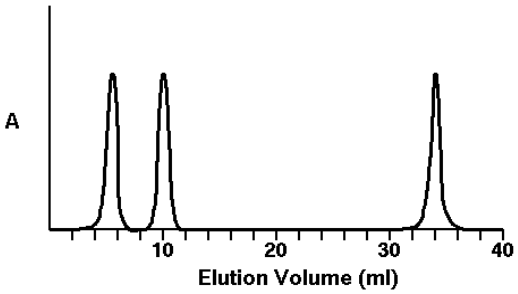
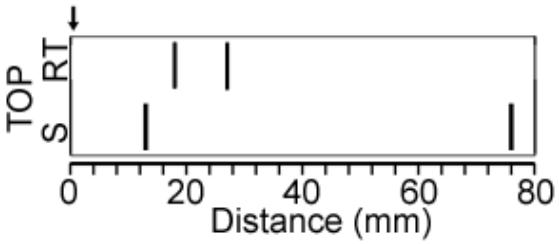
Problem Set 8

1. (17 pts, 25 min) Two experiments were performed to determine the quaternary structure of HIV reverse transcriptase. In these experiments, HIV reverse transcriptase was mixed with equal masses of two proteins with known molecular weights of 10 KDa and 80 KDa (i.e. molecular weight standards). Both of the standard proteins consisted of a single polypeptide chain. This mixture was then separated by size exclusion or by SDS-PAGE electrophoresis. Note that molecular weights determined by either technique are generally accurate to within ~10%.



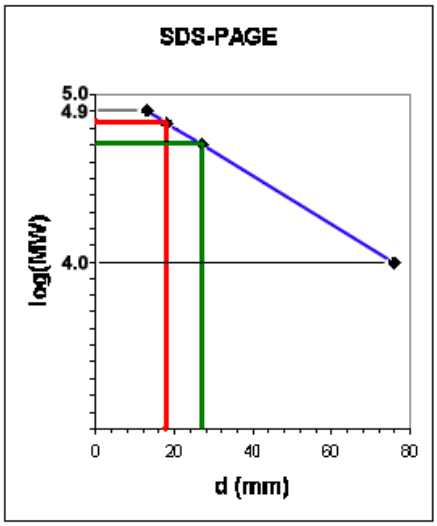
Size Exclusion Column: The absorption at 280 nm, as a function of the elution volume, is shown to the right. The 1st peak is the HIV reverse transcriptase. This was determined by incubating that fraction with RNA and the building blocks for DNA and detecting the production of DNA.



SDS-PAGE. The SDS-PAGE gel is shown on the right (turned sideways). The bands indicate regions of the gel that are stained with a stain for protein. The thicker the band, the more protein. The arrow marks the top of the gel (where the mixture of proteins would be applied to the gel before the electric field would be turned on). The lower scale gives the distance from the starting point. The lane marked S contains the standards. The same pattern was seen whether BME was present or not.

i) Would the assay that was used on the size exclusion column (DNA synthesis) work to determine which bands are reverse transcriptase in the SDS-PAGE gel? Why or why not? (2 pts)

No, because treatment of the protein with SDS will denature the enzyme, inactivating it.



ii) Use the SDS-PAGE gel to determine the molecular weight(s) of the polypeptide chains that are present in this enzyme. Be sure to show your work (5 pts).

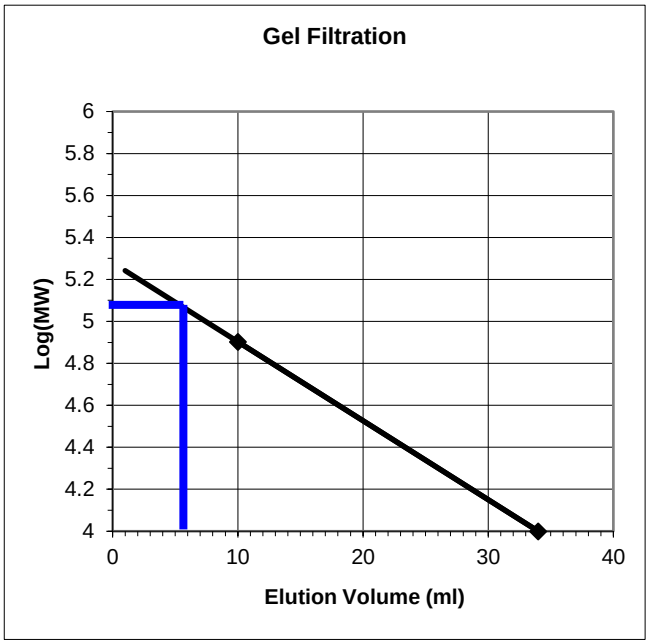
The steps are:

1) Generate a plot of log(MW) versus distance migrated using the known molecular weights as standards.
2) Draw a line through these points to generate a calibration curve. The log(MW) for the 80,000 Da standard is 4.9 and that for the 10,000 Da standard is 4.0.
3) Use the distance migrated of the unknown to find its log(MW), take antilogs to find MW.
   - The protein that migrated 18 mm has a log MW of 4.83 or a **MW of about 66 KDa**.
   - The protein that migrated 27 mm has a log MW of 4.7, giving a **molecular weight of 50 KDa**.
   - The relative intensity of the two bands is 1:1, **so the ratio of α:β is 1:1**.

iii) Use the elution profile from the size exclusion column to determine the native molecular weight of reverse transcriptase (5 pts).

The steps are:

1) Generate a plot of log(MW) versus elution volume using the known molecular weight standards (10,000 and 80,000 Da).
2) Draw a line through these points to generate a calibration curve.



3) Use the elution volume of the unknown protein to find its log(MW), take antilog to get the molecular weight.

The 10 KDa protein elutes at 34 mls and the 80 KDa protein elutes at 10 mls. The plot of log(MW) versus elution volume is shown to the right.

- The reverse transcriptase elutes at 6ml, giving a molecule weight of about **120 KDa**.

iv) Determine the quaternary structure of HIV reverse transcriptase by combining the information from the size exclusion data with that from SDS-PAGE. Indicate the presence of any disulfide bonds. Please show your work (5 pts).

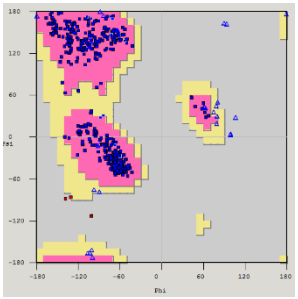
Possible quaternary structures and their molecular weights are:

$\alpha_1\beta_1$ = 116 kDa

$\alpha_2\beta_2$ = 232 kDa, etc.

The only model that is also consistent with the size exclusion data is αβ – HIV reverse transcriptase is a **heterodimer** – it has two subunits of different size.

2. (5 pts, 10 min) An electron density map can be viewed on a Jmol page by selecting the link to jmol_xray on the Jmol link page. The buttons on this page will trace the main-chain through this electron density as well as give you some choices regarding the sidechain of the residue. Determine the amino acid sequence that best fits the experimental electron density. Briefly justify your answer.



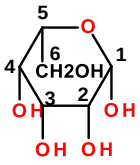
You should have selected residues whose side chains best fit the experimental density. The correct sequence is: **Gly-Leu-Phe-Ala-Gly** (Asp is a close fit for the second position)

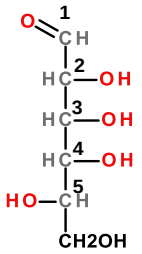
You had to give the correct amino acid name, plus a brief description that the sidechain fits the density.

3. (5 pts, 5 min) We recently determined the structure of a protein in our lab and the Ramachandran plot for the final fitted structure is shown on the right. Is our model of the structure likely correct, or not? Briefly justify your answer.

Yes – the structure is likely correct because the phi and psi angles for most of the residues fall in the low energy region of the Ramachandran plot, as you would expect if the model represented a folded protein in its lowest energy configuration. An incorrect structure would have many points in the gray (high energy) region.

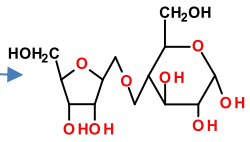
4. (6 pts, 5 min) Draw the reduced Haworth representation for the **L form** of the sugar shown on the right. Assume the configuration of the anomeric carbon is α. Diagram is shown on the left, note that the C6 carbon is pointing down because this is an L-sugar.





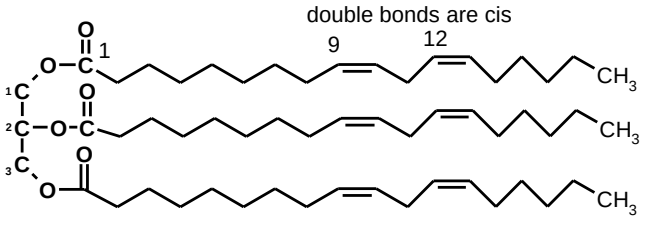
5. (5 pts, 5 min) Name the disaccharide shown to the right.

β-ribofuranosyl-(1-4)-α-glucopyranose



6. (5 pts, 5 min) Corn oil is a triglyceride with a high concentration of linoleic acid (C18:2 *cis*,*cis*-Δ^9,Δ^{12}) as the fatty acid component. Draw the triglyceride and explain why corn oil is a liquid at 20 C.

