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MDPI

Supplementary

Molecular dynamic model of tryptophan overproduction in *Escherichia coli*

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File S1. Matlab code developed for the molecular dynamic model.

This code is also available at https://gitlab.com/amalib/trp-biot-dynmod

Part I

This part shows the implementation of the model proposed by Santillán and Zeron [18] in MATLAB R2020b:

```
%-------------------------------------------------------------------------------------------

%A Representation of the tryptophan operon model developed by Santillan and Zeron (2004)

%Glossary of terms:

%RNAP: RNA polymerase

%Œ°M: Micromolar

%RBA: Ribosome binding site

%TrpE: Anthranilate synthase enzyme

%tRNA-Trp:

mu=0.01; %1/min: Growth rate

kT=7.3*10^4; %1/min: Tryptophan production rate constant

KI=4.1; %µM: TrpE inhibition constant

p=240; %µM/min: Maximum metabolic tryptophan consumption, as estimated by Bliss (1982)

Kp=10; %µM: Metabolic tryptophan consumption constant: It is equal to or less than one-tenth of the steady

state tryptophan concentration, as estimated by Bliss (1982).

kM= 5.1; %1/min: Rate constant of trpE expression

Ot= 4*10^-3 %µM: Operon concentration
```

```
P=3; %µM: RNAP concentration

kP=4.5*10^-2; %µM: Dissociation constant of the reaction between RNAP and operon promoter

Rt=0.8; %µM: Concentration of repressor dimers

kR=2*10^-4; %µM: Dissociation constant of the binding reaction between repressor (TrpR) and operator

KT=40; %µM: Dissociation constant of the activation reaction between TrpR and tryptophan

KG=5; %µM: Dissociation constant of the charging reaction between uncharged tRNA-Trp and tryptophan

alfa=18.5 %Dimensionless: The reason between total tRNA-Trp (charged and uncharged) concentration and KG

gamM=0.69; %1/min: mRNA degradation rate

kE=30; %1/min, mRNA translation rate

2ame= 0; %min, TrpE degradation constant

Time=25000; %Simulation time

h=0.01; %Step size

x = 0; %Timer

yTrp = zeros(1,Time); %A matrix that stores 2ame2cellular Tryptophan concentration values

Tf= zeros(1,Time); %A matrix that stores 2ame2cellular Free Tryptophan concentration values

yTrp(1) =100*10;  %Initial intracellular tryptophan concentration for a derepression experiment     41.958

yMF = zeros(1,Time); %A matrix that stores free mRNA concentration values

yMF(1) =0;% Initial free mRNA concentration for a derepression experiment

yEnz = zeros(1,Time); %A matrix that stores total trpE concentration values

yEnz(1) =0; % Initial total enzyme concentration for a derepression experiment

KeaT= zeros(1,Time);%A matrix that stores enzymatic activity values

mins=0:0.01:250; %Minutes

%----------------------------Calculation-Loop-Runge-Kutta-4th-order-----------------------

for i=1:Time

Tf(i)=(0.5*(((KI+(2*yEnz(i))-yTrp(i))^2)+(4*KI*yTrp(i)))^0.5)-(0.5*(KI+(2*yEnz(i))-yTrp(i)));  %Free  trypto-

phan

F_xy = (@(t,T)(kT*yEnz(i)*(KI/(KI+Tf(i)))^2)-((p)*(Tf(i)/(Tf(i)+Kp)))-(mu*T)); %Total tryptophan

    k_1 = F_xy(x,yTrp(i));
```

```
    k_2 = F_xy(x+0.5*h,yTrp(i)+0.5*h*k_1);

    k_3 = F_xy((x+0.5*h),(yTrp(i)+0.5*h*k_2));

    k_4 = F_xy((x+h),(yTrp(i)+k_3*h));

    yTrp(i+1) = yTrp(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h); %Main equation, tryptophan concentration.

%-------------------------------------------------------------------------------------------

 F_xy                                                                                    =

@(t,Mf)(kM*Ot*((P/kP)/(1+(P/kP)+((Rt/kR)*(Tf(i)/(Tf(i)+KT))^2))))*((1+(2*alfa*(Tf(i)/(KG+Tf(i)))))/((1+(alfa*

(Tf(i)/(KG+Tf(i)))))^2))-(gamM+mu)*Mf;

    k_1 = F_xy(x,yMF(i));

    k_2 = F_xy(x+0.5*h,yMF(i)+0.5*h*k_1);

    k_3 = F_xy((x+0.5*h),(yMF(i)+0.5*h*k_2));

    k_4 = F_xy((x+h),(yMF(i)+k_3*h));

    yMF(i+1) = yMF(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  % main equation, free mRNA production

%-------------------------------------------------------------------------------------------

if i<=100 %Equation rule before reaching enough time to apply the time delay

F_xy = (@(t,Enz)(0.5*kE*yMF(1))-((3ame+mu)*Enz));

    k_1 = F_xy(x,yEnz(i));

    k_2 = F_xy(x+0.5*h,yEnz(i)+0.5*h*k_1);

    k_3 = F_xy((x+0.5*h),(yEnz(i)+0.5*h*k_2));

    k_4 = F_xy((x+h),(yEnz(i)+k_3*h));

    yEnz(i+1) = yEnz(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  % main equation, trpE production

else %Equation rule after reaching enough time to apply one time delay

 F_xy = (@(t,Enz)(0.5*kE*yMF(i-100))-((3ame+mu)*Enz));

    k_1 = F_xy(x,yEnz(i));

    k_2 = F_xy(x+0.5*h,yEnz(i)+0.5*h*k_1);

    k_3 = F_xy((x+0.5*h),(yEnz(i)+0.5*h*k_2));

    k_4 = F_xy((x+h),(yEnz(i)+k_3*h));

    yEnz(i+1) = yEnz(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  % main equation, trpE production
```

```
end
    KeaT(i)=(yEnz(i)*(KI/(KI+Tf(i)))^2)/0.002633; %Normalized enzyme activity
    x=x+h;
end
figure
plot(mins(1:length(mins)-1),KeaT,'k','LineWidth',2)
xlabel('Time (min)')
ylabel('Normalized enzyme activity')
ylim([0 1.2])
```

