

CHEME 7770: Advanced

Biomolecular Engg.

Prelim #1

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2.

a) For the case of Fig 1B, we see that there is no expression for Y .

From Star Methods secⁿ, we get,

$$\frac{d\tilde{x}}{dt} = \frac{\tilde{\alpha}_x + \tilde{\beta}_x S}{1 + S + (\tilde{z}/\tilde{z}_x)^{n_{xz}}} - \tilde{\delta}_x \tilde{x}$$

$$\frac{d\tilde{z}}{dt} = \frac{\tilde{\alpha}_z}{1 + (\tilde{x}/\tilde{x}_z)^{n_{xz}}} - \tilde{\delta}_z \tilde{z}$$

The above set of ODEs in dimensional form shows the network diagrams for a signal S .

b) The small error in the STAR METHODS

$$\text{is } \rightarrow t = \tilde{t} \tilde{\delta}_x \text{ and not } = \tilde{t} \delta_x.$$

Taking $\alpha_x = \frac{\tilde{x}_x}{\tilde{x}_z}$, $\beta_x = \frac{\tilde{\beta}_x}{\tilde{x}_z}$.

$$\delta_y = \frac{\tilde{\delta}_y}{\tilde{x}} \quad \delta_z = \frac{\tilde{\delta}_z}{\tilde{x}}$$

$$* z_x = \frac{\tilde{z}_x \tilde{\delta}_x}{\tilde{x}_z} \quad x_z = \frac{\tilde{x}_z \tilde{\delta}_x}{\tilde{x}_z}$$

$$x = \frac{\tilde{x} \tilde{\delta}_x}{\tilde{x}_z} \quad z = \frac{\tilde{z} \tilde{\delta}_x}{\tilde{x}_z}$$

Using the above eqns, and the eqns of

Part a-a, we can get

Multiplying numerator & denominator of LHS by $\tilde{\delta}_x$, and dividing both sides by \tilde{x}_z ,

$$\frac{d \frac{\tilde{x} \tilde{\delta}_x}{\tilde{x}_z}}{d \tilde{t} \tilde{\delta}_x} = \frac{\tilde{x}_x}{\tilde{x}_z} + \frac{\tilde{\beta}_x}{\tilde{x}_z} S - \frac{\tilde{x}_x \tilde{x}}{\tilde{x}_z}$$

$$1 + S + \left(\frac{\tilde{z} \tilde{\delta}_x}{\tilde{x}_z} / \frac{\tilde{z}_x \tilde{\delta}_x}{\tilde{x}_z} \right)$$

$$\Rightarrow \frac{dx}{dt} = \frac{\alpha_x + \beta_x s}{1 + s + (z/z_r)^{n_{zx}}} - x$$

Similarly, by simple manipulation.

$$\frac{dz}{dt} = \frac{1}{1 + (x/x_z)^{n_{xz}}} - \cancel{\beta_z z}$$

- c) We want stable steady-state values of x
vs s .

→ steady state:

$$\frac{dx}{dt} = 0 = \frac{\alpha_x + \beta_x s}{1 + s + (z/z_r)^{n_{zx}}} - x$$

$$x = \frac{\alpha_x + \beta_x s}{1 + s + (z/z_r)^{n_{zx}}}$$

$$\frac{dz}{dt} = 0 = \frac{1}{1 + (x/x_z)^{n_{xz}}} - \cancel{\beta_z z}$$

$$z = \frac{1}{\delta_z + \delta_z(x/x_z)^{n_{xz}}}$$

$$\delta_z + \delta_z(x/x_z)^{n_{xz}}$$

Substituting,

$$x = \frac{\alpha_x + \beta_x s}{1 + s + \left(\frac{1}{\delta_z z_x (1 + x/x_z)^{n_{xz}}} \right)}$$

we know all parameters here.

\rightarrow Stable:

For this, we first calculate the Jacobian matrix:

$$\bar{J} = \begin{bmatrix} -1 & \frac{-(\alpha_x + \beta_x s) \cdot n_{xz} (\frac{z}{z_x})^{n_{xz}-1}}{(1 + s + (z/z_x)^{n_{xz}})^2} \\ -n_{xz} (x/x_z)^{n_{xz}-1} & -1 \end{bmatrix}$$

$$f_2(\bar{J}) = -2$$

$$\det(\bar{J}) = 1 - \frac{(\alpha_x + \beta_x s) (x/x_z)^{n_{xz}-1} (\frac{z}{z_x})^{n_{xz}-1} n_{xz} n_{zx}}{z_x x_z (1 + (x/x_z)^{n_{xz}})^2 (1 + s + (z/z_x)^{n_{xz}})^2}$$

Since $\text{Tr}(\bar{J}) < 0$, for x to be stable, we need $\det(\bar{J}) < 0$.

