Saturday, May 9, 2020 12:33 PM

Problem 1

P_{lac} = promotor

specific volume = $\frac{\langle n \rangle}{\beta}$

(a) Assuming $OD600 = 0.1 \sim 1 \times 10^8$ cells/mL

$\beta = \langle m_c \rangle \hat{N}_c V$ $\langle n \rangle \rightarrow \beta$

water fraction

mass of e.coli cell

$V = 1\,mL$

$\langle n \rangle = \frac{\text{lacZ messenger RNA copy \#}}{\text{cell}}$ $\langle m_c \rangle = \frac{gDW}{cell}$ $\hat{N}_c = \frac{\#\,cells}{mL}$

$$\beta = \frac{2.8 \times 10^{-13}\,gDW}{cell}\left(\frac{1 \times 10^8\,cell}{mL}\right)\left(1\,mL\right) = 2.8 \times 10^{-5}\,gDW$$

$$\langle n \rangle = \frac{lacZ\ mRNA}{cell}\left(\frac{mol}{6.022 \times 10^{23}\ molecules}\right)\left(\frac{10^9\,nmol}{1\,mol}\right)$$

converted <n>	IPTG (nm)
1.12682E-09	0
1.24543E-09	5E-10
2.43156E-09	0.000000005
3.97353E-09	0.000000012
5.10035E-09	0.000000053
5.51549E-09	0.000000216
5.51549E-09	0.000001

See excel sheet for calculations.

(b)

$$\dot{m}_i = \underbrace{r_{x,i}}_{\frac{nmol}{gDW \cdot hr}} \bar{u}_i - (\mu + \theta_{m,i}) m_i$$

promotor activity function

rate of transcription of gene i

show that $m^* = K_x(g_i, \theta)\bar{u}(I, \kappa)$

$\frac{nmol}{gDW}$

lacZ gene abundance

parameters

S.S. $0 = r_{x,i}\bar{u}_i - (\mu + \theta_{m,i}) m_i$

$$m^* = \frac{r_x \bar{u}}{\mu + \theta_{m^*}} = \underbrace{\left(\frac{r_x}{\mu + \theta_{m^*}}\right)}_{K_x} \bar{u}$$

$u(I, \kappa)$

$$\boxed{K_x = \frac{r_x}{\mu + \theta_m}}$$

$$u = \frac{W_1 + W_2 f_1}{1 + W_1 + W_2 f_1} \qquad f_1 = \frac{I^n}{K_d^n + I^n}$$

$$r_X = k_E^X R_{X,T}\left(\frac{G}{\tau_X K_X + (\tau_X+1)G}\right) \quad \text{(Eqn 26 LN)}$$

(c)

We need: k_E^X, $R_{X,T}$, G, τ_X, K_X

we have:
IPTG = inducer concentration (I)
$\mu = \frac{1}{t_{half}}$ (LN2)

k_E^X: elongation rate constant = $\frac{\text{transcription elongation rate}}{\text{gene length}}$ $\quad k_I = 1/\text{characteristic initiation time}$

τ_1 characteristic initiation time (initiation limited)

$$K_X = \frac{k_- + k_1}{k_+}$$

$$u(I) = \frac{W_1 + W_2 f_I}{1 + W_1 + W_2 f_I} \qquad f_I = \frac{I^n}{K_D^n + I^n} \qquad K_D = \cancel{49600}\text{ nM}$$

0.049

At IPTG = 0:

$$m^* = K_{gain}\bar{u} \rightsquigarrow 1.13\times10^7 = 2.05\times10^7\ \bar{u} \quad \bar{u} = 0.38$$

$k_2 = k_I \quad k_- = k_{-1} \quad k_1 = k_+$

$$\frac{W_1}{1+W_1} = \bar{u}$$

$$k_I = \frac{4\times10^{-2}}{s} = \frac{1}{k_{on}} \quad k_{on} = 25 \quad 9\times10^6\ \frac{1}{M\cdot s}\left(\frac{1M}{10^9}\right) = 0.009$$

$$2\times10^8 = \frac{4\times10^{-2}}{k_{off}}$$

$\tau_{obs} = 400$

$$\tau_{obs} = \frac{1}{k_I} + \underbrace{\left(\frac{k_- + k_I}{k_+ k_I}\right)}_{\frac{K_X}{k_I}}\frac{1}{R_{X,T}}$$

slope 0.12 μM·s
intercept 25 s

$$f_I = \frac{1^n}{49600 + 1^n}$$

Ignore my random scratch work

$k_{on} = 4\times10^{-4}$

$$G = \frac{2\text{ copies}}{\text{cell}}$$

$^{-}$cell

Bionumbers:
$K_d = 49.6 \times 10^{-8}$

$\boxed{K_x = 5.15 \times 10^{-9}}$

Background $= \frac{W_1}{1+W_1} = 0.22$ $\boxed{W_1 = 0.28}$

I started out finding a bunch of parameters to solve for Kx, But I could never get the units to match up when solving for u (to get w1), since u is between 0 and 1. I ended up basing my Kx value from my graph of the converted data. At fully induced transcription, u=1 so I used the same logic as the lecture notes.

