BE/APh 161: Physical Biology of the Cell, Winter 2023
Homework #3
Due at the start of lecture, 2:30 PM, February 1, 2023.

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 statistical mechanical skills. You will also gain a much deeper insight into the deceptively simple, but ubiquitous, ligand-receptor binding.

Problem 3.1 (Ligand-receptor binding and small numbers of molecules, 40 pts)**.** In this problem, we will explore the effect of having small number of ligands and receptors in a small volume, as is often the case in cells. Imagine we have a cell with volume V_{cell} that contains L total ligands and R total receptors. (Of course here we mean copies of specific ligand-receptor pair; cells have lots of ligands and receptors of different type.) The receptors and ligands are all free to move about in the cell. Each receptor can bind a single ligand. Let n be the number of receptors that are bound to ligands.

a) Compute the expected number of bound receptors, n, as a function of L, R, and $W \equiv K_{\text{d}} V_{\text{cell}}$. In doing the calculation, assume that R and L are large, which enables you to use

$$K_{\text{d}} = \frac{c_L c_R}{c_{LR}}. \tag{3.1}$$

b) W is a dimensionless number. What is its physical meaning?

c) When L and R are not large, just knowing the expected number of bound receptors is not enough to fully understand what the molecules are doing in our system. We therefore would like to know $P(n)$, the probability mass function of n. I.e., $P(n)$ is the probability that there are n bound receptors at equilibrium. Show that

$$P(n) = \frac{\left[W^n\, n!(R-n)!(L-n)!\right]^{-1}}{\displaystyle\sum_{n=0}^{\min(R,L)} \left[W^n\, n!(R-n)!(L-n)!\right]^{-1}}. \tag{3.2}$$

d) Plot $P(n)$ for various values of L, R, and W. Comment on what you see, especially for small L and R. By "small," I mean between 1 and 100. (Are there ligands and receptors with these sorts of copy numbers in cells?) Think carefully about how to represent your plot so that you can highlight the important physical consequences of your analysis. Be sure to discuss your plots. *Hint:* It will be difficult to compute the statistical weights and the partition function.

Work with logarithms of the statistical weights when you can. If you are using Python, `scipy.special.gammaln()` and `scipy.misc.logsumexp()` might be useful functions.

e) The coefficient of variation is the ratio of the standard deviation of a distribution to its mean. Plot the coefficient of variation of $P(n)$ for $W = 1000$, R going from 1 to 10^5, and $L = 2R$. What does this say about variability in number of species? When can you just use your result from part (a), and when should be think more carefully about the full distribution?

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 have 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.