5440 Final exam

Part 2 Question 1

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a) from notes $\tau = \gamma_N t$, $\nu = \gamma_D, \gamma_N$

$$\frac{\partial N_1}{\partial \tau} = f(D_2) - N_1 \quad (2)$$

$$\frac{\partial D_1}{\partial \tau} = (g(N_1) - D_1)\nu \quad (3)$$

$$\frac{\partial N_2}{\partial \tau} = f(D_1) - N_2 \quad (4)$$

$$\frac{\partial D_2}{\partial \tau} = (g(N_2) - D_2)\nu \quad (5)$$

given $\gamma_D / \gamma_N = \nu << 1$

from (3) and (5) we get

$$\frac{\partial D_1}{\partial \tau} \approx 0, \quad \frac{\partial D_2}{\partial \tau} \approx 0$$

as $\nu \ll 1$ and ν multiplies across all terms.

$\therefore$ $D_1, D_2 \approx$ constant

$\therefore$ $f(D_1), f(D_2) \approx$ constant

looking @ (1) if $N_1 > f(D_2)$ then $\frac{\partial N_1}{\partial t} < 0$ causing N_1 to decrease until it reaches S.S. @ $N_1 = f(D_2)$.

if $N_1 < f(D_2)$ then $\frac{\partial N_1}{\partial t} > 0$ causing N_1 to increase until it reaches S.S. @ $N_1 = f(D_2)$.

1a continued

The same can be said looking @ eqn (3)

as because $\frac{\partial D_1}{\partial t} \approx 0$ $f(D_1) \approx$ constant $\therefore$

if $N_2 > f(D_1)$ then $\frac{\partial N_2}{\partial \tau} < 0$ and N_2 decreases until it reaches s.s. @ $N_2 = f(D_1)$.

if $N_2 < f(D_1)$ then $\frac{\partial N_2}{\partial \tau} > 0$ and N_2 increases until it reaches s.s. @ $N_2 = f(D_1)$.

$\therefore$ if $\nu = \frac{\gamma_D}{\gamma_N} << 1$ both the delta and Notch activity approach a s.s.

And we get

$$\frac{\partial D_1}{\partial \tau} = \nu(g(N_1) - D_1) \approx 0, \quad \frac{\partial D_2}{\partial \tau} = \nu(g(N_2) - D_2) \approx 0$$

$$g(N_1) \approx D_1, \quad g(N_2) \approx D_2$$

$\therefore$

$$\frac{\partial N_1}{\partial \tau} = f(g(N_2)) - N_1, \quad \frac{\partial N_2}{\partial \tau} = f(g(N_1)) - N_2$$

and because $D_i \approx$ constant $\rightarrow g(N_i) \approx$ constant and $\therefore$ $f(g(N_i)) \approx$ constant and thus N_i goes to approx. s.s. and $\therefore$ $f(g(N_i)) \approx$ constant and thus N_i also goes to approx. s.s.

$\therefore$ as described above $\frac{\partial D_1}{\partial \tau}, \frac{\partial D_2}{\partial \tau}, \frac{\partial N_1}{\partial \tau}, \frac{\partial N_2}{\partial \tau} \approx 0$

ADDENDUM to Problem 1 (why we can assume $g(N_i) \simeq D_i$)

$$\frac{\partial D_i}{\partial \tau} = \left(g(N_i) - D_i\right)\nu, \quad \nu = \frac{\gamma_0}{\gamma_N}$$

for $\nu \ll 1$

$$\frac{1}{\nu}\frac{\partial D_i}{\partial \tau} = g(N_i) - D_i$$

Note 1 D_i must be non-negative

Note 2 $g(N_i)$ has a range of $[0,1]$

$\therefore$ $\frac{1}{\nu}\frac{\partial D_i}{\partial \tau}$ has a max of 1 (if $g(N_i)=1$, $D_i=0$)

Because $\nu \ll 1 \rightarrow \frac{1}{\nu} \gg 1$

$\therefore$ $\frac{\partial D_i}{\partial \tau}$ must be $\ll 1$ such that

$$\frac{1}{\nu}\frac{\partial D_i}{\partial \tau} = 1 \text{ @ max}$$

$\therefore$ $\frac{\partial D_i}{\partial \tau} \simeq 0$ and both

D_1, D_2 can be assumed to be @ S.S. as this analysis applies to both.