$$\text{i.e. } \frac{(K_x + \beta_x s)(x/x_c)^{n_{xz}-1}(z/z_c)^{n_{zx}-1}}{z_c x^2 \left(1 + (x/x_c)^{n_{xz}}\right)^2 \left(1 + s + (z/z_c)^{n_{zx}}\right)^2} > 1$$

Substituting values, and rearranging, we get

$$\frac{32 - 0.5 (58 + 1.5) \left(\frac{1}{1 + \left(\frac{2x^{2.7}}{3}\right)} \right)^{1.7} \times 0.904 x^{1.7}}{\left(1 + s + 11.87 \left(\frac{1}{1 + \left(\frac{2x^{2.7}}{3}\right)}\right)\right)^2 \left(1 + 0.34 x^{2.7}\right)^2} > 1$$

The graph was plotted on Desmos. There seemed to be some recursion issue while trying on Julia.

(BONUS), for ~~unstable~~ ^{unstable} state, $\underline{\det(\bar{J}) > 0}$.

In graph, the curve outside the green shaded region refers to stable values of x , and the curve segment within the shaded region is for ~~unstable~~ unstable values of x .

Whole curve is given in red.

d) Graph attached separately.

e) * Using code from Part 2 D (see the attached code).

i) The steady state values of X, Y, Z for $S = 0.2$ (the case where S is just below the Hopf bifurcation point) was calculated as

$$X = 0.000644$$

$$Y = 0.584295$$

$$Z = 0.000340$$

These values were used for Cell 1.

∴ For Cell 2

Cell 3

$$X = 0.000805$$

$$X = 0.000483$$

$$Y = 0.730369$$

$$Y = 0.438221$$

$$Z = 0.000425$$

$$Z = 0.000255$$

In this case, we see that the oscillations are incoherent.

(ii) For the case of S near but above saddle node bifurcation, I chose $S = 50,000$.

For this case,

	Cell 1 (25%↑)	Cell 2 (25%↓)	Cell 3 (25%↓)
X	5.73411	7.1676	4.3005
Y	0.00514	0.00643	0.00386
Z	0.00042	0.00052	0.00031

In this case, we see that oscillations are coherent.

As the authors explain, in Hopf bifurcation, an attracting spiral loses its stability and becomes a repulsive spiral, leading to oscillations. So, in this case, if oscillations begin close to this point, a small perturbation can lead to large differences in final oscillation phase, since the ~~on the other hand~~, small initial disturbances get amplified. On the other hand, for saddle node bifurcation, the expression levels of the cells at this point are far removed from that required for attractive or repulsive regime. Therefore,

 they pass through without any phase difference.

f) No. We won't expect them to show no out-of-phase behavior (i.e. coherence) for the same model parameters. This is because the initial value $S=105$ is too close to the Hopf bifurcation region, and so incoherence is expected unless they change concentrations or parameters.

I. 

a) Given $B = \langle m_c \rangle N_c V$

$$N_c = 10^8 \text{ cells/mL}$$

$$V = 1 \text{ mL}$$

From Biomumbus (103904), avg. wt of E.Coli cells $\langle m_c \rangle = 280 \text{ fg} = 2.8 \times 10^{-13} \text{ g DW/cell}$

$$\begin{aligned} \therefore B &= 2.8 \times 10^{-13} \times 10^8 \times 1 \\ &= 2.8 \times 10^{-5} \text{ g DW.cell} \end{aligned}$$

To get $\langle n \rangle$ in terms of nmol of mRNA per gDW cell, we therefore need to convert as follows, where $\langle n^* \rangle$ is the new units.

$$\langle n^* \rangle = \frac{\langle n \rangle \times V \times N_A}{B \times N_A}$$

↪ Avogadro's number

$$= \frac{\langle n \rangle}{\langle m_c \rangle N_A} \frac{\text{no./cell}}{\text{g DW/cell} \times \frac{\text{no.}}{\text{mol}}} = \frac{\text{mol}}{\text{g DW}}$$

$$= \frac{10^9 \langle n \rangle}{\langle m_c \rangle N_A} \frac{\text{n mol}}{\text{g DW}}$$

Tabulated in Excel sheet 'Problem1.xlsx'.

f) $\dot{m} = \gamma_x \bar{u} - (\mu + \theta_m) m$ (Removing subscript)

For pseudo steady state, $\dot{m} = 0$

$$\therefore m = \frac{\gamma_x \bar{u}}{(\mu + \theta_m)}$$

we know (from class notes)

$$\gamma_x = K_E R_{x,T} \left(\frac{G}{C_x K_x + (E_x + 1) G} \right)$$

$$\bar{u} = \frac{w_1 + w_2 f_I}{1 + w_1 + w_2 f_I}$$

$$\text{where } f_I = \frac{I^n}{K^n + I^n}$$

Substituting,

$$m^+ = \left(\frac{k_E R_{x,T}}{\mu + \theta_m} \right) \left[\frac{G}{E_x K_x + (E_x + 1) G_1} \right] \cdot \left[\frac{w_1 + w_2 f_I}{1 + w_1 + w_2 f_I} \right]$$

