Prelim Chem 5440 Q1

a) $B = \langle m_c \rangle \hat{N}_c V$

$[gDW/cell]\left[\frac{\#\ cells}{mL}\right][mL]$

from the problem assumptions OD600 = 0.1 ≈ 1×10^8 cells/mL
mRNA transcript length = 1000nt

from bionumbers
ID 103904 "Dry weight of E.coli B/r cell in exponential phase on glucose medium, mass doubling time of 40min"
280 fg (280×10^{-15} g)

$$B = \left(\frac{280\times10^{-15}\ gDW}{cell}\right)\left(\frac{1\times10^8\ cells}{mL}\right)(1mL) = 2.8\times10^{-5}\ gDW$$

$$19\left[\frac{mRNA}{cell}\right]\left[\frac{1\times10^8\ cells}{mL}\right]\left[\frac{1\ mol}{6.02\times10^{23}\ mol}\right]\left[\frac{1000\ mL}{L}\right] = 3.156\times10^{-12}\ M$$

see excel file for the rest of the conversions $= 3.156\times10^{-9}$ mM

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

pseudo steady state assumption

$\dot{m}_i = 0$, for lacZ mRNA $m_i = m^*$, $\theta_{m,i} = \theta$

$$0 = r_x^*\bar{u} - (\mu + \theta)m^*$$

$$(\mu + \theta_{m,i})m^* = r_x^*\bar{u}$$

$$m^* = \frac{r_x}{\mu + \theta}\bar{u}$$

from class, $r_x = k_E^x R_{x,T}\left(\frac{b}{\tau_x k_x + (\tau_x + 1)b}\right)$

where k_E^x = elongation rate constant for b_i
$R_{x,T}$ = total abundance of RNAP
τ_x = time constant
b = lac Z gene abundance

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$$m^* = K_E^x R_{x,T} \left(\frac{b}{\tau_x k_x + (\tau_x + 1) b} \right) \left(\frac{1}{\mu + \theta} \right) \bar{u}$$

$$m^* = \frac{K_E^x R_{x,T} b}{(\mu+\theta)(\tau_x k_x + (\tau_x+1) b)} \bar{u}$$

since $m^* = k_x(b, \theta) \bar{u}(I, K)$

gain function

gain function $\boxed{k_x(b,\theta) = \frac{K_E^x R_{x,T} b}{(\mu+\theta)(\tau_x k_x + (\tau_x+1) b)}}$

c) model for $\bar{u}$

$$\bar{u}(\ldots) = \frac{\text{sum of micro states leading to transcription}}{\text{sum of all possible microstates}}$$

from problem assume P_{lac} is a positively inducible promoter that responds to IPTG

I = IPTG

$$\bar{u}(I) = \frac{W_1 + W_2 f_I}{1 + W_1 + W_2 f_I}$$

induced (regulated)

background (unregulated)

model used in class for f_I matches shape in the holding paper

$$f_I = \frac{I^n}{k^n + I^n}$$

full model: to simplify model add background / unregulated as determined in experiments for P_{lac}

$$m^* = \frac{K_E^x R_{x,T} b}{(\mu+\theta)(\tau_x k_x + (\tau_x+1) b)} \left[\frac{I^n}{k^n + I^n} \right] + b$$

↳ b = background

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K_E^x ▌ elongation rate constant

from notes :

$$K_{E,i}^x = \langle K_E^x \rangle \left(\frac{L}{L_i}\right)$$

L_i → length of gene i

where $\langle K_E^x \rangle = \frac{\ell_x}{L}$ → ℓ_x → elongation rate of polymerase, L → characteristic length

characteristic transcript length = 1000 nt

from Bionumbers (ID = 103021) ℓ_x = 3.72 kb/min

$$\frac{3.72\ \cancel{kb}}{\cancel{min}}\left(\frac{1\ \cancel{min}}{60\ s}\right)\left(\frac{1000\ nt}{1\ \cancel{kb}}\right) = 62\ nt/s$$

$$\langle K_E^x \rangle = \frac{62\ nt/s}{1000\ nt} = 0.062\ s^{-1}$$

assume lac Z gene ≈ 3069 nt

$$K_{E,i}^x = 0.062\ s^{-1}\left(\frac{1000\ nt}{3069\ nt}\right) = 0.0202\ s^{-1}$$