if $D_i > g(N_i)$

↳ $\frac{\partial D_i}{\partial \tau} < 0$, $\therefore$ $\frac{\partial D_i}{\partial \tau}$ is either $\simeq 0$ or causes D_i to plummet until $D_i = g(N_i)$ and $\therefore$ $\frac{\partial D_i}{\partial \tau} = 0$ irrespective of

if $D_i < g(N_i)$

↳ $\frac{\partial D_i}{\partial \tau} > 0$, $\therefore$ $\frac{\partial D_i}{\partial \tau}$ is either $\simeq 0$ or causes D_i to rise until $D_i = g(N_i)$.

1B

from notes

D of other cell

$$f(D') = \frac{F(D')}{\gamma_N} = \frac{D'^2}{0.1 + D'^2} \quad (6)$$

N of the same cell

$$g(N) = \frac{G(N)}{\gamma_D} = \frac{1}{1 + 10N^2} \quad (7)$$

$$\frac{\partial N_1}{\partial \tau} = f\left(g(N_2)\right) - N_1 = f\left(\frac{1}{1+10N_2^2}\right) - N_1$$

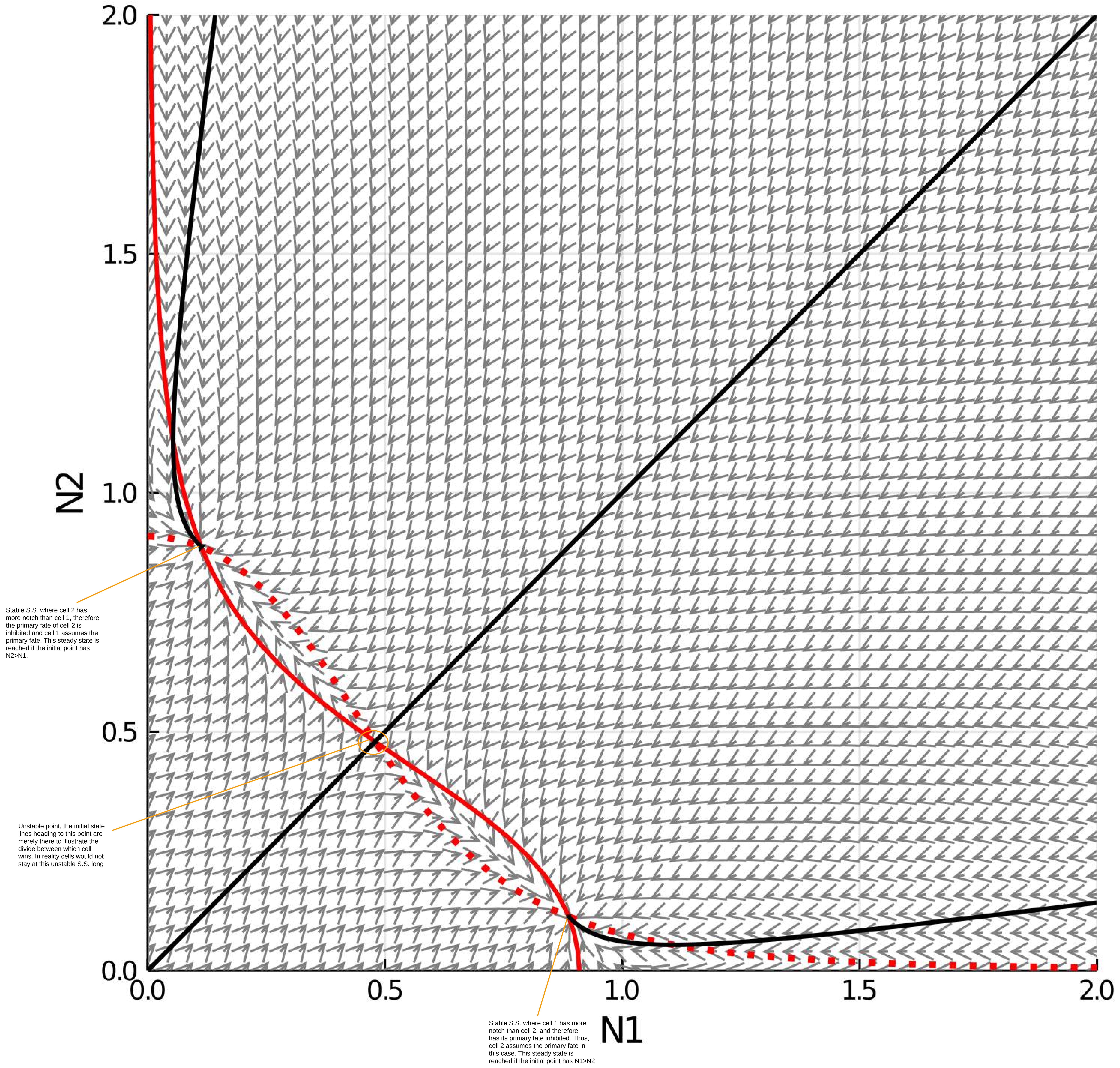
$$\frac{\partial N_1}{\partial \tau} = \frac{\left(\frac{1}{1+10N_2^2}\right)^2}{0.1 + \left(\frac{1}{1+10N_2^2}\right)^2} - N_1 \quad (E1)$$

