CHEME 5440 - Final Exam
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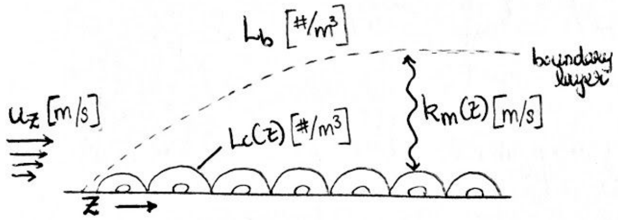
Problem 2 - Autocrine signaling and proliferation in the presence of forced convection.



→ EGF transport from bulk to the surface of the cells in the monolayer:-

• flux = $k_m(z)\left[L_b - L_c(z)\right]$ (#/m²·s)

→ Cells generate EGF (L) at a rate.-

• q [#/cell·s]

→ Initial seeding density:-

• n_c [cell/m²]

a) Conduct a local steady-state species balance for EGF in the growth medium.

At some point $z > 0$, construct a M·B for EGF (L) in the growth medium.

ACCUMULATION = IN − OUT + GENERATION − $\cancel{\text{CONSUMPTION}}^{0}$

$$\boxed{\frac{dL}{dt} = k_r R_s^{\star} - k_f L_c(z) R_s + k_m(z)\left[L_b - L_c(z)\right]\cdot\frac{1}{n_c} + q}$$

Unit check → $\left[\frac{1}{s}\cdot\frac{\#}{cell}\right]$ $\left[\frac{m^3}{\#\cdot s}\cdot\frac{\#}{m^3}\cdot\frac{\#}{cell}\right]$ $\left[\frac{m}{s}\cdot\frac{\#}{m^3}\cdot\frac{m^2}{cell}\right]$ + $\left[\frac{\#}{s\cdot cell}\right]$ ✓ UNIT CHECK IS A SUCCESS.

At steady state ⇒ $\boxed{\frac{dL}{dt} = 0}$

$$\therefore\ 0 = k_r R_s^{\star} - k_f L_c(z) R_s + \frac{k_m(z)}{n_c}\left[L_b - L_c(z)\right] + q$$

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

$$\therefore\ \boxed{L_c(z) = \frac{k_r R_s^{\star} + \left(\frac{k_m(z)\cdot L_b}{n_c}\right) + q}{k_f R_s + \left(\frac{k_m(z)}{n_c}\right)}}$$

b) Find expressions for $L_c(z)$ in the transport limited and binding limited regimes.
[$k_m(z)$ is very small] [$k_m(z)$ is very large]

$$\boxed{L_c(z) = \frac{k_r R_s^* + \left(\frac{k_m(z) \cdot L_b}{n_c}\right) + q}{k_f R_s + \frac{k_m(z)}{n_c}}} \quad \text{— (1)}$$

→ When $L_c(z)$ is transport limited; $k_m(z)$ is very small. Thus, the $L_c(z)$ expression (1) can be simplified to the form:-

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

In this transport limited regime, the volumetric concentration of EGF at the surface depends only on the amount generated by the cell – ie. the 'q' and amt. produced from binding with receptors on the cell surface. Convection/Diffusion does not have an impact here.

→ When $L_c(z)$ is binding limited; $k_m(z)$ is very large. Thus, the $L_c(z)$ expression (1) can be simplified to the form:-

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

$$\therefore \boxed{L_c(z) \approx L_b}$$

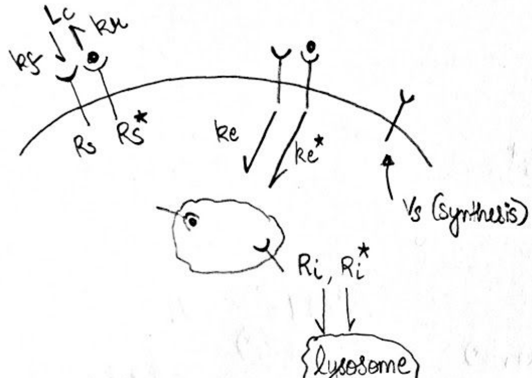
In this binding limited regime, the volumetric concentration of EGF at the surface depends on the bulk concentration of the ligand. This indicates that the binding production of EGF and generation (q) is quickly diffused away into the bulk ligand concentration. – diffusion is seen to have a large impact on $L_c(z)$

c) Use Knauer's model to find an expression for $R_T^*(z)$.