$R_{x,T}$ ▌ total abundance of RNAP

from Bionumbers (ID : 101440)

for τ_D = 40 min, 5×10^3 RNAP / e coli cell

convert units : $\frac{5\times10^3\ RNAP}{cell}\left(1\times10^8\ cells\right)\left(\frac{1\ mole}{6.02\times10^{23}\ RNAP}\right)$

$$= \frac{8.306\times10^{-10}\ mmol}{mL}\left(\frac{1000\ mL}{L}\right)$$

$$= 8.306\times10^{-7}\ mM$$

G ▌ amount of lac Z gene

from problem description, 2 lac Z / e. coli cell

$$\frac{2\ lac\ Z}{cell}\left(1\times10^8\ cells\right)\left(\frac{1\ mol}{6.02\times10^{23}\ lacZ}\right) = 3.32\times10^{-13}\ mol$$

$$3.32\times10^{-10}\ mM$$

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μ ▮ specific growth rate of E.coli

figure out from given doubling time $\tau_D = 40\,min$
$= 2400\,s$

$$\ln 2 = \mu \tau_D \rightarrow \mu = \frac{\ln 2}{2400\,s} = 2.89 \times 10^{-4}\,s^{-1}$$

θ ▮ degradation rate constant

treat as first order and use given
half life = 5 min

$$\text{half life} = \frac{\ln 2}{\theta} \rightarrow \theta = \frac{\ln 2}{\text{half life}}$$

$$\theta = \frac{\ln 2}{300\,s} = 0.00231\,s^{-1}$$

τ_x and K_x ▮ time + saturation constants

use Mclune paper
comparing the models

our model	mclune
K_I	K_2
K_-	K_{-1}
K_+	K_1
K_A	K_{-2}

Use Mclune values for A2 promoter

from mclune Table 3

$K_I = 4 \times 10^{-2}\,s^{-1}$

$\frac{K_+ K_I}{K_-} = >9 \times 10^{6}\,M^{-1}\,s^{-1}$

$K_A = 1.7 \times 10^{-4}\,s^{-1}$

$$\text{slope} = \frac{K_- + K_I}{K_+ + K_I} = \frac{K_x}{K_I}$$

slope = (0.12 μM s)

$K_x = 4.8 \times 10^{-6}\,mM$

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$$K_x = \frac{k_- + k_E}{k_+} \qquad \tau_y = \frac{k_A + k_E^x}{k_I}$$

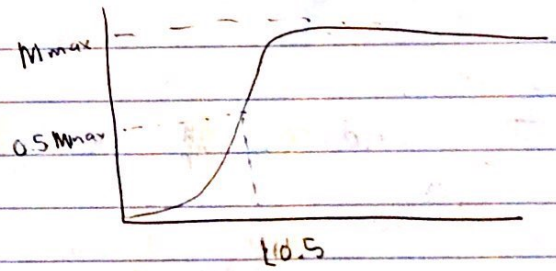
Strong

$$\tau_x = \frac{1.7 \times 10^{-4} s^{-1} + 0.0202 s^{-1}}{4 \times 10^{-2} s^{-1}}$$

$$\tau_x = 0.50925$$

Determine remaining parameters from shape of data → resembles Hill equation

$$m = \frac{m_{max} [I]^n}{(K_{0.5})^n + [I]^n}$$



in this problem

$V_{max} = 1.545 \times 10^{-8}$ mM

$0.5 V_{max} = 7.725 \times 10^{-9}$ mM

graphically determine $K_{0.5} \approx 0.007$

$K = 0.007$ mM

$$\log \frac{m}{1-m} = \frac{\log [I]^n}{K_{0.5}^n}$$

$$\log\left(\frac{m}{1-m}\right) = n \log[I] - n \log(K_{0.5})$$

plot $\log\left(\frac{m}{1-m}\right)$ vs $\log [I]$ → slope $= n = 0.2605$

b) background noise $[I] = 0$ $m^* = 3.156 \times 10^{-9}$

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parameter estimates

parameter	description	value	units	source
K_E^x	elongation rate constant	0.0202	s^{-1}	bio#, calculated ID 103021
$R_{x,T}$	total abundance of RNAP	8.306×10^{-7}	mM	bio #, converted ID 101440
G	amount of lacZ gene	3.32×10^{-10}	mM	problem description converted
μ	specific growth rate of E. coli	2.89×10^{-4}	s^{-1}	problem description
θ	degradation rate constant	0.00231	s^{-1}	problem description
τ_x	time constant	0.50925	n/a	McIune
K_x	saturation constant	4.8×10^{-6}	mM	McIune
n	exponent	0.2605	n/a	estimated
K	inducer constant	0.007	mM	estimated
b	background noise	3.156×10^{-4}	mM	exp data

