1. (26 pts, 25 min). The following 37 residue peptide can adopt a stable fold in solution.

Ser-Ala-Cys-Val-Asp-Val-Asn-Pro-Gly-Ser-Ser-Lys-Asn-Ala-Phe-Glu-Asp-Val-Ser-Lys-Arg
-Phe-Val-Asp-Asp-Asn-Pro-Gly-Ser-Arg-Lys-Val-Glu-Phe-Lys-Tyr-Ala

i) Calculate the conformational entropy change, ΔS^conf, that occurs when the peptide unfolds from its stable (and unique) structure; assume each residue has 9 independent conformations in the unfolded state and only one conformation in the folded state (4 pts).

The relationship between entropy and the number of conformation is; S=RlnW. For one residue S = R ln 9, for two residues S = R ln 9 × 9, for n-residues: S=Rln9^n. S_f = R ln9^37 = 37 ln 9 = 8.31 J/mol-K × 37 × 2.2 = +676 J/mol-K.
Therefore ΔS = S_f-S_o = 676 - 0 = +676 J/mol-K (Note that the sign is positive, because of the direction that the reaction was written: N → U).

ii) Calculate the ΔH° associated with unfolding of this peptide, assuming that the ΔH° is due entirely to the breaking of hydrogen bonds. You can assume that all 37 residues in this peptide form some sort of intramolecular hydrogen bond in the folded state. You should also assume that each hydrogen bond contributes 2.5 kJ/mol to the stability of the native state (2 pts).

Assuming each hydrogen bond contributes 2.5 kJ/mol, ΔH = 2.5 × 37 = 92.5 kJ/mol (H_o - H_n). This is positive, since heat must be added to break the H-bonds to unfold the peptide. Note that the 2.5 kJ/mol represents the DIFFERENCE in energy between a hydrogen bond in secondary structure and a hydrogen bond to water; H-bond broken during unfolding are reformed with water.

iii) Navigate to the “dry” lab for this problem and “measure” the fraction unfolding versus temperature for this protein. The links to the dry labs are found on the Jmol page. From your data: (6 pts, 2 pts each).

a) Plot a melting curve and indicate T_M on your curve.

The T_m is when fraction unfolded = 0.5, which is at T=313K.

b) Plot a van’t Hoff plot to obtain the ΔH°.

I used temperatures that gave fraction unfolded between 0.2 and 0.8. Slope is -17,118, therefore ΔH° = 142 kJ/mol

c) Obtain the ΔS° (observed) from the ΔH° and T_M.

ΔS° = ΔH° / T_M = 454 J/mol-K

iv) Explain, in terms of molecular interactions, the difference between the ΔS° and ΔH° that you calculated in part i and ii and the experimentally determined values that you obtained from part iii (6 pts).

The calculated ΔH°, based on H-bonds is +92.5 kJ/mol. The measured is +144 kJ/mol. The additional ~50 kJ/mol is due to van der Waals interactions that will also stabilize the folded form.

The calculated ΔS°, based on conformational entropy, is +676 J/mol-deg. The experimental is 457. The experimental entropy is smaller because of the reduction in the entropy of the solvent when non-polar groups are exposed during unfolding, therefore the overall entropy change of the system (protein + solvent) is smaller.
v) Suggest a structure for the folded state of this peptide and briefly justify your answer. [Hint, consider the frequency, or periodicity, of polar and non-polar residues along the peptide chain and which type of residues are found in tight turns.] (4 pts).

The structure is a β-α-β fold, the beginning and end of the peptide have the following pattern of polar and non-polar residues (non-polar in bold) suggesting that they form a two stranded amphipathic β-sheet, with one face showing polar residues and facing the solvent, and the other non-polar side facing the α-helix.

Ser-Ala-Cys-Val-Asp-Val-Asn-Pro-Gly-Ser-Ser-Lys-Asn-Ala-Phe-Glu-Asp-Val-Ser-Lys-Arg

The center residues show one or two non-polar residue about every 3-4 residues (underlined), which would make an amphipathic helix since the periodicity of a helix is 3.6 residues/turn. The non-polar side of the helix will face the non-polar side of the β-sheet.

