Nicole Newberger 5440 Final

1) (a) $\nu = \frac{\gamma_D}{\gamma_N} << 1$, notch activity quickly settles to ss

from lecture

$$\frac{dN_1}{d\tau} = f(D_2) - N_1 \qquad \frac{dN_2}{d\tau} = f(D_1) - N_2$$

$$\frac{dD_1}{d\tau} = (g(N_1) - D_1)\nu \qquad \frac{dD_2}{d\tau} = (g(N_2) - D_2)\nu$$

quasi steady state assumption

$$\frac{dN_1}{d\tau}, \frac{dN_2}{d\tau} \approx 0 \Rightarrow \begin{matrix} f(D_2) \approx N_1 \\ f(D_1) \approx N_2 \end{matrix}$$

$$\frac{dD_1}{d\tau} = (g(f(D_2)) - D_1)\nu \quad , \quad f(D_2) = \frac{D_2^2}{0.1 + D_2^2}$$

$$\frac{dD_2}{d\tau} = (g(f(D_1)) - D_2)\nu \quad , \quad f(D_1) = \frac{D_1^2}{0.1 + D_1^2}$$

$$\frac{dD_1}{d\tau} = \left(\left(\frac{1}{1 + 10\left(\frac{D_2^2}{0.1 + D_2^2}\right)}\right)^2 - D_1\right)\nu$$

$$\frac{dD_2}{d\tau} = \left(\left(\frac{1}{1 + 10\left(\frac{D_1^2}{0.1 + D_1^2}\right)}\right)^2 - D_2\right)\nu$$

(b) see "5440 Final Plots.pdf"

2) (a) local steady state species balance for EGF in the growth medium

EGF = L

transport into monolayer term

$$\underbrace{k_m(z)\,[L_b - L_c(z)]}_{\# \, s^{-1} m^{-2}}$$
$$[m\,s^{-1}] \quad [\#\,m^{-3}] \quad [\#\,m^{-3}]$$

secretion term

$$\underbrace{q \quad n_{cell}}_{\# \, s^{-1} m^{-2}}$$
$$[\#\,s^{-1}\,\cancel{cell^{-1}}] \quad [\cancel{cell}\,m^{-2}]$$

binding to receptor term

$$\underbrace{-\,k_f \quad R_s \quad n_{cell} \quad L_c(z)}_{\# \, s^{-1} m^{-2}}$$
$$[\cancel{m^3}\,\cancel{\#^{-1}}\,s^{-1}] \quad [\cancel{\#}\,\cancel{cell^{-1}}] \quad [\cancel{cell}\,m^{-2}] \quad [\cancel{\#}\,\cancel{m^{-3}}]$$

unbinding from receptor term

$$\underbrace{k_r \quad R_s^* \quad n_{cell}}_{\# \, s^{-1} m^{-2}}$$
$$[s^{-1}] \quad [\#\,cell^{-1}] \quad [cell\,m^{-2}]$$

units for each term match!

$$\frac{dL_c(z)}{dt} = k_m(z)[L_b - L_c(z)] + q\,n_{cell} - k_f R_s n_{cell} L_c(z) + k_r R_s^* n_{cell}$$

steady state $\frac{dL_c(z)}{dt} = 0$

$$k_m(z)L_b - k_m(z)L_c(z) + q n_{cell} - k_f R_s n_{cell} L_c(z) + k_r R_s^* n_{cell} = 0$$

solve for $L_c(z)$

$$k_m(z) L_b + q n_{cell} + k_r R_s^* n_{cell} = k_m(z) L_c(z) + k_f R_s n_{cell} L_c(z)$$

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

(b) transport limited regime
$k_m <<< 1$
$\therefore$ terms containing k_m are negligible

$$L_c(z) = \frac{q n_{cell} + k_r R_s^* n_{cell}}{k_f R_s n_{cell}}$$

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

binding limited regime
$k_m >>> 0$
$\therefore$ terms w/ k_m dominate, other terms negligible

$$L_c(z) = \frac{k_m(z) L_b}{k_m(z)}$$

$$L_c(z) = L_b$$

For the binding limited regime, the time for diffusion is negligible compared to the binding time. Therefore the concentration near the cell surface is effectively equal to the bulk concentration. For the diffusion limited regime, $L_c(z)$ is dependent on the parameters of the system

