1. (10 pts, 20 min) Two experiments were performed to determine the quaternary structure of HIV reverse transcriptase as well as the molecular size of each subunit. 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 (right diagram, upper) or by SDS-PAGE electrophoresis (right, lower).

Size Exclusion Column: The absorption as a function of the elution volume is shown to the right. The 1st peak is the HIV reverse transcriptase.

SDS-PAGE. The SDS 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.

i) 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).

ii) Determine the quaternary structure of HIV reverse transcriptase by combining the information from the gel filtration data with that from SDS-PAGE. Indicate the presence of any disulfide bonds. Please show your work (5 pts). (Note: Molecular weights determined by either technique are generally accurate to within 10-15%).

i) SDS-PAGE: 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.

ii) You want to find the native molecule weight from the gel filtration data to determine how many copies of each α and β-subunits you need.

   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.

Possible quaternary structures and their molecular weights are:

\( \alpha_1\beta_1 = 116 \text{ kDa} \)
\( \alpha_2\beta_2 = 232 \text{ kDa}, \text{ etc.} \)

The only model that is also consistent with the size exclusion data is \( \alpha\beta \)-HIV reverse transcriptase is a \textbf{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: \textbf{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 (triangles are Gly residues).

i) Is our model of the structure likely correct, or not? Briefly justify your answer (3 pts)?

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.

ii) What additional information might you like to consider before you consider the model to be correct (2 pts)?

That the free R-factor is close to the R-factor (typically within 5%).

4. (5 pts, 5 min) Name the disaccharide shown to the right. Are the two linear forms of the monosaccharides epimers of each other? (Note: the anomeric carbon is not considered when evaluating whether two sugars are epimers).

The first sugar is ribose, with its anomeric carbon in the \( \beta \) configuration, linked by a 1-4 linkage to the second sugar, which is glucose (dud). The anomeric carbon on glucose is in the \( \alpha \) configuration. Therefore the name is: \textbf{\( \beta \)-ribofuranosyl (1-4) \( \alpha \)-glucopyranose}

These cannot be epimers since they are different compounds (a C5 versus a C6 aldose). Epimers differ only in the configuration of one chiral center.

5. (5 pts, 5 min) Name the trisaccharide shown on the right.

\textbf{\( \alpha \)-glucopyranosyl-(1-5) \( \beta \)-ribofuranosyl (1-4) \( \alpha \)-glucopyranose}

6. (5 pts, 5 min) Draw \( \beta \)-fructofuranosyl (2-2) \( \alpha \)-glucopyranose using the reduced Haworth representation. \textbf{See Diagram on right.}
7. (5 pts, 5 min) When glucose is dissolved in water an equilibrium mixture that contains linear glucose, α-glucopyranose, and β-glucopyranose is formed. There is slightly more β-glucopyranose than α-glucopyranose, why? [Hint: A molecular model may be helpful].

Since the system is at equilibrium you need to think about the relative energy differences between the two forms of the sugars and not the rate of attack by the –OH on position 5.

In β-glucopyranose all of the groups are equatorial in one of the chair forms, minimizing unfavorable van der Waals (see right). In α-glucopyranose the –OH on carbon 1 is axial which generates a small amount of unfavorable van der Waals (red arrow).

8. (5 pts, 10 min) Draw the reduced Haworth representation for both the D and L forms of the monosaccharide shown on the right. You can assume the configuration of the anomeric carbon is α.

All of the –OH groups are on the right, so they will all point down at C2, C3, and C4. The –OH points down at C1 because it is alpha. The CH₂OH group coming off of C5 is up in D sugars and down in L sugars.

(Although not asked for in the question, this six carbon aldose is allose, the ring forms would be α-D-allopyranose and α-L-allopyranose.

9. (5 pts, 10 min) Navigate to the Jmol page labeled “Mystery polysaccharide”. What is this polysaccharide? Briefly justify your answer.

It is amylose, because the monomeric unit is glucose (C2=down, C3=up, C4=down), and each is linked by α 1-4 linkages. It is also helical. Starch would have additional chains coming off, with α 1-6 linkages.