units

$$m^* = \frac{k_E^x R_{x,T} G}{(\mu+\theta)(\tau_x K_x + \tau_x + 1) G} \left[\frac{I^n}{K^n + I^n} \right]$$

$$\frac{[s^{-1}][mM]}{([s^{-1}]+[s^{-1}])([mM]+[mM])} \quad [mM]$$

$$m^* = [mM]$$

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full model :

$$m^* = \frac{k_E^{\times} R_{x,T}\, b}{(\mu+\theta)(\tau_x K_x + (\tau_x+1)b)} \left[\frac{I^n}{k^n + I^n}\right]$$

$$m^* = \frac{(0.0202\,s^{-1})(8.306\times10^{-7}\,mM)(3.32\times10^{-10}\,mM)}{(2.89\times10^{-4}\,s^{-1} + 0.00231\,s^{-1})[(0.50925)(4.8\times10^{-6}\,mM) + (1.50925)(3.32\times10^{-6}\,mM)]} \left[\frac{I^{0.2605}}{0.007^{0.2605} + I^{0.2605}}\right] + 3.156\times10^{-9}$$

$$m^* = \frac{5.57034\times10^{-18}}{(0.002599)(2.4444\times10^{-6} + 5.01071\times10^{-10})} \left[\frac{I^{0.2605}}{0.007^{0.2605} + I^{0.2605}}\right] + 3.156\times10^{-9}$$

$$m^* = \underbrace{8.7662\times10^{-10}}_{\text{"max"}} \left[\frac{I^{0.2605}}{0.2746 + I^{0.2605}}\right] + 3.156\times10^{-9}$$

in the experimental data, max = 1.54485×10^{-8}
about an order of magnitude off
likely due to parameter estimation
plot separately to see if shape matches

d) See 5440 prelim Q1.xls

$\boxed{\text{Prelim 1 : Chem 5440}}$ Q2

a) Figure 1B : toggle switch

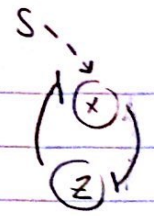
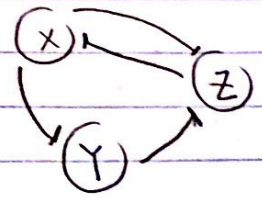


Figure 1A : AC DC circuit



Equation 2 from paper (describing 1A)

$$\frac{d\bar{X}}{d\bar{t}} = \frac{\bar{\alpha}_X + \bar{\beta}_X S}{1 + S + (\bar{Z}/\bar{Z}_X)^{n_{ZX}}} - \bar{\delta}_X \bar{X}$$

$$\frac{d\bar{Y}}{d\bar{t}} = \frac{\bar{\alpha}_Y + \bar{\beta}_Y S}{1 + S + (\bar{X}/\bar{X}_Y)^{n_{XY}}} - \bar{\delta}_Y \bar{Y}$$

$$\frac{d\bar{Z}}{dt} = \frac{\bar{\alpha}_Z}{1 + (\bar{X}/\bar{X}_Z)^{n_{XZ}} + (\bar{Y}/\bar{Y}_Z)^{n_{YZ}}} - \bar{\delta}_Z \bar{Z}$$

→ should have a bar on it small mistake in paper

for 1B, only have X + Z → drop Y terms
X doesn't change:

$$\frac{d\bar{X}}{d\bar{t}} = \frac{\bar{\alpha}_X + \bar{\beta}_X S}{1 + S + (Z/\bar{Z}_X)^{n_{ZX}}} - \bar{\delta}_X \bar{X}$$

get rid of Y terms in Z expression

$$\frac{d\bar{Z}}{dt} = \frac{\bar{\alpha} Z}{1 + (\bar{X}/\bar{X}_Z)^{n_{XZ}}} - \bar{\delta}_Z \bar{Z}$$

