

BE/APh 161: Physical Biology of the Cell, Winter 2018
Homework #3

Due at the start of lecture, 2:30 PM, January 24, 2018.

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.

But first, we'll do a quick visit to some basics of statistical mechanics.

Problem 3.1 (Boltzmann's grave, 5 pts).

Boltzmann's tomb is in Zentralfriedhoff in Vienna, a beautiful cemetery that also contains the graves of some of the world's greatest composers, including Beethoven, Brahms, Schubert, Strauss, Ligeti, and Falco. Boltzmann's tomb is shown in Fig. 1. Not the equations, $S = k \log W$, at the top of the stone.



Figure 1: Boltzmann's tomb in Zentralfriedhoff in Vienna. Photo from Daderot, licensed under [CC-BY-SA-3.0](https://creativecommons.org/licenses/by-sa/3.0/).

Here, S is entropy, k is the Boltzmann constant, \log refers to the natural logarithm, and W is the number of microstates. In class on January 17, we derived the famous Boltzmann distribution by maximizing the Shannon entropy, given that we knew an average energy of our system of interest. Derive the equation on Boltzmann's grave using the same technique. To do so, assume we do not know anything about the energy of the system.

Problem 3.2 (Ligand-receptor binding: practical calculations, loosely based on problem 6.4 of *PBoC2*, 15 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

$$p_{\text{bound}} = \frac{1}{1 + K_d/c_L}, \quad (3.1)$$

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 (3.1) holds even if we have many receptors. For concreteness of notation, assume that 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,

$$K_d = \frac{c_L c_R}{c_{LR}}, \quad (3.2)$$

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 $c_L^0 \gg c_R^0$,

$$p_{\text{bound}} \approx \frac{1}{1 + K_d/c_L^0}. \quad (3.3)$$

Problem 3.3 (Cooperative ligand-receptor binding, 40 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



- Show that by the law of mass action, $K_{d,4} = K_{d,2}K_{d,3}/K_{d,1}$.
- Consider a single receptor in a solution of ligands with concentration c_L . Write down a states and weights table.
- 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 $K_{d,1}$, $K_{d,2}$, and $K_{d,3}$. Be sure to explicitly write how the dissociation constants depend on the energies of the respective states.
- Assume $K_{d,1} = K_{d,2} \equiv K_d$. Plot p_{LRL} vs. c_L/K_d for various values of $K_{d,3}/K_d$. If $K_{d,3} < K_d$, 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.
- Assume now that only a single chemical reaction is allowed.



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).

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

$$p_{RL_n} = \frac{c_L^n}{K^n + c_L^n} \quad (3.9)$$

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

Problem 3.4 (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.

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

$$K_d = \frac{c_L c_R}{c_{LR}}. \quad (3.10)$$

- 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

$$P(n) = \frac{[W^n n!(R-n)!(L-n)!]^{-1}}{\sum_{n=0}^{\min(R,L)} [W^n n!(R-n)!(L-n)!]^{-1}}. \quad (3.11)$$

- 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?