

MCB137L/237L: Physical Biology of the Cell
Spring 2020
Homework 8: Statistical Mechanics and Cellular
Decision-Making
(Due 4/9/20 at 3:30pm)

Hernan G. Garcia

“Biology is catching up” - PAM Dirac

Note to class: You can complement these problems by reading the paper “A First Exposure to Statistical Mechanics for Life Scientists: Applications to Binding” on the course website.

1 A Feeling for the Numbers in Biology: Round two

Now that you have written your first estimate vignette in the style of *Cell Biology by the Numbers*, it's time to get ready for the second estimate! You will present this second estimate in a 5-minute presentation at the end of the semester. You're welcome to work in groups of up to three people. In that case, you will have 5 minutes *per group*.

Write a short paragraph describing the estimate you're interested in presenting about. Note that the objective at this point is not for you to have a finished estimate, but to have an outline of the calculation you plan to do so that we can give you feedback. Send this paragraph as an email to Hernan, Yang Joon and Jake by **4/16 (when Homework 9 will be due)**.

2 Ion channels and statistical mechanics

In this problem, we will derive a mathematical description of the current passing through a voltage-gated ion channel. To model this channel, we assume that it can exist in an open or closed configuration as shown in Figure 1A. The thermal fluctuations in the cell result in the channel switching between these states over time as presented in Figure 1B. Figure 1C shows how these fluctuations in channel state can be directly read out from the current flowing through the channel.

(a) Use the statistical mechanics protocol (i.e. calculating the states and weights of the system) to calculate the probability of the channel being in the open state, p_{open} . Assume that the open state has an energy ε_{open} , and that the energy of the closed state is ε_{closed} .

(b) Plot p_{open} as a function of $\Delta\varepsilon = \varepsilon_{open} - \varepsilon_{closed}$. Explain what happens in the limits $\varepsilon_{open} \ll \varepsilon_{closed}$ and $\varepsilon_{open} \gg \varepsilon_{closed}$. What significance does $\Delta\varepsilon = 0$ have for p_{open} ?

In a simple model of a voltage-gated ion channel, $\Delta\varepsilon = q(V^* - V)$. Here, V is the voltage applied to the membrane and q is the effective gating charge, which describes the movement of charges along the membrane as the channel configuration changes. You can learn more about this model in section 17.3.1 of PBoC2.

(c) What is the significance of V^* ? Namely, what happens to the probability of being open when $V = V^*$.

(d) On the website, you will find measurements of p_{open} vs. V for a sodium-gated ion channel. Write your expression for p_{open} as a function of V instead of as a function of $\Delta\varepsilon$. Estimate V^* from the data using what you learned in (c). Now that you have V^* , to estimate q , make a plot where you overlay the data and the model prediction for three different values of q corresponding to 1, 3 or 5 electron charges (note that q is positive, so here we are talking about the *absolute value* of the electron charge).

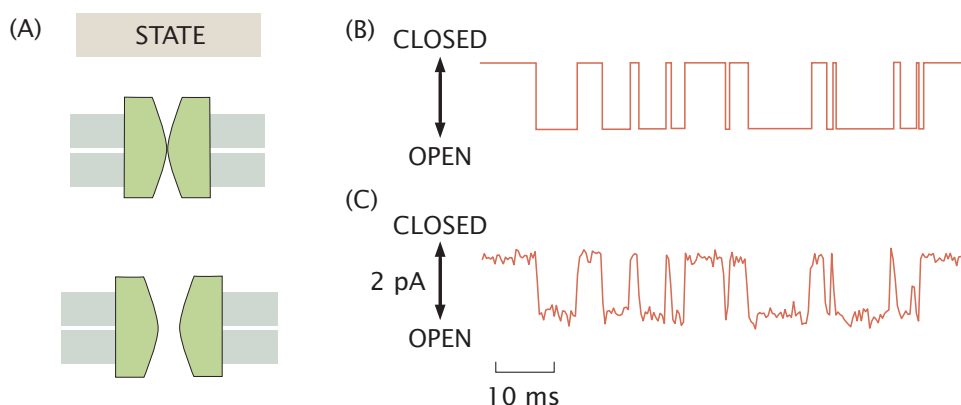


Figure 1: Current through an ion channels. (A) The ion channel can exist in a closed or open configuration, (B) fluctuating in time between these two states. (C) The current flowing through the channel is directly related to the state of the channel.

3 A feeling for the numbers: Hemoglobin

We have adopted hemoglobin as one of our molecules of interest to discuss the statistical mechanics of binding reactions. In this problem, you will perform several estimates to get a feeling for the numbers for hemoglobin.

(a) Do problem 4.1(c) from PBoC2 (shown below in Figure 2).

(b) Figure out roughly how many O_2 molecules you bring in with each breath and how many Hemoglobin molecules it would take to use each and every one of those oxygens. How does this compare with the total number of Hemoglobins in your body calculated in (a)? Hint: You will have to figure out our lung capacity and how many O_2 molecules are contained

within that volume using the ideal gas law.

