

BE/APh161: Physical Biology of the Cell

Homework 7

Due Date: Monday, March 14, 2022

“How can the events in *space and time* which take place within the spatial boundary of a living organism be accounted for by physics and chemistry?”
- Erwin Schrödinger **What is Life?**

1'. Extra Credit Read and comment on chap. 12 from our new book “The Restless Cell,” a book about the important new field of active matter. This chapter focuses on how energy consumption takes place in living organisms and touches on many of the themes from recent weeks of the course. Please submit your comments in the form of a referee report (PDF format only) which specifically refers to the page numbers in the chapter. Please mail it to me and the TAs.

1. Waiting time distributions.

One of the big messages of the course is the deep insights that come from a probabilistic assessment of biological systems. Our slogan might be: mechanistic information is hidden in the probability distributions. The binding problems that we worked out for ligands and receptors can be thought of as giving rise to a time series that looks like a so-called telegraph signal, going back and forth between 0 and 1. Because the time of switching between bound and unbound is very fast compared to the time spent in those two states, the occupancy of the receptor is either 0 or 1. Thinking about waiting time distributions is critical to the way we will think in turn about kinetic proofreading as laid out in the recorded vignettes and is the subject of the second problem on this homework.

(A) In light of this, it is interesting to explore the distribution of waiting times that we spend in the unoccupied or occupied state. To that end, we can use the interpretation of rates as follows. Consider that the receptor is currently occupied and we start a stopwatch to measure how long until a ligand hops off of it. In each instant Δt , as shown in Figure 1, there is a probability $p_+ = k_{off}\Delta t$ of hopping off of the receptor. The goal of our calculation is to work out the probability that the ligand will fall off after a time $T = n\Delta t$, where n is the number of time steps we have to wait until the ligand falls

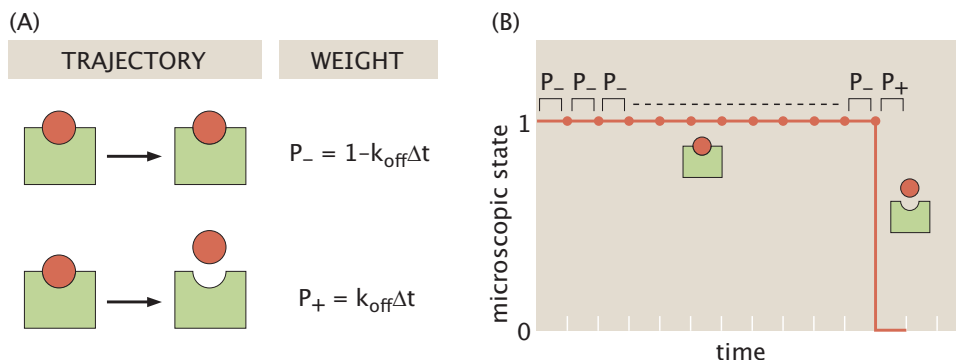


Figure 1: Computing the waiting time distribution. (A) The possible microscopic trajectories that can occur during a time step Δt . (B) Schematic of the states during all the time steps leading up to the ligand falling off of the receptor.

off. To do so, we imitate the figure by noting that to fall off at time T this means that the ligand will have to have *not* fallen off during all the previous steps. Since we have discretized time into slices of length Δt , show how to write the probability as a product of n independent probabilities. Use the insight that

$$\lim_{n \rightarrow \infty} (1 - x/n)^n = e^{-x} \quad (1)$$

to show that the probability that the ligand falls off between time T and $T + \Delta t$ is given by

$$p(T)\Delta t = k_{\text{off}}e^{-k_{\text{off}}T}\Delta t. \quad (2)$$

Show that this probability distribution is properly normalized and then compute the average waiting time

$$\langle t \rangle = \int_0^\infty tp(t)dt. \quad (3)$$

(b) When we think about molecular motors, we will be interested in molecules that transition between more than two states, but have exponential waiting times in each of those states. Consider the case of a molecular motor that has two steps, each with a waiting time distribution that is exponential like you worked out in the first part of the problem. Using that, work out an expression for the waiting time distribution for the *composite* process made

up of those two steps. That is, once again find $p(T)$ given that both t_1 and t_2 are exponentially distributed, where t_1 is the waiting time for the first step and t_2 is the waiting time for the second step. The key point in formulating your thinking is that you must respect the constraint that $t_1 + t_2 = T$. Make a plot of this kind of distribution and comment on what it means.