(c) Find expression for $R_{total}^*(z)$ from lecture (Knauer model)

$$R^*_{total} = R_s^* + R_i^* = \left(\frac{1}{k_e^*} + \frac{1}{k_{deg}}\right)\left(\frac{k_{ss}L_c}{1 + k_{ss}L_c}\right)V_s$$

problem states "work in the limit of low concentration of EGF such that $L_c k_{ss} << 1$"

where k_{ss} [=] effective binding const $= \frac{k_e^* k_f}{k_r(k_r + k_e^*)}$

$$R^*_{total} = \left(\frac{1}{k_e^*} + \frac{1}{k_{deg}}\right)(k_{ss}L_c)V_s$$

substitute in for L_c

since problem states $L_b = 0$

$$L_c(z) = \frac{q\,n_{cell} + k_r R_s^* n_{cell}}{k_m(z) + k_f R_s n_{cell}}$$

$$\boxed{R^*_{total} = \left(\frac{1}{k_e^*} + \frac{1}{k_{deg}}\right)\left(k_{ss}\left[\frac{q\,n_{cell} + k_r R_s^* n_{cell}}{k_m(z) + k_f R_s n_{cell}}\right]\right)V_s}$$

(d) find $k_m(z)$ using the sherwood number

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

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

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

$$k_m(z) = \left(\frac{10^2 \, z^2}{10^{-10}}\right)^{\frac{1}{3}} \frac{10^{-10}}{z}$$

$$k_m(z) = \left(10^{12} \, z^2\right)^{\frac{1}{3}} \frac{10^{-10}}{z}$$

$$k_m(z) \; \left(10^{12}\right)^{\frac{1}{3}} \left(z^2\right)^{\frac{1}{3}} \frac{10^{-10}}{z}$$

$$k_m(z) = 10^4 \, z^{\frac{2}{3}} \frac{10^{-10}}{z}$$

$$k_m(z) = 10^{-6} \frac{z^{\frac{2}{3}}}{z^1}$$

$$k_m(z) = 10^{-6} \, z^{-\frac{1}{3}}$$

substitute into expression for R^*_{total} along w/ other values

$$K_{ss} = \frac{k_e^* \, k_r}{k_e(k_r + k_e^*)} = \frac{5 \times 10^{-3} (s^{-1}) + 5.14 \times 10^{-21} \, m^3 s^{-1}}{10^{-4} (s^{-1}) \left(2.5 \times 10^{-2} (s^{-1}) + 5 \times 10^{-3} (s^{-1})\right)}$$

$$K_{ss} = 8.567 \times 10^{-18} \, m^3$$

Unit check

$$R^*_{total} = \left(\frac{1}{s^{-1}} + \frac{1}{s^{-1}}\right)\left(m^3\left[\frac{(\#\cancel{cell}^{-1}s^{-1})(\cancel{cell}\,m^{-2}) + (s^{-1})(\#\cancel{cell}^{-1})(\cancel{cell}\,m^{-2})}{ms^{-1} + (m^2 s^{-1})(\#\cancel{cell}^{-1})(\cancel{cell}\,\cancel{m^{-2}})}\right]\right) s^{-1}_{cell}$$

$$R^*_{total} = \cancel{s}\left(\cancel{m}^3\left[\frac{\#\ \cancel{s^{-1}}\ \cancel{m^{-2}}}{m\ \cancel{s^{-1}}}\right]\right)\cancel{s^{-1}}\,cell^{-1}$$

$$R^*_{total} = \#\ cell^{-1}\ \checkmark$$

for plotting purposes, assume $R_s^* = R_s = 1\ [\#\ cell^{-1}]$

see "5440 Final Q2d.xlsx"
for calculations and resultant plot

The plot does not match the expected trend. I would assume mitotic rate would decrease with Z. However the observed trend could be because $L_b = 0$

3) $\dot{m}_i = r_{x,i}\bar{u}_i - (\mu + \theta_{m,i})m_i \qquad i = 1,2,\ldots,N$

$\dot{p}_i = r_{L,i}w_i - (\mu + \theta_{p,i})p_i$

derive $\quad p_i^* = k_{L,i}k_{x,i}u_iw_i$

at steady state $\quad \dot{m}_i = \dot{p}_i = 0$

$0 = r_{x,i}\bar{u}_i - (\mu + \theta_{m,i})m_i^*$

$m_i = \left[\dfrac{r_{x,i}}{(\mu + \theta_{m,i})}\right]\bar{u}_i \qquad \therefore \; k_{x,i} = \dfrac{r_{x,i}}{\mu + \theta_{m,i}}$

where

$r_{x,i} = \dfrac{k^x_{E,i}R_{x,T}\,G_i}{\tau_{x,i}k_{x,i} + (\tau_{x,i}+1)G_i}$

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

$p_i^* = \left[\dfrac{r_{L,i}}{\mu + \theta_{p,i}}\right]w_i$

from derivation done in class

$$r_{L,i} = k^L_{E,i}R_{L,T}\left(\frac{m_i}{\tau_{L,i}k_{L,i} + (\tau_{L,i}+1)m_i}\right)$$

where $\quad m_i = \left[\dfrac{r_{x,i}}{\mu + \theta_{m,i}}\right]\bar{u}_i$

from the problem assumptions:

