Problem 1

Assume

i) exponentially growing population of E. coli cells

$\tau_d \simeq 40$ min

ii) $OD600 = 0.1 \Rightarrow 1\times10^8$ cells/mL

iii) write promoter function in terms of extracellular inducer

iv) LacZ gene is present @ 2 copies/cell

v) LacZ mRNA half-life = 5 min

vi) characteristic transcript length of 1000 nt

Parameters: Bionumbers and McClure study.

a) $\beta = \langle m_c \rangle \hat{N}_c V$ sample size 1mL @ OD600 = 0.1

Convert $\langle n \rangle \Rightarrow \langle n \rangle$ = Average LacZ mRNA copy # per cell for P_{lac} promoter

$\langle m_c \rangle \Rightarrow \frac{gDW}{cell} = \frac{\text{average mass}}{cell}$ $\hat{N}_c$ = cell count = $\frac{\#\text{cells}}{mL}$

Convert $\langle n \rangle$ to $\frac{\text{nmol mRNA}}{gDW}$ V = sample vol ⇒ mL

$\langle n \rangle = \frac{\text{mRNA molecules}}{cell}$ $\boxed{\text{Conversion units} \Rightarrow \frac{\langle n \rangle V}{N_A \text{ Mass}_{DW}}}$

$V = 1\,mL$

$\hat{N}_c = \frac{1\times10^8 \text{ cells}}{mL}$ N_A = Avogadro's # = $\frac{6.022\times10^{23}\text{ molecules}}{mole}$

$\hookrightarrow OD600 = 0.1 \Rightarrow 1\times10^8$ cells/mL

dry wt = 2.8×10^{-13} g
From Bionumbers

Part A: Converted Data

IPTG (nM)	< n > (nmol mRNA/gDW)	low (nmol mRNA/gDW)	high (nmol mRNA/gDW)
0	1.13E-01	1.07E-01	1.19E-01
500	1.25E-01	1.01E-01	1.54E-01
5000	2.43E-01	2.19E-01	2.61E-01
12000	3.97E-01	3.85E-01	4.09E-01
53000	5.10E-01	4.98E-01	5.22E-01
216000	5.52E-01	5.40E-01	5.63E-01
1000000	5.52E-01	5.46E-01	5.57E-01

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

$r_{x,i}$ = kinetic rate

μ: dilution

$\theta_{m,i}$: deg. rt of mRNA

Specific rate of transcription of gene i
(prod. rate of mRNA i)

$\bar{u}_i$ = promoter activity function

$0 \leq \bar{u}_i \leq 1$

m_i = concentration mRNA

κ_x = gain function

$m^* = \frac{\text{nmol}}{\text{gDW}}$] pseudo s.s

G = LacZ gene abundance

I = inducer abundance

$\bar{u}$ = promoter function

K, θ = parameter vectors (constants)

Pseudo s.s. $\dot{m}_i = 0$

$$r_{x,i}\,\bar{u}_i = (\mu + \theta_{m,i})\,m_i^* \Rightarrow m^* = \frac{r_x\,\bar{u}}{\mu + \theta_m}$$

$$m^* = \underbrace{\left(\frac{r_x}{\mu + \theta_m}\right)}_{\text{gain function}}\bar{u}$$

$\bar{u}$ function of inducer conc. and parameters (constants)

$$\boxed{m^* = \kappa_x(G, \theta)\;\bar{u}(I, K)}$$

Gain Function $\boxed{\kappa_x = \frac{r_x}{\mu + \theta_m}}$

$r_x = K_E^X (G : R_x)$

free RNA polymerase

G: LacZ gene abundance

K_E^X: elongation rate constant for gene

$$m^* = \frac{\text{nmol}}{\text{gDW}}$$

c) $r_x = k_E^x R_{x,T} \left(\frac{G}{\tau_x K_x + (\tau_x + 1) G} \right)$ Transcription.

Total # RNAP

$K_x^{-1} \equiv \frac{k_{on}}{k_{off} + k_I}$ $\quad \tau_x^{-1} \equiv \frac{k_I}{k_E^x} \Rightarrow \tau_x = \frac{k_E^x}{k_I}$

Parameters needed to be found/calculated

IPTG extracellular (inducer, I)

k_E^x	k_I	k_{on}, k_{off}
$R_{x,T}$	μ	θ

We have

I ⇒ IPTG mM given in table

told that LacZ gene present @ 2 copies/cell

$k_E = \frac{\text{transcription\_elongation rate}}{\text{characteristic transcript length}}$

$k_I = 1 / \text{characteristic initiation time}$.

