3) Written Work Regarding #3

a) Using Table 1 of Allen and Palsson

Reaction Balances

$G = -V_1 + V_2$

$G^* = +V_1 - V_2$

$RNAP = -V_1 + V_2$

$NTP = -V_2 + b_2$

$mRNA = +V_2 - V_3 - V_4 + V_5$

$P_i = +2V_2 + 2V_5 + 2V_6 - b_9$

$NMP = +V_3 - b_4$

$rib = -V_4 + V_5$

$rib^* = +V_4 - V_5$

$AAtRNA = -V_5 + V_6$

$GTP = -2V_5 + b_7$

$GDP = +2V_5 - b_8$

$tRNA = -V_6 + V_5$

$AA = -V_6 + b_1$

$ATP = -V_6 + b_5$

$AMP = +V_6 - b_6$

$protein = +V_5 - b_3$

⟹ Put into A Stoic Matrix

- See Supplemental figure 1 in Q3 folder on github link

⟹

a) After the coefficients were put into the Stoic Matrix (See Supplemental Figure 1) formulate expressions for $\hat{r}_x$ and $\hat{r}_L$

$$r_x = k_E R_{x,T}\left(\frac{G_p}{\tau_x k_x + (\tau_x + 1) G_p}\right)$$

- from Lecture + Hw

where

$$k_E = \langle k_E \rangle \frac{\ell}{\ell_j}$$

$$\langle k_E \rangle = \frac{e_x}{\ell} \Rightarrow \text{Thus } k_E = \frac{e_x}{\ell_j}$$

so

$$r_x = \left(\frac{e_x}{\ell_j}\right) R_{x,T}\left(\frac{G_p}{\tau_x k_x + (\tau_x + 1) G_p}\right)$$

G_p and $R_{x,T}$ are given as Gene plasmid

$R_{x,T}$ = RNAP Concentration

- given in table 3 -

τ_x and k_x are also given in the table 3

Thus

$$\hat{r}_{x,j} = \upsilon\, r_{x,j}$$

$$\upsilon_x = \frac{\omega_1 + \omega_2 F_I}{1 + \omega_1 + \omega_2 f_I} \quad \text{where} \quad F_I = \frac{(I^n)}{(K_I^n + I^n)}$$

putting it all together for $\hat{\Gamma_x}$

$$\hat{\Gamma_x} = \left(\frac{e_x}{\ell_j}\right) R_{x_j T}\left(\frac{G_p}{\tau_x K_x + (\tau_x + 1) G_p}\right)\left(\frac{\omega_1 + \omega_2 F_I}{1 + \omega_1 + \omega_2 F_I}\right)$$

where $$F_I = \frac{(I^n)}{(K_I^n + I^n)}$$

Told that this bound will be <u>equal</u> to R2 in the paper,

- Thus I formulated the bounds like this

$$\hat{\Gamma_x} \leq V_2 \leq \hat{\Gamma_x}$$

↑ 0 was replaced to make V_2 equal to $\hat{\Gamma_x}$

$\Rightarrow$

Now to Formulate $\hat{\Gamma_L}$

$$\Gamma_L = K_{EL} R_L \left(\frac{M_i}{\tau_L k_L + \tau_L M_i + M_i}\right) U_L$$

→ from notes

$\Rightarrow$ told Translation is at the kinetic limit

Thus

$$U_L \approx 1$$

<u>So</u>

$$\Gamma_L = \hat{\Gamma_L}$$

$\Rightarrow$

$$\hat{\Gamma}_L = \Gamma_L = K_{EL} R_L \left(\frac{M_i}{\tilde{\zeta}_L K_L + \tilde{\zeta}_L M_i + M_i} \right)$$

$M_i = ?$

from mass balance

$$\frac{dM}{dt} = Tx - K_d M_1 - \mu M_1$$

cell free so I'm assuming Dilution Due to growth is neglected

Assume steady state

$$0 = Tx - K_d M$$

Tx = rate of trasciption $= \hat{\Gamma}_x = \Gamma_x U_x$

$$0 = \Gamma_x U_x - K_d M$$

$$M = \frac{\Gamma_x U_x}{K_d} \Rightarrow M = \frac{\hat{\Gamma}_x}{K_d}$$

→ This is now able to link up to $\hat{\Gamma}_x$

→ Tells us that rate of trasciption makes the MRNA, but has some degradation K_d

Thus

$$\hat{\Gamma}_L = \left(\frac{e_L}{2_i} \right) R_L \left(\frac{\hat{\Gamma}_x / K_d}{\tilde{\zeta}_L K_L + \tilde{\zeta}_L \left(\hat{\Gamma}_x / K_d \right) + \left(\hat{\Gamma}_x / K_d \right)} \right)$$

Ribosome

b) Maximization work Done in Julia,
See Q3 folder on github for Code, Results, and operation

c) The code was run with altering the bounds of the exchages, and the resuting rate was looked at. This was Done at high and low Inducer levels.

- looking at what a shadow price is
  - how much it costs to give up to gain some amount of product/result
  - in our case, I'm applying my following logic

→ if It is sensitive, a Chage in 1 step of bounds will cause a large Change
→ if it is NOT sensitive, a smaller Chage will not cause much Chage
→ I Therefore Chaged all bounds once by the same amount and Calculated the percent reduction in Traslation rate.

Conclusion →

C) When Changing The Bounds

b_2, b_4, and b_9 caused the largest % reduction for the step reduction in Bound Made

b_1, b_3, b_6, b_5 caused little change in late inducer but large change in little inducer region

b_7 b_8 caused higher change at high inducer compared to Low inducer

Conclusion

b_2, b_4, b_9 ⇒ feed of NTP,
Sink of NMP
Sink of phosphate

b_1, b_3, b_6, b_5 ⇒ ATP and AMP exchange
AA feed
protein Sink

b_7, b_8 ⇒ GTP feed and GDP sink

Since b_2, b_4, b_9 caused the largest change, it is most sensitive to flux bounds of NTP, NMP, and phosphate seems to make sense, as these fluxes are important for the Transcription motif (V_1, V_2) which houses the control function of the cell ⇒

⇒ Continued

and Given That The rate of Traslation is Dependat on MRNA production, but lacks a control function, The Trasciption is The motif That gives highest Sensitivity and Therefore control.

I.e if feed metabolites for Trasciption are stunted, The whole production is snverely impacted however if Traslation feeds or GTP feeds are chaged, it still is affected but not bg as much as The ones That Directly limit MRNA production