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 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.

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.