CHEME 5440: Take Home Final Exam S2020

1. The take home Final Exam has four questions which are collectively worth 100 points.
2. The take home Final Exam is due by 11:59pm Fr May 22, 2020.
3. You may use your course notes, literature, the internet, or any other course materials to formulate your solutions.
4. You *cannot* consult with any other person regarding the prelim (except the TA, JV or MP). You *cannot* use any form of electronic communication to discuss the prelim questions with any other person (except the TA, JV or MP via a direct message in Slack). Violation of this policy will result in a ZERO for the prelim, and an honor code violation.
5. Mistakes/corrections/clarifications to the final exam will be made on the #general Slack channel by the TA, JV or MP.
6. In all problems, show your work and state all assumptions or simplifications. Start from the general, and work your way to the specific.
7. **Submission**: Submit a link to the #final-exam-5440 channel on Slack that points to your solutions stored on GitHub, Box, Google Drive etc. Your solutions should include all written material, source code/spreadsheets, and instructions to reproduce your calculations/figures.

Part II Questions:

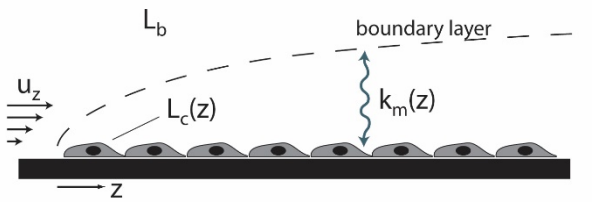
1) Lateral inhibition through Notch-Delta signaling

In lecture, we considered the dynamics of Notch and Delta in a two-cell system by taking the limit in which the decay rate of Delta is much greater than that of Notch. Here you are asked to examine the same problem in the opposite limit.

(a) Argue that in the limit $\nu = \gamma_D/\gamma_N << 1$, the Notch activity in the two cells quickly settles into a steady state. What are the resulting dynamical equations for the evolution of Delta that follow from the complete system presented in lecture (See eqns 2 – 5 in the May 5th lecture)?

(b) Using the functional forms for the Notch and Delta activation rates, F(D') and G(N) (See eqns 6, 7 in the May 5th lecture), obtain a phase portrait for the dynamics of Delta in the two cells. Based on your phase portrait, show that in the long-time limit, the system will settle into a steady state in which one cell assumes the primary fate while the other cell assumes the secondary fate. Discuss whether lateral inhibition works similarly as the case discussed in lecture (i.e. limit in which the decay rate of Delta is much greater than that of Notch).

2) Autocrine signaling and proliferation in the presence of forced convection.



Consider autocrine signaling of epithelial growth factor (EGF – ligand, L) within a monolayer of cells growing on a planar substrate in the presence of a uniaxial flow, u_z [m s^{-1}] in the growth medium. This flow defines a mass transfer coefficient, $k_m(z)$ [m s^{-1}] between the bulk at fixed concentration L_b [# m^{-3}] and the surface of the cells in the monolayer. Thus, the rate of EGF transport from the bulk to the monolayer is $k_m(z)[L_b - L_c(z)]$. The cells produce the EGF at a constant rate, q [# s^{-1} cell^{-1}] and their mitotic rate responds to receptor-bound EGF based on the mechanism discussed in class (i.e Knauer model). Assume that the cells start at a uniform seeding density, n_{cell} [cell m^{-2}]. We are interested in how the initial mitotic rate will depend on the z-position within the monolayer.

(a) Write down a local (at a given $z > 0$), steady state species balance for EGF in the growth medium in terms of concentration of bound (R_s^* [#/cell]) and unbound (R_s [#/cell] surface receptors, their on (k_f [m^3/#/s]) and off (k_r [s^{-1}]) rates, the secretion rate (q), the initial density (n_c), the bulk concentration of ligand (L_b), the volumetric concentration of ligand at the surface of the cells ($L_c(z)$ [#/m^3]), and the local mass transfer coefficient, $k_m(z)$. Solve the balance for $L_c(z)$. (Check your units!)

(**b**) Using your result in (a), find expressions for $L_c(z)$ in the transport limited and binding limited regimes (i.e. for very small and very large k_m, respectively). Explain your results.

