1. Consider the structural organization of the erythrocyte plasma membrane illustrated below, and answer all of the following questions. (The protein is often referred to as Band 3 from its relative mobility in SDS-PAGE.)

A. (10 pts.) Consider the lipid molecules illustrated, identify their parts and briefly describe how their organization is stabilized.

B. (5 pts.) Indicate clearly which side of the membrane is exterior and which side faces the cytoplasm, and briefly defend your designation.

As a strategy for answering these questions, first ask yourself 2 additional questions:

1. **What do the questions ask?**

   Question A. asks you to first identify each part of the lipid molecules in the diagram, referring specifically to them in the figure. Then, explain how the overall lipid organization is stabilized. *Note the two-part nature of the question: do the identifications in the first part aid your mechanistic explanation of their organization in the second part?*

   Question B. simply asks you to identify the exterior and interior of the membrane in the diagram, and you must provide a specific rationale for your conclusion.

2. **What questions are NOT being asked?**

   Neither question requires any functional or experimental information about red blood cells, to be answered correctly. Inclusion of such information is not only unnecessary but wastes valuable time. Focused, rather than “shotgun”, answers are better answers.

   Having first thought about the questions, reread them carefully and answer them in the space provided on the next page.

1A. Consider the lipid molecules illustrated, identify their parts and briefly describe how this type of lipid organization is stabilized.
1B. Indicate clearly which side of the membrane shown is exterior and which side faces the cytoplasm, and briefly defend your designation.

Now having answered the question for yourself; let’s examine 3 actual answers (left hand column) and marginal comments (bold type, right hand column) on the following pages. The answers have not been edited for grammar or spelling.
A. Consider the lipid molecules illustrated, identify their parts and briefly describe how this type of lipid organization is stabilized.

**Example 1.** The hydrophilic “heads” of the lipids are oriented towards the membrane surfaces, while the hydrophobic hydrocarbon “tails” interact to form a nonpolar inner layer. This type of lipid organization is stabilized by 2 forces: 1) Van der Waals forces—individual dipoles between hydrocarbon tails, 2) the exclusionary force of water forces the “burying” of the hydrocarbon tails.

A good start! More specificity is required: define “heads” and “tails” of the phospholipids to convey understanding of the lipid structure and their contribution to stabilization.

The stabilization forces are correctly identified but are not clearly related to the membrane components. Nor are they adequately described.

A “very good” answer!
Example 2. Lipids are amphipathic in nature, having polar heads (hydrophilic) and nonpolar hydrocarbon tails (hydrophobic). The lipids in the bilayer are arranged so that the polar hydrophilic heads face the polar cytoplasmic and exterior sides of the membrane with the nonpolar hydrophobic tails point toward each other and away from polar regions. This arrangement was proven by the Languimir trough experiment where lipids were dissolved in a nonpolar solvent which was placed on a film of water and then the solvent evaporated leaving the hydrophilic heads closest to the polar water and the tails (hydrophobic) pointing into the air.

This type of lipid organization is stabilized by cholesterol, polarity, and Van der Waals forces. The cholesterol acts as a mortar to fill in gaps between lipids thus stabilizing them. Polarity insures that hydrophobic heads will point toward each other, establishing a nonpolar environment. Van der Waals forces maintain the binding of the lipids. If the tails are saturated then Van der Waals forces are strong because of tight packing of the tails.

The components of lipids are first defined followed by a description of how the lipids are arranged in the membrane.

In the last sentence of the paragraph, experimental evidence for membrane structure is included although the question didn’t specifically ask for it. It is irrelevant in this context.

The first sentence is succinct and focused, but the following sentences are wordy and don’t clearly explain how “polarity” and Van der Waal interactions work. The importance of water on either side of the membrane is ignored.

A “good” but rambling answer! This student had trouble finishing the exam!
Example 3. The lipid molecules seen in this diagram are phospholipids. The parts shown in this diagram are the phosphate head and the two hydrocarbon tails. The phosphate head is the round part on the outer membrane. The tails are the squiggly lines coming from the head. The glycerol molecule is not shown in this diagram. It is the link between the head and the tails.

This organization is stabilized firstly by the amphipathic qualities of the lipids. The heads are polar. The tails are nonpolar. Thus, the two do not attract each other. Rather there is some attraction for the same kind. Therefore, heads are attracted to heads, tails to tails. Once this arrangement is established, molecular forces begin to further stabilize the structure. The chains are held together by Van der Waals forces between atoms of adjacent chains.

This answer first describes the diagram using specific language to define parts of the lipid (phosphate head, hydrocarbon tails).

The sentences used to describe how the lipids are stabilized are simple and progress logically from one thought to the next. Actually, they heads of the PL are “attracted” to water dipoles and repelled by each other.

A “much better” more focused answer!

B. Indicate clearly which side of the membrane shown is exterior and which side faces the cytoplasm, and briefly defend your designation.

