CHEME 7770: Problem Set 2

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February 18, 2019

1. The parameters involved in the transcription of mRNA can be divided into two categories: basic parameters, which depend on biological aspects, and compound parameters, which are obtained from the basic parameters. Table 1 differentiates both categories and gives a description of every parameter used in PS1.

Basic Parameters		**Compound Parameters**	
Parameter	**Units**	**Parameter**	**Equation**
Length of the gene (L)	nts	Specific elongation rate (ke)	$ke = \frac{ke*}{L}$
Elongation rate (ke*)	nts/s	Saturation constant (Sx)	$Sx = \frac{k_- + k_I}{k_+}$
Copies per cell (Gc)	copies/cell	Dimentionless tau (τ)	$\tau = \frac{ka+ke}{k_I}$
Initiation rate (k_I)	1/s	Molecular weight of plasmid (MWg)	$MWg = L * 607.1 + 157.9$
Abortion rate (ka)	1/s	Gene concentration (GP)	$Gp = \frac{\delta_c * Gc * 50 * 10^{-9}}{MWg}$
Total RNAP (Rx)	M		
Cell concentration (δ_c)	cells/mL		

Table 1: Parameters used during the solution of PS1 and their significance. All parameters can be divided into two categories, namely 'basic parameters' which are derived from biological processes, and 'compound parameters' which result from calculations involving basic parameters

To evaluate the influence of each parameter in the resulting mRNA concentration, each *basic parameter* was varied in a ± one order of magnitude basis. The variation of the final concentration of mRNA is recorded in table 2. The effects of up- and down-regulation were determined by comparing the curves obtained after the parameter variation with that one obtained as PS1 solution. Some examples of the shifting are observed in Figure 1. A set of all obtained graphs is attached to the end of this document in Appendix A. According to the results, only the gene length and the total RNA polymerase content seem to have an effect on the concentration of mRNA when varying them in one order of magnitude. This was surprising at first, since I expected much more variation coming from the other parameters, but it is easy to explain when observing the governing equations.

mRNA concentration depends on two big things: the weight function and the transcription rate. Let's ignore the weight function for a moment and look at the transcription rate given in equation 1.

$$r_{x,p} = K_{e,p} R_{x,t} \frac{G_p}{S_{x,p}\tau_{x,p} + G_p \tau_{x,p} + G_p} \tag{1}$$

As it can be observed, the transcription rate can be divided into the multiplication of three factors: the specific elongation rate, the total amount of RNA polymerase in the system and the fraction factor. It is evident that an increase in the total amount of RNA polymerase will have a positive effect on the rate of transcription, which was observed in the results as well (App. A.E). On the other hand, the specific elongation rate depends on two basic parameters: the polymerase elongation rate and the length of the gene to be transcripted. This means that an increase on the former or a decrease on the latter, by the same amount, should result in the same overall effect in terms of the transcription rate. However, it seems like there is only a measurable difference when varying the length of the gene. Why is that?

Parameter	**Up-regulation**	**Down-regulation**
Length of the gene	–	++
Elongation rate	0	0
Copies per cell	0	0
Initiation rate	0	0
Abortion rate	0	0
Total RNAP	++	–
Cell concentration	0	0

Table 2: Influence of basic parameters in the final concentration of mRNA when increased or decreased by one order of magnitude. (++) stands for increase, (–) stands for decrease and (0) stands for no-change

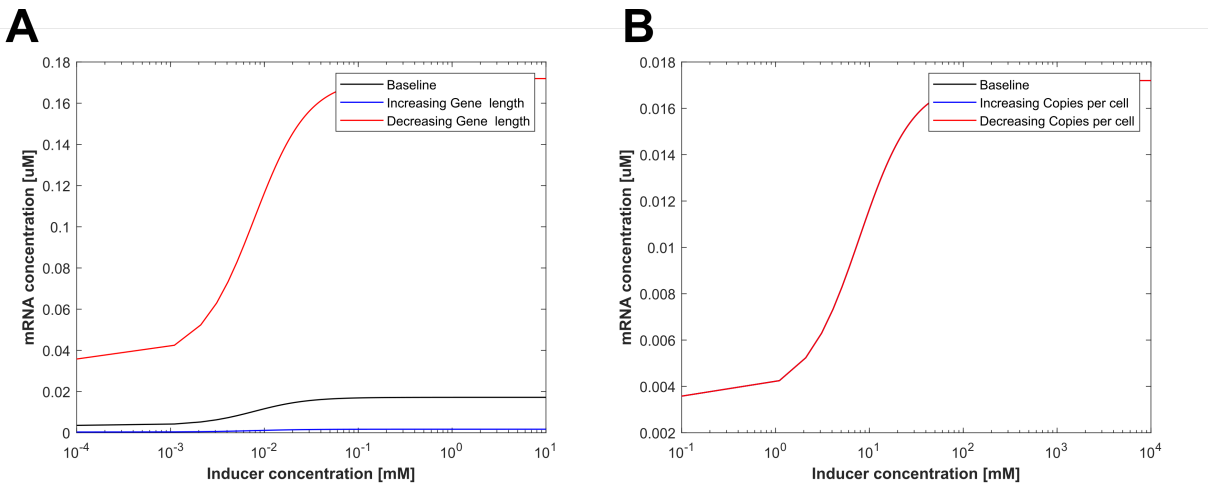

Figure 1: Changes in mRNA concentration profile at different inducer concentrations as a consequence of changes in basic parameters. (**A**) Decreasing the gene length by one order of magnitude has a positive effect on the final concentration of mRNA. Contrarily, decreasing the gene length by one order of magnitude has a negative effect on the final concentration of mRNA. (**B**) Nor the increase or the decrease of the number of copies per cell of the plasmid in one order of magnitude has an effect on the final concentration of mRNA