2. Leaky Membranes: The Cost of Defying Diffusion

As we saw in class, some ionic species are at a higher concentration inside the cell than outside the cell. As a result of this concentration gradient, there will be a flux of ions leaving the cell given by the concentration difference and the permeability which can be written as

$$\text{flux} = P(c_{in} - c_{out}) \quad (4)$$

where P is the permeability as illustrated in Figure 2.

(A) Calculate the number of ions of a species such as K^+ that leave the cell per second due to the permeability of the membrane. Essentially, this tells us about the leakiness of the cell membrane to ions which will over time lead to a complete dissipation of the gradient. You might find it useful to read up on permeability in Section 11.1.3 of PBoC2.

(B) Using ideas worked out in class about the protonmotive force, make an estimate of the power in ATP/s or $k_B T/s$ that it costs to maintain the concentration gradient against the perpetual leakiness of the membrane. Make sure you spell out the quantitative details of how you make this estimate.

(b) How does the energy necessary to maintain the K^+ gradient compare to that required to build a bacterial cell?

3. Breaking the 2nd Law and Rectifying Thermal Noise

In a great *Physics Today* article (provided on the course website), Chris Jarzynski and colleagues state that “A liter of ordinary air weighs less than half a US penny, but it contains enough thermal energy to toss a 7-kg bowling ball more than 3 m off the ground. A gadget able to harvest that abundant energy by converting the erratic movement of colliding molecules into directed motion could be very useful indeed.”

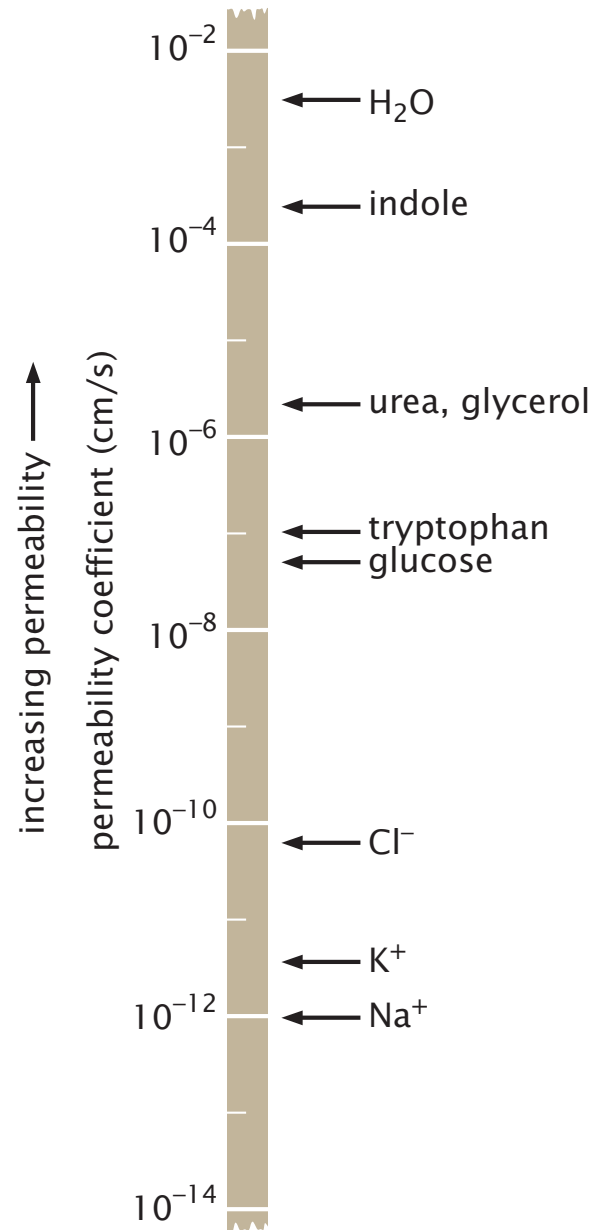


Figure 2: Permeability of various ions and molecules across membranes.