(**c**) Now, couple your result from (a) to the Knauer model (see lecture notes or original paper) to find an expression for the total level of activated receptor $R_{total}^*(z)$. Work in the limit of low concentration of EGF such that $L_cK_{ss} << 1$ and take $L_b = 0$.

(**d**) Find the predicted profile of mitotic activity with z. Use the numbers from lecture for EGFR dynamics:

$k_e = 10^{-4}$ (s^{-1}); $k_e^* = 5\times10^{-3}$ (s^{-1}); $k_f = 3.1\times10^{6}$ (M^{-1} s^{-1}) = 5.14×10^{-21} ($m^3 s^{-1}$); $k_r = 2.5\times10^{-2}$ (s^{-1}); $k_{deg} = 8\times10^{-4}$ (s^{-1}); and $V_s = 18$ (s^{-1} $cell^{-1}$).

Additionally:

$q = 10^3$ (# $cell^{-1}$ s^{-1}), and $n_c = 3\times10^8$ (cell m^{-2}).

For this problem, take the Sherwood number:

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

with shear rate, $\dot{\gamma} = 10^2$ (s^{-1}), and diffusivity, $D_L = 10^{-10}$ (m^2 s^{-1}).

3. **Estimate enzyme concentration in a growing population of *E coli* cells**. In addition to mass balance constraints, the flux balance analysis problem is subject to flux bounds constraints of the form: $0 \leq r_j \leq \mathcal{U}_j$, where $\mathcal{U}_j$ denotes an upper bound on the metabolic flux r_j (mmol gDW^{-1} hr^{-1}). Let's assume the substrates (reactants) of r_j are saturating (reactant concentration much greater than the saturation coefficient). In this case, the upper bound $\mathcal{U}_j$ can be modeled as:

$$\mathcal{U}_j = V_j^{max,\circ} \left(\frac{p_j^*}{p^\circ} \right) \tag{1}$$

where $V_j^{max,\circ}$ denotes a characteristic maximum reaction velocity for reaction j (mmol gDW^{-1} hr^{-1}), p_j^* denote the intracellular steady-state concentration of the enzyme that catalyzes reaction j (mmol gDW^{-1}), and p° denotes a characteristic enzyme concentration (mmol gDW^{-1}). Let's build a model to estimate the concentration of p_j from first principles.

Assume: (i) an exponentially growing population of *E. coli* cells with a doubling time of $\tau_d \simeq$ 40 min; (ii) the transcriptional gain $\mathcal{K}_X$ is the data-driven gain from Golding and coworkers from Q1 of Prelim-1; (iii) the characteristic intracellular enzyme concentration p° = 0.3μM; (iv) the volume of an *E.coli* cell is 1μm^3; (v) an *E.coli* cell weighs 4.3$\times$10^{-13} and is 70% water; (vi) the half-life of p_j is 24 hr while p° is constant; (vii) $(1 + \tau_{L,i})\, m_i \ll \tau_{L,i} K_L$; (viii) the translation initiation time is 1.5 s; (ix) the characteristic protein length is 333 aa; (x) the translation saturation coefficient $K_{L,i}$ = 200μM; (xi) polysome amplification constant (K_p) is unity (unless otherwise specified)

a) Starting from the mRNA (m_i) and protein (p_i) balances from the course notes:

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

$$\dot{p}_i = r_{L,i} w_i - (\mu + \theta_{p,i})\, p_i \tag{3}$$

derive an expression describing the intracellular steady-state protein concentration of the form:

$$p_i^* \simeq \mathcal{K}_{L,i} \mathcal{K}_{X,i} \bar{u}_i w_i \tag{4}$$

where $\mathcal{K}_{L,i}$ denotes the *translation gain* for protein i, $\mathcal{K}_{X,i}$ denotes the *transcription gain* for the gene encoding protein i, $\bar{u}_i$ denotes the transcriptional control

function (unregulated and regulated) for the gene encoding protein i, and w_j denotes the translational control function for protein i.

b) Using parameters estimated from `BioNumbers`, other literature or class assignments, estimate p_i^* for 300 aa protein with w_i=1 and the $\bar{u}_i$ profile taken from `Q1` of Prelim-1. Plot your estimated p_i^* as a function of $\bar{u}_i$. List parameter estimates in a table (or file) describing the value, units and source.

