k^d_{X,j} & 0 & 0 & 0 & 0 & 0 \\ 0 & -\mu_{dil} - k^d_{X,j} & 0 & 0 & 0 & 0 \\ 0 & 0 & -\mu_{dil} - k^d_{X,j} & 0 & 0 & 0 \\ 0 & 0 & 0 & -\mu_{dil} - k^d_{L,j} & 0 & 0 \\ 0 & 0 & 0 & 0 & -\mu_{dil} - k^d_{L,j} & 0 \\ 0 & 0 & 0 & 0 & 0 & -\mu_{dil} - k^d_{L,j} \end{pmatrix}. \tag{21}$$

(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,

$$\mathbf{x} = \begin{pmatrix} m_1 \\ m_2 \\ m_3 \\ p_1 \\ p_2 \\ p_3 \end{pmatrix}. \tag{22}$$

and

$$\mathbf{S} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix}. \tag{23}$$

$$\mathbf{r} = \begin{pmatrix} TX_1 \\ TX_2 \\ TX_3 \\ TL_1 \\ TL_2 \\ TL_3 \end{pmatrix}. \tag{24}$$

The following discrete evaluation was used from Problem Set 2:

) at timesteps k = 0, 1, 2, . . ., $\mathcal{T}$ (with step-size τ) is given by:

$$\mathbf{x}_{k+1} = \hat{\mathbf{A}}\mathbf{x}_k + \hat{\mathbf{S}}\mathbf{r}_k \tag{2}$$

where:

$$\hat{\mathbf{A}} = \exp \mathbf{A}\tau \tag{3}$$

and:

$$\hat{\mathbf{S}} = \mathbf{A}^{-1}\left[\hat{\mathbf{A}} - \mathbf{I}\right]\mathbf{S} \tag{4}$$

]

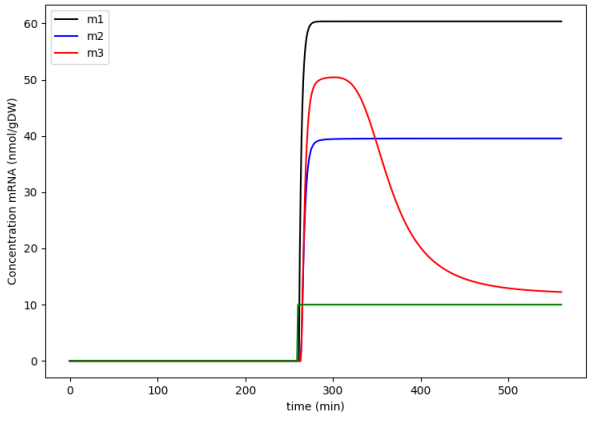
Graphs (p_j and m_j shown in graphs. $j = \{1, 2, 3\}$ where 1 = black, 2 = blue, and 3 = red.)

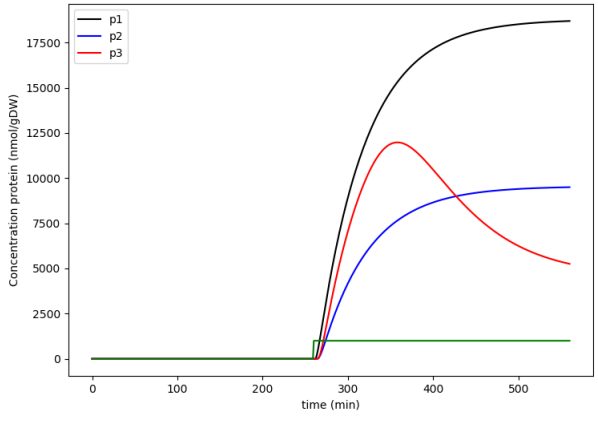
i. Run to steady-state when I = 0 (200 min).

ii. Let run for an additional 60 min. (Phase 1)

ii. Add inducer, then run for another 300 min. (Phase 2) (Inducer added at 260 min, I = 10 mM). Total time = 560 min.

The resulting graphs are below. The green unlabeled line represents step of I from 0 mM to 10 mM (not to scale on the graph).

