APh105c Homework 7 Due Date: Friday, May 19, 2006

"The mind is not a vessel to be filled but a fire to be kindled." - Plutarch

Reading: Read chaps. 18, 19, 32 and 33 of Dill and Bromberg.

A. Your Turn.

This problem must be submitted by email to RP, Dave and Frosso at the same time that the homework is due. Make sure you answer every part and give at least a brief explanation of your rationale.

(a) Explain the extent to which you have done the assigned reading. Do you look at the book?

(b) Which homework problem have you enjoyed the most? Which have you enjoyed the least? Make sure to explain.

(c) Is it useful to repeat derivations done in class during the homework? My reason for assigning these problems is this: I draw a strong distinction between knowing something and knowing about something. My sense is that the only way to really internalize stuff is to do it for yourself. Do you agree or disagree?

(d) Give a cogent statement of your opinion of the standing and role of statistical mechanics and thermodynamics in science. That is, to what extent is this stuff useful for your work? To what extent do you agree with C. P. Snow in his *The Two Cultures* who claimed that being ignorant of the 2nd law of thermodynamics is akin to being ignorant of Shakespeare's *Hamlet*?

(e) Give at least two examples of points of view or opinions or calculations or claims that have been made either in class or in homework with which you disagree. (f) If you were gossiping with a friend, what would you tell them was the biggest insight that came out of this course?

(g) Make a suggestion for a quote for the top of the homework.

A'. Beam Problem Revisited

I was disappointed to hear that many of you did not finish the beam problem (either at all or correctly). I assigned this problem because it is both interesting and important. I am happy to help anyone and everyone with how to tackle this problem and am reassigning it as extra credit for anyone that wants to have another crack at it. I think it is a total shame to punt on this problem and not see it through to the end.

1. Hydrophobic Effect: A Feeling for the Numbers

We continue with the theme of some of the interesting forces that arise in the crowded environs of the cellular interior. We have already examined depletion forces. A second hugely important class of forces are those associated with hydrophobicity. In class I gave a quick impression of the hydrophobic effect as an idea that is invoked often with great explanatory power. In this problem, you will estimate the magnitude of the interfacial energy that is assigned to having certain chemical groups in contact with water. This will give us an idea of how much free energy is gained when different molecules come into contact and sequester these hydrophobic structural elements. The essential argument is that the water molecules that surround the hydrophobic region of a molecule are deprived of some of their entropy because they can adopt fewer hydrogen bonding configurations. In particular, the water molecules are thought to form cages known as clathrate structures such as are shown in the accompanying figure.

(a) Estimate the entropy lost for each water molecule by appealing to the schematic of the tetrahedron shown in fig. 2. The basic idea is that if we think of the O of the water molecule as being situated at the center of the tetrahedron then the two H atoms can be associated with any two adjacent vertices (or, there are a total of six configurations). However, when in the presence of the hydrophobic molecule, one of the faces of the tetrahedron can be thought of as facing that hydrophobic molecule and hence all configura-



Figure 1: Schematic of the clathrate structure adopted by water molecules surrounding a hydrophobic molecule.

tions (three of the edges) facing that molecule are unavailable for hydrogen bonding. How many configurations are available now? Compute the entropy change of a single water molecule as a result of this configurational inhibition.

(b) Next, we need to estimate how many water molecules neighbor a given hydrophobic molecule. Consider the case of methane and ethane and estimate the radius of sphere that represents the hydrophobic surface area they present. Next, estimate how many water molecules neighbor these molecules and hence the total free energy difference because of the lost entropy. Convert your result into an interfacial energy and use units both of J/m^2 and cal/mol $Å^2$. Compare the result to the rule of thumb I quoted in class which is 25 cal/mol $Å^2$.

(c) Since we have said that hydrocarbons are hydrophobic, go back and examine the 20 amino acids and decide which residues are hydrophobic. Further, estimate the free energy cost for an alanine and a valine when they are put in water in isolation. Report your energies in units of kT.



Figure 2: Schematic of the arrangements available to a water molecule when in a complete network of other water molecules.

2. FRAP.

(a) In class I worked out the problem of two-dimensional FRAP. In this problem, you will carry out a similar derivation to that given in class, but we will also explore what happens when the photobleached region is not centered. Imagine a one-dimensional "cell" of length 2L (running from -L to L) and with initial concentration c_0 of the fluorescent molecule of interest which is uniformly distributed throughout the cell (note that for this onedimensional problem the concentration has units of number of molecules per unit length). How many molecules of the fluorescent molecule are there write an equation that gives this number? Now imagine that we photobleach a hole which runs from x_1 to x_2 somewhere in the interior of the cell. Before doing any calculations, explain what the final concentration (c_{∞}) will be after full relaxation and the system has returned to equilibrium. You may assume that once a molecule has been photobleached it is effectively dead and can be forgotten. Your goal now is to compute the recovery curve. What this means is that you need to work out how many fluorescent molecules are in the photobleached region as a function of time. Make graphs for two cases, one where the photobleached region is centered about the origin and one where the photobleached region is on one side of the cell. To be more precise, consider that the length of the photobleached region is $a = x_2 - x_1$. In the first case, $x_1 = -a/2$ and $x_2 = a/2$ while in the second case consider $x_1 = L - a$ and $x_2 = L$. Make plots of both cases and comment on how shifting the position of the photobleached region alters the dynamics of recovery. Make sure when you make your plots you use reasonable values for the diffusion constant - justify your choice. One of the uses of the FRAP technique is to determine the diffusion constant of various molecules within the cytoplasm of cells. Discuss how that might work on the basis of the derivation you have given here.