To answer that question, we should first analyze what is going on with the fraction term. First of all, notice that if $S_{x,p}\tau_{x,p} + G_p\tau_{x,p} << G_p$ the fraction factor will be equal to 1 and will have no effect on the overall transcription rate. This is true for the basis line, where $\tau_{x,p}$ and $S_{x,p}$ are at least two order of magnitudes smaller than G_p. Let's look now at the equations for $S_{x,p}$ and $\tau_{x,p}$ shown in

equations 2 and 3, respectively.

$$S_{x,p} = 1.04 k_I [\mu M] \tag{2}$$

$$\tau_{x,p} = \frac{k_A + k_{E,p}}{k_I} \tag{3}$$

Note that an increase in the initiation rate will have no overall effect, since the increase of $S_{x,p}$ will be countered by the decrease of $\tau_{x,p}$ and $G_p \tau_{x,p} << G_p$. Conversely, a decrease in the initiation rate will only have an effect when $G_p \tau_{x,p}$ is comparable with G_p, which is only appreciable if decreased by 5 orders of magnitude and causes a negative effect on the final mRNA concentration (App. B). Note also that this effect is relatively small for such a great change in the value of the parameter. Coming back to our initial question, why isn't there any appreciably effect on mRNA concentration with a change in the elongation rate, but there is one with a change in the gene length?

The elongation rate of RNAP appears as well in $\tau_{x,p}$, and an increase on it will cause a decrease on the fraction term, which explains why the elongation rate seems to have no effect on mRNA concentration, since the effects balance between both terms. But what's different with gene length? Well, although the effect of gene length in the specific elongation rate will have the same effect as that from the polymerase elongation rate (none), the gene length affects G_p as well. This means, that an increase in the specific elongation rate by a decrease in the gene length won't be countered by the effect of $\tau_{x,p} G_p$ being significant, since the change of G_p will keep the fraction approaching to 1.

2. The material balances for m_1, m_2, m_3 and p_1, p_2, p_3 can be written as follows:

$$\frac{dm_1}{dt} = r_{x,1} u(I)_1 - m_1 D_{x,1} \tag{4}$$

$$\frac{dm_2}{dt} = r_{x,2} [u(p_1, p_3)_2] - m_2 (D_{x,2} + \mu) \tag{5}$$

$$\frac{dm_3}{dt} = r_{x,3} [u(p_1, p_2)_3] - m_3 (D_{x,3} + \mu) \tag{6}$$

$$\frac{dp_1}{dt} = r_{L,1} w(m_1)_1 - p_1 D_{L,1} \tag{7}$$

$$\frac{dp_2}{dt} = r_{L,2} w(m_2)_2 - p_2 (D_{L,2} + \mu) \tag{8}$$

$$\frac{dp_3}{dt} = r_{L,3} w(m_3)_3 - p_3 (D_{L,3} + \mu) \tag{9}$$

Which can be written in matrix form as:

$$\frac{dx_j}{dt} = \mathbf{S} r + \mathbf{A} x \tag{10}$$

$$\mathbf{S} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

$$r = \begin{bmatrix} r_{x,1}u(I) \\ r_{x,2}u(p_2, p_3) \\ r_{x,3}u(p_1, p_2) \\ r_{L,1}w(m_1) \\ r_{L,2}w(m_2) \\ r_{L,3}w(m_3) \end{bmatrix}$$

$$\mathbf{A} = \begin{bmatrix} -D_{x,1} & 0 & 0 & 0 & 0 & 0 \\ 0 & -(D_{x,2}+\mu) & 0 & 0 & 0 & 0 \\ 0 & 0 & -(D_{x,3}+\mu) & 0 & 0 & 0 \\ 0 & 0 & 0 & -D_{L,1} & 0 & 0 \\ 0 & 0 & 0 & 0 & -(D_{L,2}+\mu) & 0 \\ 0 & 0 & 0 & 0 & 0 & -(D_{L,3}+\mu) \end{bmatrix}$$

$$x = \begin{bmatrix} m_1 \\ m_2 \\ m_3 \\ p_1 \\ p_2 \\ p_3 \end{bmatrix}$$

Where the control functions are as follows:

$$u(I) = \frac{w_1 + w_2 f_I}{1 + w_1 + w_2 f_I} \tag{11}$$

$$u(p_1, p_3) = \frac{w_1 + w_2 f_{p_1} + w_3 f_{p_3}}{1 + w_1 + w_2 f_{p_1} + w_3 f_{p_3}} \tag{12}$$