Kinetic limit = 5.15×10^{-9} $\frac{nmol}{gDW}$

$m^* = K_x \bar{u}$ @ fully induced transcription $\bar{u} = 1$ $m^* = K_x (u=1)$

background (lowest value) $\rightarrow$ $\underbrace{1.127 \times 10^{-9} = 5.15 \times 10^{-9}}_{K_{gain}} \bar{u}$ $\Rightarrow$ $u = 0.22$

I struggled to solve n and W_2 simultaneously, so I am estimating n = 1.5 for the sake of finishing this problem $\rightarrow$ $\boxed{n = 1.5}$

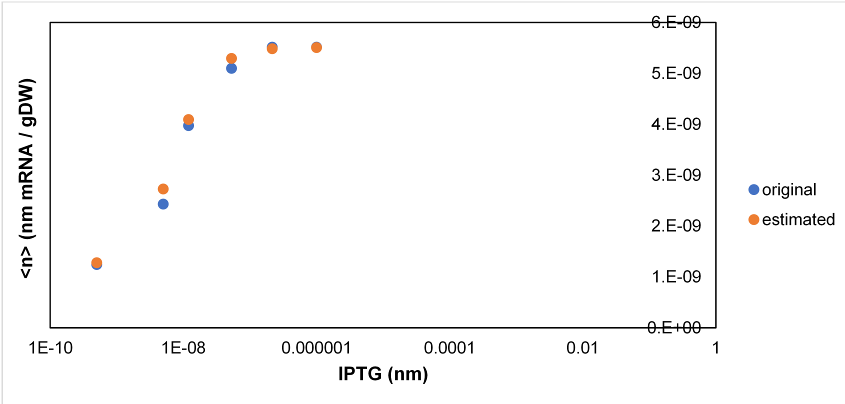
$$u(I) = \frac{0.28 + W_2\left[\frac{I^n}{K_d^n + I^n}\right]}{1 + 0.28 + W_2\left[\frac{I^n}{K_d^n + I^n}\right]} = \frac{m^*}{K_{gain}}$$

$\Downarrow$ plugging in I and $\frac{\langle m \rangle}{\beta}$ value into Wolfram Alpha

$\boxed{W_2 = 691.74}$

Eqn for estimated values: $m^* = K_{gain}\left[\frac{W_1 + W_2\left(\frac{I^n}{K_d^n + I^n}\right)}{1 + W_1 + W_2\left(\frac{I^n}{I^n + K_d^n}\right)}\right]$

(d)

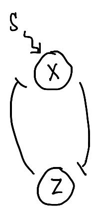


Originally, my model did not fit at ALL. My first Kd value was 49600, and I believe that I just converted my units wrong. However, I think my Kd value is wrong overall. For the sake of getting my curve to fit, I just played around with each value to see how it affected the graph, and then from there I could determine what values were wrong in my model.

I had to use a very small Kd value (i.e I just divided the original value I found (4.96 M) by factors of 10 until it fit my graph). I ended up using Kd = 4.96e-7. That being said, the Kd value has a large impact on the overall shape. I am not sure what the correct value is, but it would be on the order of 1e-7 from my guess and check method. Additionally, I suck at anything computational so I was unable to solve for n and w2 simultaneously. I guessed n=1.5, which is just the number given in the 1-30-2020 lecture notes. Changing the cooperativity affects the range of the y values, but the shape of the graph remains relatively the same. Changing w1 and w2 do affect the range of the graph, but not as drastically as Kd did.