$$(1 + \tau_{L,i})m_i \ll \tau_{L,i}k_L$$

therefore the $r_{L,i}$ expression simplifies to

$$r_{L,i} = k^L_{E,i}R_{L,T}\left(\frac{m_i}{\tau_{L,i}k_{L,i}}\right)$$

putting it all togther

$$p^* = \underbrace{\frac{K_{E,i}^{L} \; R_{L,r}}{(\mu + \theta_{pi})(\tau_{L,i} \, K_L)}}_{K_{L,i}} \; \underbrace{\frac{K_{E,i}^{x} R_{x,r} \, b}{(\mu + \theta_{mi})(\tau_x \, k_x (\tau_x + 1) b)}}_{K_{x,i}} \; \bar{u}_i \, w_i$$

b) estimating parameters. $w_i = 1$

$\bar{u}$: previously determined in Prelim 1 from solutions

$$\bar{u}(I) = \frac{W_1 + W_2 f_I}{1 + W_1 + W_2 f_I} \quad \text{where } f_I = \frac{I^n}{K_d^n + I^n}$$

$W_1 = 0.25$, $W_2 = 98.75$, $K_d = 9 \times 10^{-2}$ mM, $n = 1.85$

gain from prelim 1

$$m^* = K_{x,i} \; \bar{u}$$

at saturation $\bar{u} \approx 1$

Data indicates saturation at $I = 0.216$

$$m^*(I = 0.216) = K_{x,i}$$

convert m^*

$$m_I^* = \frac{\langle n_I \rangle \left(\frac{1 \times 10^9}{A_v}\right)}{(1-\alpha) \langle m_c \rangle}$$

$\alpha = 0.7$ for e.coli

$n_c = 93$ mRNA/cell

$\langle m_c \rangle = 4.3 \times 10^{-13}$ g/cell

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$$m_i^* = \frac{(93 \text{ mRNA/cell})\left(\frac{1\times10^9}{6.02\times10^{23}\ \frac{\text{mRNA}}{\text{mole}}}\right)}{(1-0.7)\ 4.3\times10^{-13}\ \text{g/cell}}$$

$= 1.236$ nmol/gDW

still need to find $K_{E,i}^{L}$, $R_{1,T}$, μ, θ_{Pi}, τ_{Li}, K_L

$K_{L,i} = 200\,\mu M$

$$\mu = \left(\frac{\ln 2}{\text{doubling time}}\right)60 = \left(\frac{\ln 2}{40 \text{min}}\right)60 = 1.0397 \text{ hr}^{-1}$$

$$\theta_{Pi} = \left(\frac{-\ln(0.5)}{\text{prot half life}}\right) = \frac{-\ln(0.5)}{24h} = 0.0289 \text{hr}^{-1}$$

translation rate 14 AA/s (BNID: 108487)

characteristic protein length = 333 AA

$$K_{E,i}^{L} = \frac{14 \text{ AA/s}}{333 \text{AA}} = 0.042\ s^{-1}$$

translation initiation time = 1.5 s

$$K_I = \frac{1}{1.5s} = 0.667\ s^{-1}$$

$$\tau_{LI} = \frac{K_{E,I}^{L}}{K_I} = \frac{0.042 s^{-1}}{1.5 s^{-1}} = 0.028$$

ribosome abundance = 20,000 ribosomes from bscb.org

$$20{,}000 \text{ ribosomes}\ \frac{1}{6.02\times10^{23}}\ \frac{1\times10^9}{(1-0.7)(4.3\times10^{-13}\text{ g/cell})} = 257.54 \text{ nmol/gDW}$$

$$p^* = \frac{k^L_{E,i} \, R_{L,T}}{(\mu + \theta_{pi})(\tau_{L,i} \, k_{L,i})} \, K_{X,i} \quad \bar{u}_i$$

parameter	value	unit	source
$k^L_{E,i}$	0.042	s^{-1}	used bionumbers
$R_{L,T}$	257.54	nmol/gDW	bsbc org
μ	1.0397	hr^{-1}	prelim 1
θ_{pi}	0.0289	hr^{-1}	problem statement
$\tau_{L,i}$	0.028	unitless	problem statement
$K_{L,i}$	200	μM	problem statement
$K_{X,i}$	1.236	nmol/gDW	prelim 1

change some units

$$\mu = \frac{1.0397}{hr} \cdot \frac{1\,hr}{3600\,s} = 2.89 \times 10^{-4}$$

$$\theta_{pi} = \frac{0.0289}{h} \left(\frac{1\,hr}{3600\,s}\right) = 8.028 \times 10^{-6}$$

$$k_{L,i} = 200\ \mu M$$

assume 1×10^8 cells/mL

BNID 103904 Dry weight $E.\ coli = 280 \times 10^{-15}$ gDW/cell

$$\frac{200\ \mu mol}{L} \left(\frac{1\,L}{1000\,mL}\right)\left(\frac{1\,mL}{1 \times 10^8\ cells}\right)\left(\frac{1\,cell}{280 \times 10^{-15}\,g}\right)\left(\frac{1000\,nmol}{1\,\mu mol}\right) = 7.14 \times 10^6\ \frac{nmol}{gDW}$$

see "5440 final Q3b.xlsx"

