3.

- $0 \le r_j \le U_j$, U_j [=] upper bound of metabolic flux r_j [=] $\frac{mmol}{gDW\ hr}$
- Assume substrates of r_j are saturating (reactant conc >> saturation coeff.)
- $U_j = V_j^{max,o}\left(\frac{P_j^*}{P^o}\right)$

$V_j^{max,o}$ [=] characteristic max reaction velocity for rxn j [=] $\frac{mmol}{gDW\ hr}$

P_j^* [=] intracellular S.S. conc. of enzyme that catalyzes rxn j [=] $\frac{mmol}{gDW}$

P^o [=] characteristic enzyme conc [=] $\frac{mmol}{gDW}$

Assume: (i) $T_d \approx 40$ min, (ii) K_x is data driven gain from Q1 of Prelim1 (iii) $P^o = 0.3\ \mu M$, (iv) Volume of E.coli cell is $1\ \mu m^3$ (v) E.coli cell weighs 4.3×10^{-13} g and is 70% water, (vi) half-life of p_i is 24 hr, while P^o is constant (vii) $(1+T_{L,i})\,m_i << T_{L,i}K_{L,i}$, (viii) translation time is 1.5s, (ix) characteristic protein length is 333 aaa, (x) $K_{L,i} = 200\ \mu m$, (xi) polysome amplification constant (K_P) is unity unless otherwise specified.

(a) $0 = r_{x,i}\bar{u}_i - (\mu + \theta_{m,i})m_i \qquad r_{L,j} = k_{E,j}^L R_{L,T}\left(\frac{m_j}{T_{L,j}K_{L,j} + (T_{L,j}+1)m_j}\right)$

$0 = r_{L,i}w_i - (\mu + \theta_{P,i})p_i$

using (vii), $r_{L,i} = k_{E,i}^L R_{L,T}\left(\frac{m_i}{T_{L,i}K_{L,i}}\right)$

At ss. $m_i^* = \frac{r_{x,i}\bar{u}_i}{\mu+\theta_{m,i}} \qquad p_i^* = \frac{r_{L,i}w_i}{\mu+\theta_{P,i}}$

$r_{L,i} = k_{E,i}^L R_{L,T}\left(\frac{\frac{r_{x,i}\bar{u}}{\mu+\theta_{m,i}}}{T_{L,j}K_{L,i}}\right) \qquad p_i^* = \overbrace{\left(\frac{k_{E,i}^L R_{L,T}}{(\mu+\theta_{P,i})(T_{L,i}K_{L,i})}\right)}^{K_{L,i}}\overbrace{\left(\frac{r_{x,i}}{\mu+\theta_{m,i}}\right)}^{K_{x,i}}\bar{u}\,w_i$

(b) $p_i^* = \left(\frac{k_{E,i}^L R_{L,T}}{(\mu+\theta_{p,i})\gamma_{L,i}(K_{L,i})}\right)\left(\frac{r_{x,i}}{\mu+\theta_{m,i}}\right)\bar{u}\,w_i$ — $k_{E,i}^L$: elongation rate constant for protein L; $R_{L,T}$: active ribosome; p^* [=] $\frac{mmol}{gDW}$

$\left(\frac{r_{x,i}}{\mu+\theta_{m,i}}\right) = 0.575 \frac{mmol}{gDW}$; $w_i = 1$

Parameter estimates are in excel file with plots

$\bar{u} = \frac{w_1 + w_2 f_I}{1 + w_1 + w_2 f_I}$ where $f_I = \frac{I^n}{k_d^n + I^n}$

$w_2 = 98.75$, $w_1 = 0.25$, $n = 1.85$, $k_d = 0.09$

$\theta_{p,i} = \frac{\ln 2}{t_{1/2}} = \frac{\ln 2}{24 hr} = 0.0289\ hr^{-1}$

$k_{L,i} = 200\,\mu M = 200 \times 10^{-6} \frac{mol}{L}$

$\mu = \frac{\ln 2}{T_D} = \frac{\ln 2}{40 min} = 0.0173\ min^{-1}$

$R_{L,T} = 278.14 \frac{nmol}{gDW}$

$\gamma_{L,i} = \frac{k_{E,i}}{k_I} = \frac{0.055 s^{-1}}{0.667 s^{-1}} = 0.08246$

$k_{E,i} = 0.055\ s^{-1}$

Assuming rate constant for abortive initiation is smaller than $k_{E,i}^L$ & k_I:

$\gamma_{L,i} = \frac{k_{E,i}}{k_I} = \frac{0.055\ s^{-1}}{0.667\ s^{-1}} = 0.0825$

Fraction active ribosomes: 0.8 (BIND: 102344)

Bionumbers BIND: 108603 Average ribosome number density: 27000 $\frac{Ribosomes}{\mu m^3}$

$\frac{27000\ Ribosomes}{\mu m^2} \cdot \left(\frac{1 \times 10^{18} \mu m^3}{1 m^3}\right)\left(\frac{1 m^3}{1000 L}\right)\left(\frac{1 mol}{6.02 \times 10^{23}}\right)\left(\frac{10^6 \mu mol}{1 mol}\right) = 44.85 \frac{\mu mol}{L}$