Part II

This part shows the implementation of the model proposed by Santillán and Mackey [17] in MATLAB R2020b.

```
%-------------------------------------------------------------------------------------
%*A Representation of the tryptophan operon developed by Santillan and Mackey
%Glossary of terms:
%RNAP: RNA polymerase
%µM: Micromolar
%RBA: Ribosome binding site
%TrpE: Anthranilate synthase enzyme
%List of variables for the model
Mu=0.01 %min^-1: growth rate
tauP=0.1 %min: time delay corresponding to the time after which an RNAP has moved far enough to free the operon
promoter
taup= 5*10^-2 %min: Time for a ribosome to free the RBS
taum= 0.1 %min: According to Santillan and Mackey "This is the time it takes for an RNAP to assemble a
functional TrpE-related ribosome-binding site,"
P=2.6 %µM: RNAP concentration
kP=3.9 %1/(µM min): Rate constant for the binding reaction between RNAP and operon promoter
Kr=1.2/460 %1/µM: Dissociation constant of the binding reaction between repressor (TrpR) and operator
```

```
O=3.32*10^-3 %µM: Operon concentration, considering 1.6 genome equivalents

E=0.9 %µM: Trp transport parameter, as defined by Drozdov-Tikhomirov and Skurida (1977), [19]

d=23.5 %µM/min: Maximum rate of tryptophan uptake, as defined by Drozdov-Tikhomirov and Skurida

f=380 %µM: Trp transport parameter, as defined by Drozdov-Tikhomirov and Skurida

b=0.85 %Dimensionless: this value satisfies experimental observations for attenuation

c=4*10^-2 %µM: this value satisfies experimental observations for attenuation

kp=6.9 %1/(Œ°M min): Rate constant for the binding reaction between a ribosome and an RBS.

p= 2.9 %µM: Ribosome concentration

kdD= 0.6 %1/min: mRNA degradation rate

gam= 0 %1/min: TrpE degradation rate

taue=0.66 %min: This is the time it takes for a ribosome to synthesize a fully functional TrpE

Ki=720/176 %1/µM: Dissociation constant of the inhibition reactions between tryptophan and TrpE

nH=1.2 %Dimensionless: Hill coefficient

Kt=(2.1*10^4)/348 %1/µM: Dissociation constant of the activation reaction between TrpR and tryptophan

R=0.8 %µM: Concentration of repressor dimers.

Kg= 0.2 %µM: Metabolic tryptophan consumption constant: It is equal to or less than one-tenth of the steady
state tryptophan concentration, as Bliss (1982) estimates.

g=25 %µM/min: Maximum metabolic tryptophan consumption, as estimated by Bliss (1982), [36]

K=126.4 %1/min: Tryptophan production rate constant

Time=25000 %Simulation time

h=0.01; %Step size

x=0; %Timer

yTrp = zeros(1,Time); %A matrix that stores intracellular Tryptophan concentration values

yTrp(1) = 16.47;  %Initial intracellular tryptophan concentration for a derepression experiment

yOF = zeros(1,Time); %A matrix that stores free operon concentration values

yOF(1) = 4.7611*10^-5;% Initial free operon concentration for a derepression experiment

yMF = zeros(1,Time);%A matrix that stores free mRNA concentration values

yMF(1) = 1.1656*10^-4;% Initial free mRNA concentration for a derepression experiment
```

```
yEnz = zeros(1,Time);%A matrix that stores total trpE concentration values

yEnz(1) =0.1176; % Initial total enzyme concentration for a derepression experiment

KEaT= zeros(1,Time);%A matrix that stores enzymatic activity values

Texter= zeros(1,Time);%A matrix that stores extracellular Tryptophan concentration values

Texter(1)=0;%Initial extracellular tryptophan concentration for a derepression experiment

mins=0:0.01:250; %Minutes

%-------------------------------------Calculation-Loop-Runge-Kutta-4th-order----------------

for i=1:Time

F_xy         =         (@(t,T)(K*yEnz(i)*((Ki^nH)/((Ki^nH)+T^nH)))-((g)*(T/(T+Kg)))+((d)*((Texter(i))/(E+(Tex-

ter(i)*(1+(T/f))))))-(mu*T)); %Total tryptophan

    k_1 = F_xy(x,yTrp(i));

    k_2 = F_xy(x+0.5*h,yTrp(i)+0.5*h*k_1);

    k_3 = F_xy((x+0.5*h),(yTrp(i)+0.5*h*k_2));

    k_4 = F_xy((x+h),(yTrp(i)+k_3*h));