$$\frac{\partial N_2}{\partial \tau} = f(g(N_1)) - N_2 = f\left(\frac{1}{1+10N_1^2}\right) - N_2$$

$$\frac{\partial N_2}{\partial \tau} = \frac{\left(\frac{1}{1+10N_1^2}\right)}{0.1 + \left(\frac{1}{1+10N_1}\right)^2} - N_2 \quad (E2)$$

Took E1, and E2 and Plugged them into Atum, Please see the Problem 1 file.

$\boxed{\text{It appears as if lateral inhibition works exactly the same when } \nu \ll 1 \text{ on } \nu \gg 1.}$


2.0
1.5
1.0
0.5
0.0
N2
0.0
0.5
1.0
1.5
2.0
N1
Stable S.S. where cell 2 has more notch than cell 1, therefore the primary fate of cell 2 is inhibited and cell 1 assumes the primary fate. This steady state is reached if the initial point has N2>N1.
Unstable point, the initial state lines heading to this point are merely there to illustrate the divide between which cell wins. In reality cells would not stay at this unstable S.S. long
Stable S.S. where cell 1 has more notch than cell 2, and therefore has its primary fate inhibited. Thus, cell 2 assumes the primary fate in this case. This steady state is reached if the initial point has N1>N2

Question 2

a)

S.S. balance so

$$\left.\frac{dL}{dt}\right|_{z=z>0} = 0 = \underbrace{\underbrace{\underbrace{K_m(z)\left[L_b - L_c(z)\right]}_{\left(\frac{m}{s}\right)\left(\frac{\#}{m^3}\right)}}_{\frac{\#}{s\,m^2}} \underbrace{\left(\frac{1}{\text{density of cells}}\right)}_{\frac{m^2}{cell}}}_{\frac{\#}{sec\cdot cell}} + \underbrace{q}_{\frac{\#}{sec\cdot cell}} + \underbrace{K_r R_s^*}_{\left(\frac{1}{s}\right)\left(\frac{\#}{cell}\right) \to \frac{\#}{sec\cdot cell}} - \underbrace{K_f R_s L_c(z)}_{\left(\frac{m^3}{\#\,sec}\right)\left(\frac{\#}{cell}\right)\left(\frac{\#}{m^3}\right) \to \frac{\#}{sec\cdot cell}}$$

Should be $\frac{1}{\# cells}\frac{dL}{dt}$ but

(The whole thing should be multiplied by # of cells but it just divides out so I didn't include it)

density of cells (@ t = 0) = n_c → because we are only interested in the initial mitotic response I'm assuming density of cells = n_c (constant)

from Knauer

$$\frac{\text{Mitotic rate}}{\text{max rate}} = \gamma \cdot R^*_{Total} = \gamma\left[\frac{1}{K_e^*} + \frac{1}{K_{deg}}\right]\left[\frac{K_{ss}L}{1+K_{ss}L}\right]V_s$$

where

$$K_{ss} = \frac{K_e^* K_f}{K_e(K_r + K_e^*)}$$

so

$$0 = \frac{K_m L_b}{n_c} - \frac{K_m L_c}{n_c} + q + K_r R_s^* - K_f R_s L_c$$

$$L_c\left[\frac{K_m}{n_c} + K_f R_s\right] = \frac{K_m L_b}{n_c} + q + K_r R_s^*$$

$$L_c(z) = \frac{\dfrac{K_m(z) L_b + q n_c + K_r R_s^* n_c}{n_c}}{\dfrac{K_m + K_f R_s n_c}{n_c}}$$

