Problem 3 - Enzyme concentration estimation in a growing population of E.coli cells.

Assumptions

→ $\tau_d \approx 40\,min$
→ Transcriptional gain (K_x) = $0.575\,nmol/gDW$. → Obtained from Prelim-1.
→ $p^\circ = 0.3\,\mu M$
→ Volume of E.coli cell = $1\,\mu m^3$
→ Weight of E.coli cell = $4.3 \times 10^{-13}\,g$ and is 70% water.
→ Half-life of ρ_j = 24hrs while p° is constant.
→ $(1+\tau_{L,i})m_i \ll \tau_{L,i} K_L$
→ Translation initiation time is 1.5s
→ Characteristic protein length = 333 aa
→ Translation saturation coefficient ($K_{L,i}$) = $200\,\mu M$.
→ Polysome amplification constant (K_P) is unity.

a)
$$\dot{m}_i = \alpha_{x,i}\bar{u}_i - (\mu + \theta_{m,i})m_i \qquad i = 1, 2, \ldots N$$
$$\dot{p}_i = \alpha_{L,i} w_i - (\mu + \theta_{p,i})p_i$$

⇒ At steady state :- $\boxed{\dot{m}_i = \dot{p}_i = 0}$

$$\therefore \quad 0 = \alpha_{x,i}\bar{u}_i - (\mu + \theta_{m,i})m_i^* \implies$$
$$0 = \alpha_{L,i} w_i - (\mu + \theta_{p,i})p_i^*$$

$$m_i^* = \left(\frac{\alpha_{x,i}}{\mu + \theta_{m,i}}\right)\cdot \bar{u}_i \qquad (1)$$
$$p_i^* = \left(\frac{\alpha_{L,i}}{\mu + \theta_{p,i}}\right)\cdot w_i \qquad (2)$$

⇒ From course notes:-
$$\alpha_{L,i} = k_{E,i}^L R_{L,T}\left(\frac{m_i}{\tau_{L,i}K_{L,i} + (\tau_{L,i}+1)m_i}\right)$$

→ As $\boxed{(\tau_{L,i}+1) \ll \tau_{L,i}K_L}$, this reduces to:-
$$\alpha_{L,i} = \left(\frac{k_{E,i}^L R_{L,T}}{\tau_{L,i}K_{L,i}}\right)\cdot m_i$$

$\therefore$ p_i^* from (2) can now be expressed as:-

$$p_i^* = \frac{k_{E,i}^L R_{L,T}}{(\mu+\theta_{P,i})(\tau_{L,i} K_{L,i})} \cdot m_i \cdot w_i \quad \text{— (3)}$$

$\Rightarrow$ Substituting (1) into (3) yields:-

$$p_i^* = \frac{k_{E,i}^L R_{L,T}}{(\mu+\theta_{P,i})(\tau_{L,i} K_{L,i})} \cdot \left(\frac{\alpha_{x,i}}{(\mu+\theta_{m,i})} \cdot \bar{u}_i\right) \cdot w_i$$

$\Rightarrow$ as $\alpha_{x,i} = k_{E,i}^x \cdot R_{x,T} \left(\frac{G_i}{\tau_{x,i} K_{x,i} + (\tau_{x,i}+1) G_i}\right)$, the final form of p^* can be expressed as:-

$$p_i^* = \underbrace{\frac{k_{E,i}^L \cdot R_{L,T}}{(\mu+\theta_{P,i})(\tau_{L,i} K_{L,i})}}_{K_{L,i}\ \text{(Translation gain)}} \cdot \underbrace{\frac{k_{E,i}^x R_{x,T}}{(\mu+\theta_{m,i})}\left(\frac{G_i}{\tau_{x,i} K_{x,i} + (\tau_{x,i}+1) G_i}\right)}_{K_{x,i}\ \text{(Transcription gain)}} \cdot \underbrace{\frac{W_1 + W_2 f_I}{1 + W_1 + W_2 f_I}}_{\bar{u}_i\ \text{(transcription control fxn.)}} \cdot \underbrace{w_i}_{\text{(translation control fxn.)}}$$

b) Estimation of parameter values are listed below and reflected in the attached table and Excel sheet.