Example 1. Carbohydrates are attached to the exterior surface only.

Short and sweet, and an excellent answer! But...the student failed to identify the carbohydrates in the diagram!

Resist the temptation to fill all the available space with an answer!
Example 2. The side with the carboxyl group (COO-) and the amine (NH3) face the cytoplasmic side because the transmembrane protein passes the membrane eight times, making both ends remain on the cytoplasmic side. If the transmembrane protein passes an odd amount of times, then because of polarity, the polar COO- group end will remain on the polar exterior and the nonpolar NH3 remains in the relatively less polar cytoplasmic side. A group of molecules attached to the transmembrane proteins are found on the exterior of the membrane because they are used to help erythrocytes bind to one another.

The location of the amino and carboxy terminals has no necessary relationship to which side of the membrane faces the cell’s exterior.

The cytoplasm and the cell’s exterior are aqueous and equally polar. Too much memorized detail, poorly related to the question.

What is the “group of molecules”?

Function information irrelevant!

A very long, illogical and badly “garbled” answer.

Example 3. On the diagram, the region below the membrane is the exterior. This is made clear by the branching carbohydrate attached to the integral protein.

This question is concisely answered by reference to the diagram, providing supportive evidence.

At this point you should critique your own answers to the questions and discuss them amongst yourselves. You could also try modifying the problem with additional questions of your own: for example, what level of protein organization is depicted in the box identified by the arrow? What is the name of the specific protein structure in the box? What is the function of Band III protein, and how is its overall structure related to that function?

Now on the next page, consider a more complex problem concerning plasma membrane organization? Note in particular that some questions refer to how these proteins are synthesized, a topic that you may not have covered yet. Not surprisingly, this question was taken from a final examination. Try answering those parts you can, now, and return to the question as the course progresses.
2. Carefully consider the following cross-sectional diagram of protein organization in a biological membrane, and answer all of the following questions. Note that space constraints prevent all the –amino and –carboxy terminals from being labeled.

A. (9 points) Which of the six (6) numbered proteins are peripheral membrane proteins? are integral membrane proteins?

B. (6 points) Briefly describe how you would test or verify your designations in A.

C. (12 points) Hypothesize likely functions for proteins 1, 2 and 6 and briefly explain the bases for your hypotheses.
D. (5 points) Briefly describe how you might test your hypothesis concerning any one of these proteins.

3. (30 pts) The cDNA of a gene coding for an interesting membrane protein has been cloned, amplified and sequenced, and the amino acid sequence of its probable translation product inferred from the nucleotide sequence. The protein contains 240 amino acids and exhibits an
apparent molecular weight in SDS-PAGE of approximately 30 kDa. A hydropathy plot of the protein's primary structure is presented in the figure below, numbering the amino acids from the amino terminal end to the carboxy terminal as is customary (designated "N" and "C" respectively).

Consider these data and answer all the following questions:

A. (3 pts) Why is the protein likely to be an IMP?

B. (4 pts) How many times does this IMP span the membrane? Briefly discuss the basis for your answer.

C. (4 pts) If a reagent for detecting the amino terminus labeled all of this protein in intact cells (and no additional protein was labeled in leaky cells or ghosts), where is the carboxy terminus likely located? In the cytoplasm or in the extracellular space? Why?

D. (3 pts) What is the likely secondary structure of protein in those regions spanning the membrane?
E. (4 pts) Two of the several hydrophobic regions of the protein each contain 2 clusters of polar amino acids; each of these clusters contains 1 or 2 polar amino acids. Using 4 arrows indicate the likely locations of these clusters in the figure above.

F. (8 pts) Assuming these polar clusters are functionally important, hypothesize a likely function for the protein, draw a reasonable 3-D model for its membrane organization in the space below and briefly discuss how the organization is related to its function.

G. (5 pts) How would you test your hypothesis concerning its function and what would the results show?

4. (34 pts) The current model of the plasma membrane emphasizes its asymmetric properties, in terms of both structure and function. How were these asymmetric features
Membrane Structure

**established?** Consider this question carefully, in light of what you have learned so far this term and ALSO in light of the following data, and answer A., B., C., D., and E. below.

i. Exposed tyrosine residues of membrane proteins can be labeled with $^{125}$I and membrane carbohydrate that have first been oxidized can be labeled by reduction with tritiated borohydride ($^{3}$H-BH$_4$). Both these reactions are catalyzed by enzymes that cannot cross the plasma membrane.

ii. When applied to intact mammalian erythrocytes, and the proteins extracted and analyzed by SDS-PAGE, the same bands are labeled by each technique. These represent 5 of the 20 or so major bands evident when the gel is stained for protein. Two of the 5 bands are glycophorin and the anion transporter (Band 3).

iii. All 5 bands are also stained by the Periodic Acid-Schiff reaction (PAS+); none of the PAS-negative bands is stained by either reaction.

