

PLEASE WRITE YOUR ANSWERS ON A SEPARATE SHEET

Topics:

Sugars
Polysaccharides
Lipids
Membranes
Membrane proteins
Glycoproteins and proteoglycans
Unknown Assignment

1) Fluorescence recovery after photobleaching (FRAP) is a common experiment used to study lipid bilayers and biological membranes. The experiment involves selectively labeling a molecule on the surface of a cell or within the membrane one wants to study. A laser is then used to destroy (photobleach) the fluorophore from a small spot. What happens as a function of time to the dark spot generated by the laser flash? What conclusions can be drawn from this type of experiment?

An example of a movie from a FRAP experiment involving a fluorescent membrane protein can be found at the URL below:

<http://www.bch.bris.ac.uk/staff/bigb/Movie3.htm>

2) The lipid portion of a typical membrane bilayer is about 3 nm thick.

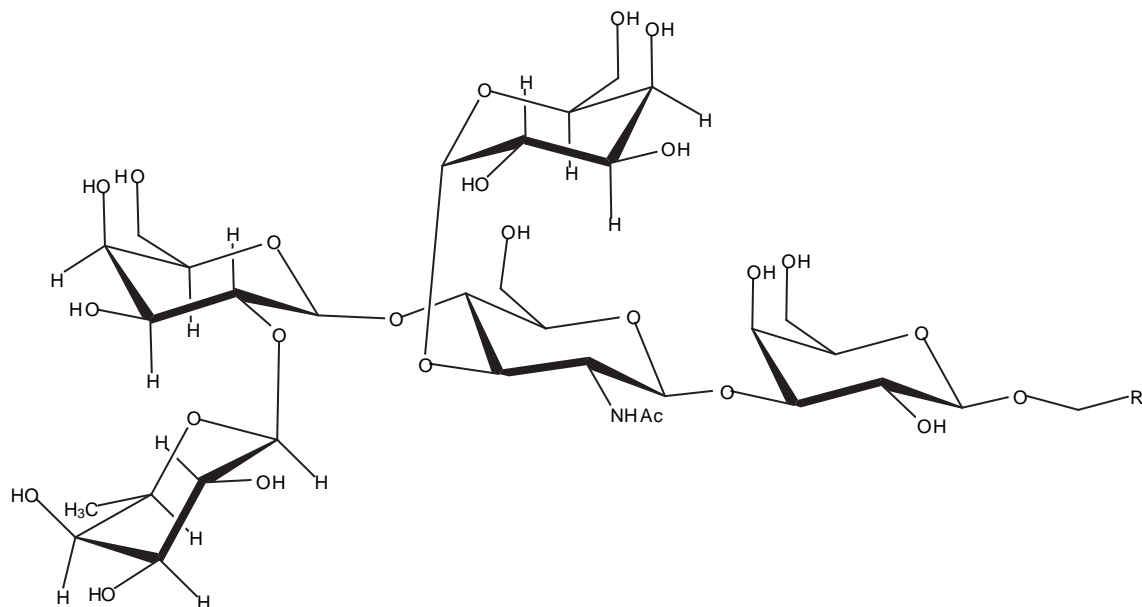
- a) Calculate how many amino acids residues of alpha helix are required to just span that distance.
- b) The epidermal growth factor receptor has a single transmembrane helix. Find it in the sequence below. Explain the reason for setting the boundaries where you have chosen them.

...RGPKIPSIATGMVGALLLVVALGIGILFMRRRH...

- c) Beta structures can also be used to span biological membranes. What features would you expect to observe in the primary structure, secondary and tertiary structures of such a protein and how would these differ from the transmembrane helix from epidermal growth factor from part b?
- d) How would you classify the *functional* differences between the two transmembrane elements you have described in parts b and c?

3) Scientists at several universities and medical schools have been exploring the idea of vaccines against certain types of cancers. The way vaccines generally work is that they challenge the body's immune systems with a non-infectious antigen derived from the natural system (such as the coat protein from an inactivated virus). In that way, when the body later encounters the

natural agent, it is already prepared to fight it with the immune response. Cancer vaccines currently in clinical trials are against a complex synthetic carbohydrate such as the one shown below.



Structure of the Le^Y antigen

- Identify the monosaccharide components of this pentasaccharide (The parent hexose of each monomeric unit can all be found in figure 7.2).
- What are the linkages between each of the monosaccharide units?
- Identify the reducing end of the pentasaccharide if there is one.
- Explain why this approach toward fighting certain types of cancers (in particular metastatic breast cancer) is plausible by describing the natural role of structures such as the one shown above and where they might be found in biological systems.

DO THE WORKSHEET ENTITLED “LEARNING TO USE CHIME AND PROTEIN EXPLORER” BEFORE PROCEEDING TO PROBLEMS 4 AND 5.

4) Learning to view biological structures. Include with your problem set the printout from p. 4 of the “Learning to Use Chime and Protein Explorer” worksheet.

5) Access the PDB file on your unknown protein. You may already have the PDB record number from your visit to the nice-zyme page on ExPASy. If you do not have it, type in the name of your protein in the line labeled “**search the archive.**” There may be multiple structures.

You may pick any of the relevant structures but questions 5 and 6 may be easier or harder depending on which one you select.

- a) Complete the worksheet, “Record of 3D Macromolecular Structure Observations for Protein Explorer” based on observations of your unknown protein.
 - b) Print out representative views of your protein in as a space filling model. Print out *equivalent views* in cartoon mode. Turn in those print outs with this problem set. In both views, change the color of the 10th residue from the N terminus (due to the way proteins are numbered in the PDB, that may or may not be residue 10) so that it can be seen distinguished from the rest of the molecule. (This part of the assignment is to assess whether or not you have learned to use the Chime plug-in to manipulate the structure).
- 6) Write a short paragraph (200 words or less – typed please) that describes the juxtaposition of secondary structure elements in your protein. Illustrate your discussion with an appropriate figure of your protein (made in Protein Explorer) as if it were to be included in a research paper. An example of a structure description can be found posted on the class web page. Please see a writing tutor if you have difficulty with this assignment. You will be graded on content and clarity as well as organizational, stylistic and grammatical considerations. (**NOTE:** This question will constitute a large portion of the total grade on this problem set. Please spend an appropriate amount of effort on this paragraph showing us that you have learned to look at and think about protein structure.)