↓

question 2) continued

a)
$$L_c(z) = \left(\frac{k_m(z) L_b + q n_c + k_r R_s^* n_c}{n_c}\right)\left(\frac{n_c}{k_m(z) + k_f R_s n_c}\right)$$

$$\boxed{L_c(z) = \left[\frac{k_m(z) L_b + q n_c + k_r R_s^* n_c}{k_m(z) + k_f R_s n_c}\right]}$$

unit check

$$\frac{\#}{m^3} = \frac{\left(\frac{m}{s}\right)\left(\frac{\#}{m^3}\right) + \left(\frac{\#}{s \cdot cell}\right)\left(\frac{cell}{m^2}\right) + \left(\frac{1}{s}\right)\left(\frac{\#}{cell}\right)\left(\frac{cell}{m^2}\right)}{\left(\frac{m}{s}\right) + \left(\frac{m^3}{\# s}\right)\left(\frac{\#}{cell}\right)\left(\frac{cell}{m^2}\right)}$$

$$\frac{\#}{m^3} = \frac{\left(\frac{\#}{s\, m^2}\right) + \left(\frac{\#}{s\, m^2}\right) + \left(\frac{\#}{s\, m^2}\right)}{\left(\frac{m}{s}\right) + \left(\frac{m}{s}\right)}$$

$$\frac{\#}{m^3} = \left(\frac{\#}{s\, m^2}\right)\left(\frac{s}{m}\right) = \frac{\#}{m^3}$$

B)

Transport Limited $\left(k_m \text{ is very small}\right)$: $\boxed{L_c(z) = \frac{q n_c + k_r R_s^* n_c}{k_f R_s n_c} = \frac{q + k_r R^*}{k_f R_s}}$

Binding Limited $\left(k_m \text{ is very large}\right)$: $\boxed{L_c(z) = \frac{k_m(z) L_b}{k_m(z)} = L_b}$

B)

Transport Limited explanation: Transport is slow ∴ the mass transfer between the boundary layers term is not present. ∴ the only thing increasing L_c is the generation (q) and the unbinding of previous ligand → ($K_r R_s^*$). For the same reason the only consumption is from the ligand binding ($K_f R_s$). Because the rate of binding is a function of ligand the expression for L_c is a ratio rather than summation.

Binding Limited explanation: Binding is slow ∴ comparatively not much ligand is bound and ∴ there is not a great impact on L_c from unbinding either. And because transport is relatively fast $L_c = L_b$ as any difference between the two (i.e. from q) is quickly redistributed evenly.

Question 2 continued

C from Knauer

$$\frac{\text{Mitotic rate}}{\text{max rate}} = \gamma \cdot R^*_{Total} = \gamma\left[\frac{1}{k_e^*}+\frac{1}{k_{deg}}\right]\left[\frac{K_{ss}L_c(z)}{1+K_{ss}L_c(z)}\right]V_s$$

where $K_{ss} = \dfrac{k_e^* k_f}{k_e(k_r+k_e^*)}$

and γ = mitogenic signal
↳ (slope of Knauer graph)

From A)

$$L_c(z) = \frac{k_m(z)L_b + q n_c + k_r R_s^* n_c}{k_m(z) + k_f R_s n_c}$$

in the Limit of Low [L] → $L_c K_{ss} \ll 1$, $L_b = 0$

gives: $$L_c(z) = \frac{q n_c + k_r R_s^* n_c}{k_m(z) + k_f R_s n_c}$$

$$\frac{\text{Mitotic Rate}}{\text{Max Rate}} = \gamma \boxed{\left[\frac{1}{k_e^*}+\frac{1}{k_{deg}}\right]\left[K_{ss}V_s\right]\left[\frac{q n_c + k_r R_s^* n_c}{k_m(z)+k_f R_s n_c}\right]}$$

