

Problem 1

Part A

convert $\langle n \rangle$ values to $B = \langle mc \rangle \hat{N}_c V$ where $\langle mc \rangle$ is $\frac{gDW}{cell}$

$$\hat{N}_c = \frac{\# cells}{mL}$$

V denotes Volume (mL)

$$V = 1 mL$$

$$B[=] gDW$$

gDW inculture

$\# mRNA/cell \rightarrow m^*$
 \nwarrow nmol/gDW of total cell in the culture

According to Bonhubs

$$\langle n \rangle [=] \frac{\# mRNA}{cell}$$

$$\langle n \rangle \left(\frac{\# mRNA}{cell} \right) \left(\frac{1 mol}{6.022 \times 10^{23}} \right) \left(\frac{10^9 nmol}{1 mol} \right) \left(\frac{1}{\langle mc \rangle} \right) \left(\frac{cell}{avg mass} \right)$$

This conversion was used in excel to calculate values given in the spread sheet.

$$\tau = 10$$

$$\frac{430 \times 10^{-15} gDW}{cell}$$

* this calculation would be the same result as calculating the volume basis for total gDW of cell

in the 1 mL and dividing the total nmol of mRNA for the gene in the culture by this number.

$$100600 \quad BIC \# 109836$$

$$\frac{0.39 gDW}{L} = 100600$$

$$0.100600 = 0.1 \times \frac{0.39 gDW}{L} \times \frac{1 L}{10^3 mL}$$

$$\times \frac{1 mL}{10^3 cells}$$

$$\frac{gDW}{cell} = 3.9 \times 10^{-13} \frac{gDW}{cell}$$

Part B

at steady state

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

$$m_{ss} = \frac{r_{x,i} \bar{u}_i}{\mu + \theta_{m,i}}$$

$$m_{ss} = \frac{r_{x,i}}{(\mu + \theta_{m,i})} \bar{a}$$

$$m_{ss} = K_x (g, \theta) \bar{a}(I, k) \quad \text{where} \quad K_x = \frac{r_{x,i}}{(\mu + \theta_{m,i})}$$

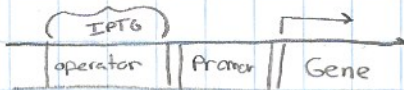
described by eqn 26
in notes on
transcription

Part C

Assume 2 copies of *gpa* cell half life for mRNA is 5 minutes $\tau_T = 1000$

Assume P_{lac} is inducible promoter that responds to IPTG μ is dilution θ_m is related to half life.

Use Moon et al. promoter modeling approach.



determining \bar{u}

there is some expression when IPTG is not bound and this function saturates with IPTG.

K_x = max value of mRNA that can be present in a cell given max growth rate and the degradation rate.

based on the moon et al. paper the positively inducible promoter is described by

$$\frac{\alpha + \beta I^n}{1 + \alpha + \beta I^n}$$

My first thought

(this is the same as the moon et al result except I^n is used in place of FTL .
(I also test this and it also works to describe data.)

The corresponding transfer function in the sum for the type of system we have in N.A

eqn (4)

$$P_{TAC} = F_{TAC}^{max} \left(\frac{\bar{u}}{1 + K_1 + K_2 f_{TL}} \right)$$

$$\text{where } f_{TL} = \frac{L^n}{K_D^n + L^n}$$

$$\text{where } L = [I] [=] \frac{\text{mmd}}{L}$$

Part C take 2

we have

$$m^* = k_x (G, \Theta) \bar{u}(I, K)$$

$$\frac{r_{x,j}}{(u + \Theta_{m,i})}$$

$$= \frac{k_1 + k_2 f_{TL}}{1 + k_1 + k_2 f_{TL}}$$

$$\text{where } f_{TL} = \frac{I^n}{K_0^n + I^n}$$

where $I = [\text{IPTG}]$

we need to determine the associated biological parameters

we can calculate the parameter values for μ and Θ based on growth rate and doubling time

$$-r_{deg} = \Theta_{m,i} \cdot m^*$$

$$\text{for first term: } t_{1/2} = \frac{\ln(2)}{\Theta_{m,i}} \quad \Theta_{m,i} = \frac{\ln(2)}{t_{1/2}} = \frac{\ln(2)}{5 \text{ min}} = 0.139 \text{ min}^{-1}$$

for the dilution term, the doubling time is the time it takes the cell to dilute by a factor of 2 so it can also be thought of like a half-life as well

$$\mu = \frac{\ln(2)}{\tau_0} = \frac{\ln(2)}{40 \text{ min}} = 0.0173 \text{ min}^{-1}$$

we know need to describe $r_{x,j}$. From the course notes on models of transcription eq (26) describes one such model for this term

$$r_{x,j} = k_{E,j}^* \underbrace{R_{XT}}_{\text{nmol/gdw}} \left(\frac{G_j}{\tau_{x,j} K_{x,j} + (\tau_{x,j} + 1) G_j} \right)$$

where $\checkmark k_{E,j}^*$ is elongation rate constant

$\checkmark R_{XT}$ is total RNAP concentration in cell

$\checkmark G_j$ is the copy # of the gene \Rightarrow Given as 2 in the problem

$\checkmark \tau_{x,j}$ is the time constant for transcription

$$\hookrightarrow \approx \frac{K_{E,j}^*}{K_I} \quad \checkmark \quad K_I \rightarrow \text{rate of initiation}$$