(b) Repeat the key points of the two-dimensional derivation given in class. Then, construct a graph that shows the concentration as a function of distance from the origin by using the table of zeros of the first derivative of $J_0(x)$ given in the file attached to the homework. Make sure you explain exactly what you are doing and what your results mean. Also, I want you to plot the result for different number of terms kept in the Bessel series. Try it for N = 10, 20 and 30 and compare the results. Comment on the goodness of the fit.

3. Solution to the Diffusion Equation for a Point Source.

(a) In class I sketched the solution of the diffusion equation by Fourier transformation. In this problem, I want you to repeat that derivation showing every detail to show that

$$c(x,t) = \frac{c_0}{\sqrt{4\pi Dt}} e^{-x^2/4Dt}.$$
 (1)

Use the Fourier transform convention

$$\tilde{c}(k,t) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} e^{ikx} c(x,t) dx.$$
(2)

Make sure you show all of your details. This includes demonstrating how you Fourier transform the diffusion equation (and in particular, how you Fourier transform derivatives). Once you have the Fourier transformed equation, you will need an initial condition - take $c(x, 0) = c_0 \delta(x)$.

(b) Formally derive the relation $\langle x^2 \rangle = 2Dt$ by computing the average explicitly using the solution from part (a). Then, make a plot of the relation between distance diffused and the time it takes using a log-log plot. Make plots for several choices of D - in one case use the typical diffusion coefficient for an ion species like K^+ or Na^+ , in the second case, use a typical diffusion

coefficient for a protein - justify your choice of diffusion coefficient by using the Stokes relation $D = k_B T / 6\pi \eta a$.

(c) Now imagine that you have spiked a one-dimensional "cell" (which is infinitely long!!) with a pipette that inserts GFP with a concentration c_0 between -a and a. Find the concentration everywhere at all times by integrating over the solution worked out in part (a) of this problem. Make sure you explain why this is the right thing to do using the linearity of the diffusion equation. Plot the solution for various times and show how the profile evolves over time. Also, explain why I claim the cell is infinitely long given our mathematical approach to the problem.

(d) Now consider a situation where you have the region from 0 to ∞ at concentration c_0 and the region from $-\infty$ to 0 at concentration 0. Use your Green function to write the solution in this case and get a closed form solution for the resulting spatiotemporal evolution of the concentration field. Make a plot of several time points in the life history of the system.

(e) Now derive the Green function for the three-dimensional case. Here you will take $c(\mathbf{r}, 0) = c_0 \delta(\mathbf{r})$. Make sure you explain all of the details of your calculation. Make sure you explain all of your Fourier transform conventions and that you show your work.

4. Wulff Plot.

One of the most powerful ideas in thinking about the equilibrium shapes of crystals is the so-called Wulff construction. In this problem, we will perform an elementary calculation which exhibits the key features of a Wulff calculation. Consider a rectangular prism with edge lengths a_x , a_y and a_z and corresponding surface energies γ_x , γ_y and γ_z . In addition, consider a fixed volume of material $V = a_x a_y a_z$. Write an expression for the total free energy of this material (on the assumption that the total free energy is dictated exclusively by the interfacial terms). Minimize this free energy with respect to the edge lengths and find the equilibrium shape. (Note: you can either eliminate a_z by using the volume constraint or use Lagrange multipliers). State a simple relation between the areas of the faces of the crystal and their distances from the center of the rectangular prism. Do the results jibe with your intuition that the faces with the largest surface energy should get the smaller area? If you want to read more about this, I recommend the excellent book by Jeff Tsao, Materials Fundamentals of Molecular Beam Epitaxy.

5. The Physics of the Pipette Aspiration Experiment.

In class we discussed how the pressure difference across a thin membrane balances with the surface energy density (tension) to produce a particular radius of curvature - this was called the Laplace-Young Relation. Recall the energy had two contributions, a term penalizing changes in tension over the area of the membrane and a term penalizing the pressure applied to the volume enclosed by the membrane:

$$E = \sigma A - V \Delta P \tag{3}$$

Here σ is the tension, A the membrane area, and V the volume enclosed by the membrane.

(a) Imitate the derivation given in class by assuming a spherical shape and use energy minimization to derive the Laplace-Young Relation. This relation accounts for two sources of energy - at least one other mechanical source of energy is missing - what is it? Later in this problem we will show why it can be ignored.