Assumptions

→ $\boxed{L_c K_{ss} << 1}$ → Assume no recycling of receptors within the cell.

→ $\boxed{L_b = 0}$



R_s = inactive surface receptor
R_s^* = active surface receptor
R_i = inactive internal receptor
R_i^* = active internal receptor

MASS BALANCES

surface free receptor: $\frac{dR_s}{dt} = -k_f L_c R_s + k_r R_s^* - k_e R_s + V_s$ —— ①

bound surface receptor: $\frac{dR_s^*}{dt} = k_f L_c R_s - k_r R_s^* - k_e^* R_s^*$ —— ②

total internal receptor: $\frac{dR_i^T}{dt} = k_e R_s + k_e^* R_s^* - k_{deg} R_i^T$ —— ③

$R_i^T = R_i + R_i^*$

internal active receptor: $\frac{dR_i^*}{dt} = k_e^* R_s^* - k_{deg} R_i^*$ —— ④

At steady state;

Summing eqns ① & ③; $-k_f L_c R_s + k_r R_s^* \cancelto{0}{- k_e R_s} + V_s \cancelto{0}{+ k_e R_s} + k_e^* R_s^* - k_{deg} R_i^T = 0$

$V_s - k_{deg} R_i^T = \underbrace{(k_f L_c R_s - k_r R_s^* - k_e^* R_s^*)}_{=0,\ \text{from ②}}$

→ From eqn ② at S.S; the RHS equates to 0. Thus:-

$$\boxed{V_s = k_{deg} R_i^T}$$

From ②;

$$k_f L_c R_s - k_r R_s^* - k_e^* R_s^* = 0$$

$$\therefore \boxed{R_s = \frac{k_r R_s^* + k_e^* R_s^*}{k_f L_c}} \quad \text{— ⑥}$$

From ③;

$$k_e R_s + k_e^* R_s^* - \underbrace{k_{deg} R_i^T}_{= V_s \text{ from ⑤}} = 0$$

$$\therefore \boxed{R_s = \frac{V_s - k_e^* R_s^*}{k_e}} \quad \text{— ⑦}$$

Equating eqns ⑥ & ⑦ :-

$$\frac{k_r R_s^* + k_e^* R_s^*}{k_f L_c} = \frac{V_s - k_e^* R_s^*}{k_e}$$

$$k_e k_r R_s^* + k_e k_e^* R_s^* = k_f L_c V_s - k_f L_c k_e^* R_s^*$$

$$R_s^* (k_e k_r + k_e k_e^* + k_f L_c k_e^*) = k_f L_c V_s$$

$$\therefore R_s^* = \frac{k_f L_c \cdot V_s}{k_e (k_r + k_e^*) + k_f L_c k_e^*}$$

$$\times \frac{k_e^*}{k_e^*}; \quad R_s^* = \frac{k_e^* k_f L_c \cdot V_s}{k_e^* (k_e (k_r + k_e^*) + k_f k_e^* L_c)} \xRightarrow{\div k_e (k_r + k_e^*)} \frac{\frac{k_e^* k_f L_c}{k_e (k_r + k_e^*)} \cdot V_s}{k_e^* \left(\frac{k_e \cancel{(k_r + k_e^*)}}{k_e \cancel{(k_r + k_e^*)}} + \frac{k_e^* k_f L_c}{k_e (k_r + k_e^*)} \right)}$$

$\Rightarrow$ Substitute K_{ss} (effective binding constant) $= \dfrac{k_e^* k_f}{k_e (k_r + k_e^*)}$;

$$\therefore \boxed{R_s^* = \left(\frac{K_{ss} \cdot L_c}{1 + K_{ss} \cdot L_c} \right) \cdot \frac{V_s}{k_e}}$$

