PROBLEMS
(Week of 17 Feb 09)

1. Try your hand at problem 1 in Chapter 12 of the text book. And while you're there, add in the following additional issue. If the membrane you're thinking about in this problem is a biological membrane, there is protein in the membrane as well. Assume that the membrane is about 30% by weight protein. Then assume that the average molecular weight of membrane proteins is 60,000, and that the proteins are all nonhydrated cylinders with a density of 1.35 g/cm^3 and with about 1/3 of their mass imbedded in the membrane. How many molecules of protein are there per 1 μm^2, and what fraction of that surface is protein rather than lipid?

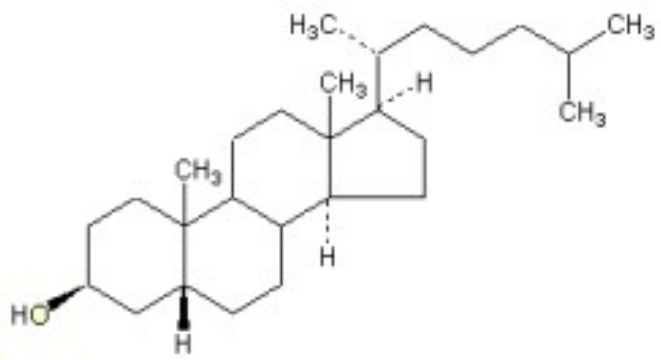
Assuming a MW for a phospholipid of 750, I got about 4 fg of phospholipid per μm^2, giving about 1.2 fg prot, or 12,000 protein molecules. If those molecules are cylinders about 10 nm high (putting 1/3 in the 3.4 nm membrane), their surface area is about 2.3 x 10^{-14} cm^2 apiece, making their total surface area about 0.03 μm^2 or about 3% of the surface area.

2. Try your hand at problems 2 and 5 as well.

3. In a competing textbook (Lehninger), the following problem is presented. A London doctor, Johann Thudicum, isolated and studied brain lipids about a century ago. Many years after his death, his vials were rediscovered, neatly labeled as, for example, "sphingomyeline" and "cerebroside". The question posed was how you might verify (using modern techniques) whether these were in fact the lipids in these vials corresponded to the names on the labels. The answer to this question does assume that you can guess at the efficacy of various separation techniques with only limited practical experience. But consider the following conundrum:. In the absence of modern techniques, what simple test might Thudichum have used to distinguish sphingomyelin from phosphatidylcholine.

The answer suggested thin layer chromatography of the samples, combined with comparisons to modern samples of known phospholipid types. However, elemental analysis (which Thudicum could probably do) would distinguish the two, and particularly the presence of twice as much N in sphingomyelin.

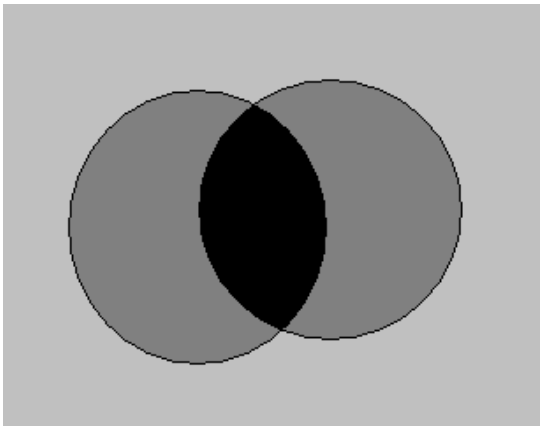
4. The formation of domains implies that cholesterol interacts more favorably with some phospholipids than with others. An ongoing argument is whether it is the headgroup or the side chains of the phospholipids that is critical for these favorable interactions. In a study of domains like those seen by Veatch and Keller, Samsonov et al showed that



enzymatic conversion of cholesterol to coprostanol (shown to the right) by cholesterol oxidase resulted in the disappearance of domains. Does this support one side or the other in the argument about what's important in the phospholipids?

Because it coprostanol is different in the body of the molecule, it suggests that the shape of the hydrophobic part of the cholesterol is important, which suggests in turn that interactions with phospholipid side chains are critical.

5. Speaking of puzzles, in looking at the dark patches in those cholesterol domain experiments, the following pattern is never observed:



Why might you expect patches to occasionally overlap in this fashion? What do you conclude from the fact that this pattern is never seen?

The composition of each leaflet of the membrane bilayer in these GUVs is the same, so you would expect that domains would form as easily in one leaflet as the other. So you might expect that some of these domains are in the outer leaflet, and some in the inner leaflet, and occasionally you would expect a domain in the inner leaflet to happen to overlap with a domain in the outer leaflet. The fact that they never do suggests that the presence of a domain in one leaflet has consequences for the ability of domains to occupy the same space in the other (either there is always another domain of the same size on the other side, or domains on opposite sides cannot approach one another laterally).