Problem 2

(a)



$$\frac{d\tilde X}{d\tilde t} = \frac{\tilde\alpha_x + \tilde\beta_x S}{1+S+\left(\tilde Z/\tilde Z_x\right)^{n_{zx}}} - \tilde\delta_x \tilde X$$

$$\frac{d\tilde Z}{d\tilde t} = \frac{\tilde\alpha_z}{1+\left(\tilde X/\tilde X_z\right)^{n_{xz}}} - \tilde\delta_z Z$$

In the paper, they should have put a tilde over the δ_x for eq. 3: $t = \tilde t\tilde\delta_x$

(b) Nondimensionalize: $\delta_z = \frac{\tilde\delta_z}{\tilde\delta_x}$ $\quad t = \tilde t\delta_x$ $\quad \alpha_x = \frac{\tilde\alpha_x}{\tilde\alpha_z}$ $\quad \beta_x = \frac{\tilde\beta_x}{\tilde\alpha_z}$

$$Z_x = \frac{\tilde Z_x\tilde\delta_x}{\tilde\alpha_z} \qquad X_z = \frac{\tilde X_z\tilde\delta_x}{\tilde\alpha_z}$$

$$X = \frac{\tilde X\tilde\delta_x}{\tilde\alpha_z} \qquad Z = \frac{\tilde Z\tilde\delta_x}{\tilde\alpha_z} \qquad \tilde X_z = \frac{X_z\tilde\alpha_z}{\tilde\delta_x}$$

$$\tilde X = \frac{X\tilde\alpha_z}{\tilde\delta_x} \qquad \tilde Z = \frac{Z\tilde\alpha_z}{\tilde\delta_x} \qquad \tilde t = \frac{t}{\tilde\delta_x} \qquad \tilde\alpha_x = \alpha_x\tilde\alpha_z \qquad \tilde Z = \frac{Z\tilde\alpha_z}{\tilde\delta_x} \qquad \tilde Z_x = \frac{Z_x\tilde\alpha_z}{\tilde\delta_x}$$

Plug in variables:

$$\left(\frac{\tilde\alpha_z\cancel{\tilde\delta_x}}{\cancel{\tilde\delta_x}}\right)\frac{dX}{dt} = \frac{\alpha_x\tilde\alpha_z + \beta_x\tilde\alpha_z S}{1+S+\left[\frac{Z\cancel{\tilde\alpha_z}}{\cancel{\tilde\delta_x}}\right]^{n_{zx}}} - \frac{\cancel{\tilde\delta_x}X\tilde\alpha_z}{\cancel{\tilde\delta_x}}$$

$$\left(\frac{\tilde{\alpha}_z \cancel{\delta_x}}{\cancel{\hat{\delta}_x}}\right)\frac{dX}{dt} = \frac{\alpha_x \tilde{\alpha}_z + \beta_x \tilde{\alpha}_z S}{1 + S + \left[\dfrac{\dfrac{Z\cancel{\tilde{\alpha}_z}}{\cancel{\hat{\delta}_x}}}{\dfrac{z_x \cancel{\tilde{\alpha}_z}}{\cancel{\hat{\delta}_x}}}\right]^{n_{zx}}} - \frac{\cancel{\delta_x} X \tilde{\alpha}_z}{\cancel{\delta_x}}$$

$$\tilde{\alpha}_z \frac{dX}{dt} = \frac{\alpha_x \tilde{\alpha}_z + \beta_x \tilde{\alpha}_z S}{1 + S + \left(\dfrac{Z}{z_x}\right)^{n_{zx}}} - X\tilde{\alpha}_z$$

Divide by $\tilde{\alpha}_z$:

$$\boxed{\frac{dX}{dt} = \frac{\alpha_x + \beta_x S}{1 + S + \left(\dfrac{Z}{z_x}\right)^{n_{zx}}} - X}$$

Plug in variables for the z-equation and divide by $\tilde{\alpha}_z$:

$$\left(\frac{\cancel{\delta_x} \tilde{\alpha}_z}{\cancel{\hat{\delta}_x}}\right)\frac{dZ}{dt} = \frac{\tilde{\alpha}_z}{1 + \left[\dfrac{\dfrac{X \cancel{\hat{\alpha}_z}}{\cancel{\hat{\delta}_x}}}{\dfrac{x_z \cancel{\tilde{\alpha}_z}}{\cancel{\hat{\delta}_x}}}\right]^{n_{xz}}} - \frac{\delta_z Z \tilde{\alpha}_z \cdot \cancel{\hat{\delta}_x}}{\cancel{\tilde{\delta}_x}}$$