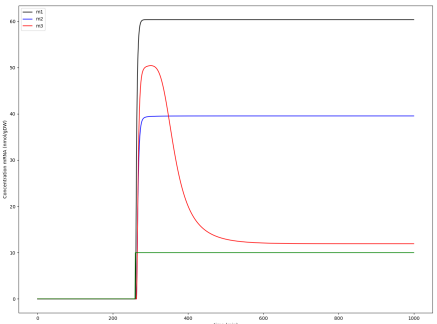


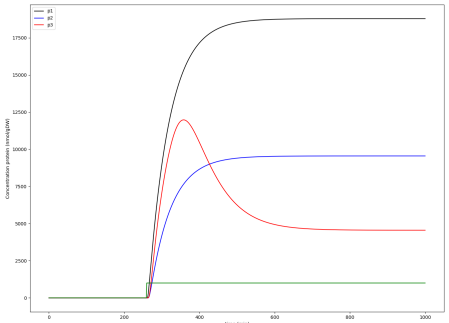


As you can see, at first there is nearly none of the mRNA and protein produced. As soon as I is added, the level of m1 jumps up, and the level of p1 slowly increases. Subsequent the levels of m2, m3, p2, and p3 increase as well. However, as seen in the schematic of the type 1 incoherent

feed forward loop p2 inhibits p3. In the graphs, you can see that once p2 reaches a critical level, the m3 decreases leading to the p3 to decrease. By the end of the time frame, you can see that the a steady-state is being approached

For reference, after running for a long time a steady-state is eventually reached:





]

(b) **ps2b_run.jl** = run file end matrix (please write "sensitivity_phase1", "sensitivity_phase2early", and "sensitivity_phase2later" in the command line.

2b_parameter_change_function.jl = includes calculations for all variables based on given parameter, contains the function that it utilized to change the parameters by certain factors for the sensitivity analysis

—For part 2b I calculated sensitivity at every time point with

$$s_{ij}(t) = \left(\frac{p_i}{x_i}\right)_\star \frac{\partial x_i}{\partial p_j}\Big|_t$$

]

where the $(.)_*$ was evaluated using the original x_i and p_j before having a p perturbation. For phase I $(.)_* = 1$ (assumption). To calculate the partial derivative, I applied the central difference theorem:

$$f'(x) = \frac{f(x+h) - f(x-h)}{2h} \tag{25}$$

each tested parameter p_j was changed by +/- 5% to make

$$h = 0.05 p_j \tag{26}$$

sensitivity_h_list.csv shows the h's used in the central difference theorem.

The s_{ij} results were time-averaged for three phases of 20 min each: –Phase 1: 230-250 minutes ; –Phase 2 early: 290-310 minutes ; –Phase 2 late: 470-490 minutes ;

The time-averaged sensitivity matrix was

$$N(i,j) = \frac{1}{T}\int_0^T |s_{ij}(t)|dt \tag{27}$$

with the integral completed via the trapezoid rule (defined in trapezoid_rule.jl), and T being the span of the window (20 min.)

Please see included csv documents with Phase 1, 2 early, and 2 late for the N matrices.

i. **"sensitivity_phase1_file.csv"**
ii. **"sensitivity_phase2early_file.csv"**
iii. **"sensitivity_phase2late_file.csv"**

The headings of the csv files show all of the parameters used to calculate sensitivity.

(c) **ps2c_run.jl** = run this file to produce perform single value decomposition of the time-averaged sensitivity matrices N from part (b).

N (MxP) was decomposed to

$$N^{(\epsilon)} = \mathbf{U}^{(\epsilon)}\mathbf{\Sigma}^{(\epsilon)}\mathbf{V}^{T,(\epsilon)} \tag{28}$$

where $\mathbf{U}^{(\epsilon)}$ is the rank-ordered sensitive species combinations (MxM), $\mathbf{\Sigma}^{(\epsilon)}$ is the singular value ranks (MxP), and $\mathbf{V}^{T,(\epsilon)}$ is the rank-ordered sensitive parameter combinations (PxP).