$\Rightarrow$ k_E^L : Elongation rate of Ribosome in E. coli (e_L) $\approx$ 18 aa/s. (obtained from bionumbers) BID: 100059

$\therefore \langle k_E^L \rangle = e_L \cdot \ell^{-1} = \frac{18\ aa \cdot s^{-1}}{333\ aa} \times 3600 \approx 194.6\ hr^{-1}$

(ℓ: characteristic protein length (given); $\langle k_E^L \rangle$: characteristic elongation rate)

$\therefore k_E^L = \langle k_E^L \rangle \cdot \left(\frac{\ell}{\ell_j}\right) = (194.5945\ldots)^{hr^{-1}} \left(\frac{333\ aa}{300\ aa}\right) = 216\ hr^{-1}$.

(ℓ_j: protein length of interest)

$\Rightarrow$ B : Specific volume Basis

$B = (1-\alpha)$(gDW of cell)
$= (1-0.7)(4.3 \times 10^{-13}\ g)$
$= 1.29 \times 10^{-13}$ gDW/cell.

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⇒ For μ: Specific growth rate.

$$\mu = \frac{\ln\left(\frac{c_1}{c_0}\right)}{t_1 - t_0} = \frac{\ln\left(\frac{2c_0}{c_0}\right)}{\tau_D} = \frac{\ln(2)}{\tau_D} = \frac{\ln(2)}{(2/3)}\ hr^{-1}$$

$\Rightarrow \tau_D = 40\,min \times \frac{1\ hr}{60\,min} = 2/3\ hrs.$

$$\mu \approx 1.03972\ hr^{-1}$$

⇒ For σ_P: First order degradation of protein (P):-

$$t_{1/2} = \frac{\ln(2)}{\sigma_P} \quad \therefore \sigma_P = \frac{\ln(2)}{t_{1/2}} = \frac{\ln(2)}{24\ hrs} \approx 0.02888\ hr^{-1}$$

⇒ For $K_{L,i}$

$$200\mu M \Rightarrow 200\times10^{-3}\frac{mol}{m^3} \times \frac{1\,m^3}{1\times10^{18}\,\mu m^3} \times \overbrace{\frac{1\,\mu m^3}{1\,cell}}^{\text{cell vol.}} \times \overbrace{\frac{1\ cell}{1.29\times10^{-13}\,gDW}}^{1/B} \times \frac{10^3\,mmol}{mol}$$

$$\therefore K_{L,i} = 1.55\times10^{-3}\ mmol/gDW$$

⇒ For τ_L: Assuming $k_E^L \gg k_I, k_A$

$$\tau_L = \frac{k_E}{k_I} = \frac{k_E^L}{\left(\frac{1}{\text{initiation time}}\right)} = \frac{216\ hr^{-1}}{2400\ hr^{-1}} = 0.090$$

↳ 1.5s

$\Rightarrow k_I = \frac{1}{1.5s} \cdot \frac{3600s}{1hr} = 2400\ hr^{-1}$

⇒ For $R_{L,T}$: Ribosome concentration in a single E.coli cell = 26,000 ribosome molecules / cell
(obtained from bionumbers - article listed)

$$\therefore 26{,}000\ \frac{\text{ribosome molecules}}{cell} \cdot \frac{1\ cell}{1.29\times10^{-13}\,gDW} \times \frac{1\ mol}{6.022\times10^{23}\ \text{molecules}} \times \frac{10^3\,mmol}{1\ mol}$$

$$\boxed{R_{L,T} \approx 3.35\times10^{-4}\ mmol/gDW}$$

⇒ For $\bar{u}_i$: $\bar{u}_i = \frac{W_1 + W_2 f_I}{1 + W_1 + W_2 f_I}$. From Prelim 1:- $W_1 = 0.25$, $W_2 = 98.75$, $K_d = 9\times10^{-2}\,mM$, $n = 1.85$

(*) Inputting these estimates along with $K_{x,i} = 0.575\ mmol/gDW$ and $\omega_i = 1$ we obtain the following graph.

