

BE/APh161: Physical Biology of the Cell

Homework 7

Due Date: Friday, March 2, 2012

Referee Report: Read the two chapters from the "Cell Biology by the Numbers" book and send the referee report in pdf form as before to my coauthor and me as well as the TAs.

1. Channel Opening Kinetics.

So far, our discussion of ion channels has focused on the equilibrium probabilities of the two states (i.e. open and closed) of the channel (note: some channels have more than two states). In this problem, we will examine the time evolution of a collection of ion channels and examine the waiting time distributions and correlation functions for a channel.

(a) Write down differential equations for the time evolution of the open and closed probabilities, $p_o(t)$ and $p_c(t)$, respectively. Assume that the kinetics of opening and closing are described by the chemical equation



where O and C are the open state and closed state, respectively. Use rate constant k_{on} to capture the rate of transition from the closed to the open state and k_{off} to capture the rate at which the system transitions from the open to the closed state.

(b) By using the fact that the sum of the probabilities is unity, write down a single differential equation for $p_o(t)$ and solve it, find the corresponding result for $p_c(t)$ and plot both of them as a function of time. Assume that at $t = 0$, $p_o(0) = 1$. Choose your units of time in some sensible way so that the plots don't require you to know the precise numerical values of the rate constants.

(c) Let's now imagine that time is discretized into a series of steps of length Δt . At the initial instant, imagine that the channel is closed. What is the probability that in the next time interval Δt that it will switch to open? What is the probability that in that same time interval Δt that it will stay closed? Using those insights, now write an expression for the probability ($p_{closed}(t)\Delta t$) that in the interval between t and $t + \Delta t$ the channel will switch from closed to open, having stayed closed the entire time until then. Make a corresponding derivation for $p_{on}(t)$. What can you say about the waiting time distributions? What are the time constants for the open and closed waiting time distributions? Using these distributions, compute the average time that the channel stays in each of the states. Make sure to compute this as an average and explain what integrals you write down and why. Given the nature of ion channel current traces, explain how you could go about determining these distributions and finding these average

times. Hint: Remember that the exponential is characterized by the interesting property

$$\lim_{N \rightarrow \infty} \left(1 - \frac{x}{N}\right)^N = e^{-x}, \quad (2)$$

and use the fact that $N = t/\Delta t$.

(d) Now we follow up on the results of part (c) to ask a more sophisticated question by considering the distribution of waiting times from one closed-open transition to the next closed-open transition. Start by sketching an ion channel current trace and show what is meant by the waiting time from one closed-open transition to the next. In particular, make a cogent argument that this waiting time distribution is given by

$$p_{\text{successive}}(t) = \int_0^t p_{\text{open}}(t - \tau) p_{\text{closed}}(\tau) d\tau. \quad (3)$$

Then, using the results of part (c) for $p_{\text{open}}(t)$ and $p_{\text{closed}}(t)$, obtain an analytic form for this distribution and plot it and explain its features and significance.

2. MWC and ion channels.

In class, we argued that the treatment of ligand-gated ion channels is a fascinating problem in statistical mechanics and that the Monod-Wyman-Changeux (MWC) model provides a great response to that challenge. Here, we explore this model in the context of cyclic nucleotide gated channels which are gated by cGMP.

(a) Write down an MWC model for the ligand-gated channel and calculate the probability of the channel being open as a function of the ligand concentration. Do separate versions of this model by considering channels with 1, 2, 3 and 4 binding sites.

(b) For each version of the model compare the resulting curve to the data shown in Figure ?? for the bovine retinal CNG channel. In order to do this, perform a “fit by eye”. It might be useful to understand how each model behaves in the limits $L \rightarrow 0$, $L \rightarrow +\infty$ and how the sharpness of the curve is affected by the choice of the various model parameters. What do you conclude about the number of binding sites for cGMP in this channel?

3. Gaussian chain, diffusion equation and force-extension.

(a) Do problem 8.4 from PBoC. This problem basically asks you to show that the equation for polymer conformations is the diffusion equation. What plays the role of time in your equation? What plays the role of the diffusion constant? Make the mapping between diffusion and polymer conformations explicit by precisely describing the relation between the two equations.

(b) Use the results from the earlier homework in which we found the Green function for the diffusion equation to write down the probability function $p(x, N)$

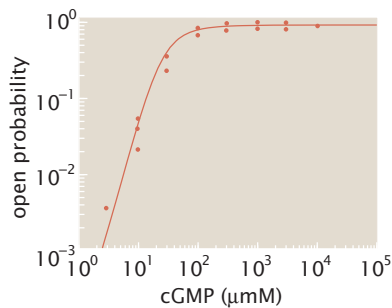


Figure 1: Open probability of the bovine retinal CNG channel. The probability of the channel being opened is measured as a function of the concentration of cGMP. The fit corresponds to an MWC model for the case of four binding sites per channel. (Adapted from E. H. Gouling et al. *Nature* 372:369, 1994.)

which gives the probability that a polymer of N segments will have its endpoint at position x (for a one-dimensional polymer).

(c) As you can see by looking at chap. 8 of PBoC, one of the interesting modern techniques for looking at molecules is to use “force-spectroscopy” in which individual molecules are grabbed and pulled and the resulting force-extension properties are evaluated. Your job is to compute the force-extension properties of the simple model of statistical conformations implied by the “Gaussian chain” model you worked out above. In this model, the entire free energy is due to entropy, hence, we have

$$\Delta G = -T\Delta S \quad (4)$$

where

$$S = k_B \ln (\text{number of configurations}). \quad (5)$$

To work this out, use the probability $p(x, N)$ and note that the number of states when the end-end distance is x is proportional to $p(x, N)$. Hence, the change in the entropy when you go from the unstretched state with $x = 0$ to the stretched state of length x can be calculated from our knowledge of the probability. What is the spring constant for the “entropic spring” that emerges from this calculation?