McClure Table 2 ⇒ τ_{obs} = lag time = 400 s

Author's state that they found lag time to be the rate-limiting initiation step

↳ So, I chose to use this value as the characteristic initiation time.

Dilution due to growth, μ units $\Rightarrow$ 1/s

$\mu = \tau_d^{-1}$ From Lecture 2 Notes

E. coli cells growing τ_d = doubling time

$U(I, K) \Rightarrow$ Promoter Function

↳ Use promoter modeling Moon et. al.
↳ Assume P_{lac} is a positively inducible promoter that responds to IPTG

Adding IPTG $\Rightarrow$ Activate Transcription

$$\boxed{P_{lac} = u(I,K) = \frac{K_1 + K_2 f_I}{1 + K_1 + K_2 f_I}}$$

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

I = IPTG concentration , n = cooperativity

$\Rightarrow$ Now, Need to estimate K_1, K_2, and n values with the given data

I = 0 $\Rightarrow$ No inducer $\Rightarrow$ 1.13×10^{7} nmol mRNA / gDW

$$u = \frac{K_1}{1+K_1} = \frac{1.13 \times 10^{-1} \text{ nmol mRNA / gDW}}{\mathcal{K}_x}$$

$m^* = \mathcal{K}_x u$

$\Rightarrow u = 0.251425$

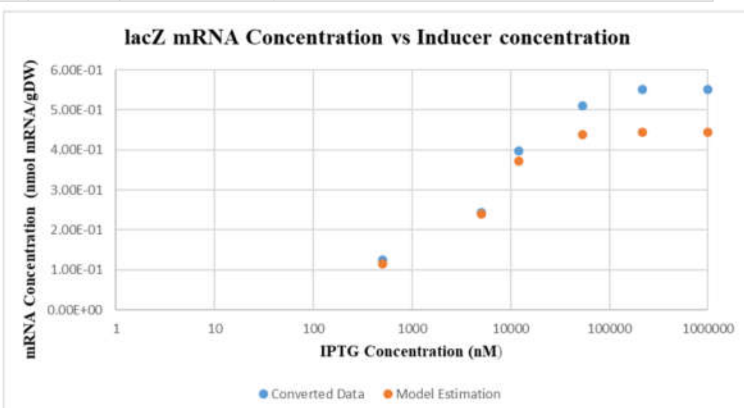
$\mathcal{K}_x = 0.4494382$

$$0.251425 = \frac{K_1}{1+K_1} \Rightarrow \boxed{K_1 = 0.33687}$$

Parameters Found/Calculated				
Parameters	Value	Units	Description	Source
mass DW	2.80E-13	gDW	dry weight E.coli cell	Bionumbers:103904
RX,T	100	nM	total RNA polymerase concentration	McClure Table 2
mu	0.0004167	1/s	dilution factor	Lecture 2 Notes (1/Tau_d)
tI	400	s	characteristic initation time	McClure Table 2
e	19	nts/s	transcription elogation rate	Bionumbers: 108491
theta	0.0033333	1/s	degradation rate of mRNA	Half-life given in Problem
kon	9.00E-03	1/(nM*s)	on rate, RNA polymerase	McClure Table 3
koff	1.70E-04	1/s	off rate RNA polymerase	McClure Table 3
kI	0.0025	1/s	initiation rate constant	McClure
kE	1.90E-02	1/s	elongation rate constant	McClure
Tau_x	7.60E+00	none	time constant for gene	Lecture 2 Notes Eqn. 21
Kx	2.97E+08	nM	Saturation constant for gene	Lecture 2 Notes Eqn. 20
rx	1.69E-03	1/s	rate of transcription	Lecture 2 Notes Eqn. 26
Gain Funct.	0.4494382	none	the gain function	Expression Derived in part b
KD	4.96E+04	nM	dissociation constant	Bionumbers: 101976
K1	0.33687	none	constant for promoter function	calculated using I=0 case
K2	80	none	constant for promoter function	trial and error to best fit the data
n	2	none	cooperativity coefficient	trial and error to best fit the data
G	2.00E+06	nM	lacZ gene abundance	calculated using data given (copies/ cell , cell #)

Given in Problem Statement			
Parameter	Value	Units	Description
G_copy	2.00E+00	copies/cell	number of copies of lacZ gene present in each cell
Nc_hat	1.00E+08	cell/ml	cell count
V	1	ml	sample volume
NA	6.02E+14	molecules/nmol	avogadro's #
Tau_d	2400	s	doubling time of E. coli cells
L	1.00E+03	nt	characteristic transcript length
Half-life	300	s	half-life of lacZ mRNA

d)



Plot made using Excel.