} ODE system for Figure 1B system (dimensional term)

b) Nondimensionalization

from STAR methods eqn 3 (temporal variables)

$$\delta_Y = \frac{\bar{\delta}_Y}{\bar{\delta}_X}, \quad \delta_Z = \frac{\bar{\delta}_Z}{\bar{\delta}_X}, \quad t = \bar{t}\,\delta_X$$

error in paper! should be $t = \bar{t}\,\bar{\delta}_X$

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(concentrations and rates)

eqn 4:

$$\alpha_x = \frac{\bar{\alpha}_x}{\bar{\alpha}_z}, \quad \alpha_Y = \frac{\bar{\alpha}_Y}{\bar{\alpha}_z}, \quad \beta_x = \frac{\bar{\beta}_x}{\bar{\alpha}_z}, \quad \beta_Y = \frac{\bar{\beta}_Y}{\bar{\alpha}_z}$$

eqn 5:

$$Z_x = \frac{\bar{Z}_x \bar{\delta}_x}{\bar{\alpha}_z}, \quad X_Y = \frac{\bar{X}_Y \bar{\delta}_x}{\bar{\alpha}_z}, \quad X_z = \frac{\bar{X}_z \bar{\delta}_x}{\bar{\alpha}_z}, \quad Y_z = \frac{\bar{Y}_z \bar{\delta}_x}{\alpha_z}$$

eqn 6:

$$X = \frac{\bar{X}\bar{\delta}_x}{\bar{\alpha}_z}, \quad Y = \frac{\bar{Y}\bar{\delta}_x}{\bar{\alpha}_z}, \quad Z = \frac{\bar{Z}\bar{\delta}_x}{\bar{\alpha}_z}$$

Since X expression didn't change, we should obtain the same nondimensional form:

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

check by subbing in non dimensional quantities in eqn 3-6

$$\frac{d\bar{X}}{d\bar{t}} = \frac{\bar{\alpha}_x + \bar{\beta}_x S}{1 + S + (\bar{Z}/\bar{Z}_x)^{n_{zx}}} - \bar{\delta}_x \bar{X}$$

$$\frac{d\frac{X\bar{\alpha}_z}{\bar{\delta}_x}}{d\frac{t}{\bar{\delta}_x}} = \frac{\alpha_x \bar{\alpha}_z + \beta_x \bar{\alpha}_z S}{1 + S + \left(\frac{\frac{Z\bar{\alpha}_z}{\bar{\delta}_x}}{\frac{Z_x \bar{\alpha}_z}{\delta_x}}\right)^{n_{zx}}} - \frac{\bar{\delta}_x X \bar{\alpha}_z}{\bar{\delta}_x}$$

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

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

matches expression in paper

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do the same for the Z expression

$$\frac{d\bar{Z}}{dt} = \frac{\bar{\alpha}_Z}{1+(\bar{X}/\bar{X}_Z)^{nxz}} - \bar{\delta}_Z \bar{Z}$$

$$\frac{d\,\frac{Z\bar{\alpha}_Z}{\bar{\delta}_X}}{d\,\frac{t}{\bar{\delta}_X}} = \frac{\bar{\alpha}_Z}{1+\left(\frac{\frac{X\bar{\alpha}_Z}{\bar{\delta}_X}}{\frac{X_Z\bar{\alpha}_Z}{\bar{\delta}_X}}\right)^{nxz}} - \frac{\delta_Z \bar{\delta}_X Z\bar{\alpha}_Z}{\bar{\delta}_X}$$

$$\bar{\alpha}_Z \frac{dZ}{dt} = \bar{\alpha}_Z\left(\frac{1}{1+(X/X_Z)^{nxz}} - \delta_Z Z\right)$$

$$\dot{Z} = \frac{dZ}{dt} = \frac{1}{1+(X/X_Z)^{nxz}} - \delta_Z Z$$

c) Plot steady state values of X vs S

@ SS $\dot{X} = 0$

$$\frac{\alpha_X + \beta_X S}{1+S+\left(\frac{Z}{Z_X}\right)^{nzx}} - X = 0$$

$$0 = \frac{1}{1+(X/X_Z)^{nxz}} - \delta_Z Z$$

$$\delta_Z Z = \frac{1}{1+(X/X_Z)^{nxz}}$$

$$Z = \frac{1}{\delta_Z\left(1+(X/X_Z)^{nxz}\right)}$$

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$$\frac{\alpha_x + B_x S}{1 + S + \frac{1}{z_x \delta_z \left(\left(1 + (x/x_z)^{n_{xz}}\right)\right)^{n_{zx}}}} - X = 0$$