Proline-Glycine residues (boxed) could be turns.

vi) **Calculate** the fraction unfolded at 45° C using the experimentally determined values for enthalpy and entropy. Show your work. How does this compare to the experimental value? (4 pts)

You need to use the experimental enthalpy and entropy in your calculation. You also need to convert from Celsius to Kelvin.

\[ \Delta G = - RT \ln K_{eq} = \Delta H - T \Delta S \]

\[ T = 45 + 273 = 318 \]

\[ \Delta G = 142,000 - (318)454 = -2372 \text{ J/mol} \]

\[ K_{eq} = e^{-\frac{\Delta G}{RT}} = 2.43 \]

\[ F_{U} = \frac{K_{eq}}{1+K_{eq}} = 0.71, \text{ very similar to experimental value, as you would expect since we obtained these from the experimental data.} \]

2. (10 pts, 20 min) A wild-type protein contains an Ile at position 18 in the chain. You can view the structure of this protein on the Jmol page associated with this problem set. Three different mutant proteins are studied, with the Ile replaced by Phe, Val, or Ala. The thermodynamic parameters for unfolding of the wild-type protein are: \( \Delta H^0 = +200 \text{ kJ/mol} \), \( \Delta S^0 = +600 \text{ J/mol-deg} \). The denaturation curves for all four proteins are shown on the right.

i) Estimate the \( \Delta S^0 \) for unfolding of all three mutant proteins (Ala, Val, Phe) using the data (enthalpy change due to transfer to water) given in lecture 8 and the \( \Delta S^0 \) observed for the wild-type protein. (4 pts)

The figure gives the entropy change for different amino acids when they are transferred to water (i.e. unfolding). The difference between Ala and Ile is 2 J/mol-deg. Therefore, the entropy change for unfolding the Ala protein should be +2 J/mol-deg larger (less water is ordered when the Ala unfolds), giving a total entropy of +602 J/mol-deg. In the case of valine, the entropy change in transferring valine to water is -1.5, a difference of 1 from Ile, so the observed entropy will be +601 J/mol/K. Ile and Phe have about the same transfer entropy, so the overall entropy for denaturation will remain the same (see recitation for more details).

<table>
<thead>
<tr>
<th></th>
<th>( \Delta H \text{ (kJ/mol)} )</th>
<th>( \Delta S \text{ (J/mol-K)} )</th>
<th>( T_m \text{ (K)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ile</td>
<td>200000</td>
<td>600</td>
<td>333.3</td>
</tr>
<tr>
<td>Ala</td>
<td>190000</td>
<td>602</td>
<td>315.6</td>
</tr>
<tr>
<td>Val</td>
<td>195000</td>
<td>601</td>
<td>324.5</td>
</tr>
<tr>
<td>Phe</td>
<td>185000</td>
<td>600</td>
<td>308.3</td>
</tr>
</tbody>
</table>

ii) Using your estimate from part i) calculate the \( \Delta H^0 \) for the unfolding for all three mutants mutant [Hint: No van’t Hoff analysis is required, \( \Delta H^0 = T_m \Delta S^0 \)]. (3 pts)

iii) Explain the differences in enthalpy and entropy between the wild-type and all three mutants in terms of the structural differences. (3 pts)

The entropy differences are explained above (part i). In
3. (10 pts, 15 min) Use the next two ‘dry labs’ to measure the fraction unfolded as a function of temperature for a 100 residue protein (A) and a 200 residue protein (B). Obtain the ΔH° for unfolding using van’t Hoff plots.

i) Which protein shows a more cooperative transition, i.e. it unfolds over a narrower temperature range? Briefly justify your answer (2 pts). The larger protein unfolds over a narrower temperature range, completing the transition over ~20 deg, while the smaller protein completes its transition over ~30 deg.

ii) Which protein has the smallest enthalpy for denaturation? Which has the largest? (4 pts)

The slope of the van’t Hoff plots are:

ΔH° =  -17961 x 8.31 J/mol-K = 149 kJ/mol

ΔH° =  35369 x 8.31 J/mol-K = 293 kJ/mol

The smaller protein has the smaller enthalpy.

iii) Explain the relationship between cooperativity, enthalpy, and protein size in terms of the enthalpic thermodynamic factors that affect protein folding (4 pts).