In our discussion of continuum mechanics a few classes ago, we tabulated the possible modes of deformation (i.e. stresses) to which a body can be subjected. Unlike the materials we discussed then, things like soap bubbles and biological membranes have the interesting property that their in-plane shear modulus is zero - what does that mean physically? In particular, let's examine biological lipid membranes. In the early 1970's E. Evans began the first direct mechanical measurements of the bending and area-stretch modulus of lipid membranes (he was examining red blood cells). A small glass pipe (a micropipette) a few microns in diameter was used to apply suction to a large spherical membrane. He realized the membrane's shearless mechanical properties meant the tension over the entire surface of the membrane had to be equal. By applying suction to the micropipette, the membrane measurably deforms into an azimuthally symmetric combination of a sphere, a cylinder and a hemisphere.

(b) Using the nomenclature on the figures below derive an expression for the tension as a function of the pressures and geometrical features of this closed membrane. Keep in mind we only have direct control over the pressure difference $p_3 - p_1$. (HINT: write two separate equations for the tension in the spherical sections).



Figure 3: Diagram of the geometrical features which characterize a deformed lipid membrane in a micropipette experiment. Assume this shape is azimuthally symmetric along the axis of the micropipette.

In order for the membrane to deform into this shape its area must change with respect to a fixed volume. Let us define the areal strain as:

$$\alpha = \frac{A - A_o}{A_o} \tag{4}$$

which simply quantifies the change in area and normalizes this by the original area A_o . It turns out lipid membranes are wonderfully linear elastic materials, which means the stress (tension) and the strain (areal strain) are related by a constant. This constant is the area-stretch modulus, $K_A \cong 58 \frac{k_B T}{nm^2}$:



Figure 4: Diagram of pressures in a membrane-micropipette experiment.

$$\sigma = K_A \alpha \tag{5}$$

(c) Using the fact that surface energy is tension times area:

$$E_{stretch} = \int \sigma(A) \, \mathrm{d}A \tag{6}$$

derive the Hookean energy from tension in the membrane as a function of the areal strain.

Something we have neglected up to this point is that the membrane has a preferred flat orientation (this is not always true, but this is the simplest case to examine), therefore some energy must be spent to bend it into this azimuthally symmetric shape. In the simplest cases, the bending energy is dependent on the *mean curvature* - a measure of the average degree of



Figure 5: Picture from an actual micropipette experiment using fluorescent lipids for visualization.

bending at each point on the surface. The mean curvature, $\overline{\kappa}$, is the sum of the minimum and maximum curvatures at each point on the surface:

$$\overline{\kappa} = \frac{1}{2} \left(\frac{1}{R_{min}} + \frac{1}{R_{max}} \right) \tag{7}$$

We said that lipid membranes were a linear elastic material, hence we expect the bending energy to be quadratically related to the mean curvature through the bending modulus (κ_b):

$$E_{bend} = \frac{\kappa_b}{2} \int (2\overline{\kappa})^2 \,\mathrm{d}A \tag{8}$$

On simple surfaces, like spheres and cylinders (hint hint), the mean curvature is constant.

(d) Use these facts to derive an expression for the bending energy stored in the deformed shape of fig. 3 as a function of the geometrical parameters. Apply conservation of volume to eliminate L_p and make an actual numerical estimate of the bending energy as compared to the tensile energy in Fig. 5. Show that you do not actually need the scale bar to determine the bending energy. A typical lipid membrane has a bending modulus of $20k_BT$ and a typical micropipette experiment produces areal strains on the order of $\alpha = 0.01$ - do we need to account for bending energy - why or why not? You may appreciate the fact that in general $R_v >> R_p$.

Next let's take a closer look at the failure mechanism of such membranes. Recall we did a homework problem where we calculated nucleation of a defect in a crystal by accounting for surface and bulk energies. Under tension, lipid membranes fail because holes that form in the membrane will grow unbounded once they reach a certain radius - consider this hole analgous to the crystal defect, only now we are working in 2D. The two competing energetic effects are the benefit of opening a hole in the presence of tension and the penalty of exposing the hydrophobic core of the membrane to water. The first terms scales like the area and the second like the perimeter of the hole:

$$E_{hole} = -\sigma \Delta A + l\gamma \tag{9}$$

where γ is the perimeter energy penalty ($\cong 1k_BT/nm$) and l is the perimeter length.

(e) Make a plot of this energy using an appropriate reaction coordinate for tensions corresponding to areal strains of 0.01, 0.03 and 0.05. Describe what happens to the hole nucleation barrier and what this would mean for the integrity of a membrane. In reality this is a kinetic process - find the height of the energy barrier and let's presume that when the barrier is $1k_BT$ high the process spontaneously occurs. To what critical tension and areal strain does this correspond? Using a nominal lipid membrane thickness of 4nm, find an everyday material that has roughly the same Young's Modulus. Using the membrane thickness and critical tension, what everyday material fails in this range of stress and areal strain (that has two distinct answers)?