$\checkmark K_{x,j}$ saturation constant for transcription

$$\rightarrow k_{E,j}^* = \langle k_E^* \rangle = \underbrace{e_x}_{\text{elongation rate nt/s}} \times \underbrace{\frac{1}{L}}_{\text{length of gene (we will take as 1000 nt)}} \quad \checkmark$$

Based on the McCle paper

$$k_{II} = \frac{k_1}{k_2} = 4 \times 10^{-2} s^{-1}$$

$$K_{x,ij} = \frac{k_{-1} + k_{II}}{k_1}$$

in his notation

$$K_{x,ij} = \frac{k_{-1}}{k_1} + \frac{k_{II}}{k_1}$$

The value of ex the transcription elongation rate

$i_{ex} = 25 \text{ nt/s}$ Bio# 112325

$$L = 1000 \text{ nt}$$

The avg total RNAP [J] in a cell R_{XT} is 1500 molecules/cell Bio#

11400 we will use 1500

with Bio#s are 1500 as well.

101440

$$\frac{1500 \text{ molec}}{\text{cell}} \frac{1 \text{ cell}}{3.9 \times 10^{-13} \text{ GOW}} \left(\frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ mol}} \right) \left(\frac{10^9 \text{ nmol}}{1 \text{ mol}} \right) = 6.38 \text{ nmol/GOW}$$

Based on the values we use in problem set 2

$$K_{x,ij} \approx 0.0136 \mu\text{M}$$

we need

g in units of μM to agree with the units of $K_{x,ij}$

$$\frac{2 \text{ gen}}{\text{cell}} \frac{1 \text{ cell}}{1 \mu\text{m}^3} \left(\frac{1 \mu\text{m}^3}{1 \times 10^{-15} \text{ L}} \right) \left(\frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ molec}} \right) \left(\frac{10^6 \mu\text{mol}}{1 \text{ mol}} \right) = 0.0033 \mu\text{M}$$

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we can do calculations now with these parameters

$$k^* E_j = ex \times \frac{1}{L} = \frac{25 \text{ nt}}{s} \times \frac{1}{1000 \text{ nt}} = 0.025 s^{-1}$$

$$\frac{0.025}{s} \times \frac{60 s}{1 \text{ min}} = 1.5 \text{ min}^{-1}$$

$$Z_{x,i} = \frac{k^* E_j}{K_{II}} = \frac{0.025 s^{-1}}{4 \times 10^{-2} s^{-1}} = 0.625$$

$$\left(\frac{g_j}{Z_{x,i} K_{x,ij} + (Z_{x,i} + 1) g_j} \right) = \frac{0.0033 \mu\text{M}}{0.625 \times 0.0136 \mu\text{M} + (1.625) 0.0033 \mu\text{M}} = 0.238$$

$$r_{x,ij} = K^* E_j R_{XT} \left(\frac{g_j}{Z_{x,i} K_{x,ij} + (Z_{x,i} + 1) g_j} \right)$$

$$1.5 \text{ min}^{-1} (6.38 \text{ nmol/GOW}) (0.238) = 2.28 \frac{\text{nmol}}{\text{GOW min}}$$

$$K_x = \frac{r_{x,ij}}{g_{m,ic} + g} = \frac{2.28}{0.139 \text{ min}^{-1} + 0.0173 \text{ min}^{-1}} = 14.6 \text{ nmol/GOW}$$

We can now fit to the parameters in the \bar{u} function

→ K_1

we have $m^* = K_1 \bar{u}$

at $[IPTG] = 0$ $I = 0$, $f(L) = 0$

$$\bar{u} = \frac{K_1}{1 + K_1}$$

we have the data point

@ $[IPTG] = 0$

$m^* = 0.0809 \text{ nmol/gdw}$ from this we can

Solve for K_1 given the calculated K_1

$$m^* = K_1 \bar{u}$$
$$0.0809 = 14.6 \times \frac{K_1}{1 + K_1}$$

$$K_1 = 0.0056$$

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C.J. Wilsen et al. / Biophysical Chemistry 26

A paper report (in Table 1)

K_D for IPTG binding to LacI
of $2.8 \times 10^{-6} \text{ M}$

↳ which is 0.0028 mM

↳ we will use this for the value of
 K_D in the $f(L)$ function

See Excel sheet p91
for the requested table

We will assume $n=1$, the result of assuming only 1 molecule of IPTG
binds the repressor, and there is only 1 site for the repressor to bind on the DNA

Based on all of these parameters I used a non-linear Least Squares fit to determine
an appropriate value of K_2 , and Excel Solver calculated a value of $0.0199 = K_2$

Part D

The model fits the data surprisingly well, it has the correct shape,
the only predominant errors are in the value to which the calculated
concentrations of m^* saturate to.

All that is necessary to improve the fit is to fit both K_2 and K_D to the data,
instead of just K_2 , this result can be seen in the second figure, where
both K_2 and K_D were fit to the data given all other parameters. This is enough to
eliminate the offset.

Based on this I would say the value of K_D is the parameter that is controlling
the fit of the data preventing a more exact fit of the model.

The graphs can all be recreated by entering these parameters into the equations for K_1 and m
and plotting in Excel. See the Excel sheet. (Problem 1 Excel workbook)