Represents gain $f^n K(G, \theta)$ Represents promoter $f^+ u(I, k)$

c) Given parameters:

~~$\theta_m = 2 \text{ copies/cell}$~~

$\text{lacZ gene conc.} = 2 \text{ copies/cell}$

$\text{cell doubling time} = 40 \text{ mins}$

$\text{lacZ mRNA } t_{1/2} = 5 \text{ mins}$

$\text{Transcript length} = 1000 \text{ nt}$

$\text{We know } \theta_m = \frac{\frac{\ln 2}{\text{doubling time}}}{\frac{\ln 2}{\text{mRNA } t_{1/2}}} = \frac{\frac{\ln 2}{40}}{\frac{\ln 2}{5/60}} = \frac{5/60}{40} = \frac{5}{240} = \frac{1}{48}$

$= 12 \ln 2 = 8.32 \text{ h}^{-1}$

$$\mu = \frac{\ln 2}{\text{cell doubling time}} = \frac{\ln 2}{40/60} \\ = \underline{1.039 \text{ hr}^{-1}}$$

G_1 = Lac Z gene conc. in nmol/g DW cell

$$= \frac{2}{\langle m \rangle N_A} = \frac{2 \times 10^9}{2.8 \times 10^{13} \times 6.023 \times 10^{23}} \\ = \underline{0.0118 \text{ nmol/g DW}}$$

Now, to calculate k_E , K_s , R_x , \bar{t}_x , we use Bio numbers.

k_E : We know characteristic elongation rate const.

$$\langle k_E \rangle = \frac{\bar{t}_x}{\text{Characteristic transcript length}}$$

From Bio numbers, (112325), $\bar{t}_x = 25 \text{ nt/s}$
(mRNA elongation rate)

$$\therefore \langle k_E^* \rangle = \frac{25}{1000}$$

$$= 0.025 \text{ s}^{-1}$$

Now $k_E^* = \langle k_E^* \rangle \frac{\text{Transcript length}}{\text{lacZ gene length}}$

From Biomimics 102070,

$$= 3075 \text{ nt}$$

$$\therefore k_E^* = 0.025 \frac{1000}{3075}$$

$$= 8.13 \times 10^{-3} \text{ s}^{-1}$$

$$= 29.26 \text{ hrs}^{-1}$$

From BioN 100194, free RNAP conc $R_x = 30 \text{ nM}$

We have to convert into nmol/g DW

so we divide by $\langle m_c \rangle + N_c$.

$$R_x = 30 \text{ nM} = 30 \text{ nmol/mL} = 0.0$$

$$= \frac{30 \text{ nmol}}{\cancel{\text{mL}}} \times 10^{-3}$$

$$\frac{10^8 \text{ cell}}{\text{mL}} \times 2.8 \times 10^{-13} \frac{\text{g DW}}{\text{cell}}$$

$$= 1.07 \times 10^3 \text{ nmol/g DW}$$

Tx This can be calculated using

$$\text{Tx} \approx \frac{k_E}{k_I}$$

where k_I = Transcription Initiation rate const.
 $= 0.024 \text{ s}^{-1}$ (McClellan Paper)

$$\therefore \text{Tx} = \frac{0.00813}{0.024} \\ = \underline{\underline{0.338}}$$

Now, we know $0 < \bar{n}(I, \kappa) < 1$,

i.e. gain of " determines the max value of
~~loss~~ $\langle n^+ \rangle$, which is 0.551 nmol/g DW.

$$\therefore K_x = 0.551 = \frac{k_E R_x G}{(M + \theta_m)(\text{Tx} K_x + (\text{Tx} f_1) G)}$$

Substituting all values, we get

$$K_x = \underline{\underline{211.90 \text{ nmol/g DW}}}$$

Now, in order to find w_1 , w_2 , K , & n ,
for $\bar{u}(I, K)$ term,

I used solver from excel. See attached file.

Parameter	Value	Source
k_E^x	29.26 hr^{-1}	BioNumbers (codes in text)
R_x	$1.07 \times 10^3 \text{ nmol/gDW}$	Calculated BioNumbers
G	0.0118 nmol/g DW	Calculated
I_x	0.338	BioNumbers
K_x	211.4 nmol/gDW	Calculated
M	1.039 hr^{-1}	Calculated
θ_m	8.32 hr^{-1}	Calculated.
w_1	0.25	Excel Solver
w_2	225	"
K_g	0.25	"
n	1.6	"

(d) See plot attached.

Also see Excel sheet.