As $K_{ss} L_c \ll 1$; R_s^* now becomes:-

$$\boxed{R_s^* = \frac{K_{ss} \cdot L_c \cdot V_s}{k_e}} \quad \text{— ⑧}$$

$\Rightarrow$ $L_c(z)$ expression still has R_s term. Thus substitute eqn. ⑥ into the $L_c(z)$ expression, where $\boxed{L_b = 0}$:-

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

$$\therefore L_c k_m(z) = \cancel{n_c k_r R_s^*} + n_c q - \cancel{n_c k_r R_s^*} - n_c k_e^* R_s^*$$

$$\therefore \boxed{L_c = \frac{n_c (q - k_e^* R_s^*)}{k_m(z)}} \quad \text{— ⑨}$$

→ Plugging (9) into (8);

$$\therefore R_s^* = \frac{K_{ss} \cdot V_s}{ke^*} \cdot \frac{n_c(q - ke^* R_s^*)}{km(z)} \Rightarrow \frac{n_c K_{ss} V_s q - n_c K_{ss} V_s ke^* R_s^*}{ke^* \, km(z)}$$

$$ke^* km(z) R_s^* + n_c K_{ss} V_s ke^* R_s^* = n_c K_{ss} V_s q$$

$$\therefore \boxed{R_s^* = \frac{n_c K_{ss} V_s \cdot q}{ke^* km(z) + n_c K_{ss} V_s \cdot ke^*}}$$

From (4) @ s.s;

$$ke^* R_s^* - k_{deg} R_i^* = 0$$

$$\therefore \boxed{R_i^* = \frac{ke^*}{k_{deg}} R_s^*}$$

Thus;

$$R_T^* = R_s^* + R_i^*$$

$$= R_s^* + \frac{ke^*}{k_{deg}} R_s^*$$

$$= R_s^* \left(1 + \frac{ke^*}{k_{deg}}\right)$$

$$\times \frac{ke^*}{ke^*}; \; R_T^* = R_s^* \cdot ke^* \left(\frac{1}{ke^*} + \frac{1}{k_{deg}}\right)$$

$$= \frac{n_c K_{ss} \cdot V_s \cdot q}{\cancel{ke^*}(km(z) + n_c K_{ss} V_s)} \cdot \cancel{ke^*} \cdot \left(\frac{1}{ke^*} + \frac{1}{k_{deg}}\right)$$

$$\therefore \boxed{R_T^* = \underbrace{\left(\frac{1}{ke^*} + \frac{1}{k_{deg}}\right)}_{\text{resistance to activation.}} \cdot \underbrace{\left(\frac{K_{ss} V_s n_c}{km(z) + K_{ss} V_s n_c}\right)}_{\text{analogous to bound fraction}} \cdot q}$$

d) An expression for $k_m(z)$ can be found using the Sherwood number (Sh_z) to help plot the predicted profile of mitotic activity with z.

$$\Rightarrow Sh_z = \frac{k_m(z)}{D_L/z}$$

$$k_m(z) = \frac{Sh_z \cdot D_L}{z}$$

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

$$\therefore k_m(z) = \gamma^{1/3} \cdot z^{-1/3} \cdot D^{2/3}$$

$$\boxed{k_m(z) = \left(\frac{\dot{\gamma} \cdot D^2}{z}\right)^{1/3}}$$

⇒ The mitotic activity and total activated receptor concentration are linearly propotional as shown by plot A in Fig. 12 of (Knauer, 1984) or the plot in the lecture notes:-

$$\boxed{\text{Mitotic rate} = \gamma \cdot R_T^*}$$

The slope is estimated (as shown in the following pages) resulting in the following propotionality constant:-

$$\gamma = \frac{y_2 - y_1}{x_2 - x_1} \approx \frac{80 - 60}{22 - 17} \approx \underline{\underline{4}}.$$

$$\therefore \boxed{\text{Mitotic rate} = 4 \cdot R_T^*}$$

⇒ The corresponding plot is listed in the following pages and attached in the Excel file sent along with the exam.

