BE/APh 161: Physical Biology of the Cell, Winter 2019
Homework #3
Due at the start of lecture, 2:30 PM, January 30, 2019.

In this homework, we will explore ligand-receptor binding in depth, using many of the skills from statistical mechanics we learned last week. It may seem a bit redundant, but this is a great model system to hone your skills.

Problem 3.1 (Growth curves, based loosely on page 103 of *PBoC2*, 40 pts)**.**
In chapter 3 of *PBoC2*, the authors considered growth curves of *E. coli*. The logistic equation is written as

$$\frac{\mathrm{d}N}{\mathrm{d}t} = rN\left(1 - \frac{N}{K}\right), \tag{3.1}$$

where N is the number of bacteria, r is the growth rate, and K is the carrying capacity, or the maximum number of bacteria that can be present and still have growth. In the analysis in *PBoC2*, the values of r and K were assumed to be constant. For bacteria growing in media, r and K could also be functions of the the concentration of food in the media, which we will call $F(N,t)$ (not to be confused with $f(N,t)$ in equation 3.10 in *PBoC2*).

Since we will be taking N as a continuous variable, and since F is a concentration, we can write the logistic equation in terms of concentration of bacteria, c, by dividing the entire equation by the volume V of the vessel containing the medium.

$$\frac{\mathrm{d}c}{\mathrm{d}t} = rc\left(1 - \frac{c}{K'}\right), \tag{3.2}$$

where $K' \equiv K/V$. We will drop the prime henceforth for notational convenience.

a) Write down an expression for $\mathrm{d}F/\mathrm{d}t$. You should try to keep your expression simple. Give your reasoning for how you chose this expression.

b) Sketch functional forms that you think are reasonable for $r(F)$ and $K(F)$. Again, try to keep them simple.

c) Based on what you know about bacterial growth, give reasonable values of the parameters you defined in your expressions for $\mathrm{d}F/\mathrm{d}t$, $r(F)$ and $K(F)$. Also give reasonable values for the initial bacteria concentration, c_0, and the initial food concentration, F_0. Explain how you came up with these values. *Hint*: Working through problem 2.5 of *PBoC2* will help you.

d) Solve the differential equations (numerically or analytically) and plot the results. You can use whatever numerical integration software you like. If you would like to use Python with NumPy/SciPy, the Jupyter notebook accompanying lecture 3 might serve as a useful reference.

e) Explain the shape of the curves.

f) Comment on any enhancements you would propose to this model for bacterial growth.

Problem 3.2 (Ligand-receptor binding: practical calculations, loosely based on problem 6.4 of *PBoC2*, 10 pts)**.**
In lecture, we considered simple ligand-receptor binding. We considered a single receptor with many ligands and found that the equilibrium probability of a receptor having a ligand bound to it is

$$p_{\text{bound}} = \frac{1}{1 + K_{\text{d}}/c_{\text{L}}}, \tag{3.3}$$

where c_{L} is the concentration of free ligand and K_{d} is the dissociation constant, expressed in the same units as c_{L}.

a) Show that the expression given in equation (3.3) holds even if we have many receptors. For concreteness of notation, assume that the the *total* receptor concentration (including both bound and unbound) is c_{R}^0, with c_{L}^0 being similarly defined for ligands. You may take the law of mass action,

$$K_{\text{d}} = \frac{c_{\text{L}} c_{\text{R}}}{c_{\text{LR}}}, \tag{3.4}$$

as given, though it may be derived from statistical mechanics.

b) As I mentioned in lecture, it is not always easy to measure c_{L}. This is especially true when we do binding experiments with purified proteins in a test tube, where we know c_{L}^0 and c_{R}^0. Derive an expression for p_{bound} as a function of the total ligand concentration c_{L}^0, the total receptor concentration, c_{R}^0, and the dissociation constant, K_{d}.

