1) A wild-type protein contains an Ile residue that is buried in a non-polar core of a protein. This Ile residue has been changed to Ala in a mutant protein, without any other significant changes to the structure of the protein, generating a small cavity.

Predict the $\Delta S^o$ for unfolding of the alanine protein, using the transfer entropies shown on the right. You may find it helpful to use the “entropy vector diagram” that summarizes the relationship between calculated and measured entropy changes. You can assume that the chain entropy, $\Delta S^o_{\text{chain}}$ is the same for both proteins. The transfer entropies given in the graph are in J-mol/K. The partial energy diagrams for the two proteins are also given for you. In the case of the Ile protein, the contribution of the other residues in the non-polar core, plus the Ile are indicated:

$$\Delta S^o_{\text{conf}} = \Delta S_{\text{solv}} + \Delta S_{\text{exp}}$$

**Construct an entropy diagram that accounts for the positive conformational entropy (red arrow) and the opposing hydrophobic effect (blue arrow).**

The contribution of Ile and Ala to the hydrophobic effect can be read off of the graph. Ala has 2.0 J/mol-K smaller transfer entropy, so the overall entropy should be 2 higher.

**V Explain your answers to a TA.**

2) A 37 residue protein transitions from its folded state (a β-β-α structure), to its unfolded state. **Open the Jmol for this recitation to view this protein.**

This question calculates some theoretical values for $\Delta H^o$ and $\Delta S^o$ values due to unfolding. You will compare these to experimental values in Q3.

i) Where would you expect to find the sequence Leu-Glu-Leu-Glu-Leu-Glu, in one of the β-strands or the helix? Verify your expectation by inspection of the Jmol structure. At what residue in the protein does this sequence start?

*Sheet*. The sheet must present a non-polar surface on one face, and a polar surface on the other, the non-polar and polar residues must alternate: Leu-Glu-Leu-Glu-Leu-Glu. This sequence is found beginning at residue 3 in the protein.

ii) List the residues that compose the core of this protein, what do they all have in common (if you can’t find the core, or center of the protein, there is a “hint” button on the Jmol page).

Four leucines, two alanines, one phenylalanine. The non-polar part of a tyrosine and a threonine also participate. The sidechain of these residues are either all non-polar (leu, ala, phe) or contribute the non-polar part of their sidechain to the non-polar core.

iii) Calculate the change in conformational entropy between the two states unfolded and folded states, assuming that each residue in the unfolded state has 9 different conformations ($S = R \ln W$), $R = 8.31$ J/mol-deg.

Assume that each residue in the folded state has a single configuration: $S_F = R \ln 1 = 0$

Assume that each residue in the unfolded state has 9 different configurations: $S_U = R \ln 9^{37}$

Therefore $\Delta S^o = S_U - S_N = R \ln 9^{37} - 0 = 8.31 \times 37 \times \ln 9 = 675$ J/mol-K
iv) Assuming only H-bonding enthalpic interactions, calculate the expected ΔH° for denaturation of this protein. You can assume hydrogen bond that is formed has a ΔH° of -2.5 kJ/mol. You need to determine the number of h-bonds in the protein, which can be obtained from simply counting them in the Jmol structure.

The number of hydrogen bonds is 9 for the β-strand and 12 for the helix, plus one from sidechains - giving a total of 22. ΔH° = 22 × (-2.5 kJ/mol) = -55 kJ/mol

v) Were all of the Hbonds you identified between mainchain atoms?

No, there is one between the sidechain of lys 13 and the mainchain of Gly 9

V Explain your answer to the TA. Enter your calculated values for ΔS° and ΔH° into the table below. 

3) i) Use the dry lab for recitation to experimentally determine the values of ΔH° using a van’t Hoff plot (plot ln Keq versus 1/T):

a) Set-up an Excel spreadsheet with the following columns (make sure you use the LN(x) function in excel.

<table>
<thead>
<tr>
<th>Temp</th>
<th>Fu</th>
<th>Keq</th>
<th>1/T</th>
<th>lnKeq</th>
</tr>
</thead>
</table>

b) Vary temperature and collect fraction unfolded (fu), plot fu versus temperature - enter these data into the first two columns of your sheet and plot them to obtain T_M. Start at 300K & try 5 K increments.

c) Calculate the equilibrium constant at each temperature, using the second column: Keq=fu/f_n.

d) Now populate the 4th (1/T) and 5th column (lnKeq). Plot ln Keq versus 1/T (van’t Hoff plot), Use the add trend line in Excel to obtain the slope of the plot.

e) ΔH° can be obtained from the slope of the line: ΔH° = -slope x R. Enter ΔH° in the table below.

ΔG° = ΔH°-TΔS° = -RT ln Keq

\[ \ln K_{eq} = -\frac{\Delta H^°}{R} - \frac{\Delta S^°}{R} \]

The slope is -18445

Therefore ΔH° = 18445 × 8.31 = +153 kJ/mol

ii) Determine the experimental value of ΔS°. The ΔS° can be calculated using the following formula:

ΔS° = ΔH° / T_M, where T_M is the temperature where f_u=0.5.

Enter this value in the table. V Have the TAs check all four of your entries.

<table>
<thead>
<tr>
<th>Calculated (Q2)</th>
<th>Experimental (Q3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔS° = +675 J/mol-K</td>
<td>+460 J/mol-K</td>
</tr>
<tr>
<td>ΔH° = +55 kJ/mol</td>
<td>+153 kJ/mol</td>
</tr>
</tbody>
</table>

iii) Why is the experimental value for the change in entropy due to the unfolding of the protein smaller than the value calculated in question 2? What interaction(s) have we not taken into account.

The overall entropy change of unfolding contains a contribution from the conformational entropy of the chain and the change in the entropy of the solvent: ΔS_observ = ΔS_chain + ΔS_solv.

Since the observed entropy change is smaller than that calculated from the conformational change, the entropy of the solvent must have decreased, i.e. ΔS_SOLV is negative in the direction of native to unfolded. This is due to the ordering of water molecules around exposed non-polar residues in the unfolded state that were originally buried between the β-sheet and the helix in the folded state.

V Explain your answer to the TA.

iv) Why is the experimental value for the change in enthalpy due to unfolding of the protein larger than the value calculated in question 2? What interaction(s) have we not taken into account.

The additional heat is required to break the van der Waals interactions between the helix and the β-sheet (and any potential H-bonds between sidechain groups)