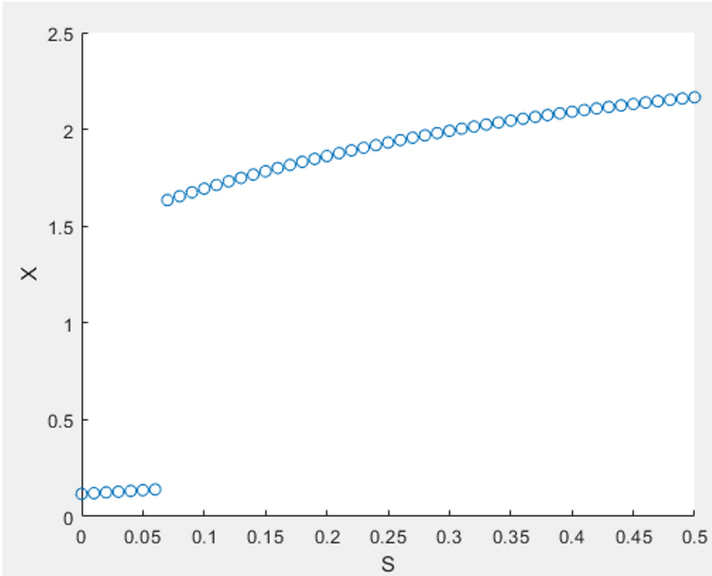
$$\boxed{\frac{dZ}{dt} = \frac{1}{1 + \left(\dfrac{X}{x_z}\right)^{n_{xz}}} - \delta_z Z}$$

(c) Given: $\alpha_x = 1.5$ $\quad z_x = 0.4$ $\quad x_z = 1.5$ $\quad \delta_z = 1.0$

$\beta_x = 5.0$ $\quad n_{zx} = 2.7$ $\quad n_{xz} = 2.7$

$$\frac{dX}{dt} = \frac{\alpha_x + \beta_x S}{1 + S + \left(\dfrac{Z}{z_x}\right)^{n_{zx}}} - X = 0 \quad @ \text{ S.S.}$$

$$\frac{dZ}{dt} = \frac{1}{1 + \left(\dfrac{X}{x_z}\right)^{n_{xz}}} - \delta_z Z = 0 \quad @ \text{ S.S.}$$



The bottom S.S line in the paper is more extended than mine, but I believe it is just an error in my code, and that the figure can be reproduced. I used fsolve, so I'm not sure if this is the reason it won't plot the rest of the bottom line.

(d)

$$\dot{X} = \frac{\alpha_x + \beta_x S}{1 + S + (Z/z_x)^{n_{zx}}} - X$$

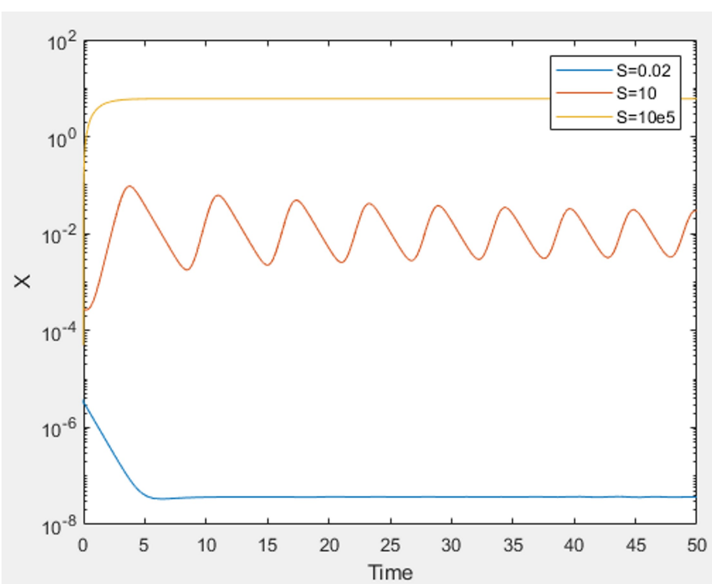
$$\dot{Y} = \frac{\alpha_y + \beta_y S}{1 + S + (X/x_y)^{n_{xy}}} - \delta_y Y$$

$$\dot{Z} = \frac{1}{1 + \left(\frac{X}{X_z}\right)^{n_{xz}} + \left(\frac{Y}{Y_z}\right)^{n_{yz}}} - \delta_z Z$$

I.C: $X_0 = Y_0 = Z_0 = 0$

parameter	mode
α_x	3.9×10^{-2}
α_y	4.3×10^{-3}
β_x	6.1
β_y	5.7
δ_y	1.05
δ_z	1.04
z_x	1.3×10^{-5}
y_z	11×10^{-3}
x_z	12×10^{-2}
x_y	7.9×10^{-4}
n_{zx}	2.32
n_{xy}	2
n_{yz}	2