6. Compare the time required for a lipid to diffuse in the membrane from one edge of a fibroblast to the other edge (ca 30 micrometerss) if the membrane contains cholesterol compared to if it doesn't (and is not in the solid phase).

Using the expression $t = s^2/4D$, and a diffusion coefficient of about 10^{-7} cm^2/sec for a liquid leaflet without cholesterol, the time is about 25 seconds. For a D of 10^{-9} cm^2/sec for a liquid leaflet containing cholesterol, the time is about 2500 seconds, or about 40 minutes.

7. As noted in lecture, a difference in the area of the two leaflets of the bilayer will result in membrane bending. In real cells, the tightest turn that membranes seem to take has a radius of

about 25 nm. Calculate the fraction of phospholipids that would have to be moved from the outer to the inner layer in such a bent membrane to accommodate (or induce) such a curvature. (To make it simpler, do the calculation in two dimensions, not in three).

8. Draw the DSC profiles you would expect for mixtures of dilauroylPC (C12) and distearoylPC (C18) at molar ratios of 1:3, 2:2, and 3:1.

You would expect double peaks, one at ca 7º, the other at ca 67º, with the size of each determined by the amount of the C12 (7º peak) and C18 (67º peak).

9. The following sequence appears in the Ca-ATPase from rabbits (the one whose structure can be found as 1SU4.pdb, for example).

...STEIGKIRDQMAATEQDKTPLQQKLDEFGEQLSKVISLICVAVWLINIGHFNDPVHGGS
WIRGAIYYFKIAVALAVAAIPEGLPAVITTCLALGTRRMAKKNAIVRSLPSVETLGC...

a) Calculate the hydrophobicity of this sequence, using a window size of 19. You can do this by hand (or using Excel) starting from the solvent transfer energies given in lecture, or you can have it done for you using the hydrophobicity plotter at

http://athena.bioc.uvic.ca/tools/Hydrophobicity

b) From this plot, predict the parts of the sequence which are transmembrane domains.

The region from about residue 35 to 60, and the region from about 65 to 93

c) Recalculate the profile using a window size of 8 (in the plotter, there is a box on the plotting page that allows you to select the window size). Explain the difference in the profile that results from the change in window size.

The second TM domain is now divided in half, because of the presence of a charged amino acid (E) in the center of the sequence

d) The amino acids ...PEGL... in this sequence are highly conserved among cation-transporting P-type ATPases. Suggest why this might be so.

That's the E in the second transmembrane domain. A conserved anionic side chain in the middle of the bilayer might be involved in binding the transported cation when it is in the membrane.

10. The small unilamellar vesicles (SUVs) used by Hamilton's lab have a diameter of about 30 nm.

a) Calculate the surface area of these vesicles at the outer surface of the membrane, at the center

of bilayer (where the tips of the phospholipids meet), and at the inner surface of the bilayer.

About 900 nm^2, 700 nm^2, and 500 nm^2.

b) The phospholipids that make up biological membranes (with interesting and important exceptions) are generally shaped like cylinders (rather than cones). What does this fact suggest about the spacing of the headgroups in the outer and inner leaflets of SUVs?

They must be very crowded in the inner leaflet, and spaced quite far apart in the outer leaflet.

c) Given your answer to the last question, suggest an explanation for the increased flip rate of oleate in SUVs compared to LUVs (Large Unilamellar Vesicles).

The spacing between the lipids in the outer leaflet will mean that water can penetrate part of the way into the outer leaflet, which would make it easier for the hydrophilic headgroup of the oleate to get to the center of the membrane.

11. If you haven't already brought up the structure of the K channel (1BL8.pdb) in Protein Explorer on your own, it's too late - now this problem requires it. So bring it up, and compare what you see with Figure 13.21 in the text.

a) There are 4 objects in the selectivity filter, but one of them seems to be different from the other three. What is the different one?

It's a water molecule.

b) There's something really strange about two of the potassium ions. How could this strange situation be explained while retaining what we know about the laws of chemistry and physics?

The two overlapping ions can't really overlap, so there must be K+ ions at one of the positions in some of the molecules of the crystal, and at the other position in the remaining molecules of protein in the crystal.

c) Reconsider the final sentence in the legend to Figure 13.21 in light of the observed distribution of ions in the structure of the channel. Is that conclusion still valid? Is there any interesting differences in the conclusion which are suggested by the observed structure?

The basic idea in that last sentence (that repulsion between K+ ions keeps things moving in the channel) is still valid. But the observed distribution of ions suggests that one ion has two stable positions, one far enough away that the single ion may be comfortable, and one closer (with a water molecule between them as a cushion). If the first ion jumps to the closer position, it will tend to kick the comfortable ion out, which then leaves a vacant position which the first ion can move to occupy. Another K+ sneaking into the place with two possible stable positions begins the cycle again.