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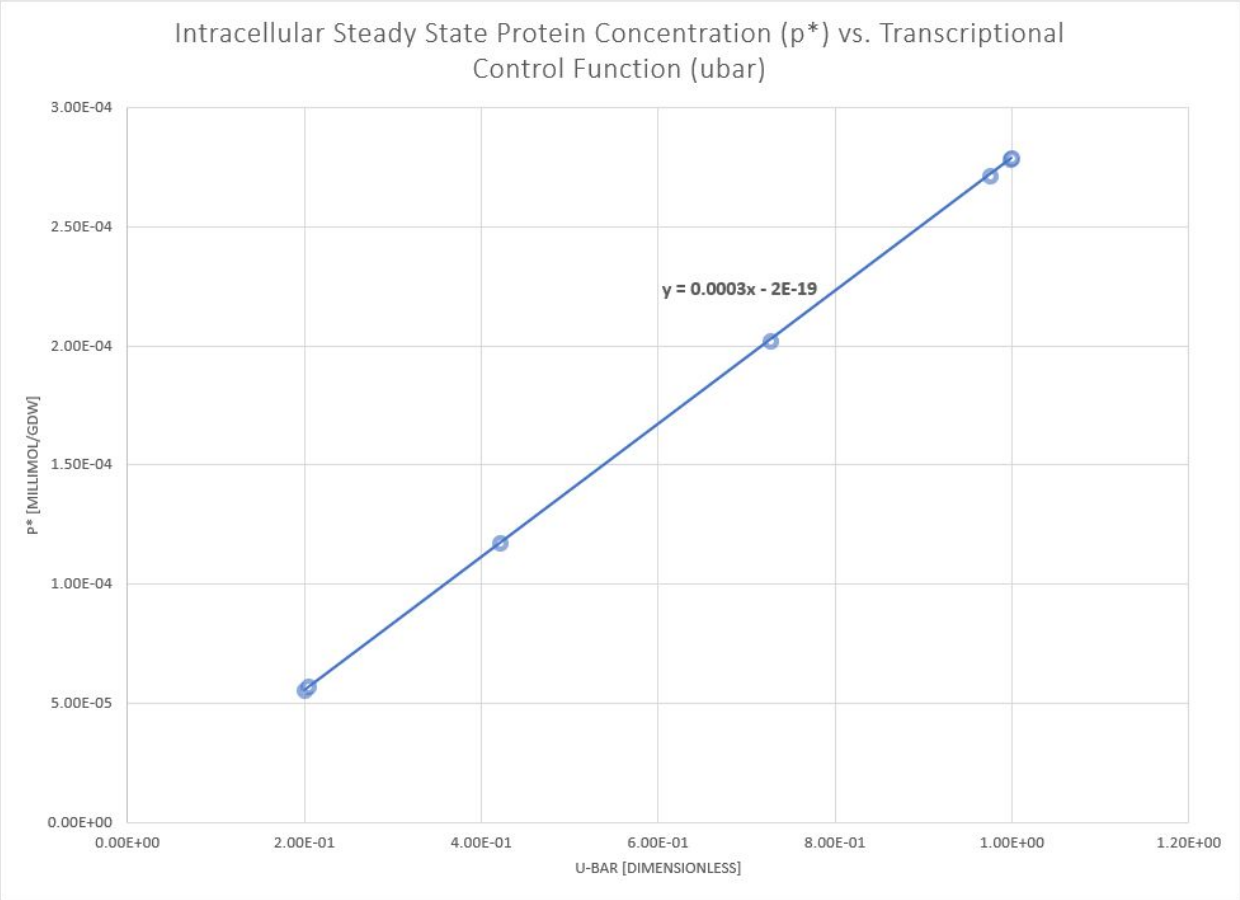
Plot for p* vs. ū for 300 aa protein with wi = 1:



As we see from the plot above, we obtain a linear relationship between the steady-state intracellular protein concentration (p*) and the transcriptional control function (ū) . This intuitively makes sense, as increasing ū, allows for more transcription, which when coupled with complete translational control (w = 1), results in increased translation and therefore an increasing concentration of protein (p_i).

