Problem 1

Part A

convert $\langle n \rangle$ values to $\beta = \langle m_c \rangle \hat{N}_c V$ where $\langle m_c \rangle$ is $\frac{gDW}{cell}$ gDW/culture

$\hat{N}_c = \frac{\#\ cells}{mL}$ V denotes Volume (mL)

$V = 1mL$ β[=] gDW

gDW in culture

#mRN/cell

$\langle n \rangle \rightarrow m^*$ ← nmol/gDW of total cell in the culture

According to Bionumbers

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

$$\frac{\langle n \rangle}{\left(\frac{\#\ mRNA}{cell}\right)\left(\frac{1\ mol}{6.022\times10^{23}}\right)\left(\frac{10^9\ nmol}{1\ mol}\right)\left(\frac{cell}{avg\ gDW\ mass}\right)} \qquad \frac{1}{\langle m_c \rangle}$$

$\tau = 40$

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

[This conversion was used in excell to calculate Values given in the spread sheet.

BIO# 109836

* this calculation would be the same result as calculating the volume basis for total gDW of cell in the 1mL and dividing the total nmol of mRNA for the gene in the culture by this number.

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

$0.100600 = 0.1 \times \frac{0.39\ gDW}{L} \times \frac{1L}{10^3 mL} \times \frac{1mL}{10^8\ cells}$

$\frac{gDW}{cell} = 3.9\times10^{-13}\ \frac{gDW}{cell}$

Part B

at steady state

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

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

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

$$\boxed{m_* = K_x(G,\theta)\;\bar{u}(I,k)} \quad \text{where} \quad \boxed{K_x = \frac{r_{x,i}}{(\mu + \theta_{m,i})}}$$

$r_{x,i}$ described by each TL in notes on transcription

Part C

Assume 2 copies of gene per cell half life for mRNA i, 5 minutes $n_T = 1000$

Assume P_{lac} is an indu 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 and the degredation rate.

based on the moon et al. paper the positively indu promotr is described by some function of the approximate form

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

(this is the same as the moon et al result except I^n is used in place of f_{TL}, (I also test this and it also works to describe the data.

my first thought

The corresponding transfer function in the same form as this type of system we saw in N.A

eqn (4)

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

$$\text{where } f_{TL} = \frac{L^n}{K_D^n + L^n} \quad \text{where } L = [I]\ [=]\ \frac{mmol}{L}$$

Part C take 2

we have

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

$$\bar{u} = \frac{k_1 + k_2 f_{TL}}{1 + k_1 + k_2 f_{TL}} \quad \text{where } f_{TL} = \frac{I^n}{K_D^n + L^n}$$

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

where $I = [IPTG]$

we need to determine the associated biological parameters

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

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

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

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 and to calculate

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

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

$$r_{x,j} = k^x_{E,j} \underbrace{R_{x,T}}_{nmol/gDW} \left(\frac{G_j}{\tau_{x,j} K_{x,j} + (\tau_{x,j} + 1) G_j} \right)$$

where $k^x_{E,j}$ is elongation rate constant

R_{x_T} is total RNAP concentration/cell

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

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

$$\hookrightarrow \simeq \frac{K_E{}^x_{,j}}{K_I}$$ $K_I \rightarrow$ rate of initiation

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

$$k^*_{E,j} = \langle k_E{}^x \rangle = \underbrace{e_x}_{\text{elongation rate nt/s}} \times \frac{1}{L}$$ length of gene (we will ballpark this #)

Based on the McClure paper

$K_I = \widetilde{K_2}^{\text{thats}} = 4\times10^{-2}\ s^{-1}$

$K_{x,j} = \frac{K_- + K_I}{K_+}$

in his notation

$K_{x,j} = \frac{K_{-1}}{K_1} + \frac{K_2}{K_1}$

$\frac{K_{-1}}{K_1} \sim \frac{K_{off}}{K_2}$

The value of ex the transcription elongation rate is $ex = 25\ nt/s$ BIO#: 112325

$L = 1000\ nt$

The avg total RNAP [] in a cell RXT is 1500 molecules/cell BIO#: 101440

(with BIO #s are ~1500 as well.)

~1400 we will use 1500

$$\frac{1500\ molec}{cell}\ \frac{1\ cell}{3.9\times10^{-13}\ GDW}\left(\frac{1\ mol}{6.022\times10^{23}\ molec}\right)\left(\frac{10^9\ nmol}{1\ mol}\right) = 6.38\ nmol/GDW$$

Based on the values we use in problem set 2

$K_{x,j} \approx 0.0136\ \mu M$

we need

$\mathcal{G}$ in units of μM to agree with the units of $K_{x,j}$

$$\frac{2\ gen}{cell}\ \frac{1\ cell}{1\ \mu m^3}\left(\frac{1\ \mu m^3}{1\times10^{-15}\ L}\right)\left(\frac{1\ mol}{6.022\times10^{23}\ molec}\right)\left(\frac{10^6\ \mu mol}{1\ mol}\right) = 0.0033\ \mu M$$

BID: 100014

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we can do calculations now with these parameters

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

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

$\tau_{x_i} = \frac{k_e^{x_j}}{K_I} = \frac{0.025\ s^{-1}}{4\times10^{-2}\ s^{-1}} = 0.625$

$$\left(\frac{\mathcal{G}_j}{\tau_{x_i} K_{x_j} + (\tau_{x_i}+1)\mathcal{G}_j}\right) = \frac{0.0033\ \mu M}{0.625 \times 0.0136\ \mu M + (1.625)\ 0.0033\ \mu M} = 0.238$$