Box = R^*_{Total}

$$\frac{\text{Mitotic Rate}}{\text{max Rate}} = \rightarrow \gamma\, R^*_{Total}$$

issue R_s^*, R_s

question 2 continued

d) given $K_e = 10^{-4}\ (s^{-1})$, $K_e^* = 5 \cdot 10^{-3}\ (s^{-1})$

$K_f = 3.1 \cdot 10^{6}\ (M^{-1} s^{-1}) = 5.14 \cdot 10^{-21}\ (m^3 s^{-1})$

$K_r = 2.5 \cdot 10^{-2}\ (s^{-1})$, $K_{deg} = 8 \cdot 10^{-4}\ (s^{-1})$

$V_s = 18\ (s^{-1} cell^{-1})$, $q = 10^3\ (\# cell^{-1} s^{-1})$

$n_c = 3 \cdot 10^{8}\ (cell \cdot m^{-2})$

$$Sh_z = \frac{K_m(z)}{D_L/z} = \left(\frac{\dot{\gamma} z^2}{D_L}\right)^{1/3}$$

$$\swarrow$$

$$K_m(z) = \left(\frac{\dot{\gamma}^{1/3} z^{2/3}}{D_L^{1/3}}\right)\left(\frac{D_L}{z}\right)$$

$$\boxed{K_m(z) = \frac{\dot{\gamma}^{1/3} D_L^{2/3}}{z^{1/3}} = \left(\frac{\dot{\gamma} D_L^2}{z}\right)^{1/3}}$$

$\dot{\gamma} = 10^2\ (s^{-1})$, $D_L = 10^{-10}\ (m^2 s^{-1})$

5440 Problem 3

a)

$$\frac{dm_i}{dt} = r_{x,i}\, u_i - (\mu + \theta_{m,i})\bar{m}_i \quad (1)$$

$$\frac{d\rho_i}{dt} = r_{L,i}\, w_i - (\mu + \theta_{\rho,i})\rho_i \quad (2)$$

@ S.S. $\frac{d}{dt} = 0$ $\therefore$ from (1)

$$(\mu + \theta_{m_i}) m_i^* = r_{x_i} \bar{u}_i$$

$$m_i^* = \frac{r_{xi}}{\mu + \theta_{m_i}} \bar{u}_i = K_x \bar{u}_i$$

and from (2)

$$\rho_i^*(\mu + \theta_{\rho,i}) = r_{L_i} w_i$$

$$r_{L_i} = K^L_{E,i} R_{LT} \left(\frac{m_i}{\nu \tau_{L,i} K_{L,i} + (\nu \tau_{L,i} + 1) m_i} \right)$$

$\therefore$ @ S.S.

$$r_{L_i} = K^L_{E,i} R_{LT} \left(\frac{K_x \bar{u}_i}{\nu \tau_{L_i} K_{L_i} + (\nu \tau_{L_i} + 1) K_x \bar{u}_i} \right)$$

$\therefore$

$$\rho_i^* = \frac{w_i}{\mu + \theta_{\rho_i}} \left(K^L_{E_i} R_{LT} \right) \left(\frac{K_x \bar{u}_i}{\nu \tau_{L_i} K_{L,i} + (\nu \tau_{L,i} + 1) K_x \bar{u}_i} \right)$$

$$\rho_i^* = \left[\left(\frac{K^L_{E_i} R_{LT}}{\mu + \theta_{\rho,i}} \right) \left(\frac{1}{\nu \tau_{L_i} K_{L_i} + (\nu \tau_{L,i} + 1) K_x \bar{u}_i} \right) \right] (K_x)(\bar{u}_i)(w_i)$$

↓

3 a) continued

$$p_i^* = \left[\left(\frac{K_{E_i}^L R_{LT}}{\mu + \theta_{p,i}}\right)\left(\frac{1}{\tau_{L,i} K_{L,i} + (\tau_{L,i}+1) K_X \bar{\mu}}\right)\right](K_X)(\bar{u}_i)(w_i)$$

by assumption #7 $\tau_{L,i} K_{L,i} \gg (\tau_{L,i}+1) K_X \bar{\mu}$

$\therefore$

$$\boxed{p_i^* = \underbrace{\left[\left(\frac{K_{E_i}^L R_{LT}}{\mu + \theta_{p,i}}\right)\left(\frac{1}{\tau_{L,i} K_{L,i}}\right)\right]}_{= K_L} (K_X)(\bar{u}_i)(w_i) \qquad p_i^* = K_L K_X \bar{u}_i w_i}$$