I used the absolute magnitude values in the first column of $\mathbf{U}$ (shown below, rows are m1, m2, m3, p1, p2, p3 from top to bottom) as the basis to rank-order the importance of model species.

```
julia> U_phase1
6×6 Array{Float64,2}:
 -0.00161686  -0.00396905  -0.000945957   0.440678      0.864737      0.24086
 -0.00206971  -0.00223421   0.00256734    0.611299     -0.485591      0.6249
 -0.0014488   -0.00152489  -0.00196314    0.657337     -0.128152     -0.742615
 -0.643898    -0.725825    -0.241957     -0.00490856   -0.00213421   -0.000590337
 -0.546448     0.214939     0.809436     -0.000604579   0.00181676   -0.00236372
 -0.535519     0.653418    -0.535031      0.00104741    0.000324909   0.00198848

julia> U_phase2early
6×6 Array{Float64,2}:
 -7.84552e-6   3.93152e-6   0.000399419   0.779372     -0.328452    -0.533572
 -3.44929e-6   4.68335e-6   0.00023571    0.324126      0.940137    -0.105281
 -5.74227e-6   1.28175e-5   0.000273305   0.536211     -0.0908912    0.839176
 -0.820582    -0.561108    -0.108638      5.60055e-5    8.09746e-6   3.42799e-6
 -0.189156     0.0872603    0.978062     -0.000525475  -6.43278e-5   7.63167e-6
 -0.539319     0.82313     -0.177741      7.99671e-5    9.97429e-6  -8.39206e-6

julia> U_phase2late
6×6 Array{Float64,2}:
 -1.78286e-6  -1.47499e-5   0.000215161   0.926365     -0.0414142   -0.374343
 -7.74039e-7  -7.59809e-6   0.000105951   0.373948      0.21949      0.901103
 -2.36559e-6  -1.57258e-6  -7.68663e-6   -0.0448463     0.974735    -0.218815
 -0.914648     0.339093    -0.220078      5.65554e-5    1.89257e-7   4.43418e-6
 -0.240509    -0.0188842    0.970463     -0.000232967  -7.27623e-6  -1.60211e-5
 -0.324922    -0.940563    -0.0988274     7.59373e-6   -2.53903e-6   8.77254e-7
```

Higher abs values are more important. Here are the results:

Phase 1: The species from most important to least important are [p1,p2,p3,m2,m1,m3].

Specifically p1 was most important, followed by p2 then p3. p1 is the effector of both p2 and p3, and hence it is reasonable that at low-levels it would be the most important (because the of the k_{12} and k_{13} are 1000, with means the fraction f would be higher at lower p1 concentrations compared to the f containing p2 and p3). p2 is slightly more than p3 which in this case is reasonable because p2 is an inhibitor for p3, but the opposite is not true. Both p2 and p3 are pretty similar though, which is reasonable because at low levels of p2 the effect of it on p3 is not that significant. The levels of the mRNA are generally not that important because they are simply precursors to the protein levels (and the protein levels are the actual effectors of each other.)

Additionally, the mRNA levels are much lower than those of the proteins so their changes have very little effect. Within the mRNA levels, we see that m2 is most important because that is the first mRNA effected by values in the system without inducer (it is effected by p1, but m1 is only effected by inducer which is not present.) m1 is next important within the mRNA because it is the inducer for m2 and m3. m3 does not have much induction at this point nor is it an effector, which is why it is last.

Phase 2early: The species from most important to least important are [p1,p3,p2,m1,m3,m2].

p1 is the most important, followed by p3, then p2. With inducer, p1 is very changed by I, with a large weight of W_{I1} ans small $k_{I1} = 0.30$. p2 is also an effector (inhibitor of p3.) Although $W_{13} < W_{23}$, $k_{13} << k_{23}$ which is why p1 ends up more important than p2 (m3 has greater affinity for p1.) Despite not being an inducer, p3 is more important than p3 because

it is changing dramatically in this region in this "balancing act" between induction by p1 and inhibition by p2.

As for phase 1, all of the proteins are more important than the mRNA (for the same reasons.)

m3 like p3 moves up in the ranking here because now its value is limited by the both the values of p1 and p2 having a "balancing act" (p1 is an inducer and p2 is a inhibitor.) m1 like p1 is most important due to its induction by inducer. m2 like p2 is last because of $k_{13} << k_{23}$ and different in affinity to m3.

Phase 2late: The species from most important to least important are [p1,p3,p2,m3,m1,m2].

Like before, p1 is the most important, followed by p3, then p2. With inducer, p1 is very changed by I, with a large weight of W_I1 and small $k_I1 = 0.30$. Although $W_13 < W_23$, $k_13 << k_23$ which is why p1 ends up more important than p2 (there is greater affinity for p1.) p3 is in the middle because it is still in this balance between being effected by p1 and p2 at this stage, despite not being an effector itself. This leaves p2 last, as with Phase 2early. (To note, p2 and p3 are very close in this phase, similar to how they were in Phase 1. This suggests that, like in phase 1, since they are reaching steady-state and are not directly influenced by the inducer, they are both pretty insignificant in this stage.)

As for phase 1, all of the proteins are more important than the mRNA (for the same reasons)

m3 is most important now because it is even more in the balance act (in the late region m3 is decreasing since p2 inibition ¿ p1 induction, – small differences in m3 will therefore have a big effect on p3 since of this sensitive balance of induction my p1 and inhibition by p2.) m1 is second because it is induced in this region and p1 an effector for both m2 and m3. m2 is only an inhibitor which is why it is last (as it was in Phase2early). m2 being last can also be explained by the lower affinity of p3 for p2 compared to affinity for p1 (as explained before, $k_13 << k_23$).

