

BE/APh 161: Physical Biology of the Cell, Winter 2025

Homework #4

Due at the start of lecture, 2:30 PM, February 5, 2025.

The first three problems of this homework are all related. They deal with a single experiment involving unfolding an RNA hairpin using optical tweezers. Interesting, all three problems related to this experiment can be solved using the machinery of statistical mechanics, some with two-state models, that we have been working on in class.

Problem 4.1 (Unfolding an RNA hairpin by pulling, 30 pts).

In a paper in 2001, Liphardt and coworkers ([Liphardt, et al., Science, 292, 733–737, 2001](#)) investigated the energetics of the base pairing in an RNA hairpin by mechanically pulling the hairpin apart. The setup of the experiment is shown in Figure 1. The ends of an RNA hairpin are tethered to two beads. One bead is held on the end of a pipette through suction. The other bead is held in an **optical trap** that enables the researcher to move the bead and exert and measure force. The experiment was done at room temperature.

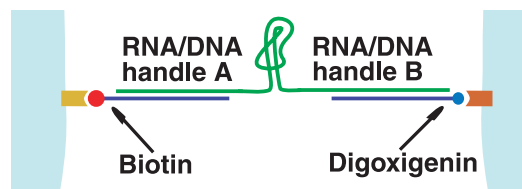
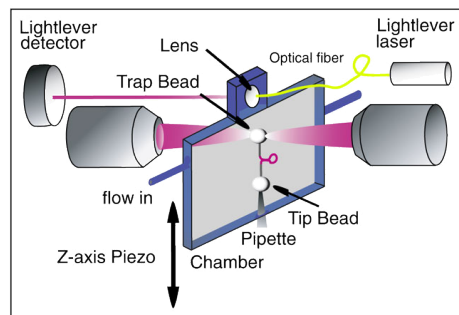


Figure 1: Schematic of the experimental setup for the pulling experiment of Liphardt and coworkers. Top, the setup for the optical trap. Bottom, detail of RNA hairpin and tethers. Figures taken from [Liphardt, et al., Science, 292, 733–737, 2001](#).

The RNA hairpin they tested is P5ab, taken from a *Tetrahymena thermophila* ribozyme, which consists of approximately 22 base pairs. Its sequence is ACAGCCGUUCAGUACCAAGUCUCAGGGGAAACUUUGAGAUGGGGUGCUGACGGACA, in case you are interested in investigating it further, for example with [NUPACK](#).

In Fig. 2, I show a typical force-extension curve from the experiment in black. In red is a force-extension curve of the DNA tethers alone, without the RNA hairpin. So, the first part of the force-extension curve involves pulling out the fluctuations in the DNA tethers (see Problem 4.3). We see that at about 14 or 15 pN, the extension suddenly jumps. This corresponds to the unfolding of the hairpin.

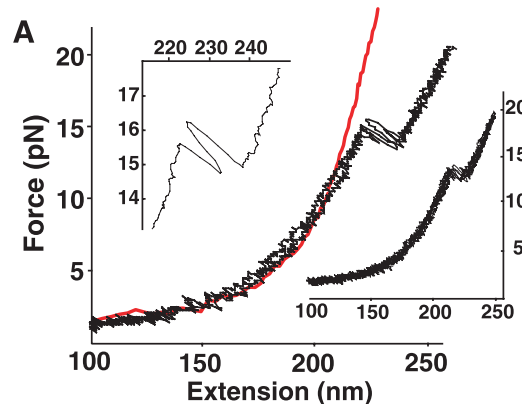


Figure 2: Main plot, black: trace of pulling an RNA hairpin. Main plot, red: trace of pulling DNA tethers alone, without an RNA hairpin. Upper left inset: Detail of folding and unfolding events of the hairpin. Lower right inset: A similar trace with different solvent conditions. Figure taken from [Liphardt, et al., Science, 292, 733-737, 2001](#).

- Provide an order-of-magnitude estimate of what you think the force should be to unfold the hairpin as a sanity check of the experiment. A useful number to have in your head: an RNA base pair has an energy of about 1.6 kcal/mol.
- We will now consider a two-state model for hairpin formation, where we have a hairpin state, “h,” and an unfolded state, “u.” Derive an expression for $p_h(F)$, the probability that the RNA strand is in a hairpin configuration, as a function of the applied force F , and the displacement Δx , the difference in the distance between the ends of the sequence of interest in the hairpin state versus the unfolded state. *Hint*: The force and the displacement of the ends of the hairpin are thermodynamic conjugate variables.
- By holding the beads in the optical trap at a constant force, Liphardt and coworkers could observe many folding and unfolding events. They could then compute the amount of time the RNA strand was in the hairpin state versus unfolded state. You can download these data for various pulling forces [here](#). Perform a regression to obtain the difference in energy between the hairpin and unfolded state.

Problem 4.2 (Calibrating an optical trap, based on problem 6.2 of *PBoC2*, 20 pts).