• **4.1 Structure of hemoglobin and myoglobin**

(a) As in Problem 2.6, obtain the atomic coordinates for hemoglobin and myoglobin. Measure their dimensions, identify the different subunits and the heme groups.

(b) Expand the analysis of hemoglobin on p. 143 by calculating the mean spacing between hemoglobin molecules inside a red blood cell. How does this spacing compare with the size of a hemoglobin molecule?

(c) Typical results for a complete blood count (CBC) are shown in Table 4.1. Assume that an adult has roughly 5 L of blood in his or her body. Based on these values, estimate:

- (i) the number of red blood cells;
- (ii) the percentage in volume they represent in the blood;
- (iii) their mean spacing;
- (iv) the total amount of hemoglobin in the blood;
- (v) the number of hemoglobin molecules per cell;
- (vi) the number of white blood cells in the blood.

Table 4.1: Typical values from a CBC. (Adapted from R. W. Maxwell, Maxwell Quick Medical Reference, Maxwell Publishing, 2002.)

Test	Value
Red blood cell count (RBC)	Men: $\approx (4.3-5.7) \times 10^6$ cells/ μ L Women: $\approx (3.8-5.1) \times 10^6$ cells/ μ L
Hematocrit (HCT)	Men: $\approx 39-49\%$ Women: $\approx 35-45\%$
Hemoglobin (HGB)	Men: $\approx 13.5-17.5$ g/dL Women: $\approx 12.0-16.0$ g/dL
Mean corpuscular hemoglobin (MCH)	$\approx 26-34$ pg/cell
MCH concentration (MCHC)	$\approx 31-37\%$
Mean corpuscular volume (MCV)	$\approx 80-100$ fL
White blood cell count (WBC)	$\approx (4.5-11) \times 10^3$ cells/ μ L
Differential (% of WBC):	
Neutrophils	$\approx 57-67$
Lymphocytes	$\approx 23-33$
Monocytes	$\approx 3-7$
Eosinophils	$\approx 1-3$
Basophils	$\approx 0-1$
Platelets	$\approx (150-450) \times 10^3$ cell/ μ L

Figure 2: Problem 4.1 from PBoC.

4 Dimoglobin: A Toy Model of Hemoglobin

In Homework 7, you derived the probability of a receptor being bound by a ligand using a lattice model from the statistical mechanics perspective. This resulted in

$$p_{bound} = \frac{\frac{L}{\Omega} e^{-\beta \Delta \epsilon}}{1 + \frac{L}{\Omega} e^{-\beta \Delta \epsilon}}, \quad (1)$$

where L is the number of ligands in the solution and $\Delta \epsilon = \epsilon_b - \epsilon_{sol}$ with ϵ_b being the binding energy of a ligand to the receptor and ϵ_{sol} the energy of a ligand when in the lattice. Further, Ω is the number of lattice sites.

(a) Write p_{bound} in terms of the concentration of ligands $\frac{[L]}{\Omega v}$, where v is the volume of a lattice box. Now, note that we can think of the inverse of v as a concentration c_0 corresponding to each lattice site being occupied by a ligand such that $v = 1/c_0$. If the volume of a lattice site is 1 nm^3 , what is the corresponding c_0 ? In biochemistry this c_0 is called the concentration of the standard state. How does this concentration compare to those you'd usually pipette in an experiment? What do you conclude about how dilute the solutions you usually deal with in the lab are?

In class, we discussed how cooperativity in oxygen binding to hemoglobin makes it possible for the binding curve to be switch-like. Now that we are experts at ligand-receptor binding,

we want to mathematically explore the consequences of cooperativity in the context of a toy model of hemoglobin: dimoglobin. Unlike hemoglobin, which binds four oxygen molecules, dimoglobin binds only to two oxygen molecules.

Figure 3 features a lattice model of dimoglobin. Here, oxygen molecules in solution have an energy ε_{sol} , oxygen binds to either dimoglobin site with energy ε_b . Finally, when two oxygen molecules are bound, they also interact with energy ε_{int} .

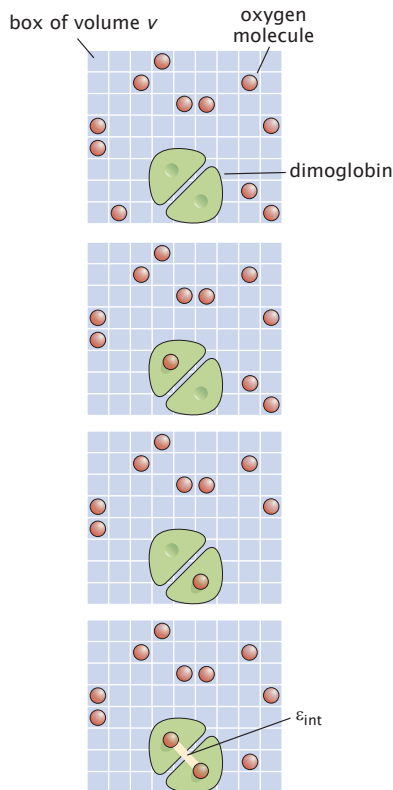


Figure 3: Cooperativity model of dimoglobin in a lattice. Different states the dimoglobin molecule and the oxygen molecules in the lattice can be found in. An oxygen molecule in solution has energy ε_{sol} , while it has a binding energy to dimoglobin of ε_b . Two oxygen molecules bound to dimoglobin interact with an energy ε_{int} .