$$u(p_1, p_2) = \frac{w_1 + w_2 f_{p_1} + w_3 f_{p_2}}{1 + w_1 + w_2 f_{p_1} + w_3 f_{p_2}} \tag{13}$$

$$f_I = \frac{I^n}{K^n + I^n} \tag{14}$$

$$f_{p_1} = \frac{p_1^n}{K^n + p_1^n} \tag{15}$$

$$f_{p_2} = \frac{p_2^n}{K^n + p_2^n} \tag{16}$$

$$f_{p_3} = \frac{p_3^n}{K^n + p_3^n} \tag{17}$$

The linear system can be solved as a system of differential equations. However, since it is expected to encounter fast changes in the concentration of some species after the inducer is turn off, it is better

to solve the problem as having a stiff system of differential equations. The results obtained with the numerical solver for a complete and a broken system are shown in Figure 2. The code used to obtain the solution is shown in Appendix D. Unless stated differently in the code, all parameters were obtained from Bionumbers and are used in mol/gDW.

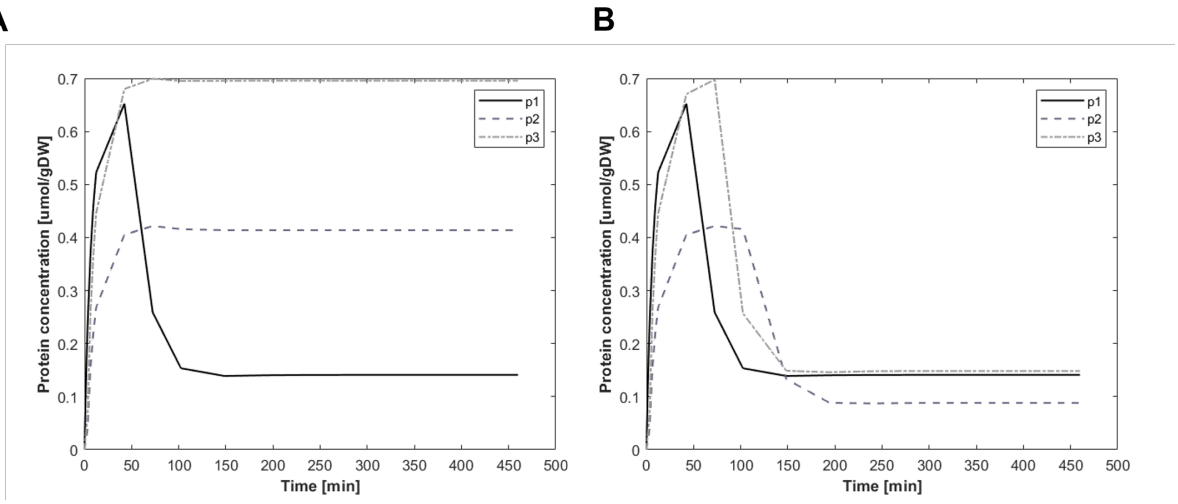

Figure 2: Concentration of proteins 1, 2, 3 in the memory circuit after induction for 60 minutes when (**A**) protein 2 induces transcription of protein 3 and protein 3 induces transcription of protein 2, and when (**B**) only protein 3 induces transcription of protein 2. Note that only in the first case the system behaves as a memory system

The properties of the linear system allow for an approximate numerical solution of the form:

$$x_{k+1} = \hat{\mathbf{A}} x_k + \hat{\mathbf{S}} r_k \tag{18}$$

$$\hat{\mathbf{A}} = exp\mathbf{A}\tau \tag{19}$$

$$\hat{\mathbf{S}} = \mathbf{A}^{-1}[\hat{\mathbf{A}} - \mathbf{I}]\mathbf{S} \tag{20}$$

The results obtained by this approximation are shown in Figure 3. Although the approximation treats the concentration values as step functions, where the change happens instantaneously, they correlate exactly with the ones shown in Figure 2 for both the complete and the broken system.