The model fits pretty well until IPTG conc. is over 10,000 nM. It does have the correct shape.

$n \Rightarrow$ controls the shape
K_1 and $K_2 \Rightarrow$ control the fit

I solved for K_1, but found n and K_2 through trial and error, in order to best fit to the converted data.

f_I, u, m^α values on next page

Found using excel

Excel Sheet

IPTG (nM)	< *n* > (nmol mRNA/gDW)	low (nmol mRNA/gDW)	high (nmol mRNA/gDW)	fI	u (promoter function)	Model Estimation m* (nmol mRNA/gDW)
0	1.13E-01	1.07E-01	1.19E-01	0	0.251984112	0.113251285
500	1.25E-01	1.01E-01	1.54E-01	0.000101609	0.256504872	0.115283088
5000	2.43E-01	2.19E-01	2.61E-01	0.010059714	0.533069676	0.239581875
12000	3.97E-01	3.85E-01	4.09E-01	0.055296142	0.826405806	0.371418337
53000	5.10E-01	4.98E-01	5.22E-01	0.53310205	0.977264994	0.439220219
216000	5.52E-01	5.40E-01	5.63E-01	0.949911394	0.987068372	0.443626231
1000000	5.52E-01	5.46E-01	5.57E-01	0.997545878	0.987675704	0.44389919

Inducer concentration

f_I

Problem 2

a) STAR METHOD - equation 2

Dimensional Form

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

$$② \quad \frac{d\tilde{Z}}{d\tilde{t}} = \frac{\tilde{\alpha}_z}{1 + (\tilde{X}/\tilde{x}_z)^{n_{xz}}} - \tilde{\delta}_z \tilde{Z}$$

$\tilde{\delta}_x, \tilde{\delta}_z \Downarrow$ degradation rates

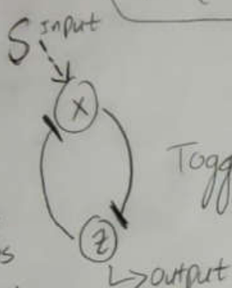
$\tilde{X}, \tilde{Z}$ = genes

$\tilde{\alpha}_x, \tilde{\alpha}_z$ = basal prod. rates

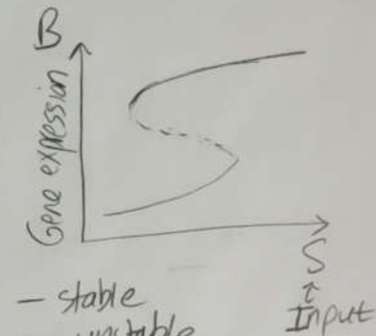
$\tilde{\beta}_x \Rightarrow$ controls signal transduction

$\tilde{z}_x, \tilde{x}_z \Rightarrow$ control strength of repressions

$n_{zx}, n_{xz} \Rightarrow$ control shape of repressions



Toggle switch



b) Non-dimensionalize eqns from part A.

Use Eqns 3-6 of STAR METHODS

deg. rt rel. to deg. of gene X $\Rightarrow \delta_z = \frac{\tilde{\delta}_z}{\tilde{\delta}_x}$ $\quad \boxed{t = \tilde{t}\tilde{\delta}_x}$] temporal variables

This was the error the paper wrote $t = \tilde{t}\delta_x$ instead of $t = \tilde{t}\tilde{\delta}_x$

timescale $\tilde{\delta}_x$

prod. rt. $\tilde{\alpha}_z$

Concentrations and Rates

$$\alpha_x = \frac{\tilde{\alpha}_x}{\tilde{\alpha}_z} \qquad \beta_x = \frac{\tilde{\beta}_x}{\tilde{\alpha}_z}$$

$$z_x = \frac{\tilde{z}_x \tilde{\delta}_x}{\tilde{\alpha}_z} \qquad x_z = \frac{\tilde{x}_z \tilde{\delta}_x}{\tilde{\alpha}_z} \qquad X = \frac{\tilde{X}\tilde{\delta}_x}{\tilde{\alpha}_z} \qquad Z = \frac{\tilde{Z}\tilde{\delta}_x}{\tilde{\alpha}_z}$$

Plug in non-dimensionalized variables

$$\boxed{t = \tilde{t}\tilde{\delta}_x}$$

corrected, paper incorrectly wrote

$$t = \tilde{t}\,\delta_x$$

dimens.

nodim.