    yTrp(i+1) = yTrp(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h); %Main equation, tryptophan concentration.

%-----------------------------------------------------------------------------------------

Ra= (R)*(yTrp(i)/(yTrp(i)+Kt)); %Active repressor concentration

if i<=10 %Equation rule before reaching enough time to apply the time delay

    F_xy = (@(t, Of)((Kr/(Kr+Ra))*((mu*O)-(kP*P*(Of-((yOF(1))*exp(-mu*tauP))))))-(mu*Of));

    k_1 = F_xy(x,yOF(i));

    k_2 = F_xy(x+0.5*h,yOF(i)+0.5*h*k_1);

    k_3 = F_xy((x+0.5*h),(yOF(i)+0.5*h*k_2));

    k_4 = F_xy((x+h),(yOF(i)+k_3*h));

    yOF(i+1) = yOF(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  %Main equation, Free operator concentration.

else %Equation rule after reaching enough time to apply the time delay

F_xy = (@(t, Of)((Kr/(Kr+Ra))*((mu*O)-(kP*P*(Of-((yOF(i-10))*exp(-mu*tauP))))))-(mu*Of));

    k_1 = F_xy(x,yOF(i));

    k_2 = F_xy(x+0.5*h,yOF(i)+0.5*h*k_1);
```

```
    k_3 = F_xy((x+0.5*h),(yOF(i)+0.5*h*k_2));
    k_4 = F_xy((x+h),(yOF(i)+k_3*h));
    yOF(i+1) = yOF(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h); %Main equation, Free operator concentration.
end
%-------------------------------------------------------------------------------------------
A=b*(1-(exp(-yTrp(i)/c))); %Attenuator strength
if i<=5 %Equation rule before reaching enough time to apply the time delay
 F_xy  =  (@(t,Mf)((kP*P*(yOF(1))*exp(-mu*taum))*(1-A))-((kp*p*(Mf-(yMF(1)*exp(-mu*taup)))))-((kdD+mu)*Mf)); %
change the function as you desire
    k_1 = F_xy(x,yMF(i));
    k_2 = F_xy(x+0.5*h,yMF(i)+0.5*h*k_1);
    k_3 = F_xy((x+0.5*h),(yMF(i)+0.5*h*k_2));
    k_4 = F_xy((x+h),(yMF(i)+k_3*h));
    yMF(i+1) = yMF(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  % main equation
elseif i<=10 %Equation rule after reaching enough time to apply one time delay
    F_xy   =   (@(t,Mf)((kP*P*(yOF(1))*exp(-mu*taum))*(1-A))-((kp*p*(Mf-(yMF(i-5)*exp(-           mu*taup)))))-
((kdD+mu)*Mf)); % change the function as you desire
    k_1 = F_xy(x,yMF(i));
    k_2 = F_xy(x+0.5*h,yMF(i)+0.5*h*k_1);
    k_3 = F_xy((x+0.5*h),(yMF(i)+0.5*h*k_2));
    k_4 = F_xy((x+h),(yMF(i)+k_3*h));
    yMF(i+1) = yMF(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  % main equation
else  %Equation rule after reaching enough time to apply two-time delays
F_xy        =        (@(t,Mf)((kP*P*(yOF(i-10))*exp(-mu*taum))*(1-A))-((kp*p*(Mf-(yMF(i-5)*exp(-mu*taup)))))-
((kdD+mu)*Mf)); % change the function as you desire
    k_1 = F_xy(x,yMF(i));
    k_2 = F_xy(x+0.5*h,yMF(i)+0.5*h*k_1);
    k_3 = F_xy((x+0.5*h),(yMF(i)+0.5*h*k_2));
```

```
    k_4 = F_xy((x+h),(yMF(i)+k_3*h));

    yMF(i+1) = yMF(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  % main equation

end

%-------------------------------------------------------------------------

if i<=66 %Equation rule before reaching enough time to apply the time delay

F_xy = (@(t,Enz)(0.5*kp*p*yMF(1)*exp(-mu*taue))-((gam+mu)*Enz));

    k_1 = F_xy(x,yEnz(i));

    k_2 = F_xy(x+0.5*h,yEnz(i)+0.5*h*k_1);

    k_3 = F_xy((x+0.5*h),(yEnz(i)+0.5*h*k_2));

    k_4 = F_xy((x+h),(yEnz(i)+k_3*h));

    yEnz(i+1) = yEnz(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  % main equation

else %Equation rule after reaching enough time to apply the time delay

 F_xy = (@(t,Enz)(0.5*kp*p*yMF(i-66)*exp(-mu*taue))-((gam+mu)*Enz));

    k_1 = F_xy(x,yEnz(i));

    k_2 = F_xy(x+0.5*h,yEnz(i)+0.5*h*k_1);

    k_3 = F_xy((x+0.5*h),(yEnz(i)+0.5*h*k_2));

    k_4 = F_xy((x+h),(yEnz(i)+k_3*h));

    yEnz(i+1) = yEnz(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  % main equation

end

    KEaT(i)=K*yEnz(i)*((Ki^nH)/((Ki^nH)+((yTrp(i))^nH)))/24.159;  %Normalized enzyme activity

    x=x+h;

end

figure

plot(mins(1:length(mins)-1),KEaT, 'k','LineWidth',2)

title('ANTA synthase activity)

xlabel('Time (min)')

ylabel('Enzyme activity (x normal value)')

ylim([0 1.19])
```