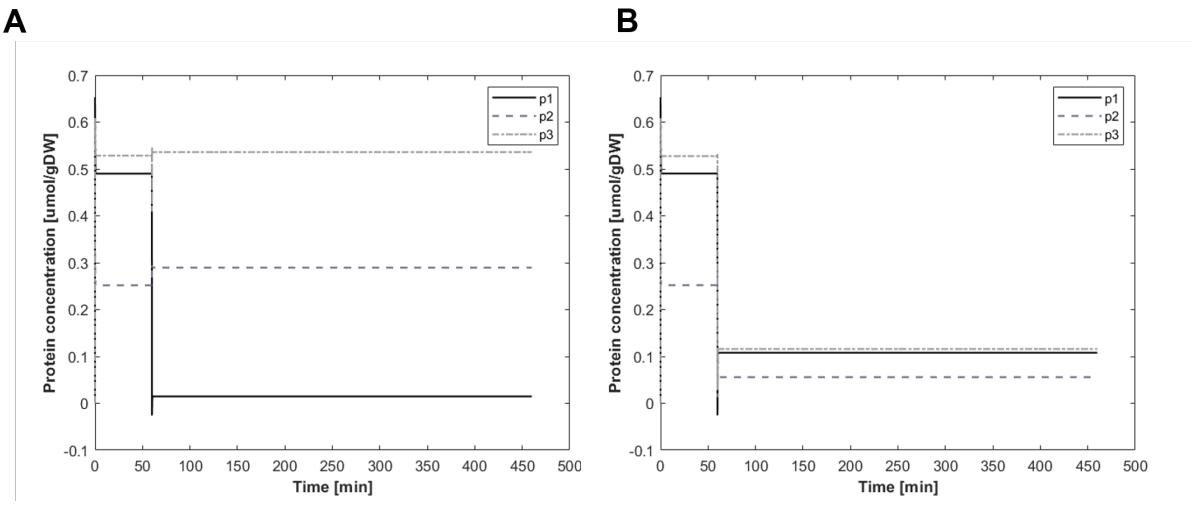

Figure 3: Numerical approximation of the protein concentration for a (**A**) complete circuit and a (**B**) broken system

3. Appendix A: Influence of basic parameters in PS1

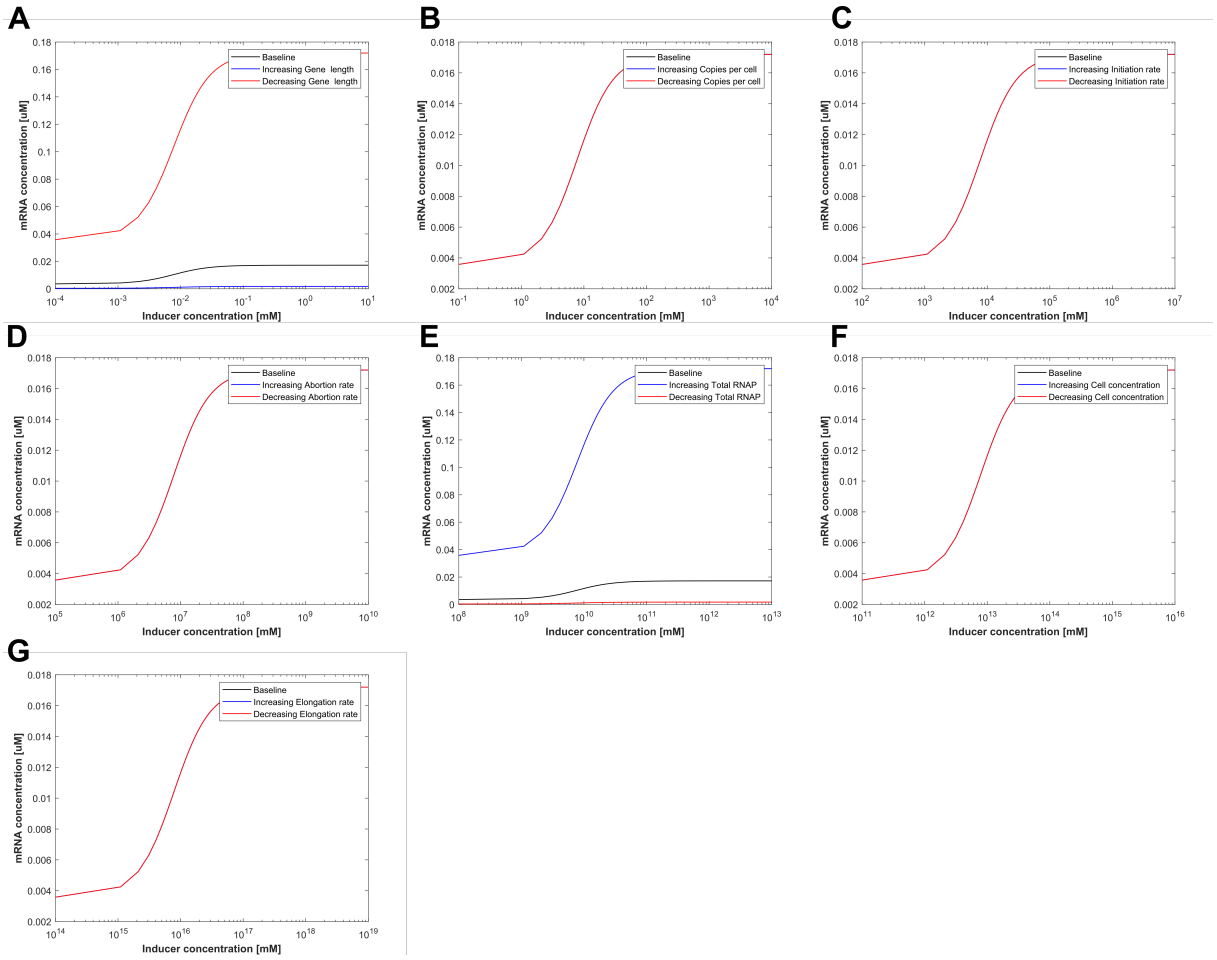

Figure 4: Changes in mRNA concentration profile at different inducer concentrations when increasing and decreasing in one order of magnitude (**A**) gene length, (**B**) copies per cell, (**C**) initiation rate, (**D**) abortion rate, (**E**) total RNA polymerase content, (**F**) cell concentration and (**G**) elongation rate. Only gene length and total RNA polymerase content seem to have an effect on the concentration profile of mRNA

