Prelim, Q3

✲ want concentrations in μM

gene length = 924 nt = L_{mRNA}

protien length = 308 aa = L_p

Gene Concentration = $5 \times 10^{-9} M \cdot \frac{10^6 \mu M}{1 M} = 5 \times 10^{-3} \mu M = G$

↑ from assumption (i)

from assumption (ii) cell free volume = $15 \times 10^{-6} L$ = vol

from assumption (iii) Protien expression is induced by inducer I §

RNAP concen. $\Rightarrow R_X = 0.15 \mu M$

Ribosome concen. $\Rightarrow R_L = 1.6 \mu M$

Transcription elongation rate $\Rightarrow \dot{V}_X = 60 \frac{nt}{s}$ $\quad K_E = 234 hr^{-1}$

⇑ by (***) § (****)

$\frac{1}{s} \cdot \frac{60 s}{1 min} \cdot \frac{60 min}{1 hr} = \frac{3600}{hr}$ $\quad \Rightarrow K_E = \frac{\dot{V}_X}{L_{mRNA}} = 0.0649 s^{-1}$

$\Rightarrow 3600 s = 1 hr$

$\Rightarrow \frac{3600 s}{1 hr} = 1$ (****)

Translation elongation rate $\Rightarrow \dot{V}_L = 16.5 \frac{aa}{s}$

$\Rightarrow K_{EX} = \frac{\dot{V}_L}{L_p} = 0.0536 s^{-1}$

⇓ by (***) and (****)

$K_{EX} = 193 hr^{-1}$

✲✲ Assume polysome amplification number = 1

Transcription saturation constant $\Rightarrow K_X = 0.3 \mu M$

Translation saturation constant $\Rightarrow K_L = 57.0 \mu M$

Time constant transcription $\Rightarrow \tau_X = 2.7$

Time constant translation $\Rightarrow \tau_L = 0.8$

mRNA Degradation constant $\Rightarrow K_{d,X} = 8.35 hr^{-1}$

Protien Degradation constant $\Rightarrow K_{d,L} = 9.9 \times 10^{-3} hr^{-1}$

Prelim. Q3. Continued.

from assumption (iii) continued:

Characteristic gene length $\Rightarrow L_{mRNA,char.} = 1000\ nt$

Characteristic protien length $\Rightarrow L_{p,char.} = 330\ aa$

from assumption (iv), translation operates at the kinetic limit $\Rightarrow \hat{r}_L = v_L u_L(\ldots)$, $u_L(\ldots) = 1$ (from assumption (iv)) $\Rightarrow \hat{r}_L = v_L$.

for; v_L: kinetic limit for translation; $u_L(\ldots)$: regulation function for translation

*** not explicitly stating what $\hat{r}_L$ or v_L depend on, i.e. I am not stating they are constants

from assumption (v), $(*)[e] \leftrightarrows (*)$, exchange reaction are reversible with

[e]: external species

bounds $-100{,}000.0 \leq b_* \leq 100{,}000.0$ in units of $\mu M/hr$ (***)

want our time scale in hr

from appendix of paper and our parameters

*a = 308 aa
n = 924 nt

reactions:

Transcription init: $G + RNAP \xrightarrow{V_1} G^*$

Transcription: $G^* + n\ NTP \xrightarrow{V_2} mRNA + G + RNAP + 2n\ P_i$

mRNA decay: $mRNA \xrightarrow{V_3} n\ NMP$

Translation init: $mRNA + rib \xrightarrow{V_4} rib^*$

Translation: $rib^* + a\ AAtRNA + 2a\ GTP \xrightarrow{V_5} a\ tRNA + 2a\ GDP + 2a\ P_i + rib + mRNA + peptide$

tRNA charging: $AA + tRNA + ATP \xrightarrow{V_6} AMP + 2P_i + AAtRNA$

Exchange fluxes: $AA[e] \xrightarrow{b_1} AA$, $NTP[e] \xrightarrow{b_2} NTP$, $peptide \xrightarrow{b_3} peptide[e]$, $NMP \xrightarrow{b_4} NMP[e]$, $ATP[e] \xrightarrow{b_5} ATP$, $AMP \xrightarrow{b_6} AMP[e]$, $GTP[e] \xrightarrow{b_7} GTP$, $GDP \xrightarrow{b_8} GDP[e]$, $P_i \xrightarrow{b_9} P_i[e]$

Prelim. Q3. Continued.

a.) & b.)