Part III

This part shows the implementation of the model proposed by Santillán and Zeron (2004) [18] in MATLAB R2020b, modified to include the effects of the enzymes TnaA and TnaB, as well as the transport system proposed by Drozdov-Tikhomirov et al. (1977) [19]. The attenuator modeling was exchanged for the modeling proposed by Santillán and Mackey (2001) [17].

```
%-------------------------------------------------------------------------------------

%*A modified representation of the tryptophan operon model developed by Santillan and Zeron (2004)

%accounting for the effect of TnaA and TnaB

%Glossary of terms:

%RNAP: RNA polymerase

%µM: Micromolar

%RBA: Ribosome binding site

%TrpE: Anthranilate synthase enzyme

%tRNA-Trp:

mu=0.01; %1/min: Growth rate

kT=7.3*10^4;  %1/min: Tryptophan production rate constant

KI=4.1; %Œ°M: TrpE inhibition constant

p=240; %Œ°M/min: Maximum metabolic tryptophan consumption, as estimated by Bliss (1982)

Kp=10; %Œ°M: Metabolic tryptophan consumption constant: It is equal to or less than one-tenth of the steady

state tryptophan concentration, as estimated by Bliss (1982).

kM= 5.1; %1/min: Rate constant of trpE expression

Ot= 4*10^-3 %Œ°M: Operon concentration

P=3; %Œ°M: RNAP concentration

kP=4.5*10^-2; %Œ°M: Dissociation constant of the reaction between RNAP and operon promoter

Rt=0.8; %Œ°M: Concentration of repressor dimers

kR=2*10^-4; %Œ°M: Dissociation constant of the binding reaction between repressor (TrpR) and operator

KT=40; %Œ°M: Dissociation constant of the activation reaction between TrpR and tryptophan

gamM=0.69; %1/min: mRNA degradation rate

b=0.85 % attenuation constant, as defined by Santillán y Mackey (2001)
```

```
c=4*10^-2  % attenuation constant, as defined by Santillán y Mackey (2001)

kE=30; %1/min, mRNA translation rate

gamE= 0; %min, TrpE degradation constant

ka=65 %1/min: Expression rate of tnaAB operon (it appears as kA in text)

kb=ka %1/min: Expression rate of tnaAB operon (it appears as kB in text)

EE=8.7066 %1/min: Tryptophanase rate of indol production (in text appears as ktnaA)

beta=1750 %1/min: TnaB rate of tryptophan uptake (in text appears as ktnaB)

KGG=11 %Œ°M: Half saturation constant of tnaAB repression by glucose.

KW=60 %Œ°M: Half saturation constant of tnaAB induction by tryptophan

ng=4; %Dimensionless: Hill coefficient of tnaAB repression by glucose.

nw=4; %Dimensionless: Hill coefficient of tnaAB induction by tryptophan

K1=14;%Dimensionless: empirically determined by Orozco-Gomez (2019)

K2=9; %Dimensionless: empirically determined by Orozco-Gomez (2019)

lambda=7;%Dimensionless: empirically determined by Orozco-Gomez (2019)

Ge=0 %Extracellular Glucose concentration

kmb=70 %Œ°M: Michaelis constant of TnaB, as experimentally determined elsewhere.

Time=50000; %Simulation time

mins=0:0.01:Time/100; %Minutes

h=0.01;  %Step size

x = 0; %Timer

yTrp = zeros(1,Time); %A matrix that stores intracellular Tryptophan concentration values

yTrp(1) =16;  %Œ°M: Initial intracellular tryptophan concentration

yMF = zeros(1,Time); %A matrix that stores free mRNA concentration values

yMF(1) =1.1656*10^-4; %Œ°M: Initial free mRNA concentration

yEnz = zeros(1,Time); %A matrix that stores total trpE concentration values

yEnz(1) =0.1176; %Œ°M: Initial total enzyme concentration

FluxTnaB=zeros(1,Time);%A matrix that stores TnaB flux values

We = zeros(1,Time)+4; %A matrix that stores external tryptophan concentration values
```

```
A = zeros(1,Time); %A matrix that stores tryptophanase concentration values

B = zeros(1,Time); %A matrix that stores TnaB concentration values

d=23.5 %Œ°M/min: Maximum rate of tryptophan uptake according to Drozdov-Tikhomirov and Skurida (1977)

dout=d;%Œ°M/min: Maximum rate of tryptophan export according to Drozdov-Tikhomirov and Skurida (1977)

Kt=0.9 %Œ°M: Michaelis constant of tryptophan transport

Kf=380 %Œ°M: Tryptophan transport inhibition constant

%----------------------------Calculation-Loop-Runge-Kutta-4th-order-----------------------

for i=1:Time

    pgG=((KGG^ng)/((KGG^ng)+(Ge^ng))); %stands for transcriptional regulation via catabolite repression of the

operon genes

    pwW=(((yTrp(i)))^nw)/((KW^nw)+(yTrp(i)^nw)); %Accounts for tryptophan-mediated regulation via premature

transcriptional termination

    x1=(lambda*Ge)+We(i);

    PGeWe= 1-(((x1/K1)^3)*exp(-x1/K2)); %Denotes the fraction of active tryptophanase enzymes in terms of

external glucose and tryptophan levels.

F_xy = @(t,A)ka*Ot*pgG*pwW-(mu*A);

    k_1 = F_xy(x,A(i));

    k_2 = F_xy(x+0.5*h,A(i)+0.5*h*k_1);

    k_3 = F_xy((x+0.5*h),(A(i)+0.5*h*k_2));

    k_4 = F_xy((x+h),(A(i)+k_3*h));

    A(i+1) = A(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h); %main equation, Total tryptophanase concentration

F_xy = @(t,B)kb*Ot*pgG*pwW-(mu*B);

    k_1 = F_xy(x,B(i));

    k_2 = F_xy(x+0.5*h,B(i)+0.5*h*k_1);

    k_3 = F_xy((x+0.5*h),(B(i)+0.5*h*k_2));

    k_4 = F_xy((x+h),(B(i)+k_3*h));

    B(i+1) = B(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  %Main equation, TnaB concentration

%-------------------------------------------------------------------------------------------
```

```
Tf=(0.5*(((KI+(2*yEnz(i))-yTrp(i))^2)+(4*KI*yTrp(i)))^0.5)-(0.5*(KI+(2*yEnz(i))-yTrp(i))); %Free tryptophan

F_xy                    =                    @(t,T)(kT*yEnz(i)*(KI/(KI+Tf))^2)-((p)*(Tf/(Tf+Kp)))-(mu*T)-
(A(i)*PGeWe*T*EE)+(d*((We(i))/(We(i)*(1+(Tf/Kf))+Kt)))+(beta*B(i)*(We(i)/(We(i)+(kmb))))-
(dout*(Tf)/(Tf*(1+(We(i)/Kf))+Kt)); %Total tryptophan
    k_1 = F_xy(x,yTrp(i));
    k_2 = F_xy(x+0.5*h,yTrp(i)+0.5*h*k_1);
    k_3 = F_xy((x+0.5*h),(yTrp(i)+0.5*h*k_2));
    k_4 = F_xy((x+h),(yTrp(i)+k_3*h));
    yTrp(i+1) = yTrp(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  %Main equation, total tryptophan concentration

%-------------------------------------------------------------------------------------------
 AA=b*(1-(exp(-yTrp(i)/c))); %Attenuator
 F_xy                    =                    @(t,Mf)(kM*Ot*((P/kP)/(1+(P/kP)+((Rt/kR)*(Tf/(Tf+KT))^2))))*(1-AA)-
(gamM+mu)*Mf;          %*(yOF(1))*exp(-mu*taum))*(1-A))-((kp*p*(Mf-(yMF(1)*exp(-mu*taup)))))-((kdD+mu)*Mf)); %
change the function as you desire
    k_1 = F_xy(x,yMF(i));
    k_2 = F_xy(x+0.5*h,yMF(i)+0.5*h*k_1);
    k_3 = F_xy((x+0.5*h),(yMF(i)+0.5*h*k_2));
    k_4 = F_xy((x+h),(yMF(i)+k_3*h));
    yMF(i+1) = yMF(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  % main equation, free mRNA concentration

%-------------------------------------------------------------------------------------------

if i<=100 %Equation rule before reaching enough time to apply the time delay
F_xy = (@(t,Enz)(0.5*kE*yMF(1))-((gamE+mu)*Enz));
    k_1 = F_xy(x,yEnz(i));
```

```
    k_2 = F_xy(x+0.5*h,yEnz(i)+0.5*h*k_1);

    k_3 = F_xy((x+0.5*h),(yEnz(i)+0.5*h*k_2));

    k_4 = F_xy((x+h),(yEnz(i)+k_3*h));

    yEnz(i+1) = yEnz(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);   % Main equation, trpE production
else %Equation rule after reaching enough time to apply one time delay

 F_xy = (@(t,Enz)(0.5*kE*yMF(i-100))-((gamE+mu)*Enz));

    k_1 = F_xy(x,yEnz(i));

    k_2 = F_xy(x+0.5*h,yEnz(i)+0.5*h*k_1);

    k_3 = F_xy((x+0.5*h),(yEnz(i)+0.5*h*k_2));

    k_4 = F_xy((x+h),(yEnz(i)+k_3*h));

    yEnz(i+1) = yEnz(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h); % Main equation, trpE production
end

    FluxTnaB(i)=(beta*B(i)*(We(i)/(We(i)+(kmb)))); %TnaB flux

    x=x+h;
end

figure

plot(mins(1:length(mins)-1),FluxTnaB,'k','LineWidth',2)

xlabel('Time (min)')

ylabel('\mu M ')

hold off
```