Extra

For completeness, the $\mathbf{\Sigma}^{(\epsilon)}$ were

```
julia> S_phase1
6-element Array{Float64,1}:
 37711.859571341534
     2.0911055898476523
     0.15708766121161744
     0.0007429319821608885
     1.1031634022197682e-5
     3.7120075828953443e-6

julia> S_phase2early
6-element Array{Float64,1}:
    1.7106380118502l1e8
    2.791930494807283e6
    1.9556893785395562e6
 3995.73294538513
   61.108830445694046
    7.041030906586345

julia> S_phase2late
6-element Array{Float64,1}:
    7.547700447085937e8
    3.847304617763139e7
    7.422274409220887e6
 2942.3029260920516
   95.12941272889007
   50.217200103296236
```

and the $\mathbf{V}^{T,(\epsilon)}$ can be printed from the command line as "Vt_phase1", "Vt_phase2early", and "Vt_phase2late"

3 Problem 3

(a) **"ps3_run.jl"** = contains all of the math associated with this problem to make the stoichiometric matrix, and define r_X and r_L as a function of time

Using the Table 1 in the paper, (where n = length of the gene (nt), and a = length of the protein (aa)), the stoichiometeric matrix is

$S =$

-1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0	-1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	-1000.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	1.0	-1.0	-1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	2000.0	0.0	0.0	660.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-1.0
0.0	0.0	1000.0	0.0	0.0	0.0	0.0	0.0	0.0	-1.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	-1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	1.0	-1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	-330.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	-660.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
0.0	0.0	0.0	0.0	660.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-1.0	0.0
0.0	0.0	0.0	0.0	0.0	-1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	330.0	-1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	-1.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	-1.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	-1.0	0.0	0.0	0.0	0.0	0.0	0.0

where the columns are v1-v6,b1-b9, and the rows are the different metabolites:

$$\textbf{rows} = \begin{pmatrix} G \\ RNAP \\ G* \\ NTP \\ mRNA \\ Pi \\ NMP \\ rib \\ rib* \\ AAtRNA \\ GTP \\ GDP \\ AA \\ tRNA \\ ATP \\ AMP \\ protein \end{pmatrix}. \tag{29}$$

r_X for gene j was defined as:

$$r_X = v_X R_{X,T} \frac{G}{\tau_X K_x + (1 + \tau_X) G} \tag{30}$$

and the actual transcription rate (with a control term is)

$$TX(I) = r_X * u_X(I) \tag{31}$$

where v_X is the transcription elongation rate, $R_{X,T}$ is the total number of RNAP, K_X is the RNAP saturation constant, τ_X is the time constant, I is the inducer concentration (mM), G is the gene concentration (uM),

$$u_X(I) = \frac{W_1 + f(I)W_2}{1 + W_1 + f(I)W_2}, \tag{32}$$

$$f(I) = \frac{I^n}{k^n + I^n}, \tag{33}$$

n = binding order, and k = binding parameter. Note, the "(I)" means "as a function of I." At steady-state, the balance for mRNA j is:

$$0 = \frac{dm_j(I)}{dt} = TX_j(I) - k^d_{X,j} m_j(I) - m_j \beta^{-1} \beta \tag{34}$$

where $k^d_{X,j}$ is the degradation rate, and

$$\beta^{-1}\beta = \mu_{dilution} = 0.693/doublingtime. \tag{35}$$

In this cell-free system, $\mu_{dilution} = 0$ because volume is constant and there is no cells present that can grow. So, simplified:

$$0 = \frac{dm_j(I)}{dt} = TX_j(I) - k^d_{X,j} m_j(I) \tag{36}$$

By rearrangement, we find that the steady-state mRNA concentration is:

$$m_j(I) = \frac{TX_j(I)}{k^d_{X,j}} \tag{37}$$

From this, the maximum protein production rate for protein j at steady-state is

$$r_{L,j}(I) = v_{L,j} R_L \frac{m_j(I)}{\tau_{L,j} K_{L,j} + (\tau_{L,j} + 1) m_j(I)} \tag{38}$$

I then set the bounds for the flux balance analysis. I set $v_2 = TX(I)$ and the $v_{5,upperbound} = r_L(I)$. the $v_{5,lowerbound} = 0$. All other fluxes in the system were bounded by $0 \leq v \leq \infty$ because they were assumed irreversible and there was no upper-bound. For the fluxes to external, I set the bounds as $-100000 \leq b \leq 100000$.