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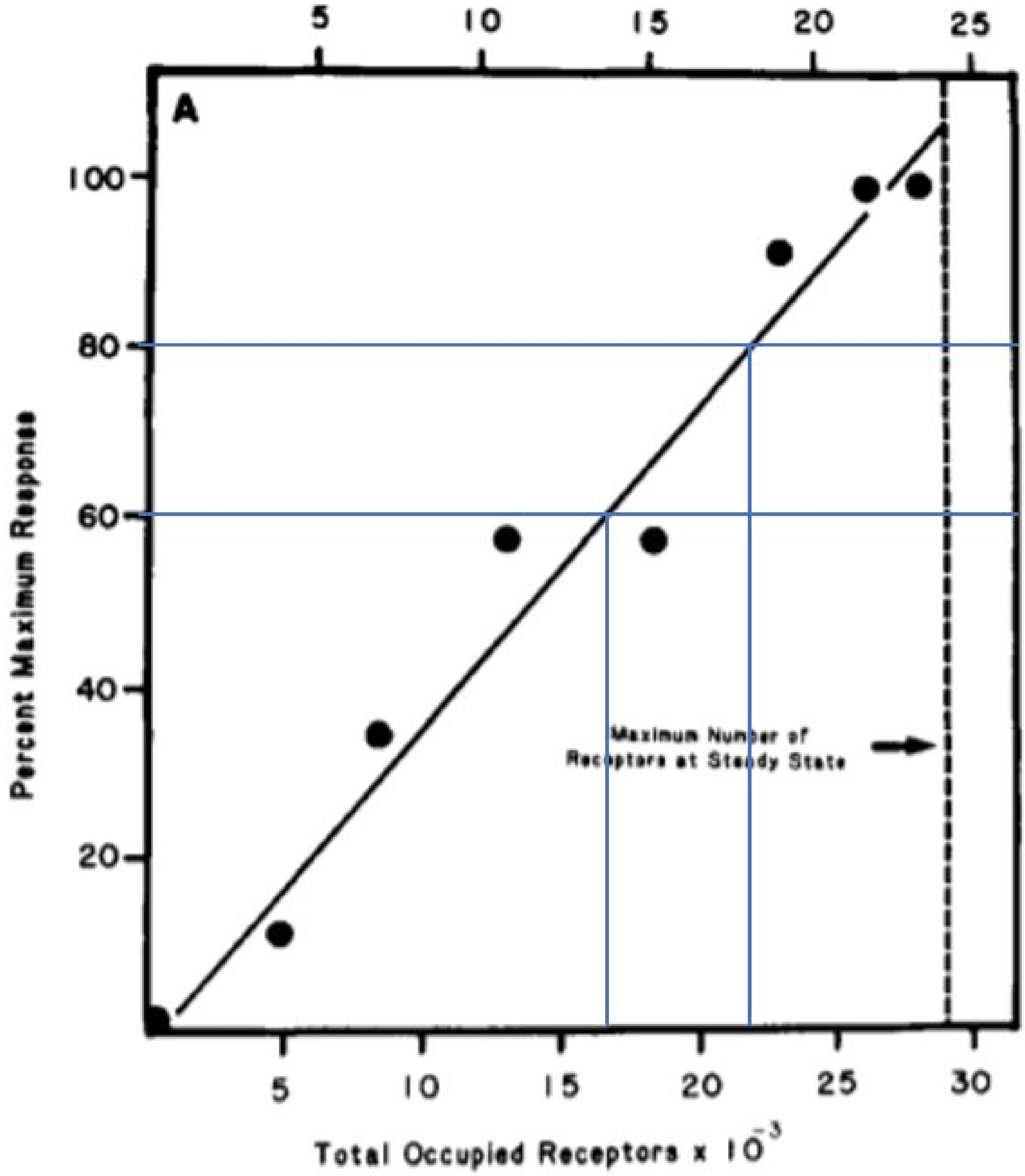
Estimation of the slope to convert R^*_T to Mitotic activity:



The above linear relationship was obtained from Knauer's paper and the lecture notes. Note that this relationship was used for human fibroblast (HF) cells and so this assumption is taken for our analysis as well. From the above estimation, the slope (intrinsic mitogenic signal generation) had a value of about **4**.