The larger protein unfolds more cooperatively (over a narrower temperature range). This would be due to increased van der Waals/H-bonding because the protein is larger.

4. (5 pts, 5 min) A drug binds to an enzyme with a K_D of 100 μM. The enzyme-drug complex is inactive because this drug binds to the active site. The concentration of this drug in a patient’s blood is 10 μM. What % of the enzyme is active under these conditions?

The fraction of active enzyme will be 1-Y, since the fractional saturation, Y, is the amount of enzyme with drug bound.

Y=[L]/(K_D+[L]) = 10μM/(100 μM + 10 μM) = 10/110=0.09

1-Y=0.91, or **91% of the enzyme will be active**. This concentration of the drug is not particularly effective.

5. (21 points, 20 min) The Jmol page for this question contains structures for the complex between two different Fv fragments (A & B) bound to the same dinitrophenyl ligand (amino-DNP). The chemical structure of this hapten is shown on the right. In answering the following questions you should focus on the two nitro groups (NO_2) as well as the aromatic ring on the DNP, the ethylamine group (-CH_2-CH_2-NH_3^+) is not important for binding.

i) Give the residue name and number (position in sequence) of one residue in the antibody that would have a significant van der Waals interaction with the hapten. Select a residue that is **common** to both antibodies (2 pts). Either Trp90 on the light chain, or Trp100 on the heavy chain.

...
ii) What other type of interaction that is associated with the residue you picked in part I would also stabilize the complex? (2 pts)

Hydrophobic effect, both Trps and the hapten are somewhat non-polar and would release ordered water molecules when the complex is formed, increasing the entropy of the system. Although the Trp residues have potential H-bond donors (N-H), the geometry is not optimal for a stable H-bond.

iii) You are using equilibrium dialysis to measure the binding constant of DNP to both F_v fragments. The concentration of the F_v inside the dialysis bag is 5 μM (μM=10^-6 M). Use the “dry-lab” associated with this problem to obtain the amount of ligand in the dialysis bag. From these data obtain the fractional saturation for both proteins and use these values to plot a binding curve (Y versus [L]). Determine the dissociation constant (K_d) for each F_v using both a binding curve and a Hill plot. Submit all plots. (6 pts).

The fractional saturation at each ligand concentration (L_OUT) is just Y= (L_IN-L_OUT)/M_T = (L_IN-L_OUT)/5. This is because Y=[ML]/[M_T] and [ML] is the additional ligand in the bag that is bound to the macromolecule. L_OUT=L_Free. K_D is obtained from the binding constant as the ligand concentration to give Y=0.5. This is 5 uM for Fv-A and 20 uM for Fv-B.

The Hill plots are shown on the right, they give a slope of 1 (non-cooperative) with essentially the same K_D values as the binding curves (the x-axis is in units of uM, so the K_d values will be in units of uM).

FvA. LogK_D = 0.69, K_D=10^{0.69}=4.89 uM
FvB. LogK_D = 1.30, K_D=10^{1.30}=19.9 uM

iv) Calculate the standard energy of binding for each K_D value (T=300K). Based on the K_D values and the ΔG° values, which Fv fragment binds the DNP with higher affinity? (2 pts)

ΔG°=-RT ln(K_EQ): Fv-A: -8.31 X 300 X ln(1/(5x10^-6))=-30.4kJ/mol. Fv-B: =-27.0 kJ/mol

Fv-A has the larger ΔG°, so the binding is better to Fv-A.

v) Explain the difference in affinity, based on sequence/structural differences between the two Fv proteins. Clearly explain which type of interaction(s) were affected by the sequence difference. (5 pts)

A Glutamine residue in Fv-A has been replaced by an Alanine in Fv-B, causing the loss of a hydrogen bond, plus some van der Waals, thus less heat is require to dissociate the bound ligand from Fv-B.