(b) Use the statistical mechanics protocol to calculate p_0 , p_1 and p_2 , the probabilities of having no, one, or two oxygen molecules bound to dimoglobin. Use these probabilities to show that the average number of bound molecules is given by

$$\langle N \rangle = \frac{2 \frac{[L]}{c_0} e^{-\beta \Delta \varepsilon} + 2 \left(\frac{[L]}{c_0} \right)^2 e^{-\beta(2\Delta \varepsilon + \varepsilon_{int})}}{1 + 2 \frac{[L]}{c_0} e^{-\beta \Delta \varepsilon} + \left(\frac{[L]}{c_0} \right)^2 e^{-\beta(2\Delta \varepsilon + \varepsilon_{int})}}, \quad (2)$$

where $[L]$ is the oxygen partial pressure (which is a measure of concentration) and $c_0 = 760$ mmHg is the standard state partial pressure. Make sure to include and explain all steps

in your derivation.

(c) Plot the average number of bound molecules as a function of oxygen partial pressure for $\varepsilon_{int} = -5 K_B T$ and for $\varepsilon_{int} = 0$ on a linear-log plot in order to show the effect of ε_{int} on the sharpness of the occupancy curve. Use $\Delta\varepsilon = -5 K_B T$ for both curves.

(d) Plot p_0 , p_1 and p_2 as a function of oxygen partial pressure. Make one plot for $\varepsilon_{int} = -5 K_B T$ and one for $\varepsilon_{int} = 0$ in order to show sharpness is achieved through ε_{int} by draining probability from p_1 .

5 Dimerization of Lambda repressor

Lambda repressor monomers dimerize in solution before they can bind to the DNA. In class, we explored how two adjacent Lambda repressor dimers on the DNA can interact with each other leading to switch-like behavior. However, we did not take into account the effect of the initial dimerization of Lambda repressor on the input-output function. In this problem, we explore how this dimerization can also contribute to the Lambda switch.

(a) Let's start by writing a model of Lambda repressor dimerization *in solution*. Use the dynamics protocol to write a rate equation for the concentration of dimers $[D]$ in terms of the concentration of monomers $[M]$. Assume steady state and show that you can define a dissociation constant given by

$$K_{dimer} = \frac{[M]^2}{[D]}. \quad (3)$$

Explain how K_{dimer} is determined by the rates of dimerization and monomerization.

Note that in the equation above, $[M]$ and $[D]$ correspond to the *free* concentration of monomers and dimers. The total amount of Lambda repressor monomers (that makes up both free monomers and dimers) is given by $[M]_{tot} = [M] + 2[D]$.

(b) Calculate the concentration of free monomer $[M]$ as a function of the total monomer concentration $[M]_{tot}$. To make this possible, you'll have to make use of Equation 3 and of the condition $[M]_{tot} = [M] + 2[D]$, and solve a quadratic equation.

(c) Calculate and plot the fraction of total monomers in monomeric and dimeric form as a function of the total monomer concentration $[M]_{tot}$ on the same graph on linear-log axis. Make independent plots for a K_{dimer} of 0.1 nM, 1 nM and 10 nM. What feature of the curves is controlled by the value of K_{dimer} ?

In class, we calculated the fold-change in gene expression for simple repression and obtained

$$\text{fold-change} = \frac{1}{1 + \frac{R}{N_{NS}} e^{-\beta\Delta\varepsilon}}, \quad (4)$$

where R is the number of repressor molecules in the cell, $\Delta\varepsilon$ the difference between the specific and non-specific binding energy, and N_{NS} the number of non-specific binding sites.

In the language of biochemistry, this same expression can be written as

$$\text{fold-change} = \frac{1}{1 + \frac{[R]}{K_{DNA}}}. \quad (5)$$

Here, $[R]$ is the concentration of free repressor and K_{DNA} the dissociation constant that characterizes its binding to the DNA. With this equation in hand, we want to compare the sharpness of the repression input-output function for a repressor that dimerizes in solution such as Lambda repressor and for one that doesn't such as Lac repressor.

(d) Plot the fold-change for regulation by Lac repressor. Define $[R] = [I]$ as the Lac repressor concentration and plot the fold-change as a function of $[I]$ for $K_{DNA} = 1 \text{ nM}$ on linear-log axes.

(e) On the same axis, plot the fold-change for regulation by a Lambda repressor dimer as a function of the total concentration of Lambda repressor monomers $[M]_{tot}$. To make this possible, assume that $[R] = [D]$, $K_{DNA} = 1 \text{ nM}$ and $K_{dimer} = 10 \text{ nM}$. Show graphically how the fact that Lambda repressor dimerizes in solution contributes to sharpening the input-output function.