$r_{x_j} = K_E^{x_j} R_{x_T}\ (\quad)$

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

$K_x = \frac{r_{x_j}}{\Theta_{mix}} = \frac{2.28}{0.139\ min^{-1} + 0.0173\ min^{-1}} = 14.6\ nmol/GDW$

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

→ K_1

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

at [IPTG] = 0 $I = 0$, $ftL = 0$

$$\bar{u} = \frac{k_1}{1 + k_1}$$

we have the data point @ [IPTG] = 0

$m^* = 0.0809$ nmol/60w from this we can solve for K_1 given the calculated K_x

$m^* = K_x \bar{u}$

$0.0809 = 14.6 \times \frac{K_1}{1 + K_1}$

$K_1 = 0.0056$

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A paper reports (in Table 1)

K_D for IPTG binding to LacI of 2.8×10^{-6} M

↳ which is 0.0028 mM

↳ we will use this for the value of K_D in the fTL function

See Excell Sheet pg1 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 excell solver calculated a value of $0.0199 = K_2$

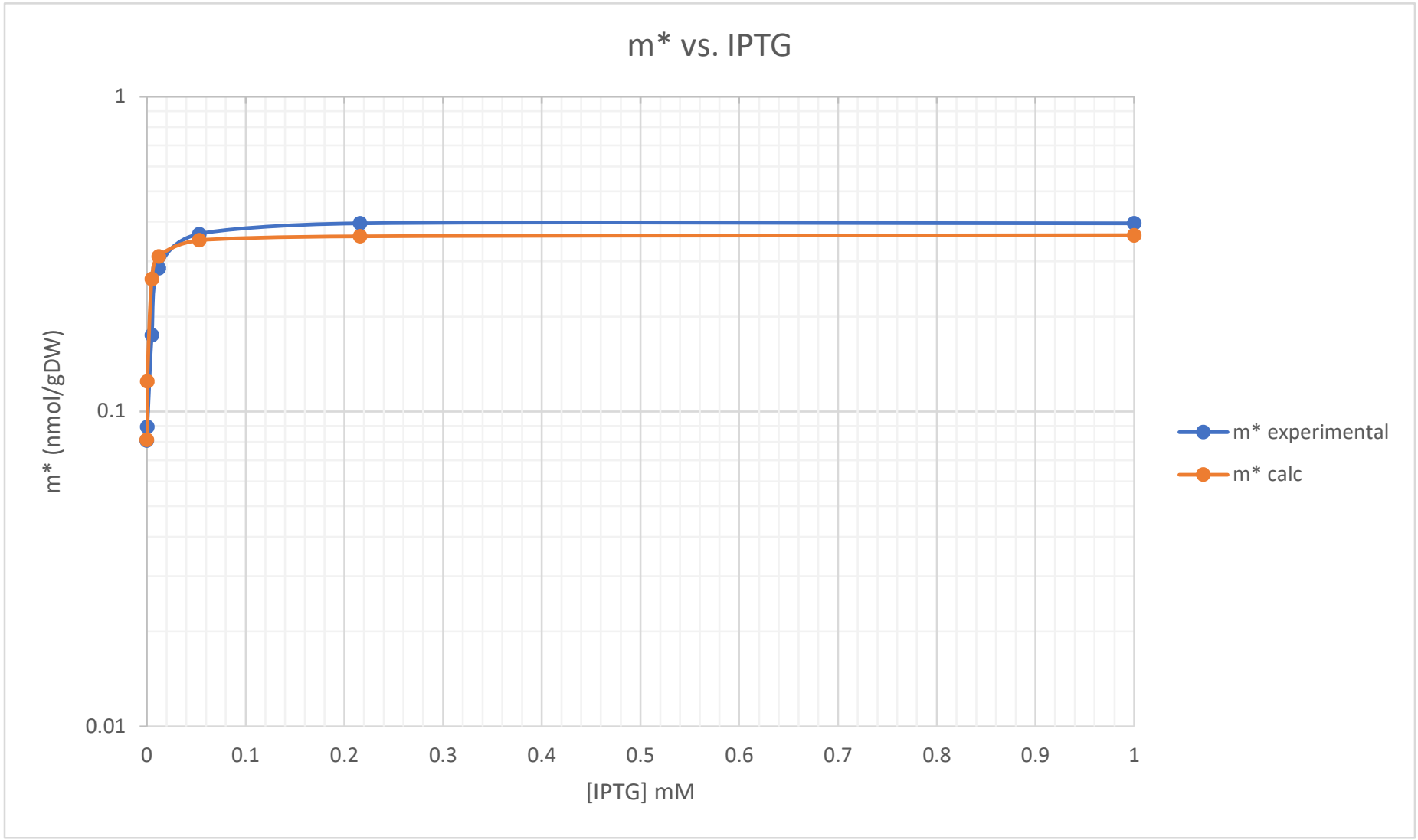
Part D

The model fits the data surprisingly well, it has the correct shape, the only predominate 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_x and $\bar{u}$ and plotting in excell See the excell sheet.) (Problem 1 Excell Workbook)


m* vs. IPTG
m* (nmol/gDW)
1
0.1
0.01
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
[IPTG] mM
m* experimental
m* calc