Solving for $S = 0.02, 10, 10^5$

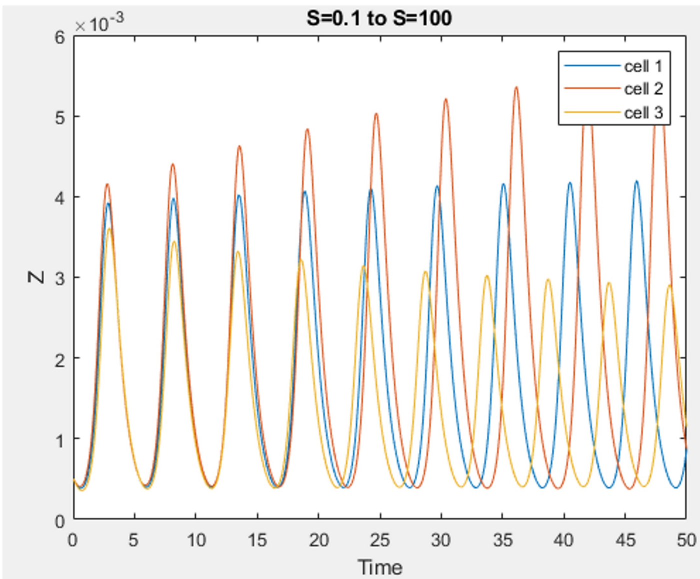


(e)

Steady-state for a value of signal, S, near but below the Hopf bifurcation point = 0.1

Corresponding S.S values: X = 0.0001
Y = 0.4839
Z = 0.0005

I found the S.S. values for S = 0.1, plugged those in as the initial conditions for S = 100 and multiplied each equation by the correct multiplier, 0.75, 1.25, or 1.

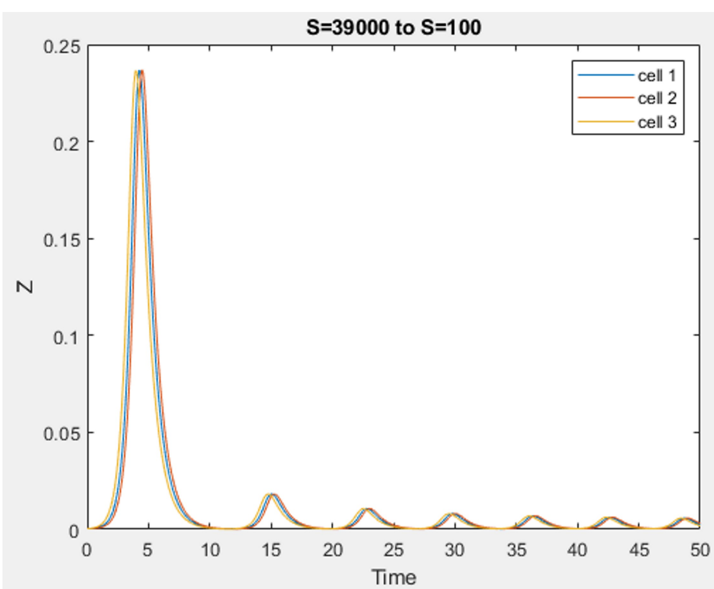


The oscillations are incoherent for an abrupt change from S=0.1 to S=100. They start out coherent, but then lose their coherence like the figure in the paper.

Steady-state for a value of signal, S, near but above the saddle node bifurcation - I started at 10000 and basically just kept guessing large values until I found guessed one that led to one steady state.

S = 40000

Corresponding S.S values: X = 5.6024
Y = 0.0043
Z = 0.0004



plugged in S.S. values for S = 40,000 to S = 100 and solved like above graph

The change from S=40000 to S=100 is coherent.

In a Hopf bifurcation, the oscillations occur because a spiral loses stability. The oscillations near this point are already near unsteady centers, so the initial conditions will play a large role in stability and oscillations. For example, the steady-state for a value of signal, S, near but below the Hopf bifurcation point = 0.1 to S=100 is incoherent because small differences in the initial conditions are amplified over time, which is what causes the asynchronous oscillations.

When the cells are passing through the saddle-node bifurcation, they are expressed at levels that are farther from the unstable oscillatory region - this means that the initial conditions won't be amplified over time and the cells won't oscillate out of sync like in the Hopf bifurcation example. Therefore, the change from S=40000, which is near but above the saddle bifurcation results in coherent oscillations. The authors state that the saddle-node bifurcation also allows the rapid establishment of constant amplitude oscillation after the signal is reduced, which is verified by the graph.

(f) Using this same logic, I would make an educated guess that abruptly changing the signal from S=105 to S=100 would still be coherent, since decreasing the signal in the second example was coherent. When I tried to graph the change from S=105 to S=100, S=105 was still oscillatory so I'm not sure how to determine steady-state values to plug in. It is possible that they use different parameter values, but this would only affect the range of the oscillations or shift the oscillations on the x-axis for the most part. In conclusion, I trust that they used the values that they claimed.