In Problem 4.1, we mentioned that an optical trap was used to hold a bead in place and exert a force to unfold an RNA hairpin. In this problem, we will explore how we might calibrate the trap to enable determination of force. Specifically, we know the center of the trap, since we can aim the optics, and we know the center of the bead, since we can measure it through imaging. Knowing these two things, we would like to compute the force.

We define the energy of the bead in the trap as $E(x)$, where x is the displacement of the bead from the center of the trap. The force exerted by the trap on the bead, is

$$f = -\frac{\partial E}{\partial x}. \quad (4.1)$$

In general, $E(x)$ can be a complicated function of x , but if x is small, we might approximate $E(x)$ as its truncated Taylor series expansion about $x = 0$, where the $E(x)$ is minimal.

$$E(x) = E_0 + \frac{kx^2}{2} + \mathcal{O}(x^3), \quad (4.2)$$

where k is called the trap stiffness and is formally given by

$$k = \left. \frac{d^2 E}{dx^2} \right|_{x=0}. \quad (4.3)$$

So, the force is $f = -kx$ for small displacements. Thus, if we know k , we can determine the force from the center of the trap and the position of the bead, the difference of which gives x .

To calibrate the trap, we will let a bead sit in the trap and rattle around due to thermal motion. We will take many many images to get a set of values for x . Show that if we average the square of these displacements, we can get an estimate for the trap stiffness using

$$k = \frac{k_B T}{\langle x^2 \rangle}. \quad (4.4)$$

Hint: Define $p(x)$ as the probability that the bead shows displacement x at equilibrium. Then,

$$\langle x^2 \rangle = \sum_x x^2 p(x) = \int \frac{dx}{m(x)} x^2 p(x), \quad (4.5)$$

where $m(x)$ is the density of states. This is necessary to convert the sum into an integral. We will assume that $m(x) = m_0$, a constant, meaning that the density of states is uniform in x . You might also want to look up Gaussian integrals to help you evaluate the definite integral you will encounter.

Problem 4.3 (Pulling DNA using a two-state model, 30 pts).

Later in the class, we will delve into polymer physics. In this problem, we will dip our toe in those waters using a two-state model.

We saw in Problem 4.1 that the early portion of the force extension curve involved pulling the DNA tethers until they were taut. We will develop a model to describe pulling a single segment of double-stranded DNA. We model the DNA polymer as a 1-D random walk, as described on page 341 of *PBoC2*. It consists of N segments, each of length a . Each segment can either point right or left. We stretch the DNA segment by holding the ends and pulling with a total force f . We take each segment to be independent of the others. We will ignore end effects and consider the end segments to be the same as all others.

- Write a states and weights diagram for a single segment of the polymer. *Hint:* Remember our discussion in lecture about deriving the statistical weights for generic thermodynamic potentials. The force should enter into your weights.
- From your states and weights diagram, derive the probability that a given segment points to the right.
- We want to find the probability that the end-to-end distance of a DNA segment under a force f is L . It is easier to note that $L = (2N_r - N)a$, where N_r is the number of segments that point to the right, and then find the probability of observing N_r . Write an expression for $P(N_r)$. Your expression from part (b) will be useful, and the binomial theorem may be useful as well.
- Show that

$$\langle L \rangle = \frac{1}{Z} \frac{\partial Z}{\partial(\beta f)}, \quad (4.6)$$

where Z is the partition function that appears in the denominator of $P(N_r)$ that you derived in part (c). Compute $\langle L \rangle$.

- Compute the magnitude of the fluctuations in L . I.e., compute the variance $\sigma_L^2 = \langle L^2 \rangle - \langle L \rangle^2$. How does the ratio $\sigma_L / \langle L \rangle$ depend on N ?

Problem 4.4 (Hill functions and the word cooperativity (20 pts)).

An activating **Hill function** is of the form

$$f(x) = \frac{(x/k)^n}{1 + (x/k)^n}, \quad (4.7)$$

where the parameter n is called the **Hill coefficient**. A repressive Hill function is

$$f(x) = \frac{1}{1 + (x/k)^n}. \quad (4.8)$$

Hill functions are often used to phenomenologically model systems we have been treating by using the machinery of statistical mechanics to mathematize cartoons of molecular interactions.

The word “cooperativity” is typically used in two different senses in molecular biology. In one meaning, “cooperativity” is the value of a phenomenological Hill coefficient. Another use is related to the added energy of binding a second ligand after a first is bound.

Consider cooperative binding of two repressors. Specifically, let J be the extra energy beyond the energy of the repressor-DNA interaction that is involved in the binding of the second repressor. Using the statistical thermodynamical approach as we have in class, write down an expression for fold change under the weak promoter approximation. In what limit is this expression equivalent to the phenomenological Hill function, (4.8) with $n = 2$? What is the value of k in equation (4.8) in terms of the values used in the expression derived from states and weights using statistical thermodynamics? Based on this analysis, how are the two different definitions of cooperativity related, if at all?