3B K_X from Prelim 1 Solutions $= \dfrac{\left(93 \frac{\text{mRNA}}{\text{cell}}\right)\left(\frac{10^9 \text{ nmol/mol}}{6.022 \cdot 10^{23} \frac{\text{mRNA}}{\text{mol}}}\right)}{(1 - 70\%_{\text{water}})\left(4.3 \cdot 10^{-13} \frac{g}{\text{cell}}\right)} = \underline{1.197 \frac{\text{nmol}}{\text{gdw}}}$

given $\underline{w_i = 1}$

$\bar{u}_i$ from Prelim 1 $= \dfrac{w_1 + w_2 f_I}{1 + w_1 + w_2 f_I}$, from Prelim 1 Solns. $\underline{w_1 = 0.25}$, $\underline{w_2 = 98.75}$

f_I from Prelim 1 $= \dfrac{I^n}{K_d^n + I^n}$, from Prelim 1 Solns. $\underline{n = 1.85}$, $K_d = 9 \cdot 10^{-2}\ mM$, $\rightarrow \underline{K_d = 9 \cdot 10^{-5}\ nM}$

Translation Elongation

Translation Total Ribosomes,

$\therefore$

$$p_i^* = \left[\left(\frac{K_{E_i}^L R_{LT}}{\mu + \theta_{p,i}}\right)\left(\frac{1}{\tau_{L,i} K_{L,i}}\right)\right]\left(1.197 \frac{\text{nmol}}{\text{gdw}}\right)\left(\frac{0.25 + 98.75\left(\frac{I^{1.85}}{(9 \cdot 10^{-5}_{\text{nM}})^{1.85} + I^{1.85}}\right)}{1 + 0.25 + 98.75\left(\frac{I^{1.85}}{(9 \cdot 10^{-5}_{\text{nM}})^{1.85} + I^{1.85}}\right)}\right)(1)$$

dilution factor

Protein degredation

Translation time constant

Translation saturation constant

3 B continued

from PS2, from Part B

Translation Elongation: $K_{E_i}^{L} = \left(\frac{\text{Translation elongation rate}}{}\right)\left(\frac{1}{\underbrace{\text{Protein length}}_{300aa}}\right) = \left(16.5\frac{aa}{sec}\right)\left(\frac{1}{300\ aa}\right) = 0.055\ sec^{-1}$

Translation TOTAL Ribosome?: $R_{LT} = (2.3\ \mu mol)$ - from PS2

dilution factor: $\mu = \frac{\log(2)}{\tau_D}$ -doubling time $= \frac{0.301}{\frac{40 min}{60 m/Hr}}$ from assumption 1 $= 0.4515\ Hr^{-1}$

Protein degregation: $\theta_{P_i} = \frac{\log(\frac{1}{2})}{P_i \text{ half life}} = \frac{\log(\frac{1}{2})}{24 Hrs} = -0.012543\ Hr^{-1}$ from assumption 6

Translation time constant: $\tau_{L,i} \approx \frac{K_E^L}{K_I} = \frac{0.055 sec^{-1}}{\left(\frac{1}{1.5 sec}\right)} = 0.0825$ unitless

assumes $K_A \ll K_I, K_E^L$

translation initiation time from assumption 8.