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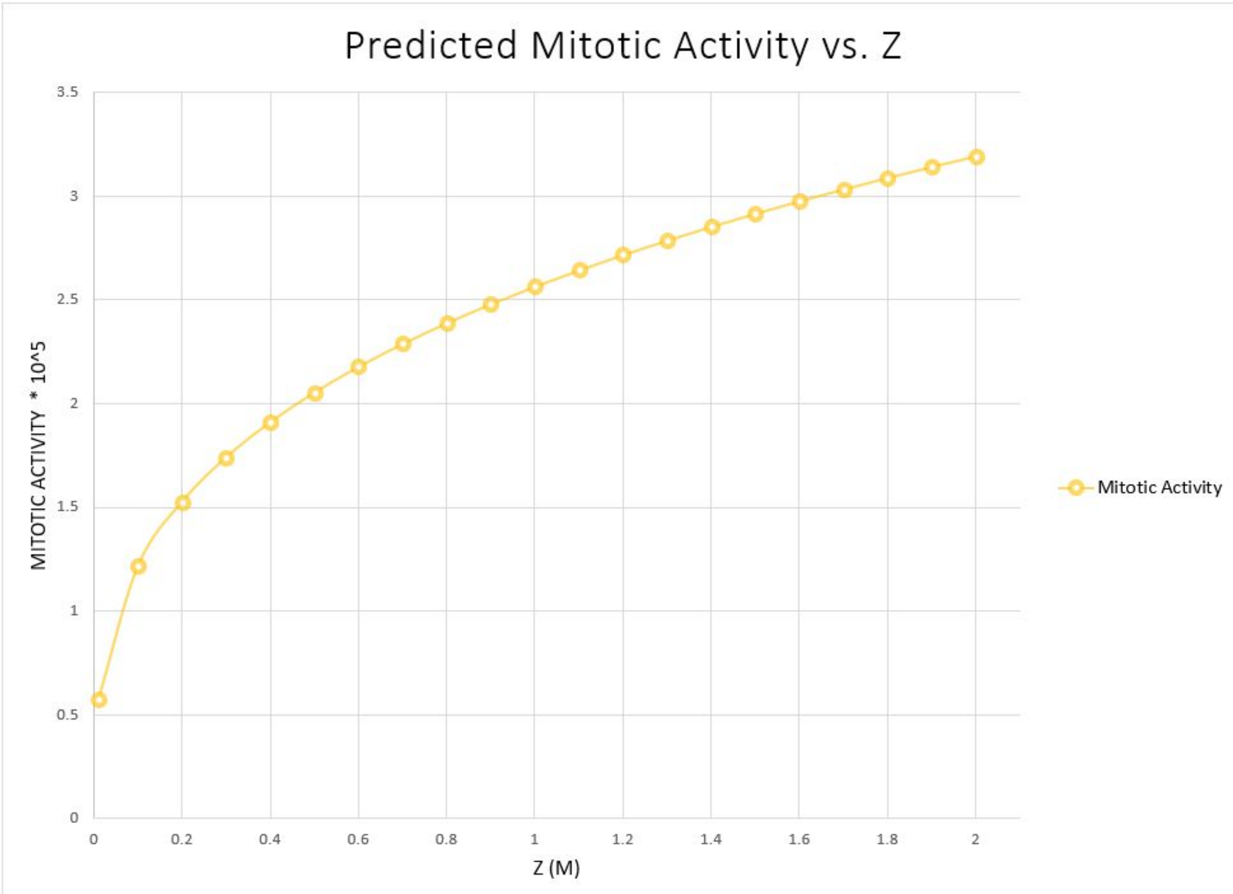
A plot of the predicted Mitotic activity vs. z:



*(Note: Mitotic Activity is plotted as Value*10^5)*

As seen in the plot above, the mitotic activity is seen to increase with increasing z, until it gradually beings to plateau. This seems to intuitively make sense as when moving along and increasing the number of cells, an increase in mitotic activity is expected. However, it is quite interesting to note that the saturation of mitotic activity only occurs when is z is extremely large.