c) What happens to the p_i^* curve if the polysome amplification constant $K_p > 1$? Hint: the polysome amplification constant describes the average number of ribosomes reading a message at any time. We can model the effect of K_p as:

$$\mathcal{K}'_{L,i} = K_p \mathcal{K}_{L,i} \tag{5}$$

If $K_p > 1$ does the p_i^* curve move up, down left or right as a function of $\bar{u}_i$? (explain)

4. **Can we use a statistical mechanical approach to describe allosteric regulation?** Allosteric regulation is a fast mechanism cells use to regulate the catalytic activity of metabolic enzymes. In this type of regulation, the ability of an enzyme to catalyze chemistry depends upon the concentration of metabolite effector molecules which do not directly participate in the chemical reaction. For example, Phosphofructokinase (PFK), a key glycolytic enzyme which catalyzes the conversion of D-fructose 6-phosphate (F6P):

$$\mathrm{ATP} + \mathrm{D-fructose\ 6-phosphate} \longrightarrow \mathrm{ADP} + \mathrm{D-fructose\ 1,6-bisphosphate} \quad (6)$$

is strongly activated in the presence of 3′-5′-AMP, a signal metabolite produced when glucose is transported into cells.

Let's apply the statistical mechanical promoter idea of Moon et al to a completely different situation, namely the description of allosteric regulation. In particular, let's build a model of the allosteric regulation of PFK and estimate the model parameters using the experimental dataset for PFK activity (3′-5′-AMP versus reaction rate) posted in the #final-exam-5440 channel in Slack.

Model: Let the model take the form $\hat{r}_j = r_j v\left(\ldots\right)_j$ where $\hat{r}_j$, which denotes the overall rate of the PFK reaction (μM h^{-1}), is the product of kinetic limit r_j (μM h^{-1}), i.e., the maximum rate of conversion in the absence of allosteric regulation, and a control variable $0 \leq v\left(\ldots\right)_j \leq 1$ (dimensionless) that describes the influence of effectors molecules. Based on the Moon et approach, let the allosteric control variable take the form:

$$v\left(\ldots\right)_j = \frac{\sum\limits_{i \in \{\mathcal{X}_j\}} W_i f_i\left(\ldots\right)}{\sum\limits_{j \in \mathcal{C}_j} W_j f_j\left(\ldots\right)} \quad (7)$$

where W_i (dimensionless) denotes the weight of configuration i, while $f_i\left(\cdots\right)$ (dimensionless) is a hill-binding function $f_i = (x/K_i)^{n_i}/(1 + (x/K_i)^{n_i})$ which describes the fraction of bound activator/inhibitor (x) for configuration i; K_i denotes a binding constant (mM), and n_j denotes an order parameter (dimensionless). The summation in the numerator of $v\left(\ldots\right)_j$ is over those configurations that lead to activity (denoted by set $\mathcal{X}_j$), while the summation in the denominator is over all possible configurations for enzyme j (denoted as $\mathcal{C}_j$). Let the kinetic limit for PFK (E_1, μM) be given

by:

$$r_1 = k_{cat} E_1 \left(\frac{F6P}{K_{F6P} + F6P} \right) \left(\frac{ATP}{K_{ATP} + ATP} \right) \tag{8}$$

Assume: (i) the concentration of F6P in the assay equals 0.1 mM and is constant; (ii) the concentration of ATP in the assay equals 2.3 mM and is constant; (iii) the concentration of PFK (E_1) in the assay equals 0.12 μM and is constant; (iv) K_{F6P} = 0.11 mM and K_{ATP} = 0.42 mM. (v) k_{cat} = 0.4 s^{-1}.

a) Let the activator be Estimate the parameter(s) W_1 (no 3′-5′-AMP) and W_2 (with 3′-5′-AMP) directly from the dataset. **Note**: this can be done analytically, but need not be.

b) Estimate the binding constants and order parameters for the 3′-5′-AMP binding function from the dataset.

c) Plot your estimated overall rate (y-axis), and the measured rate (with error-bars), versus the 3′-5′-AMP concentration (x-axis) on the same plot. Can the proposed model describe the data?