Explore the assertion about the weight of the air in the room and the energy within it. Remember the meaning of $k_B T$!

4. Dissecting ATP Consumption.

One of the most important topics discussed in this course is that of biological batteries, the many different ways that cells fuel reactions that maintain them in nonequilibrium steady states. The symporter problem we worked out last week gave a concrete example of how the concentration gradient biological battery could be used to move sugars up their gradient. In this problem, your goal is to essentially recreate in your own words and doing all the algebraic steps yourself, the story of ATP consumption in the context of phosphorylation-dephosphorylation reactions.

(A) In class, we considered an enzyme E that can be phosphorylated resulting in the state E^* . We can think of the resulting nonequilibrium steady state as arising from two coupled reactions given by



and



Write a dynamical equation for $d[E]/dt$. Yes, I know I did this in class. The goal here is not for you to regurgitate what I did, but for you to now become the instructor (see the final problem on this homework) and to write up completely transparent, pedagogical notes that truly explain all steps in the logic.

(B) Using the constraint that $[E] + [E^*] = [E_{tot}]$, solve for the steady-state values of $[E]^{ss}$ and $[E^*]^{ss}$. Make sure you explain the entire set of steps resulting in these steady state values and produce a formula for

$$\text{fraction active} = \frac{[E^*]^{ss}}{[E^*]^{ss} + [E]^{ss}}. \quad (7)$$

Explain the trends in the active fraction as a function of the ratio of ATP to ADP, for example.

(C) Compute the next flux in the reaction of eqn. 5. That is, find a formula that shows the difference between the number of right going reactions and the number of left going reactions. Once again, comment on how this flux depends upon the relative proportion of ATP and ADP. Under what circumstances will there be no net flux? Compute the same quantity for the reaction in eqn. 6. Are the fluxes passing through those two reactions the same?

(D) Carefully reconstruct the argument for the rate of energy consumption that starts from the expression

$$\text{rate of energy consumption} = J_{net}\Delta\mu \quad (8)$$

that I gave in class. Explain what this expression means? What is $\Delta\mu$ (don't just tell me "it is the chemical potential difference") - I need some insight and intuition. Explain the particular choice of $\Delta\mu$ and write an explicit expression for it in terms of the various rate constants and concentrations of ATP, ADP and P_i . Explain why we think of the chemical potential difference as a "driving force." Driving force for what? Towards what?

(E) Building on the results from the previous part of the problem, show that the energy consumption rate can be written as

$$\frac{\partial F}{\partial t} = k_B T \left((J_1 - J_{-1}) \ln \frac{J_1}{J_{-1}} + (J_2 - J_{-2}) \ln \frac{J_2}{J_{-2}} \right). \quad (9)$$

Comment on the meaning of the various symbols and what it implies about the free energy consumption rate when the backwards fluxes go to zero.

(F) Summarize your understanding of driving forces and nonequilibrium steady states in the context of biological reactions.

5. MWC Ion Channel: One Equation that Rules Them All

In class, we introduced the idea of allosteric proteins as those that have a regulatory binding site that cause the protein to switch between inactive and active states. In this problem, we will take the same ideas developed in class and apply them to the so-called ligand-gated ion channels. These channels are relevant in contexts ranging from our neuromuscular junctions to the

photoreceptors in our eyes to olfactory neurons. Figure 3 shows two classic examples of these channels.

(a) Write a paragraph that summarizes the function of the two ion channels shown in Figure 3. The point here is just to make sure you have a little understanding of their physiological function before we start working out their statistical mechanical properties.

(b) Make a diagram with your version of the statistical mechanics protocol showing the states and weights for the nAChR ion channel. Make sure you explain all of your notation for the parameters that appear here.

(c) Write an equation for the probability that the channel is open $p_{open}(c)$, where c is the concentration of acetylcholine.