4. Appendix B: Changing the initiation rate

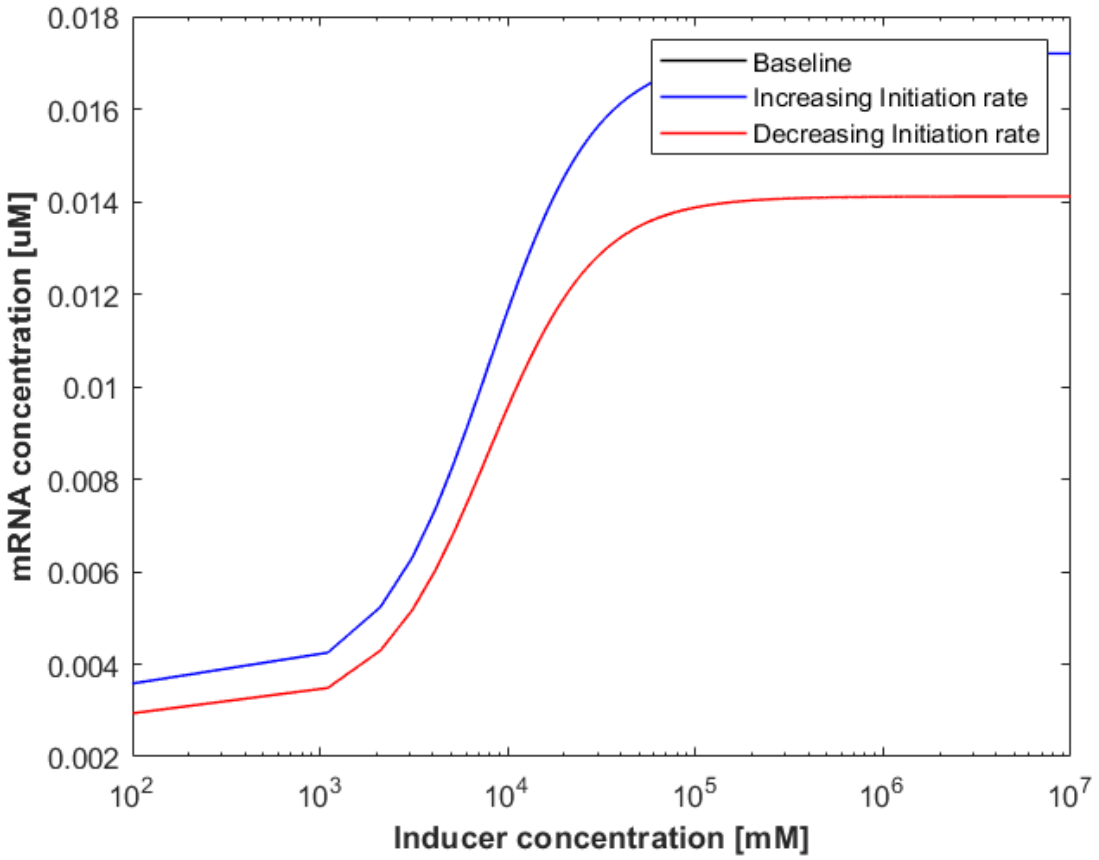

Figure 5: A decrease on the final concentration of mRNA can be observed after decreasing the initiation rate by five order of magnitudes with respect to PS1. The black and blue lines in the figure are overlapping