$$t \neq \tilde{t}\,\delta_x$$

$$d\tilde{x} = dX \frac{\tilde{\alpha}_z}{\tilde{\delta}_x} \qquad d\tilde{t} = \frac{1}{\tilde{\delta}_x} dt$$

$$\tilde{\alpha}_x = \alpha_x \tilde{\alpha}_z \qquad \tilde{\beta}_x = \beta_x \tilde{\alpha}_z \qquad \tilde{z} = \frac{Z\tilde{\alpha}_z}{\tilde{\delta}_x}$$

$$\tilde{z}_x = z_x \tilde{\alpha}_z / \tilde{\delta}_x$$

① $$\frac{dX}{dt}\left(\frac{\tilde{\alpha}_z}{\cancel{\tilde{\delta}_x}}\cancel{\tilde{\delta}_x}\right) \Rightarrow \tilde{\alpha}_z \frac{dX}{dt}$$

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

$$\tilde{\delta}_x = \frac{\delta_z}{\tilde{\delta}_z} \qquad \cancel{\tilde{\alpha}_z}\frac{dX}{dt} = \frac{\cancel{\tilde{\alpha}_z}(\alpha_x + \beta_x S)}{1 + S + (Z/z_x)^{n_{zx}}} - X\cancel{\tilde{\alpha}_z}$$

$$\tilde{x} = \frac{X\tilde{\alpha}_z}{\tilde{\delta}_x} \qquad \boxed{\frac{dX}{dt} = \frac{\alpha_x + \beta_x S}{1 + S + (Z/z_x)^{n_{zx}}} - X}$$

② $$\frac{dZ}{dt}\left(\frac{\cancel{\tilde{\alpha}_z}}{\tilde{\delta}_x}\cancel{\tilde{\delta}_x}\right) = \frac{\cancel{\tilde{\alpha}_z}}{1 + \left(\frac{X\cancel{\tilde{\alpha}_z}}{\cancel{\tilde{\delta}_x}} \Big/ \frac{x_z\cancel{\tilde{\alpha}_z}}{\cancel{\tilde{\delta}_x}}\right)^{n_{xz}}} - \frac{\cancel{\tilde{\delta}_x}\delta_z Z\cancel{\tilde{\alpha}_z}}{\cancel{\tilde{\delta}_x}}$$

$$\tilde{x}_z = \frac{x_z\tilde{\alpha}_z}{\tilde{\delta}_x} \qquad \boxed{\frac{dZ}{dt} = \frac{1}{1 + (X/x_z)^{n_{xz}}} - \delta_z Z}$$

c) Create plot X vs. S

$\uparrow$ non-dim. $\uparrow$ input

Steady-State

$\Downarrow$

time derivatives equal zero

$\alpha_x = 1.5$

$\beta_x = 5.0$

$z_x = 0.4$

$n_{zx} = 2.7$

$x_z = 1.5$

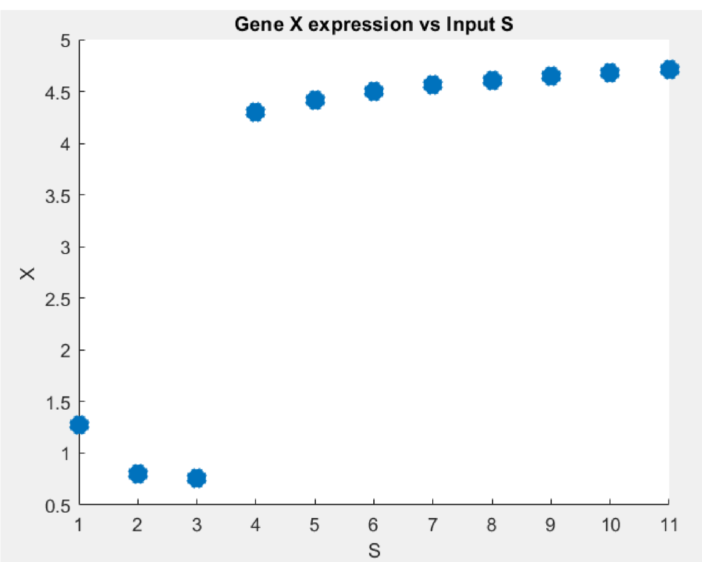
$n_{xz} = 2.7$

$\delta_z = 1.0$

$$\frac{dX}{dt} = \frac{dZ}{dt} = 0$$

Initial conditions $\Rightarrow X_0 = Z_0 = 0$

Code in Github Repository



yes, solid Black Lines of Figure 1B are qualitatively reproducible

$\uparrow$ Plot of the stable steady state values of X vs. S

S = input

d) AC-DC circuit Analyzed in Figure 2 of Paper

Equation 1 $\Rightarrow$

$$\begin{cases} \dfrac{dX}{dt} = \dfrac{\alpha_X + \beta_X S}{1+S+(Z/z_X)^{n_{zx}}} - X \\[2ex] \dfrac{dY}{dt} = \dfrac{\alpha_Y + \beta_Y S}{1+S+(X/x_Y)^{n_{xy}}} - \delta_Y Y \\[2ex] \dfrac{dZ}{dt} = \dfrac{1}{1+(X/x_Z)^{n_{xz}} + (Y/y_Z)^{n_{yz}}} - \delta_Z Z \end{cases}$$