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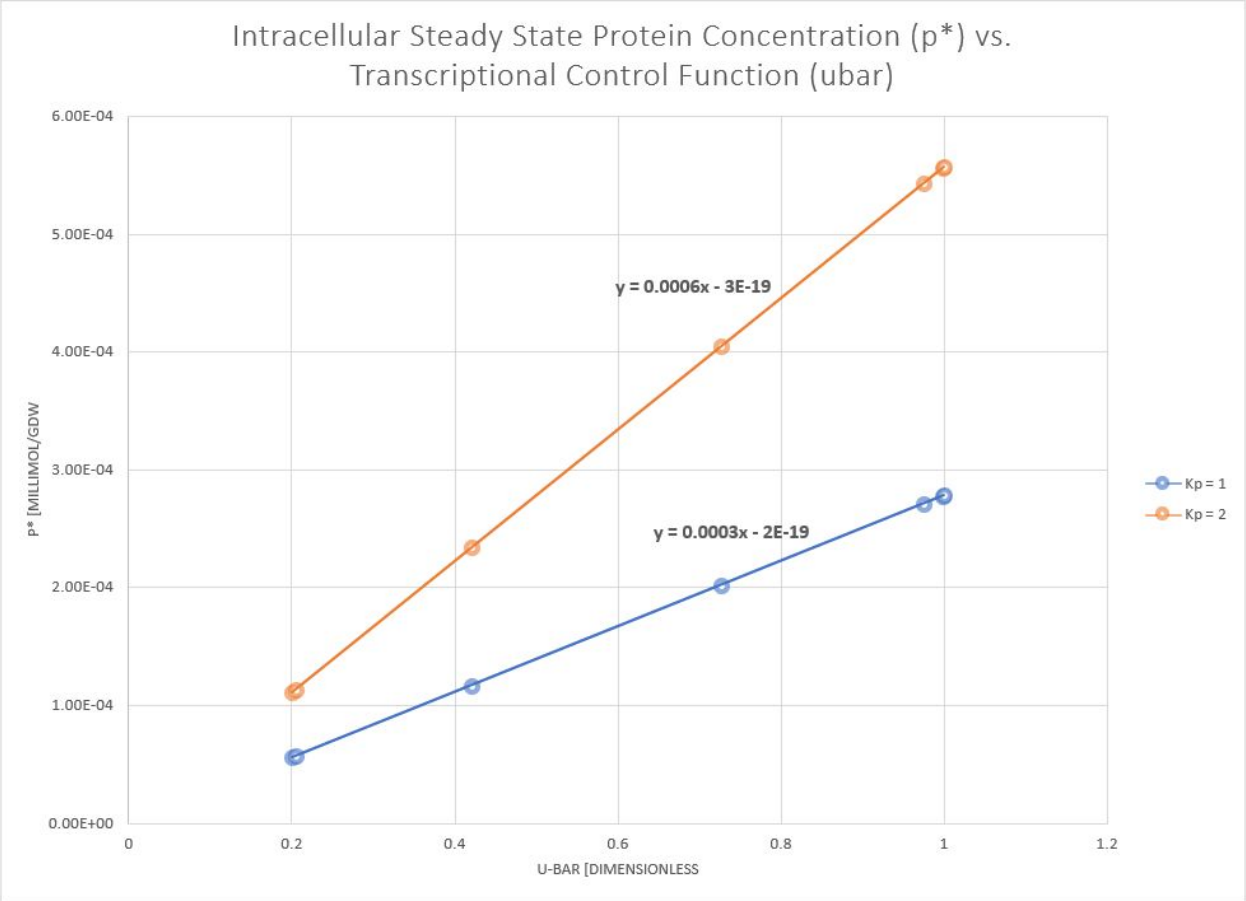
Part c

Increasing the polysome amplification constant (K_p) beyond 1, results in a plot as seen below. The polysome amplification constant can be modeled as:-

$$\mathcal{K}'_{L,i} = K_p \mathcal{K}_{L,i}$$

Where κ'_L is the translational gain that accounts for K_p. The plot below represents the relationship between p* and ū, when K_p = 1(blue line) and K_p = 2 (orange line)



As seen from the graph above, increasing K_p to a value of 2 shifts the linear plot up, and also results in a plot with a steeper gradient (i.e. a larger slope). This intuitively makes sense, as increasing the polysome amplification constant results in a larger translational gain, and thus an increased generation of intracellular protein. This trend continues when K_p is increased to 5, as shown in the plot on the following page.

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