$R_{L,T} = 44.85\ \mu M$

What is k_I? Rate constant for initiation assuming 0th order reaction

use (viii) Initiation time: $k_I = \frac{1}{1.5 s} = 0.667 \frac{1}{s}$

$\langle k_E^L \rangle = ex\ L^{-1}$ $\quad k_{E,j}^L = \langle k_E^x \rangle \left(\frac{L}{L_j}\right)$

translation elongation rate: $16.5 \frac{aa}{s} = ex$ } BIND: 114271

$L = 330\ aa$ $\quad L_j = 300 aa$ $\quad \langle k_E^L \rangle = \left(16.5 \frac{aa}{s}\right)\left(\frac{1}{330 aa}\right) = 0.05 \frac{1}{s}$

$k_{E,j}^L = (0.05 s^{-1})\left(\frac{330\ aa}{300\ aa}\right) = 0.055 s^{-1}$ $\quad \gamma_{L,i} = \frac{0.055 s^{-1}}{0.667 s^{-1}} = 0.082$

$\theta_{p,i} = \frac{0.0289}{hr} \cdot \frac{1 hr}{3600 sec} = 8.03 \times 10^{-6} \frac{1}{s}$ $\quad \mu = 0.0173 \frac{1}{m} \cdot \frac{1 m}{60 s} = 2.883 \times 10^{-4} \frac{1}{s}$

$k_{L,i} = \left(\frac{(0.055 s^{-1})(44.85\ \mu M)}{(8.028 \times 10^{-6} s^{-1} + 2.883 \times 10^{-4} s^{-1})(0.0825)(200\ \mu M)}\right) = 504.45$

$p_i^* = (504.45)(0.575 \frac{nmol}{gDW})(1)\left(\frac{0.25 + 98.75 f_I}{1 + 0.25 + 98.75 f_I}\right)$

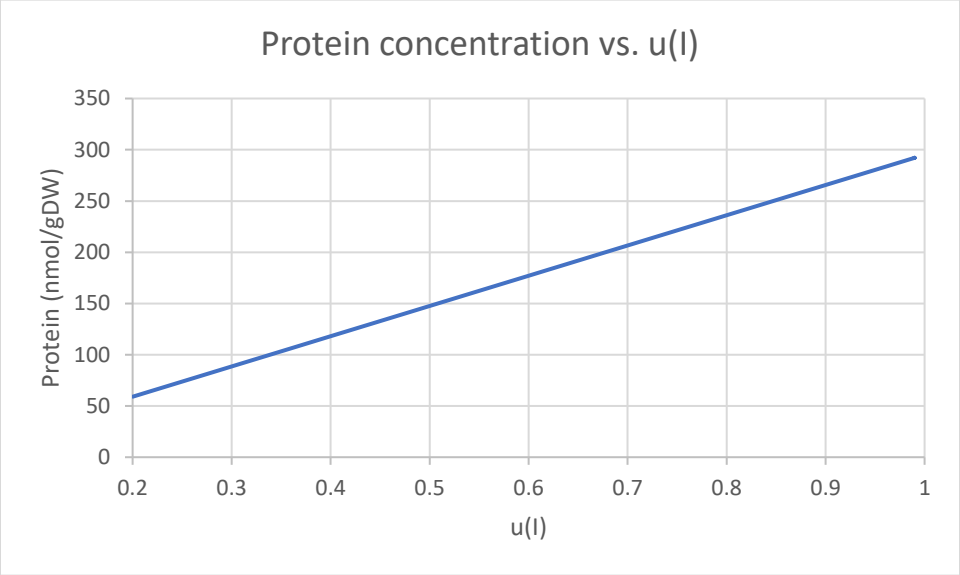
Where $f_I = \frac{I^{1.85}}{(0.09)^{1.85} + I^{1.85}}$

(c) Let's say the value of k_P used = 2 (plot later)

Plot the values, and the curve shifts up. This is because k_P is a constant multiplying $\bar{u}(I)$ and is greater than 1.

This also makes physiological sense, since amplifying polysome concentration would amplify protein concentration.

(b) The parameters used to calculate KL,i = 505 and plot the function are in the excel sheet are shown below. They are also stored in the sheet where the function was plotted.



Parameter	Value	Units	Source
Mass of single ecoli cell	4.3E-13	g	BIND: 106437
Fraction of water in ecoli cell	0.7	fraction	BIND: 100044
W1	0.25	unitless	Q1 Prelim
W2	98.75	unitless	Q1 Prelim
n	1.85	unitless	Q1 Prelim
K	0.09	unitless	Q1 Prelim
Kx,i	0.585	nmol/gDW	Q1 Prelim
Ave ribosome number density	27000	ribsomes/microm^3	BIND: 108603
Translation elongation rate	16.5	aa/s	BIND: 114271
Doubling time of ecoli	40	min	Given
Volume of ecoli cell	1	microm^3	Given
translation initiation time	1.5	s	Given
characteristic protein length	333	aa/s	Given
translation saturation coefficient	200	microM	Given

(c)

An arbitrary value Kp = 2 was chosen greater than 1. This plot clearly shows that the curve shifts upward. This makes mathematical sense because multiplying KL,i by Kp is equivalent to multiplying p*(I) by a constant greater than 1, which would cause p*(I) to increase. This also makes physiological sense since amplifying polysome concentration would increase translation to protein and protein concentration.