Tasks

#1] Solve time varying values of X, Y, Z for $S = 0.02, 10, 10^5$]

Initial Conditions $\Rightarrow$ $X_0 = Y_0 = Z_0 = 0$

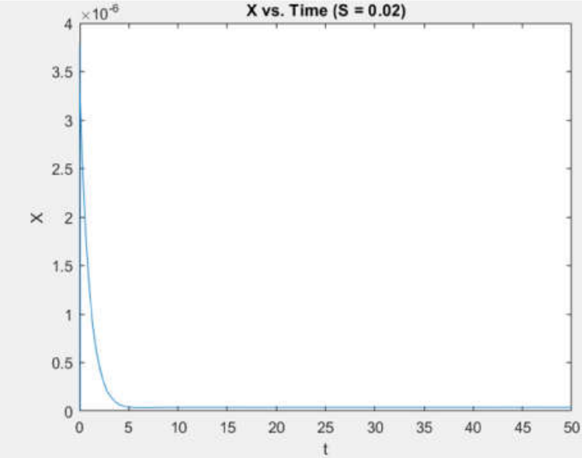
#2] Provide plots of X vs. t for all three S values

Table S.1

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

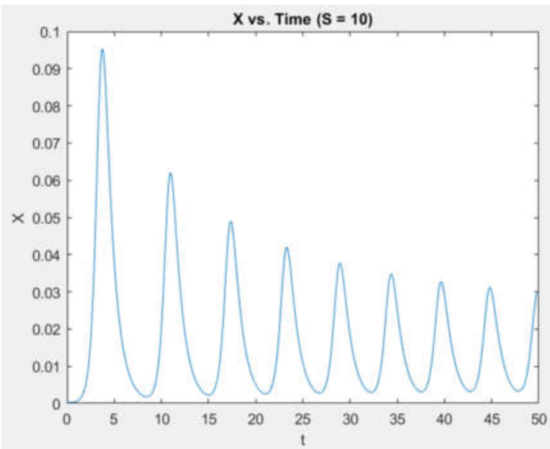
$n_{xy} = 2.0$
$n_{yz} = 2.0$
$n_{xz} = 2.0$

Solved using Ode45 in Matlab, code in github respository

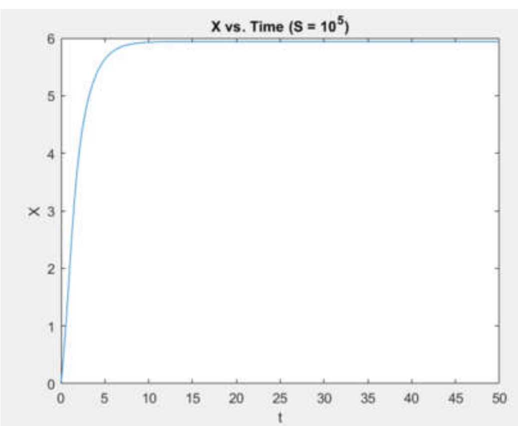


↳ Solved for X, Y, Z as function of time using Matlab.

↳ Codes in github repository



↳ Here are plots of X vs. t for three diff. S values



e) Figure 3 Authors conclude ⇒ oscillations arising by passing through the Hopf bifurcation are incoherent
& those passing through saddle node bifurcation are coherent

Goals:

↳ Find stable steady state value of signal S near but below the Hopf bifurcation point

↳ where stable spiral becomes unstable giving rise to stable oscillations

Figure 3C ⇒ Lose of coherence $\overbrace{S=0.1 \rightarrow S=100}^{\Delta S_1}$
Increased to level above Hopf bifurcation threshold.

Figure 3D ⇒ starting from the stable expression state, oscillatory state reached by reducing S below the saddle-node bifurcation ΔS_2

Stable steady state

$$S = 0.1 \Rightarrow \boxed{\begin{aligned} X &= -0.0047 \\ Y &= 0.0137 \\ Z &= 0.3738 \end{aligned}}$$

Code used to solve for S.S. values included in repository

cell 1 ⇒ X, Y, Z same as those found for $S=0.1$

cell 2 ⇒ X, Y, Z are 25% higher than expected

cell 3 ⇒ X, Y, Z 25% lower than expected.