The two cis double bonds in each fatty acid are effective at disrupting the van der Waals interactions between the fatty acid chains, decreasing the melting temperature. **Note**: they must be cis to cause a kink in the acyl chain of the fatty acid (the drawing at the right does not show the kink, but has the cis configuration).



7. (5 pts, 5 min) Corn oil margarine, a substitute for butter, can be made by chemically altering corn oil. The reaction does not change the number of carbons, but raises the melting temperature so that margarine is a solid at 20 C. What is the chemical alteration?

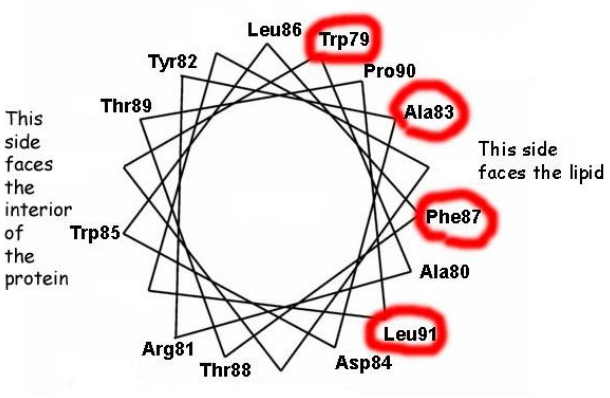
The double bonds are reduced to single bonds by the addition of hydrogens, a process known as hydrogenation. This increases the van der Waals attraction between the chains, raising the melting

temperature so that margarine is a solid at room temperature. (A by-product of this modification is the production of trans-fatty acids, which have negative health consequences.)

8. (8 pts, 15 min) View the Jmol page that displays the membrane protein bacteriorhodopsin by following the link to ***jmol_br***. The following is the polypeptide sequence of part of one of the α-helices in this protein.

Trp_{79}-Ala_{80}-Arg_{81}-Tyr_{82}-Ala_{83}-Asp_{84}-Trp_{85}-Leu_{86}-Phe_{87}-Thr_{88}-Thr_{89}-Pro_{90}-Leu_{91}

i) Beginning with Ala_{80} as the second residue, write the name of the amino acid on the 'helical wheel' that is shown on the right (Trp_{79} has been done for you). The helical wheel represents a "top view" and shows the projection of amino acid sidechains from the helix with a line connecting sequential residues.

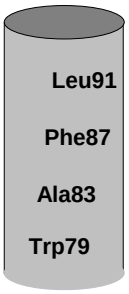


ii) The angle between successive spokes or positions on this wheel is 100°. Why? (Hint: Consider the geometric properties of an alpha helix.) There are 3.6 residues per turn in an alpha helix, so when you go from one residue to the next, the sidechains will be 100 degrees apart: 100 x 3.6 = 360 degrees = one turn.

iii) View the Jmol page and circle the residues names on the wheel that point outward from the protein towards the lipid and indicate whether they are predominately polar (p) or non-polar (np).

$\underline{Trp_{79}}$-Ala_{80}-Arg_{81}-Tyr_{82}-$\underline{Ala_{83}}$-Asp_{84}-Trp_{85}-Leu_{86}-$\underline{Phe_{87}}$-Thr_{88}-Thr_{89}-Pro_{90}-$\underline{Leu_{91}}$

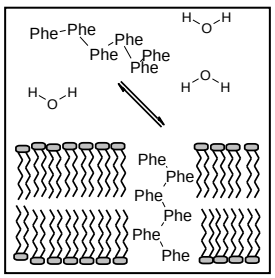
The residues that are exposed to the lipid are underlined in the sequence and circled in the diagram. All are non-polar, as you would expect because they are interacting with the non-polar acyl chains.



iv) In what way does the pattern of polar and non-polar residues on the helical wheel relate to the orientation of the helix with respect to the lipid acyl chains? Why is this arrangement energetically favorable?

The underlined residues in part iii are all on one side of the helical wheel. Since this is a "top-view" of the helix this means they will all be on the same side of the helix – facing the non-polar lipids, as shown in the "side view" to the right. This is energetically favorable because of the hydrophobic effect – the release of ordered water that occurs when the protein was inserted into the membrane.

9. (8 pts, 15 min) A peptide that is 20 residues in length is mixed with pure lipid bilayers in aqueous solution. The concentration of the peptide in the membrane and in solution is measured. From this measurement it is possible to calculate an equilibrium constant for the transfer of peptide from the solvent to the membrane (this type of equilibrium constant is often called a partition coefficient): $K_{EQ} = [P_{Membrane}]/[P_{H2O}]$. Note that the peptide is either completely in solution or completely buried in the membrane, as indicated in the diagram on the right that shows the equilibrium for Phe_6 (*part ii assumes a different composition, i.e. Ala+Phe*).



i) What secondary structure will a 20 residue peptide most likely assume in the membrane? Why? (2 pts).