Part IV

The following values in the code of Part III were changed.

- The following variables were declared:

```
We = zeros(1,Time);

Ge=5550; %Equivalent to 0.1% glucose

Time=90000;
```

- Just after yTrp(i+1) = yTrp(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h), on line 190, the following was added:

```
if yTrp(i+1)<0
yTrp(i+1)=0;
end
```

- Immediately after the start of the "for" cycle that implements the Runge–Kutta method, the following fragment was added, which introduces dynamic values for cell concentration and specific growth rate in minimal medium:

```
if i<=6000 %0 hours -> 1 hour
mu=0.0001;
    dcellnum=8*10^6-7*10^6;
    dtime=6000;
     cellnum=(7*10^6)+((dcellnum/dtime)*(i));
elseif i>6000 & i<=12000 %2 hours
   mu=0.0115;
     dcellnum=16*10^6-8*10^6;
    dtime=6000;
    cellnum=(8*10^6)+((dcellnum/dtime)*(i-6000));
elseif  i>12000 & i<=18000 %3 hours
   mu=0.015;
  dcellnum=40*10^6-16*10^6;
    dtime=6000;
    cellnum=(16*10^6)+((dcellnum/dtime)*(i-(12000)));
elseif i>18000 & i<=24000  %4 hours
      mu=0.0159;
      dcellnum=104*10^6-40*10^6;
    dtime=6000;
    cellnum=(40*10^6)+((dcellnum/dtime)*(i-(18000)));
elseif  i>24000 & i<=30000 %5 hours
   mu=0.01;
```

```
     dcellnum=200*10^6-104*10^6;
     dtime=6000;
     cellnum=(104*10^6)+((dcellnum/dtime)*(i-(24000)));
elseif  i>30000 & i<=36000 %6 hours
       mu=0.013;
     dcellnum=440*10^6-200*10^6;
     dtime=6000;
     cellnum=(200*10^6)+((dcellnum/dtime)*(i-(30000)));
     %--------------------------------
     elseif i>36000 & i<=42000 %7 hours
    mu=0.006;
       dcellnum=640*10^6-440*10^6;
     dtime=6000;
     cellnum=(440*10^6)+((dcellnum/dtime)*(i-36000));
elseif  i>42000 & i<=48000  %8 hours
    mu=0.0033;
   dcellnum=784*10^6-640*10^6;
     dtime=6000;
     cellnum=(640*10^6)+((dcellnum/dtime)*(i-(42000)));
elseif i>48000 & i<=54000 %9 hours
       mu=0.0019;
       dcellnum=880*10^6-784*10^6;
     dtime=6000;
     cellnum=(784*10^6)+((dcellnum/dtime)*(i-(48000)));
elseif  i>54000 & i<=60000 %10 hours
    mu=0.00074;
     dcellnum=920*10^6-880*10^6;
     dtime=6000;
```

```
    cellnum=(880*10^6)+((dcellnum/dtime)*(i-(54000)));
elseif  i>60000 & i<=66000 %11 hours
       mu=0.00035;
    dcellnum=940*10^6-920*10^6;
    dtime=6000;
    cellnum=(920*10^6)+((dcellnum/dtime)*(i-(60000)));
elseif i>66000 & i<=72000 %12 hours
      mu=0;
      dcellnum=940*10^6-928*10^6;
    dtime=6000;
    cellnum=(940*10^6)-((dcellnum/dtime)*(i-(66000)));
elseif  i>72000 & i<=78000 %13 hours
   mu=0.00028;
    dcellnum=944*10^6-928*10^6;
    dtime=6000;
    cellnum=(928*10^6)+((dcellnum/dtime)*(i-(72000)));
elseif  i>78000 & i<=84000 %14 hours
       mu=0.00028;
    dcellnum=960*10^6-944*10^6;
    dtime=6000;
    cellnum=(944*10^6)+((dcellnum/dtime)*(i-(78000)));
else  %15 hours
    mu=0.00013;
    dcellnum=968*10^6-960*10^6;
    dtime=6000;
    cellnum=(960*10^6)+((dcellnum/dtime)*(i-(84000)));
end
```

- Just before "x=x+h" within the "for" loop shown in the code in appendix three, the following fragment was introduced:

```
%-----------------------------------------------------------------------------------------

    F_xy = @(t,We)(-d*((We)/(We*(1+(Tf/Kf))+Kt)))-(beta*B(i)*(We/(We+(kmb))))

+(dout*(Tf)/(Tf*(1+(We/Kf))+Kt)); %Transport equation

    k_1 = F_xy(x,We(i));

    k_2 = F_xy(x+0.5*h,We(i)+0.5*h*k_1);

    k_3 = F_xy((x+0.5*h),(We(i)+0.5*h*k_2));

    k_4 = F_xy((x+h),(We(i)+k_3*h));

    We(i+1) = ((We(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h)))*(8*10^-16)*((cellnum*50)/0.05); %Main equation, ex-

ternal tryptophan concentration

if We(i+1)<0

    We(i+1)=0

end

%-----------------------------------------------------------------------------------------
```

Part V

The following values were replaced in the code of Part IV.