$\alpha_x = 1.5$, $\beta_x = 5.0$, $z_x = 0.4$, $n_{zx} = 2.7$, $x_z = 1.5$
$n_{xz} = 2.7$, $\delta_z = 1.0$

$$\frac{1.5 + 5.0 S}{1 + S + \frac{1}{(0.4)(1.0)\left(\left(1 + \frac{x}{1.5}\right)^{2.7}\right)^{2.7}}} - X = 0$$

$$\frac{1.5 + 5.0 S}{1 + S + \frac{1}{0.4\left(1 + \frac{x}{1.5}\right)^{7.29}}} - X = 0$$

Use excel to solve + plot
for some reason I am only
able to reproduce what resembles
one of the stable solutions (black solid
lines) of figure 1. Probably related
to my solving + plotting method
in excel, other program may be more
suitable.

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d) Equation 1 (from paper)

$$\dot{X} = \frac{\alpha_X + \beta_X S}{1 + S + (Z/Z_X)^{n_{ZX}}} - X$$

$$\dot{Y} = \frac{\alpha_Y + \beta_Y S}{1 + S + (X/X_Y)^{n_{XY}}} - \delta_Y Y$$

$$\dot{Z} = \frac{1}{1 + (X/X_Z)^{n_{XZ}} + (Y/Y_Z)^{n_{YZ}}} - \delta_Z Z$$

mode parameters from table 5.1

$\alpha_X = 3.9 \times 10^{-2}$, $\alpha_Y = 4.3 \times 10^{-3}$, $\beta_X = 6.1$, $\beta_Y = 5.7$

$\delta_Y = 1.05$, $\delta_Z = 1.04$, $Z_X = 1.3 \times 10^{-5}$, $Y_Z = 11 \times 10^{-3}$

$X_Z = 12 \times 10^{-2}$ $X_Y = 7.8 \times 10^{-4}$ $n_{ZX} = 2.32$

solve ODE system for $S = 0.02, 10, 10^5$

see prelim 2d.jl and 5440 prelim1.pdf

comparing the X graphs to figure 2b, we see that they match

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e) from figure 2, Hopf bifurcation occurs at
$S \approx 0.6$
$\longrightarrow$ choose $S = 0.3$
find SS
@ SS $\dot{X} = \dot{Y} = \dot{Z} = 0$
use parameters from part c (found in supplemental materials)
solve for SS values in MATLAB (prelim.m)
get:

$X = 0.000964131$
$Y = 0.585306 39$
$Z = 0.000339494$
} cell 1

multiply by 1.25
$X = 0.001205$
$Y = 0.731633$
$Z = 0.0004244$
} cell 2

multiply by 0.75
$X = 0.000723098$
$Y = 0.43898$
$Z = 0.00025462$
} cell 3

use Prelim2e.jl to solve ODE with $S = 100$
use the values above as initial conditions

see pdf for plot

repeat process for saddle bifurcation
from figure 2, occurs at $S \approx 700$

choose $S = 1000$
solve for steady state values using MATLAB
$X = 0.1065$
$Y = 0.2829$
$Z = 0.0015$

calculate values for cell 2 + cell 3 by multiplying by 1.25 and 0.75 (see Prelim2e.jl)

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again run Prelim2e.jl to solve at
S = 100 with initial conditions determined
see pdf for plot

For the first plot, the oscillations are incoherent. For the second plot, the oscillations are coherent (essentially align with one another). This is consistent with the results of the paper. When increasing the signal from low levels to a level above the Hopf bifurcation, a small limit cycle arises around the initial steady state. In the second case (move from stable expression state at high levels of S to below saddle bifurcation) a large amplitude limit cycle is already in place. These conditions give rise to the different patterns shown. The differences can be attributed to the initial gene expression states in relation to the oscillatory spiral center. In the Hopf bifurcation, small variations are amplified as the attracting spiral loses stability and becomes a repulsing spiral. Cells passing through the saddle node are immune to these changes as they are operating at expression levels far from the oscillatory regime

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f) It seems unlikely that given the parameters listed, that coherent oscillations would appear as the signal is decreased from 105 to 100 because no bifurcation point is being passed over this range.

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