(c) Work out the leakiness, dynamic range and the EC50. Leakiness refers to the probability that the channel is open in the absence of ligand and can be thought of as p_{min} , the minimum probability the channel is open. Dynamic range refers to the difference between p_{max} and p_{min} , where p_{max} is the probability of being open at saturating concentrations of ligand. Find explicit expressions for both p_{min} and p_{max} and then use their difference to obtain the dynamic range. EC50 is the concentration of ligand at which the channel is halfway between p_{min} and p_{max} . Write expressions for each of the four properties listed above. Then, simplify your expressions for these various properties in the limit where $K_I/K_A \gg 1$.

(d) Figure 4 shows data for the wild-type nAChR ion channel from the laboratory of our own Prof. Henry Lester. With your TA, use Digitizeit to extract the data and then make a fit using the MWC model you worked out earlier in the problem. This is Figure 1B of the paper by Labarca *et al.* included with the homework. Note that unfortunately, they chose to plot “normalized current” rather than $p_{open}(c)$. As a result, your fit will have to be to the normalized current given as

$$\text{normalized current} = \frac{p_{open}(c) - p_{min}}{p_{max} - p_{min}}. \quad (10)$$

I am excited for you to learn how to use Digitizeit because it is liberating: with it, you can take figures from anyone’s papers and grab their experi-

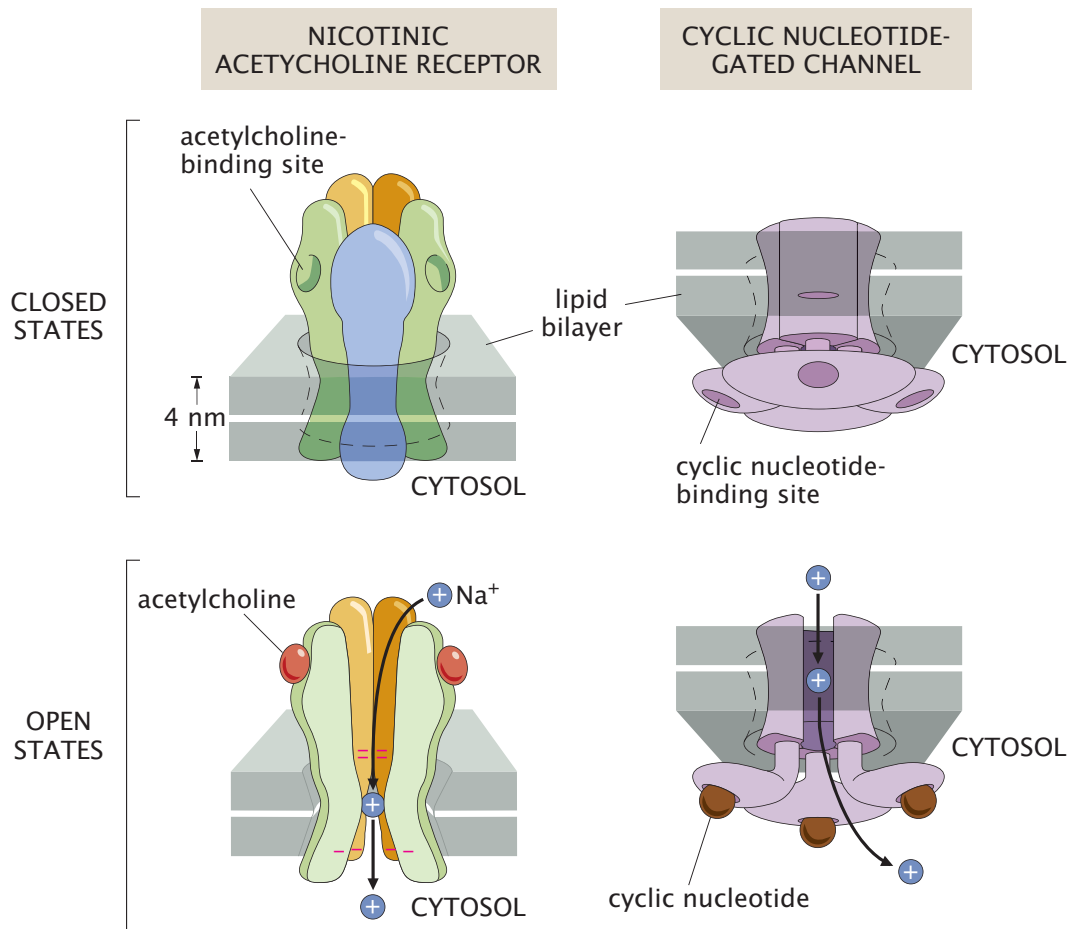


Figure 3: Key examples of ligand-gated ion channels. (left) Nicotinic acetylcholine receptor, revealing its heteropentameric structure with two binding sites for acetylcholine. (right) cGMP-gated ion channel. These channels have four cGMP binding sites.