iv. Detailed sequence analysis of glycophorin indicates the sites of both $^{3}$H and $^{125}$I labeling are located near the amino terminal end of the protein. Similar analysis of Band III presents a more complex picture: $^{125}$I is found at several, periodically separated sites some distance from either the amino or carboxy terminal ends; these sites are between 70 and 120 amino acids apart. Carbohydrate labeled with $^{3}$H in Band 3 is found exclusively at one location about 50 amino acids from the carboxy terminus.

v. When these labeling reactions are carried out on the cytoplasmic side of resealed ghosts, virtually all SDS-PAGE bands are labeled with $^{125}$I; none is labeled with $^{3}$H-BH$_4$ or stained by PAS.

A. (4 pts) Briefly describe SDS-PAGE, indicating clearly how it provides useful information in this example.
Membrane Structure

B. (10 pts) Draw a cross-section of the erythrocyte plasma membrane that accounts for its major structures and functions. Clearly label all components and indicate possible locations for the both the tritium and radioactive iodine labels.

C. (6 pts) Briefly describe the asymmetric features of your diagram and explain how they explain data i-iii and v above.
D. (8 pts) Using a higher magnification, cross-sectional diagram, describe the probable membrane organization of both glycophorin and the Band 3 protein and indicate clearly how your models explain data iv above (and are consistent with the rest of the data).

E. (6 pts) Answer either of the following two questions.

i. If the Na⁺/K⁺-pump were labeled by tritiated boron hydride, explain briefly why the label might not have been evident in a discrete SDS-PAGE band and why the labeling might not have inhibited the pump’s action.

OR

ii. If the labeling procedures had been carried out on an intact intestinal epithelial cell, briefly describe how the labeling pattern would compare (and contrast) with that obtained for erythrocytes.
5. About sixty years ago, Gorter and Grendel estimated that erythrocytes contain sufficient lipid to form a bimolecular lipid plasma membrane. With the benefits of more modern techniques we now know these pioneers made two errors in their measurements:

- Their lipid extraction procedures (with acetone) removes only about 75% of RBC lipid. More stringent extraction—using chloroform and methanol—removes 100% of the lipid.

- Their estimates of RBC surface area were made on stained blood smears (a thin, stained film of dried blood) and yielded an average value of 99.4 $\mu^2$ per cell. More recent measurements using Nomarski differential interference microscopy and living cells suspended in serum indicate a surface area of 138 ± 17.3$\mu^2$.

A. (8 pts) How did Gorter and Grendel reach their conclusions. What sorts of measurements did they perform and how did the results lead to their conclusion?

B. (5 pts) Assuming the recent determinations are more valid, how might the original inference of Gorter and Grendel be critically re-evaluated? Be specific.

C. (8 pts) Do the new data provide any additional information concerning our modern view of plasma membrane organization? Support your answer with a brief discussion.

6. Here's a slightly more complex and interesting version of Problem 5:
Membrane Structure

(The membrane of a canine red blood cell has a surface area of about 195 \( \mu^2 \), is about 75 Å thick and contains 0.7 picograms (1 pg = 10^{-12}g) of lipid and 0.8 pg of protein. The lipid consists of approximately equal numbers of phospholipid and cholesterol, which have molecular weights of about 800 and 380 respectively. In a tightly compacted, model monolayer in a Langmuir trough, each phospholipid occupies a surface area of 0.55 nm\(^2\) and each cholesterol, 0.38 nm\(^2\). In answering the questions below, show all calculations.

A. (5 pts) If one assumes an average molecular weight of 60,000 for RBC membrane protein, how many protein molecules are there associated with a single canine RBC membrane?

B. (8 pts) What is the ratio of lipid to protein, on a weight basis? On a molecular basis?

C. (5 pts) What proportion of the total canine RBC surface area is occupied by lipids?
D. (8 pts) What assumption(s) did you make in calculating your answer to C. above? How does your model of membrane organization change if the assumption(s) change?

7. (15 pts) *Tetrahymena* is a free-living, unicellular, ciliated organism that normally lives and thrives at 18-22°C. If these creatures are suddenly transferred to an environmental temperature of 8°C, they die. However, if the temperature is lowered gradually to 8°C over a period of several days, most survive and continue to grow, albeit at a slower rate.
Many changes characterize the gradual adaptation of these organisms to lower temperatures. In particular, their amphipathic lipids are found to contain fatty acids with shorter hydrocarbon chains and more unsaturated hydrocarbon bonds than found in cells growing at 18°C.

A. (10 pts) Discuss specifically how these changes in lipid composition most likely contribute to survival at the lower temperature.

B. (5 pts) If you were to extract the amphipathic lipids from *Tetrahymena* grown under the two temperature regimes and spread them out in a monolayer, how would you expect the average surface areas occupied by each lipid to compare?