CHEME 5440 prelim 1.

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Q1. (a).

$<n> = x$ no. of mRNA /cell

$$= \frac{x}{6.02 \times 10^{23}} \times 10^9 \text{ nmol of mRNA /cell}$$

On specific volume basis, $B = <m_c> \cdot \hat{N}_c \cdot V$

$\hat{N}_c = 10^8$ cell /ml. $V = 1$ml. $<m_c>$ in unit of gDW/cell. B in unit of gDW.

According to Bionumbers, cell dry weight at OD600 = 0.39 g/L

Under 1ml OD600 = 0.1 condition, $<m_c> = \frac{0.1 \times 0.39 \text{ g/L} \times 1\text{ ml}}{10^8 \text{ cells}} = 3.9 \times 10^{-13}$ gDW/cell

$<n>$ converted to nmol/gDW form (m^*)

$$= \left(\frac{x}{6.02 \times 10^{23}} \times 10^9\right) / (3.9 \times 10^{-13}) = (4.26 \times 10^{-3} \cdot x) \text{ nmol/gDW}$$

Conversion Table

IPTG (mM)	$<n>$ (mRNA/cell)	m^* (nmol/gDW)
0.	19	0.08094
5×10^{-4}	21	0.08946
0.005	41	0.17466
0.012	67	0.28542
0.053	86	0.36636
0.216	93	0.39618
1	93.	0.39618

- Note:

According to Bionumbers, dry weight of E. Coli at doubling time of 40min $= 280$ fg /cell $= 2.8 \times 10^{-13}$ gDW/cell. which is similar to the calculated $<m_c>$ value above.

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

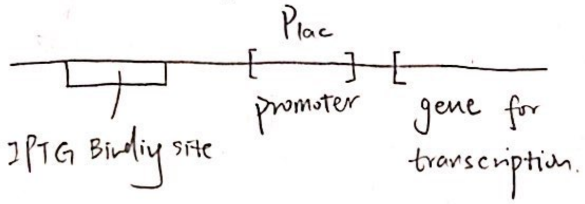
At steady state. $\dot{m}_i = 0$.

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

$$m_i^* = \frac{r_{x,i}}{\mu + \theta_{m,i}} \cdot \bar{u}_i = k_x(g,\theta) \cdot \bar{u}(I,k).$$

Therefore. $k_x(g,\theta) = r_{x,i} / (\mu + \theta_{m,i})$

(c). For lac Z gene activation.



Since we assume Plac is a positively inducible promoter, I find the similar reaction model in the supplementary file of Moon's paper, which is pLux.

According to Equation (5). $P_{Lux} = P_{Lux}^{max}\left(\frac{k_1 + k_2 f_{TL}}{1 + k_1 + k_2 f_{TL}}\right)$ where $f_{TL} = \frac{L^n}{K_D^n + L^n}$ eqn (1)

Going back to our IPTG system, Ligand can be referred as IPTG for binding. Ptac is a function of k and L, and therefore $\frac{k_1 + k_2 f_{TL}}{1 + k_1 + k_2 f_{TL}} = \bar{u}(I,k)$.

→ Consider the m^* expression in part (b).

$$m^* = k_x(g,\theta) \cdot \bar{u}(I,k).$$

$L = [I]$. $n = 1$.

$$f_{TL} = \frac{[I]}{K_D + [I]}$$

$$\frac{r_{x,i}}{\mu + \theta_{m,i}} \qquad \frac{k_1 + k_2 f_{TL}}{1 + k_1 + k_2 f_{TL}}$$

According to course lecture notes,

$$r_{X,j} = k_{E,j}^{X} R_{X,T} \left(\frac{g_j}{\tau_{X,j} K_{X,j} + (\tau_{X,j}+1) g_j} \right)$$

$k_{E,j}^{*}$: transcription elongation rate (PS2) 25 nt/s (1000 nt) $0.025\ s^{-1}$

$R_{X,T}$: total RNAP concentration (Bio [101440]) 5000 RNAP/cell (τ = 40min)

g_j : gene concentration 2 copies/cell = 8.52×10^{-3} nmol/g × 0.39 g/L = 3.32×10^{-3} nM 3.32×10^{-6} uM

$\tau_{X,j}$: time constant $= \dfrac{k_{E,j}^{*}}{k_I}$ ← $0.025\ s^{-1}$ / ← $4\times10^{-2}\ s^{-1}$ (McClure) 0.625

$K_{X,j}$: saturation constant (PS2) 0.0136 uM.

$$r_{X,j} = 0.025 \times 5000 \times \frac{3.32\times10^{-6}}{0.625\times0.0136 + 1.625\times3.32\times10^{-6}} = 0.049$$

For dilution rate $\mu = \dfrac{\ln 2}{40\,min} = \dfrac{\ln 2}{2400\,s} = 2.89\times10^{-4}\ s^{-1}$

For degradation rate $\theta = \dfrac{\ln 2}{5\,min} = \dfrac{\ln 2}{300\,s} = 2.31\times10^{-3}\ s^{-1}$

Therefore $\dfrac{r_{X,i}}{\mu + \theta_{m,i}} = 18.85$

For $\bar{\mu}(I, k)$. ∵ when [I] = 0. $f_{IL} = 0$ $\bar{\mu} = \dfrac{k_1}{1+k_1}$ $m^* = 0.08094$ nmol/gDW

$$0.08094 = 18.85 \times \frac{k_1}{1+k_1} \qquad k_1 = 4.3\times10^{-3}$$

Bionumber [101976] $K_D = 49.6$ uM $= 0.0496$ mM

when [I] = 1. $f_{IL} = \dfrac{1}{0.0496+1} = 0.953$.

$$\bar{\mu} = \frac{4.3\times10^{-3} + 0.953 k_2}{1 + 4.3\times10^{-3} + 0.953 k_2} \qquad m^* = 0.39618 = 18.85 \cdot \mu \qquad k_2 = 0.018$$

To sum up.

$$m^* = 18.85 \times \frac{4.3\times10^{-3} + 0.018 \times \frac{[I]}{0.0496+[I]}}{1 + 4.3\times10^{-3} + 0.018 \times \frac{[I]}{0.0496+[I]}} \qquad m^* = f([I])$$

(1d). The graph is generated in Excel file. "prelimQ1"

The models have the correct shape and the experimental and calculated m^* fits well, though it shows some sort of difference when [IPTG] value is low.

The overlapping of two curves can be optimized by more precise determination of K_1 and K_2 values