- The following variable was redefined:

```
        Time=48000;

        C= 8.5; %Chorismate concentration

        Kmc=5.5; %Michaelis constant of TrpE

      Ge=5550; %Equivalent to 0.1% glucose
```

- The fragment that was added in line 190 (Part III) was replaced by the following:

```
if i<=6000 %0 -> 1

mu=0.00033;

     dcellnum=16*10^6-8*10^6;

     dtime=6000;

     cellnum=(8*10^6)+((dcellnum/dtime)*(i));

elseif i>6000 & i<=12000 %2
```

```
    mu=0.001;
      dcellnum=240*10^6-16*10^6;
     dtime=6000;
     cellnum=(16*10^6)+((dcellnum/dtime)*(i-6000));
elseif  i>12000 & i<=18000 %3
    mu=0.004;
   dcellnum=720*10^6-240*10^6;
     dtime=6000;
     cellnum=(240*10^6)+((dcellnum/dtime)*(i-(12000)));
elseif i>18000 & i<=24000  %4
      mu=0.013;
      dcellnum=2240*10^6-720*10^6;
     dtime=6000;
     cellnum=(720*10^6)+((dcellnum/dtime)*(i-(18000)));
elseif  i>24000 & i<=30000 %5
    mu=0.02;
     dcellnum=3840*10^6-2240*10^6;
     dtime=6000;
     cellnum=(2240*10^6)+((dcellnum/dtime)*(i-(24000)));
elseif  i>30000 & i<=36000 %6
       mu=0.02;
     dcellnum=4240*10^6-3840*10^6;
     dtime=6000;
     cellnum=(3840*10^6)+((dcellnum/dtime)*(i-(30000)));
     %---------------------------------
     elseif i>36000 & i<=42000 %7
    mu=0.02;
      dcellnum=4280*10^6-4240*10^6;
```

```
    dtime=6000;
    cellnum=(4240*10^6)+((dcellnum/dtime)*(i-36000));
else  %8
    mu=0.02;
    dcellnum=4320*10^6-4280*10^6;
    dtime=6000;
    cellnum=(4280*10^6)+((dcellnum/dtime)*(i-(42000)));
end
```

Part VI

The following values were changed in the code of Parts IV and V, as appropriate.

- The following variables were declared:

```
Vmax=10000; %COMUN Œ°M/min: Maximum rate of tryptophan export
Kmout=100 %µM: Michaelis constant of tryptophan transport
actcoef=5000 %µM: Activation constant
```

- The equation that quantifies the intracellular production of tryptophan was modified, resulting in the following equation:

```
F_xy = @(t,T)(kT*yEnz(i)*(KI/(KI+Tf))^2)-((p)*(Tf/(Tf+Kp)))-(mu*T)-
(A(i)*PGeWe*T*EE)+(d*((We(i))/(We(i)*(1+(Tf/Kf))+Kt)))+(beta*B(i)*(We(i)/(We(i)+(kmb))))-
(dout*(Tf)/(Tf*(1+(We(i)/Kf))+Kt))-(Vmax*(Tf)/((Tf)+(Kmout*(1+(We(i)/actcoef))))));
   k_1 = F_xy(x,yTrp(i));
   k_2 = F_xy(x+0.5*h,yTrp(i)+0.5*h*k_1);
   k_3 = F_xy((x+0.5*h),(yTrp(i)+0.5*h*k_2));
   k_4 = F_xy((x+h),(yTrp(i)+k_3*h));
   yTrp(i+1) = yTrp(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  %Main equation, total tryptophan concentration
```

- The equation that quantifies the extracellular concentration of tryptophan was changed, as shown below.

```
    %--------------------------------------------------------------------
```

```
    F_xy                                =                                @(t,We)(-d*((We)/(We*(1+(Tf/Kf))+Kt)))-
(beta*B(i)*(We/(We+(kmb))))+(dout*(Tf)/(Tf*(1+(We/Kf))+Kt))+(Vout*(Tf/(Tf+Kmout))); %Transport equation


        k_1 = F_xy(x,We(i));
        k_2 = F_xy(x+0.5*h,We(i)+0.5*h*k_1);
        k_3 = F_xy((x+0.5*h),(We(i)+0.5*h*k_2));
        k_4 = F_xy((x+h),(We(i)+k_3*h));
        We(i+1) = ((We(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h)))*(8*10^-16)*((cellnum*50)/0.05);  % Main equation,
    external tryptophan concentration
     %-----------------------------------------------------------------------
```

Part VII

The following values were changed in Parts IV and V, as appropriate.

- The following variables were declared:

```
Vmax= 159030;
Kmout=0.2;
Actcoef1=168;
Actcoef11=700;
Cellnum=50;
Vol=50;
Flux=zeros(1,Time);%A matrix that stores flux out values
```

- The equation that quantifies the intracellular production of tryptophan was modified, resulting in the following equation:

```
F_xy                        =                        @(t,T)(kT*yEnz(i)*(KI/(KI+Tf))^2)-((p)*(Tf/(Tf+Kp)))-(mu*T)-
(A(i)*PGeWe*T*EE)+(d*((We(i))/(We(i)*(1+(Tf/Kf))+Kt)))+(beta*B(i)*(We(i)/(We(i)+(kmb))))-
(dout*(Tf)/(Tf*(1+(We(i)/Kf))+Kt))-(Vmax*((Tf)/(Tf*(1+(actcoef1/(We(i))))+Kmout*(1+(actcoef11/(We(i)))))));
    k_2 = F_xy(x+0.5*h,yTrp(i)+0.5*h*k_1);
    k_3 = F_xy((x+0.5*h),(yTrp(i)+0.5*h*k_2));
    k_4 = F_xy((x+h),(yTrp(i)+k_3*h));
```

```
yTrp(i+1) = yTrp(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  %Main equation, total tryptophan concentration
```