5. Appendix C: Code for part 1

```
%% Problem set 1.c
close all;
clear all;
clc;

%% Parameters
%Weight function
w1 = 0.26;
w2 = 300;
K = 0.3/1000; %In M
n = 1.5;

I_i = 0.0001/1000; %In M
I_f = 10/1000; %In M
I = I_i:0.001/1000:I_f;
```

```
f = (I.^(n))./(K^n+I.^n); %Inducer function

%Rates and initial values
%Basic parameters
L = 3075; %Gene length (nt)
Gc = 2500; %Copies per cell
ki = 0.024; %Initiation rate (s-1)
ka = 3.3*10^(-5); %Abortion rate (s-1)
Rx = 1.083*10^(-6); %Total RNAP per cell
deltaC = 5*10^7; %Cell concentration (cells/mL)
kes = 49; %Elongation rate (nts/s)

Par = [L Gc ki ka Rx deltaC kes]; %Vector with basic parameters
name = ["Gene  length", "Copies per cell", "Initiation rate", ...
    "Abortion rate", "Total RNAP", "Cell concentration","Elongation rate"];

%% Let's loop!

[a,stop] = size(Par);

for i=1:stop
    %Before changing things
    temp_Par = Par(1,i);
    ke = Par(1,7)/Par(1,1); %Specific elongation rate (s-1)
    Sx = 1.04*Par(1,3)*10^(-6); %Saturation constant (M)
    tx = (Par(1,4)*ke)/Par(1,3); %Dimentionless tau
    MWg = (Par(1,1)*607.4)+157.9; %Molecular Weight of plasmid (Da)
    Gp = (Par(1,6)*Par(1,2)*50*10^(-9))/MWg; %Gp in M. 50ng of plasmid are
                                        %suggested per transformation
    temp_rx = (ke*Par(1,5)*Gp)/(Sx*tx+Gp*tx+Gp);
    mp = temp_rx*((w1+w2.*f)./(1+w1+w2.*f)); %mRNA in M
    mp = mp.*10^6; %uM
    I = I.*10^3; %mM

    Par(1,i) = 10*Par(1,i); %Increase parameter 1 order of magnitude
    ke = 49/Par(1,1); %Specific elongation rate (s-1)
    Sx = 1.04*Par(1,3)*10^(-6); %Saturation constant (M)
    tx = (Par(1,4)*ke)/Par(1,3); %Dimentionless tau
    MWg = (Par(1,1)*607.4)+157.9; %Molecular Weight of plasmid (Da)
    Gp = (Par(1,6)*1000*Par(1,2)*50*10^(-9))/MWg; %Gp in M. 50ng of plasmid are
                                        %suggested per transformation
    in_rx = (ke*Par(1,5)*Gp)/(Sx*tx+Gp*tx+Gp);
    in_mp = in_rx*((w1+w2.*f)./(1+w1+w2.*f)); %mRNA in M
    in_mp = in_mp.*10^6; %uM

    Par(1,i) = Par(1,i)/100000; %Decrease parameter 1 order of magnitude
    ke = 49/Par(1,1); %Specific elongation rate (s-1)
```

```
    Sx = 1.04*Par(1,3)*10^(-6); %Saturation constant (M)
    tx = (Par(1,4)*ke)/Par(1,3); %Dimentionless tau
    MWg = (Par(1,1)*607.4)+157.9; %Molecular Weight of plasmid (Da)
    Gp = (Par(1,6)*1000*Par(1,2)*50*10^(-9))/MWg; %Gp in M. 50ng of plasmid are
                                        %suggested per transformation
    de_rx = (ke*Par(1,5)*Gp)/(Sx*tx+Gp*tx+Gp);
    de_mp = de_rx*((w1+w2.*f)./(1+w1+w2.*f)); %mRNA in M
    de_mp = de_mp.*10^6; %uM

    figure(i)
    p = semilogx(I,mp);
    hold on;
    q = semilogx(I,in_mp);
    r = semilogx(I,de_mp);
    p.Color = 'k';
    p.LineWidth = 0.85;
    q.Color = 'b';
    q.LineWidth = 0.85;
    r.Color = 'r';
    r.LineWidth = 0.85;
    xlabel('Inducer concentration [mM]','fontweight','bold');
    ylabel('mRNA concentration [uM]','fontweight','bold');
    conc1 = strcat('Increasing'," ",name(1,i));
    conc2 = strcat('Decreasing'," ",name(1,i));
    legend('Baseline',conc1,conc2);
    hold off;

    Par(1,i) = temp_Par;
end

%It looks like the [] part of r_x,p approaches 1 since Gp is much greater
%than Sx or tx, which means that the only parameters that affect this
%system are the elongation rate and RNAP concentration...
```