Translation saturation constant: $K_{L,i} = 200\ \mu m$ from assumption 10

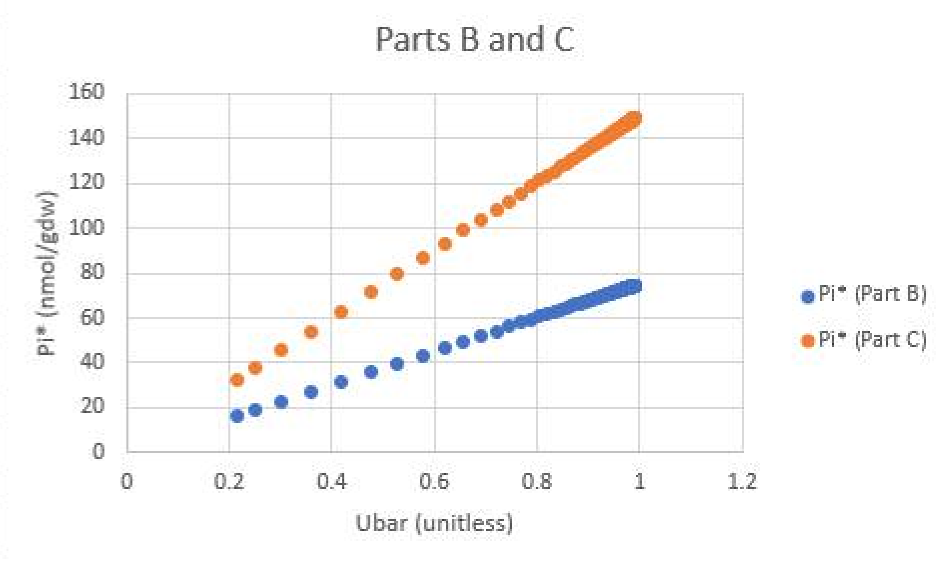
$$V_i^{max} = \left(\frac{\text{Translation elongation rate}}{\text{Protein length}}\right)\left(\text{ribosome concentration}\right)$$

$\therefore$

$$K_L = \left[\left(\frac{\left(\frac{16.5}{300}\ sec^{-1}\right)\left(\frac{3600 sec}{Hr}\right)(2.3\mu mol)}{0.4515\ Hr^{-1} + (-0.012543\ Hr^{-1})}\right)\left(\frac{1}{\underset{\text{unitless}}{(0.0825)}(200\mu M)}\right)\right]$$

$$\therefore \boxed{K_L = 62.88 \text{ unitless}}$$

Parameter Table	Value	Units	Source
Kl	62.88	unitless	Calculated on paper in my written part of 3B of this problem
Kx	1.197	nmol/gdw	gain from prelim Q1
Wi	1	unitless	Given in problem statement
n	1.85	unitless	Parameter from Prelim Q1
Kd	0.09	mmol	Parameter from Prelim Q1
W1	0.25	unitless	Parameter from Prelim Q1
W2	98.75	unitless	Parameter from Prelim Q1
KEL (Translation elongation constant)	0.055	sec^-1	From PS2/ and the problem statement of part B
RLT	2.3	micro-M	From PS2
mu (dilution factor)	0.301	hours^-1	From assumption 1 and some math shown on paper
Protein degregation thetaP	-0.0125	hours^-1	From assumption 6 and some math shown on paper
Translation time constant TauL	0.0825	unitless	From assumption 8 and using KEL
Translation saturation constant KLi	200	micro-M	from assumption 10
Polysome amplification constant	2		



Thus, it is clear to see when the polysome amplification constant is greater than 1 the curve moves up as a function of Ubar.

Explanation:
Mathematically: the polysomal constant simply multiplies the slope by its value, thus if it is greater than 1 the slope increases and the value of every point increases.

Physically: I would guess that a polysomal amplification constant greater than one means that more than 1 ribosome is working on translation at any one point, thus the sequence is read faster and the protein produced faster, ergo steeper slope.

5440 Final
Problem 4

a)

$$V_i(\cdots) = \frac{w_1 f_1 + w_2 f_2}{1 + w_1 f_1 + w_2 f_2} = \frac{\sum_{i \in \{x_j\}} w_i f_i(\cdots)}{\sum_{j \in \{c_j\}} w_j f_j(\cdots)}$$

for when no rxn occurs

State 1 = No 3'-5'-AMP

State 2 = with 3'-5'-AMP

$$f_i = \frac{\left(\frac{X}{K_i}\right)^{n_i}}{\left(1 + \left(\frac{X}{K_i}\right)^{n_i}\right)},$$