- The equation that quantifies the extracellular concentration of tryptophan was changed, as shown below.

```
%---------------------------------------------------------------------
F_xy                                =                                @(t,We)(-d*((We)/(We*(1+(Tf/Kf))+Kt)))-
(beta*B(i)*(We/(We+(kmb))))+(dout*(Tf)/(Tf*(1+(We/Kf))+Kt))+(Vout*(Tf/(Tf+Kmout))); %Transport equation

F_xy                                =                                @(t,We)(-d*((We)/(We*(1+(Tf/Kf))+Kt)))-
(beta*B(i)*(We/(We+(kmb))))+(dout*(Tf)/(Tf*(1+(We/Kf))+Kt))+(Vmax*((Tf)/(Tf*(1+(actcoef1/(We)))+Kmout*(1+(act
coef11/(We))))))
k_1 = F_xy(x,We(i));
k_2 = F_xy(x+0.5*h,We(i)+0.5*h*k_1);
k_3 = F_xy((x+0.5*h),(We(i)+0.5*h*k_2));
k_4 = F_xy((x+h),(We(i)+k_3*h));
We(i+1) = ((We(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h)))*(8*10^-16)*((cellnum*50)/0.05);  % Main equation,
external tryptophan concentration production
```

- An equation was added to quantify the flux of tryptophan into the culture medium. It was added immediately before the term "x=x+h".

```
Flux(i)= Vmax*((Tf)/(Tf*(1+(actcoef1/(We(i))))+Kmout*(1+(actcoef11/(We(i)))))))
```

Part VIII

The following elements were added and/or replaced with respect Part VII:

- The following constants were declared:

```
OP=5*2.5*10^-3 %µM: Plasmid concentration
kMlac=0.18; %µM: Lac promoter expression rate
```

```
    Ge=111000; %µM, Equivalent to 2% glucose
    pp=0.127; % probability that an RNAP molecule will bind to the promoter in the absence of the CAP activator
(catabolite activating protein)
kpc=30; % Constant value, cooperativity between CAP and lac promoter
kgg=2.6; %µM, constant value
nhh=1.3 %constant value
C= 150
```

- The fragment that calculates the concentration of free mRNA was modified. It was exchanged for the following fragment:

```
    pc=(kgg^nhh)/((kgg^nhh)+(Ge^nhh));
    PD=(pp*(1+(pc*(kpc-1))))/(1+(pp*pc*(kpc-1)));

F_xy  =  @(t,Mf)(kM*Ot*((P/kP)/(1+(P/kP)+((Rt/kR)*(Tf/(Tf+KT))^2))))*(1-AA)+  (kMlac*OtP*PD*0.3)-(gamM+mu)*Mf;%
change the function as you desire
    k_1 = F_xy(x,yMF(i));
    k_2 = F_xy(x+0.5*h,yMF(i)+0.5*h*k_1);
    k_3 = F_xy((x+0.5*h),(yMF(i)+0.5*h*k_2));
    k_4 = F_xy((x+h),(yMF(i)+k_3*h));
    yMF(i+1) = yMF(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  % main equation, free mRNA concentration
```

- The central equation that calculates the extracellular concentration of tryptophan was slightly modified to plot the concentration in g/L instead of micromoles.

```
    We(i+1) = ((We(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h)))*(8*10^-16)*((cellnum*300)/0.3);   % Main equation,
    external tryptophan concentration
```

- The cycle created in Parts IV and V was replaced by a new cycle that describes the growth and number of GPT1001 mutant cells in complex culture (Gu et al., 2012) [40].

```
if i<=36000
```

```
mu=0.00389;
dcellnum=2.6*10^9-6.4*10^8;
 dGe=116569-111000;
 dtime=36000;
 Ge=116569-((dGe/dtime)*i);
    cellnum=(6.4*10^8)+((dcellnum/dtime)*i);
elseif i>36000 & i<=72000
   mu=0.0013;
 dGe=111000-69384;
 Ge=111000-((dGe/dtime)*(i-36000));
      dcellnum=4.16*10^9-2.6*10^9;
    dtime=36000;
    cellnum=(2.6*10^9)+((dcellnum/dtime)*(i-36000));
elseif  i>72000 & i<=108000
   mu=0.00143;
 dGe=69384-64835;
 Ge=69384-((dGe/dtime)*(i-(72000)));
  dcellnum=6.96*10^9-4.16*10^9;
    dtime=36000;
    cellnum=(4.16*10^9)+((dcellnum/dtime)*(i-(72000)));
elseif i>108000 & i<=144000
      mu=0.000726;
 dGe=64835-44407;
 Ge=64835-((dGe/dtime)*(i-(108000)));
      dcellnum=9.04*10^9-6.96*10^9;
    dtime=36000;
    cellnum=(6.96*10^9)+((dcellnum/dtime)*(i-(108000)));
elseif  i>144000 & i<=180000
```

```
    mu=0.00016;
dGe=44407-13877;
 Ge=44407-((dGe/dtime)*(i-(144000)));
     dtime=36000;
     dcellnum=9.6*10^9-9.04*10^9;
     cellnum=(9.04*10^9)+((dcellnum/dtime)*(i-(144000)));
elseif  i>180000 & i<=216000
       mu=0.000248;
        dGe=13877-4440;
 Ge=13877-((dGe/dtime)*(i-(180000)));
     dcellnum=1.05*10^10-9.6*10^9;
     dtime=36000;
     cellnum=(9.6*10^9)+((dcellnum/dtime)*(i-(180000)));
else
   mu=0.000248;
   kM=1;
   kE=5;
    cellnum=1.05*10^10;
end
```

Part IX

The following item was changed from Part VIII.

- The following variables were declared:

```
Vmax2=192276000;
Kmout2=8200;
Actcoef2=123000
Actcoef21=410000;
```

```
Vmax3=64827000;

Kmout3=12300;

actcoef3=270600;

actcoef31=82000
```