6. Appendix D: Code for part 2

```
    %% Problem set 2
close all;
clear all;
clc;

%Global variables
global w1 w2 w3 rx1 rx2 rx3 Kl1 Kl2 Kl3 tl1 tl2 tl3 Sxp Rlt Dx Dl mu

%Basic Parameters
Dt = 30; %Doubling time (min)
DW = 0.3; %Percentage of dry mass per cell
Gc = 200; %Copies per cell
```

```
mRNA_h = 300/60; %mRNA half-life (min)
prot_h = 70*60; %Protein half-life (min)
Cv = 9*10^(-17); %Cell volume
Cm = 2.8*10^(-13); %Cell mass
Cc = 5*10^7; %Cell concentration (cell/mL)
Kep = 49*60; %Elongation rate (nts/min)
Klp = 16.5*60; %Translation rate (aa/min)
Ki = 0.024*60; %Initiation rate (1/min)
Rxt = 1.083*10^(-6); %Total RNAP concentration (M)
Lx1 = 1200; %gene1 length (nts)
Lx2 = 2400; %gene2 length (nts)
Lx3 = 600; %gene3 length (nts)
Ll1 = 400; %Protein 1 length (AA)
Ll2 = 800; %Protein 2 length (AA)
Ll3 = 200; %Protein 3 length (AA)
w1 = 0.26;
w2 = 30;
w3 = 30;
Av = 6.023*10^23; %Avogadro number
Rib = 20100; %Number of ribosomes per cell

%Compound Parameters
Rxt = Rxt*Cv*DW/Cm; %RNAP concentration (mol/gDW)
Rlt = (Rib*Cc*1000/Av)*(Cv*DW/Cm); %Ribosomes concentration (mol/gDW)
Dx = log(2)/mRNA_h; %Degradation rate of mRNA
Dl = log(2)/prot_h; %Degradation rate of proteins
mu = log(2)/Dt; %Dilution factor

Sxp = (1.04*Ki*Cv*DW/Cm)*10^(-6); %Saturation constant (mol/gDW)
Ke1 = Kep/Lx1; %Elongation rate for mRNA1
Ke2 = Kep/Lx2; %Elongation rate for mRNA2
Ke3 = Kep/Lx3; %Elongation rate for mRNA3
tx1 = Ke1/Ki; %Tau for mRNA1
tx2 = Ke2/Ki; %Tau for mRNA2
tx3 = Ke3/Ki; %Tau for mRNA3
MW1 = Lx1*607.4+157.9; %Molecular Weight of gene 1
MW2 = Lx2*607.4+157.9; %Molecular Weight of gene 2
MW3 = Lx3*607.4+157.9; %Molecular Weight of gene 3
Gp1 = (Cc*1000*Gc*50*10^(-9)/MW1)*(Cv*DW/Cm); %Gp for gene 1
Gp2 = (Cc*1000*Gc*50*10^(-9)/MW2)*(Cv*DW/Cm); %Gp for gene 2
Gp3 = (Cc*1000*Gc*50*10^(-9)/MW3)*(Cv*DW/Cm); %Gp for gene 3

rx1 = Ke1*Rxt*(Gp1/(Sxp*tx1+Gp1*tx1+Gp1)); %Transcription rate for gene 1
rx2 = Ke2*Rxt*(Gp2/(Sxp*tx2+Gp2*tx2+Gp2)); %Transcription rate for gene 2
rx3 = Ke3*Rxt*(Gp3/(Sxp*tx3+Gp3*tx3+Gp3)); %Transcription rate for gene 3

Kl1 = Klp/Ll1; %Translation rate protein 1
Kl2 = Klp/Ll2; %Translation rate protein 2
```

```
Kl3 = Klp/Ll3; %Translation rate protein 3
tl1 = Kl1/Ki; %Tau for protein 1
tl2 = Kl2/Ki; %Tau for protein 1
tl3 = Kl3/Ki; %Tau for protein 1

%Initial conditions
t_i = 0;
t_f = 460; %Final time (min)
step = 0.01;
t_span = t_i:step:t_f; %Time vector (min)
[m,n] = size(t_span); %Size of time
I = zeros(n+1,1);
I(1:60,1) = 10*10^(-3); %Inducer initial concentration (mol/gDW)
x0 = [0;0;0;0;0;0]; %Initial conditions for x vector

%% Case 1 - Normal circuit

[t,X] = ode15s(@(t,x) sys(t,x,I),t_span,x0);
X = X.*(10^(9));

figure(1)
q = plot(t_span,X(:,1),t_span,X(:,2),t_span,X(:,3));
q(1).LineWidth = 1.2;
q(1).Color = 'black';
q(1).LineStyle = '-';
q(2).LineWidth = 1.2;
q(2).Color = [0.4 0.4 0.5];
q(2).LineStyle = '--';
q(3).LineWidth = 1.2;
q(3).Color = [0.6 0.6 0.6];
q(3).LineStyle = '-.';
xlabel('Time [min]','fontweight','bold')
ylabel('Protein concentration [umol/gDW]','fontweight','bold')
legend('p1','p2','p3')

%% Case 2 - Broken circuit

[t,Y] = ode15s(@(t,x) sys2(t,x,I),t_span,x0);
Y = Y.*(10^(9));

figure(2)
q = plot(t_span,Y(:,1),t_span,Y(:,2),t_span,Y(:,3));
q(1).LineWidth = 1.2;
q(1).Color = 'black';
q(1).LineStyle = '-';
q(2).LineWidth = 1.2;
q(2).Color = [0.4 0.4 0.5];
q(2).LineStyle = '--';
```

```
q(3).LineWidth = 1.2;
q(3).Color = [0.6 0.6 0.6];
q(3).LineStyle = '-.';
xlabel('Time [min]','fontweight','bold')
ylabel('Protein concentration [umol/gDW]','fontweight','bold')
legend('p1','p2','p3')


%% Case 3 - Approximate solution

step = 0.01; %(min)