Bound activator for conformation i

Activator = 3'-5'-AMP

BC State 1 is No 3'-5'-AMP

$$V_1 = \frac{w_1\left(\frac{\left(\frac{X}{K_1}\right)^{n_1}}{1+\left(\frac{X}{K_1}\right)^{n_1}}\right) + w_2\left(\frac{\left(\frac{X}{K_2}\right)^{n_2}}{\left(1+\left(\frac{X}{K_2}\right)^{n_2}\right)}\right)}{1 + w_1\left(\frac{\left(\frac{X}{K_1}\right)^{n_1}}{1+\left(\frac{X}{K_1}\right)^{n_1}}\right) + w_2\left(\frac{\left(\frac{X}{K_2}\right)^{n_2}}{\left(1+\left(\frac{X}{K_2}\right)^{n_2}\right)}\right)}$$

$$\text{overall rate} = \hat{r}_1 = \underset{\substack{\text{constant} \\ \text{(see Excel)}}}{r_1} V_1$$

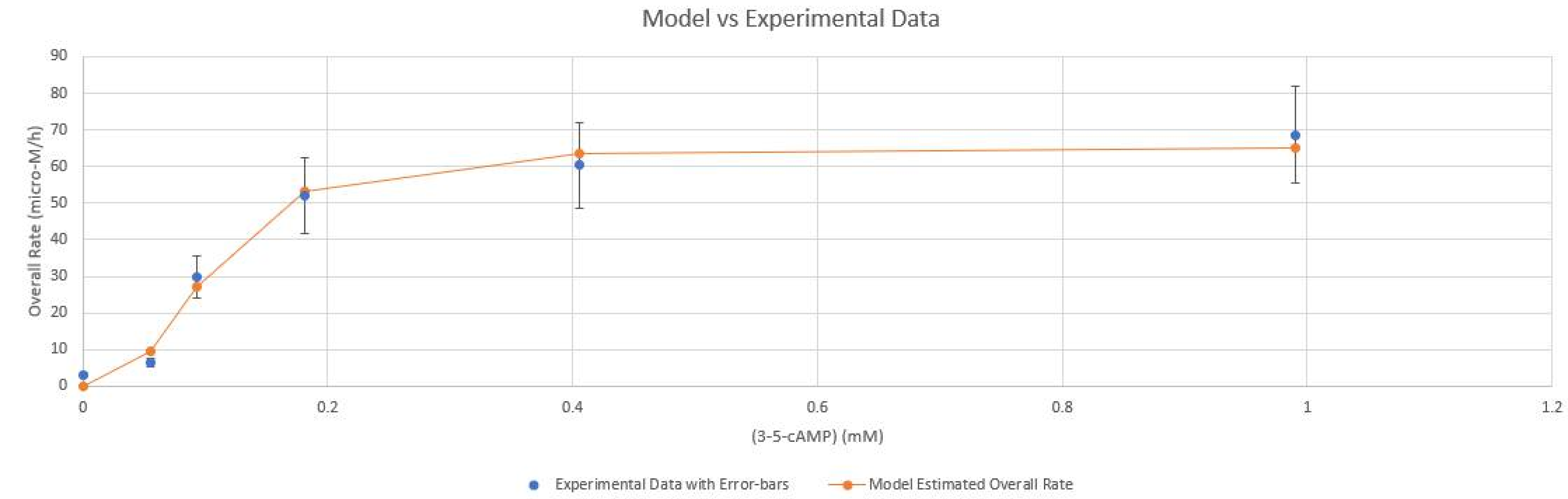
4 A/B

From the least squares fit shown on Excel

	Value from J
W_1 (unitless)	7.6
W_2 (unitless)	7.6
K_1 (mM)	0.294
K_2 (mM)	0.294
n_1 (unitless)	2.718
n_2 (unitless)	2.718

c) Please see the graph on the next page or on Excel.

Sum of Squared Error	50.88957781



The model can fit the data well at higher concentrations. But, with the given model of V the data will never be able to fit well across the whole spectrum, in particular at the lower concentrations of activator. This is because there is no term in the numerator which will allow for v to remain non zero when there is no activator.

In the model by Moon et Al. however, the term for the state where the activator is not bound is not multiplied by the dimensionless hill-binding function, fi. I.E. it is (W1+W2f2)/(1+W1 +W2f2) rather than (w1f1+w2f2)/(1+w1f1+w2f2). Thus, allows for a non zero rate when there is no activator which might lead to a better fit. (As shown below this is not the case.)