(c) since

$$P_i^* = K_{L,i} K_{xi} \bar{U}_1 W_1$$

and the effect of K_p can be modelled as $K'_{L,i} = K_p K_{L,i}$

the P_i curve is increased when $K_p > 1$, therefore moves up

for b also plot w/ respect to I

$$p_i^* = K_{L,i} K_{xi}' \left(\frac{W_1 + W_2 \left[\frac{I^n}{K_d + I^n} \right]}{1 + W_1 + W_2 \left[\frac{I^n}{K_d + I^n} \right]} \right)$$

plot also on "5440 final Q3b.xlsx"

4) $\hat{r}_j = r_j \, V(\ldots)_j$

$r_1 = \text{const} = k_{cat} E_1 \left(\frac{F6P}{K_{F6P} + F6P}\right)\left(\frac{ATP}{K_{ATP} + ATP}\right)$

from problem description

$F6P = 0.1\,mM$, $ATP = 2.3\,mM$, $E_1 = 0.12\,\mu M$

$K_{F6P} = 0.11\,mM$, $K_{ATP} = 0.42\,mM$, $k_{cat} = 0.4\,s^{-1}$

$r_1 = 0.4\,s^{-1}(0.12\,\mu M)\left(\frac{0.1\,mM}{0.11\,mM + 0.1\,mM}\right)\left(\frac{2.3\,mM}{0.42\,mM + 2.3\,mM}\right)$

$r_1 = 0.01933\ \frac{\mu M}{s}$

(a) The simplified model for the control function for regulated and unregulated activity fits this example

$V(\ldots) = \frac{W_1 + W_2 f(\ldots)}{1 + W_1 + W_2 f(\ldots)}$

to determine W_1, use the first data point

we know that $f(\ldots) = 0$ because there is no activator present

the expression therefore simplifies to

$V(\ldots) = \frac{W_1}{1+W_1}$

first data point

$\hat{r} = 3.003\ \mu M/h$

$= 8.34167 \times 10^{-4}\ \mu M/s$

$\hat{r} = r_1 \frac{W_1}{1+W_1}$

$\hat{r} + \hat{r} W_1 = r_1 W_1$

$\hat{r} = W_1(r_1 - \hat{r})$

$W_1 = \hat{r} / (r_1 - \hat{r})$

$W_1 = \frac{8.34167 \times 10^{-4}\ \mu M/s}{0.01933\ \frac{\mu M}{s} - 8.34167 \times 10^{-4}\ \mu M/s}$

$\boxed{W_1 = 0.0451}$

at saturation, $r(\ldots) = 1$

assume last point in dataset = saturation

$$\hat{r} = 68.653\ \mu M/h$$
$$= 0.01907\ \mu M/s$$

$$V(\ldots) = \frac{W_1 + W_2}{1 + W_1 + W_2}$$

$$\hat{r} = r_1 \frac{W_1 + W_2}{1 + W_1 + W_2}$$

$$\hat{r} + \hat{r}W_1 + \hat{r}W_2 = r_1 W_1 + r_1 W_2$$
$$\hat{r}W_2 - r_1 W_2 = r_1 W_1 - \hat{r}W_1 - \hat{r}$$
$$W_2 = \frac{r_1 W_1 - \hat{r} W_1 - \hat{r}}{(\hat{r} - r_1)}$$

$$W_2 = \frac{\left(0.01933 \frac{\mu M}{s}\right)(0.0451) - \left(0.01907 \frac{\mu M}{s}\right)(0.0451) - 0.01907 \frac{\mu M}{s}}{0.01907\ \mu M/s - 0.01933}$$

$$\boxed{W_2 = 73.301}$$

(b) estimate binding constants (K) + order parameters (n)

$$r(\ldots) = \frac{x^n}{K^n + x^n} \quad \text{when } x = 0.990\ mM$$
$$r(\ldots) = 1$$

set $K = 0.5\ mM$ and use trial + error to fit the data and find N

$$\hat{r} = r_1 \left[\frac{W_1 + W_2\left[\frac{x^n}{K^n + x^n}\right]}{1 + W_1 + W_2\left[\frac{x^n}{K^n + x^n}\right]} \right]$$

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(b+c) see "5440 Final Q 4c .xlsx" for calculations
to match data and comparison
of model and data

Used excel plot to facilitate trial and error
found that $n = 3.1$ works well with
$K = 0.5$ mM to match the data

part c graph shows full comparison
of model to data - good fit!