t_span = t_i:step:t_f; %Time vector (min)
[m,l] = size(t_span); %Extract the size of time
x = zeros(6,l);
r = zeros(6,1);
A = [-Dx 0 0 0 0 0;...
    0 -(Dx+mu) 0 0 0 0;...
    0 0 -(Dx+mu) 0 0 0;...
    0 0 0 -Dl 0 0;...
    0 0 0 0 -(Dl+mu) 0;...
    0 0 0 0 0 -(Dl+mu)];
S = eye(6,6);

A1 = exp(A)*step;
S1 = inv(A)*(A1-eye(6,6))*S;

n = 15;
K = 0.3*10^(-9);

for i=1:l-1
    if i<6000
        In = 10*10^(-3);
    else
        In = 0;
    end
    fi = (In^n)/(In^n+K^n); %Inducer function
    fp1 = (x(1,i)^n)/(x(1,i)^n+K^n); %P1 function
    fp2 = (x(2,i)^n)/(x(2,i)^n+K^n); %P2 function
    fp3 = (x(3,i)^n)/(x(3,i)^n+K^n); %P3 function

    ui = (w1+w2*fi)/(1+w1+w2*fi); %Control inducer function
    up13 = (w1+w2*fp1+w3*fp3)/(1+w1+w2*fp1+w3*fp3); %Control p2 function
    up12 = (w1+w2*fp1+w3*fp2)/(1+w1+w2*fp1+w3*fp2); %Control p3 function
%     up12 = (w1+w2*fp1)/(1+w1+w2*fp1); %Control p3 function broken
%     (uncomment to obtain broken graph)

    r(1,1) = rx1*ui;
    r(2,1) = rx2*up13;
```

```
    r(3,1) = rx3*up12;
    r(4,1) = Kl1*Rlt*(x(1,i)/(Sxp*tl1+x(1,i)*tl1+x(1,i)));
    r(5,1) = Kl2*Rlt*(x(2,i)/(Sxp*tl2+x(2,i)*tl2+x(2,i)));
    r(6,1) = Kl3*Rlt*(x(3,i)/(Sxp*tl3+x(3,i)*tl3+x(3,i)));


    x(:,i+1) = A1*x(:,i)+S1*r(:,1);
end

x = x.*10^9;

figure(3)
q = plot(t_span,x(1,:),t_span,x(2,:),t_span, x(3,:));
q(1).LineWidth = 1.2;
q(1).Color = 'black';
q(1).LineStyle = '-';
q(2).LineWidth = 1.2;
q(2).Color = [0.4 0.4 0.5];
q(2).LineStyle = '--';
q(3).LineWidth = 1.2;
q(3).Color = [0.6 0.6 0.6];
q(3).LineStyle = '-.';
xlabel('Time [min]','fontweight','bold')
ylabel('Protein concentration [umol/gDW]','fontweight','bold')
legend('p1','p2','p3')

function f = sys(t,x,I)
global w1 w2 w3 rx1 rx2 rx3 Kl1 Kl2 Kl3 tl1 tl2 tl3 Sxp Rlt Dx Dl mu

%x = [m1;m2;m3;p1;p2;p3];
n = 25;
K = 0.3*10^(-9);

a = round(t);
In = I(a+1,1);
m1 = x(1);
m2 = x(2);
m3 = x(3);
p1 = x(4);
p2 = x(5);
p3 = x(6);

fi = (In^n)/(In^n+K^n); %Inducer function
fp1 = (m1^n)/(m1^n+K^n); %P1 function
fp2 = (m2^n)/(m2^n+K^n); %P2 function
fp3 = (m3^n)/(m3^n+K^n); %P3 function
```

```
ui = (w1+w2*fi)/(1+w1+w2*fi); %Control inducer function
up13 = (w1+w2*fp1+w3*fp3)/(1+w1+w2*fp1+w3*fp3); %Control p2 function
up12 = (w1+w2*fp1+w3*fp2)/(1+w1+w2*fp1+w3*fp2); %Control p3 function


rl1 = Kl1*Rlt*(m1/(Sxp*tl1+m1*tl1+m1))*10^3; %Translation rate for gene 1
rl2 = Kl2*Rlt*(m2/(Sxp*tl2+m2*tl2+m2))*10^3; %Translation rate for gene 2
rl3 = Kl3*Rlt*(m3/(Sxp*tl3+m3*tl3+m3))*10^3; %Translation rate for gene 3


f = zeros(6,1);
f(1,1) = rx1*ui-m1*Dx;
f(2,1) = rx2*up13-m2*(Dx+mu);
f(3,1) = rx3*up12-m3*(Dx+mu);
f(4,1) = rl1-p1*Dl;
f(5,1) = rl2-p2*(Dl+mu);
f(6,1) = rl3-p3*(Dl+mu);

end

function f = sys2(t,x,I)
global w1 w2 w3 rx1 rx2 rx3 Kl1 Kl2 Kl3 tl1 tl2 tl3 Sxp Rlt Dx Dl mu

%x = [m1;m2;m3;p1;p2;p3];
n = 25;
K = 0.3*10^(-9);

a = round(t);
In = I(a+1,1);
m1 = x(1);
m2 = x(2);
m3 = x(3);
p1 = x(4);
p2 = x(5);
p3 = x(6);

fi = (In^n)/(In^n+K^n); %Inducer function
fp1 = (m1^n)/(m1^n+K^n); %P1 function
fp2 = (m2^n)/(m2^n+K^n); %P2 function
fp3 = (m3^n)/(m3^n+K^n); %P3 function

ui = (w1+w2*fi)/(1+w1+w2*fi); %Control inducer function
up13 = (w1+w2*fp1+w3*fp3)/(1+w1+w2*fp1+w3*fp3); %Control p2 function
up12 = (w1+w2*fp1)/(1+w1+w2*fp1); %Control p3 function

rl1 = Kl1*Rlt*(m1/(Sxp*tl1+m1*tl1+m1))*10^3; %Transcription rate for gene 1
rl2 = Kl2*Rlt*(m2/(Sxp*tl2+m2*tl2+m2))*10^3; %Transcription rate for gene 2
rl3 = Kl3*Rlt*(m3/(Sxp*tl3+m3*tl3+m3))*10^3; %Transcription rate for gene 3
```

```
f = zeros(6,1);
f(1,1) = rx1*ui-m1*Dx;
f(2,1) = rx2*up13-m2*(Dx+mu);
f(3,1) = rx3*up12-m3*(Dx+mu);
f(4,1) = rl1-p1*Dl;
f(5,1) = rl2-p2*(Dl+mu);
f(6,1) = rl3-p3*(Dl+mu);

end
```