Units of I = [mM]. All rates v or b have units [uM/sec].

(b) **"ps3_run.jl"** = contains the objective function input parameters, the flux optimization (flux balance analysis) using **flux.jl** and generates the graphs for testing the effect of various I concentrations on v_5 and protein p steady-state concentration.

For the optimization, the bounds used in part (a) were applied. Optimization was done to maximize v_5, the rate of protein production. The calculated flux array for I = 5 [mM] is below. The rows are ordered from top to bottom v_1 to v_6 then b_1 to b_9 in [uM per s]

```
julia> calculated_flux_array_5
15-element Array{Float64,1}:
 5.8584519251656626e-5
 5.8584519251656626e-5
 5.858451925165663e-5
 4.743014760093738e-5
 4.743014760093738e-5
 0.015651948708309334
 0.015651948708309334
 0.05858451925450936
 4.743014869745821e-5
 0.05858451925450936
 0.015651948706363328
 0.015651948706363328
 0.031303897412726656
 0.031303897412726656
 0.17977683333447203
```

]

The protein j production rate at steady state is concentration is:

$$0 = \frac{dp_j}{dt} = TL_j - k^d_{L,j} * p_j - p_j * \beta^{-1}\beta \tag{39}$$

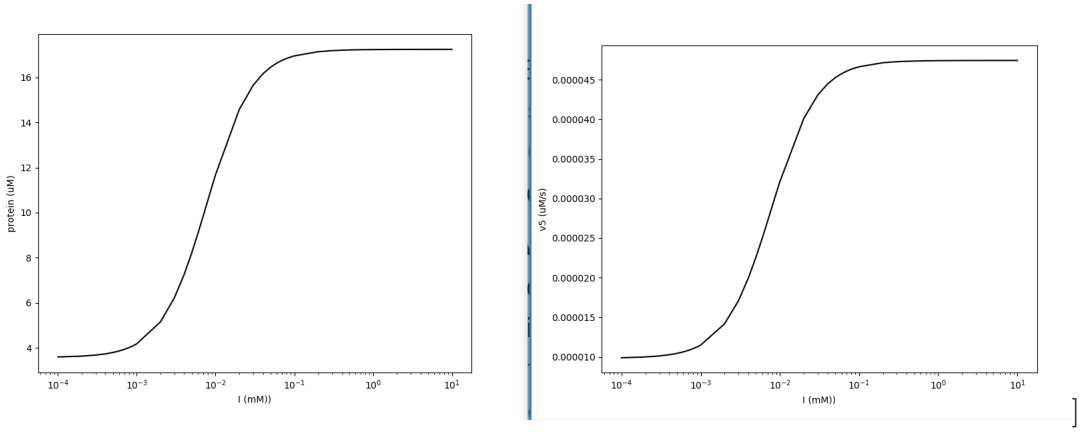
where TL_j is the protein production rate, $k^d_{L,j}$ is the protein degradation rate, and $\beta^{-1} * \beta$ is the protein dilution rate ($\beta^{-1}\beta = 0$ in this cell-free system where volume is constant and there are no cells to double.) After doing flux balance analysis with the objective function maximizing v_5 at a given I, $TL(I) = v_5(I)$ (the maximized protein production rate). Therefore, protein production at steady-state can be simplified to:

$$0 = \frac{dp_j(I)}{dt} = v_5(I) - k^d_{L,j} * p_j(I) \tag{40}$$

By rearrangement, the steady-state protein concentration as a function of I is:

$$p_j(I) = \frac{v_5(I)}{k^d_{L,j}} \tag{41}$$

The graphs below show the steady-state $p_j(I)$ and $v_5(I)$ vs. a range of I concentrations. The curve is sigmoidal in shape, suggesting there is a small range where protein production is very sensitive to small changes in I



(c) Shadow pricing is the concept that there is an additional cost associated with the

For this system, the fluxes to/from the external represent the shadow (hidden) prices associated with the corresponding metabolites. Using the concept of a shadow price, if you could increase the of a metabolite by one unit, the flux b represents how much the protein production v_5 (aka translation rate) would be increased. Therefore, the metabolite with the largest b absolute value would have the greatest effect in increasing the protein production (aka translation rate) v_5. Looking at the representative example where $I = 5$ (shown in part (b)), we see that the largest b is $b_9 = 0.179$ which corresponds to the rate at with P_i (phosphate ion) is carried away to the external. This is reasonable considering Le Chatelier's principle that product removal is will make a reaction go forward. The translation rate is most sensitive to the flux of Pi leaving.