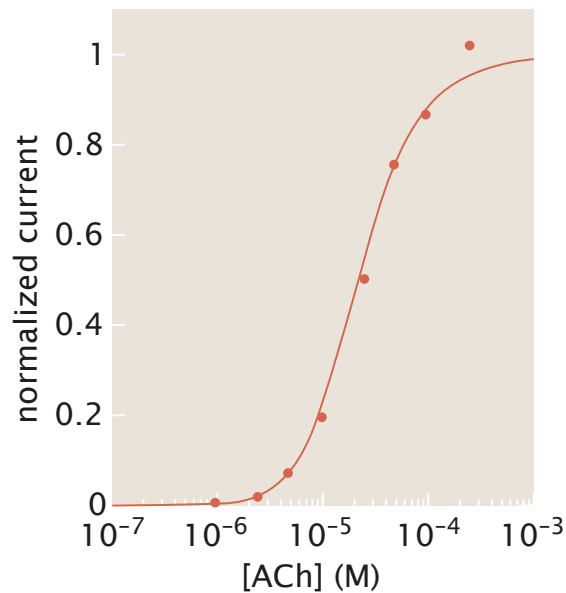


Figure 4: Ion channel currents as a function of ligand concentration. (Adapted from Labarca *et al.*, Nature, 1995).

mental data and export it into a spreadsheet so that you can unleash your theoretical analysis on it.

N-3. Your Philosophy of Biology.

Choose to answer one of the following questions in a several paragraph discussion.

a) The history of engineering has been characterized repeatedly by a transition from enlightened empiricism to rational design. For example, the flying buttresses of European cathedrals originally did not come from a deep understanding of the quantitative principles of structural mechanics. Now, companies like Boeing design their airplanes knowing very well what the stresses and strains are like within the materials from doing finite element calculations of elasticity. Give your position on the future of bioengineering and whether you think the field is currently in the enlightened empiricism stage or the rational design stage? Will medicine become a rational design topic and do you think it should?

b) The mantra of this course is that quantitative data demands quantitative models. Explain in what sense modern biology has become quantitative and defend the mantra as a tool for understanding biological phenomena.

N-2. Defiance

In a long series of vignettes, I argued that perhaps the real “secret of life” is defiance, in keeping with the Schrödinger quote that headlines this homework. Use a paragraph to explain what my argument about defiance is and give a succinct statement of how living organisms use the elements of the periodic table to make things that are clearly qualitatively different than, say, a rock.

N-1 Your Turn. A Feeling for the Organism.

Pose an order of magnitude problem about real world biology and then make the corresponding estimate. Please take this seriously and try to build on everything we have done the entire term. Formulate a particular “I wonder” question that you find exciting and that you imagine others will find interesting as well. Then, make clear statements about what assumptions you need to make in order to construct the relevant estimate. Once you obtain your estimate, make a rational discussion of why the values take the values they do and how you think this corresponds to what we know from data.

N. Your Turn.

In this final problem, I want you to construct a thoughtful syllabus for how you would teach a course on Physical Biology. You have ten weeks, two classes of 90 minutes each per week. Make sure to give a sense of whether your homeworks will involve computation, whether you will give an exam, etc. But more importantly, what is the content? What do you want students to leave the course with? What are the top five skills you want them to leave with? What are the top five insights you want them to leave with? You have 20 lectures, so I want to hear what each and every lecture will be about. How much powerpoint? How many calculations on the blackboard. For this problem, send a pdf (nothing but pdf accepted and zero credit for stuff like

powerpoint or word files) to the TAs and Rob.