c) Show that in the limit of $c_{\text{L}}^0 \gg c_{\text{R}}^0$,

$$p_{\text{bound}} \approx \frac{1}{1 + K_d/c_{\text{L}}^0}. \tag{3.5}$$

Problem 3.3 (Cooperative ligand-receptor binding, 30 pts)**.**
We continue to explore ligand-receptor binding in this problem. We consider the case where we have a receptor that has two distinct binding pockets for ligands. We will refer to the binding pockets as the left and right binding pockets. Each binding pocket can bind a single ligand, and the receptor may have either zero, one, or two ligands bound at each time. We call the compound where the left binding pocket is bound LR, the compound where the right is bound RL, and the compound where

both are bound LRL. So, written as chemical reactions with dissociation constants, we have

$$\mathrm{LR} \rightleftharpoons \mathrm{L} + \mathrm{R}, \quad K_{\mathrm{d},1} \tag{3.6}$$

$$\mathrm{RL} \rightleftharpoons \mathrm{R} + \mathrm{L}, \quad K_{\mathrm{d},2} \tag{3.7}$$

$$\mathrm{LRL} \rightleftharpoons \mathrm{L} + \mathrm{RL}, \quad K_{\mathrm{d},3} \tag{3.8}$$

$$\mathrm{LRL} \rightleftharpoons \mathrm{LR} + \mathrm{L}, \quad K_{\mathrm{d},4}. \tag{3.9}$$

a) Show that by the law of mass action, $K_{\mathrm{d},4} = K_{\mathrm{d},2}K_{\mathrm{d},3}/K_{\mathrm{d},1}$.

b) Consider a single receptor in a solution of ligands with concentration c_{L}. Write down a states and weights table.

c) Use your states and weights table to derive an expression for the probability that both binding pockets are occupied by ligands (p_{LRL}) in terms of the ligand concentration c_{L} and $K_{\mathrm{d},1}$, $K_{\mathrm{d},2}$, and $K_{\mathrm{d},3}$. Be sure to explicitly write how the dissociation constants depend on the energies of the respective states.

d) Assume $K_{\mathrm{d},1} = K_{\mathrm{d},2} \equiv K_{\mathrm{d}}$. Plot p_{LRL} vs. $c_{\mathrm{L}}/K_{\mathrm{d}}$ for various values of $K_{\mathrm{d},3}/K_{\mathrm{d}}$. If $K_{\mathrm{d},3} < K_{\mathrm{d}}$, the binding is said to be cooperative, meaning that binding a second ligand is stronger once the first ligand is bound. Use your plot to comment on the effect of cooperativity in this example.

e) Assume now that only a single chemical reaction is allowed.

$$\mathrm{LRL} \rightleftharpoons \mathrm{R} + \mathrm{L} + \mathrm{L}, \tag{3.10}$$

with an equilibrium constant we will call K. This means that the receptor may have only zero or two ligands bound to it. Write the states and weights diagram and derive an expression for p_{LRL}. Compare this result to your results in parts (c) and (d).

f) **Hill functions** are commonly used to describe cooperative binding. A Hill function for binding of n ligands to a receptor is of the form.

$$p_{RL_n} = \frac{c_L^n}{K^n + c_L^n} \tag{3.11}$$

What does the analysis in this problem say about using Hill functions to describe cooperative binding?

Problem 3.4 (Missing information in ligand-receptor binding, 20 pts)**.**
In section 6.4.1 of *PBoC2*, the authors explore the **missing information** as a characterization of ligand-receptor binding probability.

a) Explain in words what missing information is and what it means in this context.

b) Consider now the case where instead of a single receptor, we has two receptors, R1 and R2. These receptors may respectively bind ligands L1 and L2. That is, L1 and R1 may bind, and L2 and R2 may bind, but L1 may not bind R2 and L2 may not bind R1. Write down an expression for the missing information similar to equation 6.115 in *PBoC2*. Sketch a contour plot of the missing information as a function of free ligand concentration. The horizontal axis should be the concentration of L1 in units of the dissociation constant for L1-R1 and the vertical axis should be the concentration of L2 in units of the dissociation constant for L2-R2. The axes should be on a logarithmic scale. If you are having trouble sketching the contour plot, you may use plotting software. You should really try to use intuition be sketch a plot if you can, though. Be sure to explain the shape of the contour plot in words.

c) Repeat part (b), except now there are two ligands, L1 and L2, each of which may bind a single repressor.

Problem 3.5 (Analysis of Mcsl data, 10 pts extra credit).
In lecture, we analyzed data from Perozo, et al., in which they performed a patch clamp experiment to measure the probability that a single ion channel reconstituted in lipid bilayers is open under various applied pressures. We performed a maximum likelihood estimate of the parameter $\beta\,\varepsilon$ and $\beta\,\alpha\,\Delta A$, as defined in the lecture notes. Develop a statistical model and perform an analysis to get an estimate, with quantified uncertainty, of the parameters. The data are below.

```
pressure (mm Hg),p_open
5,0.008
10,0.008
15,0.008
20,0.048
25,0.126
30,0.403
35,0.734
40,0.939
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