Since it is an isolated peptide in the membrane it must form hydrogen bonds with itself; there are no donors or acceptors in the lipid. This is only possible for an α-helix or a β-barrel (but not a β-sheet). A 20 residue helix just fits across a typical membrane so a helix is more likely. 20 residues would only form a 3 or 4-stranded sheet, which can't really form a barrel. **Because the secondary structure is a helix ALL of the sidechains would be exposed to the lipid.**

ii) Assume that the 20 residue peptide contains *n* alanine residues and (20-*n*) Phe residues. Calculate the number of Ala and Phe residues in this peptide such that an approximately equal amount of the peptide will be found in solution and in the membrane (i.e. $K_{EQ} = 1$). You should find the value of *n* that is closest to giving a K_{eq} of 1. You should use the values of free energy of transfer for Ala (-0.5 Kcal/mol) and Phe residues (- 2.5 kcal/mol) that are given in lecture 26 (6 pts).

If equal amounts of peptide are found in the membrane and in solution, then ΔG^o = 0 (K_{eq} =1). The free energy for transfer of the peptide into the bilayer can be considered to be the sum of the contribution from the mainchain (+1 kcal/mol/residue- a total of +20 kcal/mol) and the sidechains. The contribution from the sidechains can be estimated from the free energy of transfer for amino acid sidechains, -0.5 kcal/mol/residue for alanine and –2.5 kcal/mol/residue for Phe. The overall free energy is:

ΔG^o = +20 – (+0.5 n + 2.5 (20-n)), where n is the number of Ala residue.

Setting this equal to zero and solving for n: 0 = +20 – 0.5n -50 + 2.5 n, 0 = -30 +2.0n , 30 = 2 n

This gives n=15. Therefore a 20 residue peptide containing 15 Ala residues and 5 Phe residues will partition equally between the aqueous phase and the lipid bilayer.

10. (10 pts, 10 min) View the Jmol page on the potassium channel by selecting the link ***jmol_k_channel*** on the Jmol links page. Please answer the following questions.

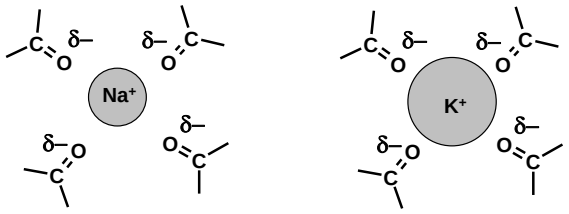
i) Potassium ions can be seen at three sites, site A, site B, and site C. Site B is within the selectively filter of the channel while A and C are at the entry and exit to the filter. Briefly describe how the ligands (groups and/or molecules) that interact with the potassium ion differ in these three environments, i.e. what happens to the K^+ as it goes through the channel?

At the entry or exit sites the ion is hydrated, it is coordinated with water molecules.

In the channel the ion has become desolvated and is only interacting with the C=O groups of the amino acid mainchain atoms in the selectivity filter.

ii) A series of buttons allow you to change the ion in the central channel, at site B. Based on the interactions between the metal ion and the groups in the central channel briefly explain, in terms of molecular interactions, why the channel is selective for potassium ions, while larger or smaller ions cannot go through the channel.

During transport of the ion through the channel, it is necessary to replace the energetically favorable interaction with the water with another favorable interaction, in this case with the C=O groups in the channel. The optimal size will provide favorable interaction with the negative partial charges on the C=O oxygen atoms and the positive charge on the ion. The closer these are together, the better the interaction. Both Li^+ and Na^+ are smaller than K^+ so the charges are more separated (more so for Li, of course). Rb^+ is too large, although the electrostatic interaction would be favorable, there is unfavorable van der Waals contact with the C=O groups.



You can view the dehydration of the ion to be the same as the activation barrier for an enzyme, and the K-channel lowers this barrier by forming more favorable interactions with the K ion than other ions.

11. (6 pt, 10 min) The rate of transfer of potassium ions as a function of $[K^+]$ is shown on the right for a solution of 1 nmol of channels (in membranes of course).

i) Calculate k_{CAT} for this "enzyme" (3 pts). Vmax is ~1.0 mmoles K/sec, so kcat = V_{max}/E_t = $1x10^{-3}/1x10^{-9}$ = 10^6/sec, i.e. a single channel can transport 10^6 potassium ions per second.

ii) How would this plot differ if Rb ions were also present in solution? Would K_M or V_{MAX} change? (3 pts). Rb, being similar in charge and only a bit larger in size than potassium would likely be able to enter the top part of the channel. But it can't fit into the selectivity filter, so it would act as a competitive inhibitor, therefore only K_M would change, V_{MAX} would remain the same.