$\underline{\underline{S}} =$

metabolite	rxns: v_1	$-v_6$	$-b_1$	$-b_9$
G	1	-1	1	0	0	0	0	0	0	0	0	0	0	0	0
G*	2	1	-1	0	0	0	0	0	0	0	0	0	0	0	0
RNAP	3	-1	1	0	0	0	0	0	0	0	0	0	0	0	0
NTP	4	0	-924	0	0	0	0	0	1	0	0	0	0	0	0
mRNA	5	0	1	-1	-1	1	0	0	0	0	0	0	0	0	0
P_i	6	0	1848	0	0	-616	2	0	0	0	0	0	0	0	-1
NMP	7	0	0	924	0	0	0	0	0	0	-1	0	0	0	0
rib	8	0	0	0	-1	1	0	0	0	0	0	0	0	0	0
rib*	9	0	0	0	1	-1	0	0	0	0	0	0	0	0	0
AA-tRNA	10	0	0	0	0	-308	1	0	0	0	0	0	0	0	0
GTP	11	0	0	0	0	-616	0	0	0	0	0	0	0	1	0
tRNA	12	0	0	0	0	308	-1	0	0	0	0	0	0	0	0
GDP	13	0	0	0	0	616	0	0	0	0	0	0	0	-1	0
peptide	14	0	0	0	0	1	0	0	0	-1	0	0	0	0	0
AA	15	0	0	0	0	0	-1	1	0	0	0	0	0	0	0
ATP	16	0	0	0	0	0	-1	0	0	0	0	1	0	0	0
AMP	17	0	0	0	0	0	1	0	0	0	0	-1	0	0	0

with bounds, units are supressed

$\underline{v} =$

$$0 \le v_1 \le \infty$$
$$v_2 = \hat{r}_x$$
$$0 \le v_3 \le 8.35$$
$$0 \le v_4 \le \infty$$
$$0 \le v_5 \le \hat{r}_L$$
$$0 \le v_6 \le \infty$$
$$-10^5 \le b_1 \le 10^5$$
$$-10^5 \le b_2 \le 10^5$$
$$-10^5 \le b_3 \le 10^5$$
$$-10^5 \le b_4 \le 10^5$$
$$-10^5 \le b_5 \le 10^5$$
$$-10^5 \le b_6 \le 10^5$$
$$-10^5 \le b_7 \le 10^5$$
$$-10^5 \le b_8 \le 10^5$$
$$-10^5 \le b_9 \le 10^5$$

$$\hat{r}_x(I) = k_E R_x \left(\frac{G}{\tau_x k_x + (\tau_x + 1) G} \right) u(I)$$

$$u(I) = \frac{w_1 + w_2 f(I)}{1 + w_1 + w_2 f(I)}$$

$$f(I) = \frac{I^n}{k^n + I^n}$$

$I = ii$ in code

$$\hat{r}_L = v_L = k_E x R_L \left(\frac{mRNA^*}{\tau_L k_L + (\tau_L + 1) mRNA^*} \right)$$

$$mRNA^* = \frac{\hat{r}_x(I)}{\lambda_{mRNA}}$$

$mRNA^*$: steady-state value of mRNA

Prelim. Q3. a. Continued & b.

λ_{mRNA} = degradation rate for mRNA + dilution rate → 0 in cell-free so cell volume is constant

$\lambda_{mRNA} = k_{d,x}$

or

$\lambda_{Protien}$ = degradation rate for protien + dilution rate

$\lambda_{Protien} = k_{d,L}$

See code Q3_runner.jl or include("Q3_runner.jl") see read me as location for plot to be saved needs to be specified.

$$Protien^* = \frac{V_5}{\lambda_p}$$ (V_5 from FBA)

For the maximum translation rate V_5 for $I = 0.0001\,mM$ to $I = 10.0\,mM$, call Max_Translation_rate(I) for the maximum rate V_5 for any I.

prelim. Q3. C. Final.

To determine the which exchange flux bounds the translation rate is most sensative to we look to the shadow prices associated with the Exchange flux bounds. To do this one relaxes the bounds on 1 exchange flux at a time and measures how this relaxation effects the optimized value, here it is the translation rate. So now our constraint is

$$a_i \le \sum_{j=1}^{15} \sigma_{ij} v_j \le b_i \quad \text{for } i = 1, \dots, 17$$

$a_i = b_i = 0$ if metabolite i does not engage in an exchange rxn

else $a_i = -4$ & $b_i = 4$

but in order to obtain information on the individual exchange rxn's shadow prices $a_i = -4$ & $b_i = 4$ is only allowed for one case at a time thus allowing us to calculate s.p for each indivual exchange rxn. To compare the change in the optimum v_5, the v_5 was obtained a(*) as well as the $\underline{v}$ (flux vector), and small pretur-bations (0.99%) was preformed on the optimum flux's so that they we bound just below the old optimum, with the new optimum and the old optimum as well as the change in the flux value for the specific Exchange flux tested, The derivative of the optimum v_5 value w.r.t the Exchange flux being test can be determined the analysis was done with $I = 10.0\,mM$. (*)

See code / run / include ("Q3.C_runner.jl") (and that gives us the shadow price)

sensative_metabolites = indexes 4, 6, 7, 11, 13, 14, 15, 16, and 17
(and corresponding ex. flux index) for exchange flux (8), (15), (10), (13), (14), (9), (7), (11), and (12)

from the code The bounds of Exchange flux for $P_i \xrightarrow[v_{15}]{b_9} P_i[e]$, are the ones which the translation rate is most sensative to, with an shadow price of 0.000271. absolute magnitude