- The equation that quantifies the intracellular production of tryptophan was modified, resulting in the following equation:

```
F_xy = @(t,T)(kT*yEnz(i)*(C/(C+trpEkm)))-((p)*(Tf/(Tf+Kp)))-(mu*T)-

(A(i)*PGeWe*T*EE)+(d*((We(i))/(We(i)*(1+(Tf/Kf))+Kt)))+(beta*B(i)*(We(i)/(We(i)+(kmb))))-

(dout*(Tf)/(Tf*(1+(We(i)/Kf))+Kt))-

(Vmax*((Tf)/(Tf*(1+(actcoef1/(We(i))))+Kmout*(1+(actcoef11/(We(i)))))))-

(Vmax2*((Tf)/(Tf*(1+(actcoef2/(We(i))))+Kmout2*(1+(actcoef21/(We(i)))))))-

(Vmax3*((Tf)/(Tf*(1+(actcoef3/(We(i))))+Kmout3*(1+(actcoef31/(We(i)))))))

(Vmax4*((Tf)/(Tf*(1+(actcoef4/(Tf)))+Kmout4*(1+(actcoef41/(Tf))))));

   k_1 = F_xy(x,yTrp(i));

   k_2 = F_xy(x+0.5*h,yTrp(i)+0.5*h*k_1);

   k_3 = F_xy((x+0.5*h),(yTrp(i)+0.5*h*k_2));

   k_4 = F_xy((x+h),(yTrp(i)+k_3*h));

   yTrp(i+1) = yTrp(i) + ((1/6)*(k_1+2*k_2+2*k_3+k_4)*h);  %Main equation, total tryptophan

concentration
```

Part X

The cycle added to Part VIII was replaced by a new cycle that describes the growth and number of GPT1002 mutant cells in complex culture (Gu et al., 2012)[40].

```
%GPT1002

if i<=36000

mu=0.00348;

dGe=108239-97138;

 dtime=36000;

 Ge=116569-((dGe/dtime)*i);

 dcellnum=2.24*10^9-6.4*10^8;
```

```
    dtime=36000;
    cellnum=((6.4*10^8)+((dcellnum/dtime)*i));
elseif i>36000 & i<=72000
   mu=0.00172;
     dGe=97138-74935;
 Ge=111000-((dGe/dtime)*(i-36000));
    dcellnum=4.16*10^9-2.24*10^9;
    dtime=36000;
    cellnum=((2.24*10^9)+((dcellnum/dtime)*(i-36000)));
elseif  i>72000 & i<=108000
   mu=0.001265;
   dGe=74935-64835;
 Ge=74935-((dGe/dtime)*(i-(72000)));
   dcellnum=6.56*10^9-4.16*10^9;
    dtime=36000;
    cellnum=((4.16*10^9)+((dcellnum/dtime)*(i-(72000))));
elseif i>108000 & i<=144000
      mu=0.000816;
       dGe=64835-41630;
 Ge=64835-((dGe/dtime)*(i-(108000)));
    dcellnum=8.8*10^9-6.56*10^9;
    dtime=36000;
    cellnum=((6.56*10^9)+((dcellnum/dtime)*(i-(108000))));
elseif  i>144000 & i<=180000
   mu=0.00041;
    dGe=41630-13877;
 Ge=41630-((dGe/dtime)*(i-(144000)));
    dcellnum=1.02*10^10-8.8*10^9;
```

```
    dtime=36000;
    cellnum=((8.8*10^9)+((dcellnum/dtime)*(i-(144000))));
elseif  i>180000 & i<=216000
       mu=0.00054;
               dGe=13877-2775;
 Ge=13877-((dGe/dtime)*(i-(180000)));
    dcellnum=1.24*10^10-1.02*10^10;
    dtime=36000;
    cellnum=((1.02*10^10)+((dcellnum/dtime)*(i-(180000))));
else
   mu=0.00054;
  kM=1*2;
  kE=7;
   cellnum=1.24*10^11;
end
```

Table S1. Growth parameters of *E. coli* cultures growing on LB medium.

Hours	OD_{600}	Cells/ml	Specific growth rate h^{-1}	Specific growth rate min^{-1}
0	0.01	8×10^6	0.010	0.00017
1	0.02	1.60×10^7	0.693	0.01155
2	0.3	2.40×10^8	2.708	0.04513
3	0.9	7.20×10^8	1.099	0.01831
4	2.8	2.24×10^9	1.135	0.01892
5	4.8	3.84×10^9	0.539	0.00898
6	5.3	4.24×10^9	0.099	0.00165
7	5.35	4.28×10^9	0.009	0.00016
8	5.4	4.32×10^9	0.009	0.00016

Absorbance (OD_{600}), cell number, and growth rate were calculated from the data reported by Baev et al. [50]. Cell concentration and growth rate were calculated as described in the text.

Table S2. Growth parameters of *E. coli* GPT1001 growing in a complex medium with glucose.

Minutes	GPT1001 OD_{600}	Cells/ml	Specific growth rate h^{-1}	Specific growth rate min^{-1}	Glucose g/L
0	0.8	6.4×10^8	<0.01	<0.0001	20.8
360	3.25	2.6×10^9	0.2336	0.00389	17.5
720	5.2	4.16×10^9	0.0783	0.00130	13.5

1080	8.7	6.96×10^9	0.0857	0.00143	9.5
1440	11.3	9.04×10^9	0.0435	0.00072	6.9
1800	12	9.6×10^9	0.0100	0.00016	2
2160	13.1	1.05×10^{10}	0.0149	0.00024	0.8

Absorbance (OD_{600}), cell numbers, glucose concentration (gl^{-1}), and specific growth rate of the GPT1001 mutant were calculated from the data provided by Gu et al. [40]. The authors report the absorbance in cultures of E. coli GPT1001 growing in a complex medium with glucose. Cell concentration and growth rate were calculated as described in the text.

Table S3. Growth parameters of *E. coli* GPT1002 growing in a complex medium with glucose.

Minutes	**GPT1002 OD_{600}**	**Cells/ml**	**Specific growth rate h^{-1}**	**Specific growth rate min^{-1}**	**Glucose g/L**
0	0.8	6.40×10^8	0	0	19.5
360	2.8	2.24×10^9	0.20879	0.00347	17.5
720	5.2	4.16×10^9	0.10317	0.00171	12.1
1080	8.2	6.56×10^9	0.07591	0.00126	9.5
1440	11	8.80×10^9	0.04896	0.00081	7
1800	12.7	1.02×10^{10}	0.02460	0.00041	2.4
2160	15.5	1.24×10^{10}	0.03255	0.00054	0.5

Absorbance (OD_{600}), cell numbers, glucose concentration (gl^{-1}), and specific growth rate of the GPT1002 mutant were calculated from the data provided by Gu et al. [40]. The authors report the absorbance in cultures of E. coli GPT1002 growing in a complex medium with glucose. Cell concentration and growth rate were calculated as described in the text.