Problem Set 3

Solutions key

1. (4 pts) □-Helix in Retinol Binding Protein
   i) **The helix starts at residue 146 (Pro) and ends at residue 158 (Glu)** (1pt).
   **ii) 13 residues** (1pt)
   iii) **18.61 A°** (□-carbon on 23 to □-carbon on 36) (1pt)
   **iv) ~3.5 turns** (1pt)
   v) 19.9A°/3.5 turns = **5.32A°/turn** (1pt)
2. (4 pts) The first residue that forms a hydrogen bond is Lys 150, donating an NH to the C=O of Pro 146. (2pts) This follows the n to n+4 rule. This particular H-bond is **3.12A°** long (2pts – accept 2.8A° to 3.5A°). Other H-bond pairs (listed from donor to acceptor) are: Ile 151 & Glu 147; Val 152 & Ala 148; Arg 153 & Gln 149; Gln 154 & Lys 150; Arg 155 & Ile 151; Gln 156 & Val 152; Glu 158 & Gln 154; H-bond length of any of these should be **3±0.5A°**.
3. (5 pts) □-Sheet in Retinol Binding Protein
   i) **Anti-parallel**. (1pt)
   ii) **8 strands** make up this □-sheet. (1pt)
   iii) H-bonds are **between strands**. (1pt)
   iv) The residues facing inward are **nonpolar/bulky/hydrophobic**. (1pt)
   v) **The inside of the barrel is lined with hydrophobic residues, creating a favorable environment for the hydrophobic retinol molecule**. The binding of retinol to RBP thus increases its solubility in blood. (1 pt)
4. (9 pts) i) The entropy is given by: S=RlnW, where W is the number of conformations. The entropy in the folded state $S_N$ is zero since there is only a single conformation. This is true regardless of the type of secondary structure. The entropy of the unfolded state is: $S_U = R \ln 9^{22} = R\,(22)\ln 9 = 8.31$ J/mol-K x 22 x 2.2 = 401.7 J/mol-K. Therefore $\Delta S = S_U - S_N = 401.7 - 0 =$ **+ 401.7 J/mol-K**. (2 pts) (Note that the sign is positive because of the direction that the reaction was written: N→U). **The entropy change would not depend on whether the peptide started out in an □-helical or □-sheet configuration because there is only one folded configuration for each**. (1 pt)

   ii) Assuming that each H-bond contributes 5 kJ/mol, $\Delta H$ = +110 kJ/mol ($H_U$-$H_N$). (The sign is positive because an input of heat was required to unfold the peptide). At 300 K, the total free energy change for the unfolding is:
   $\Delta G = \Delta H - T\Delta S$ =110,000 J/mol – 300 (401.7 J/mol-K) = -10,500 J/mol = -10.5 kJ/mol.

   $$K_{eq} = e^{-\Delta G/RT} = e^{10{,}500\,J/mol/(8.31\,J/mol\cdot K)(300\,K)} = e^{4.2} = \mathbf{66.7}$$

   $$f_U = \frac{K_{eq}}{(1 + K_{eq})} = \frac{66.7}{67.7} = 0.985.$$ Therefore **98.5% of the molecules are unfolded at this temperature.** (2 pts)

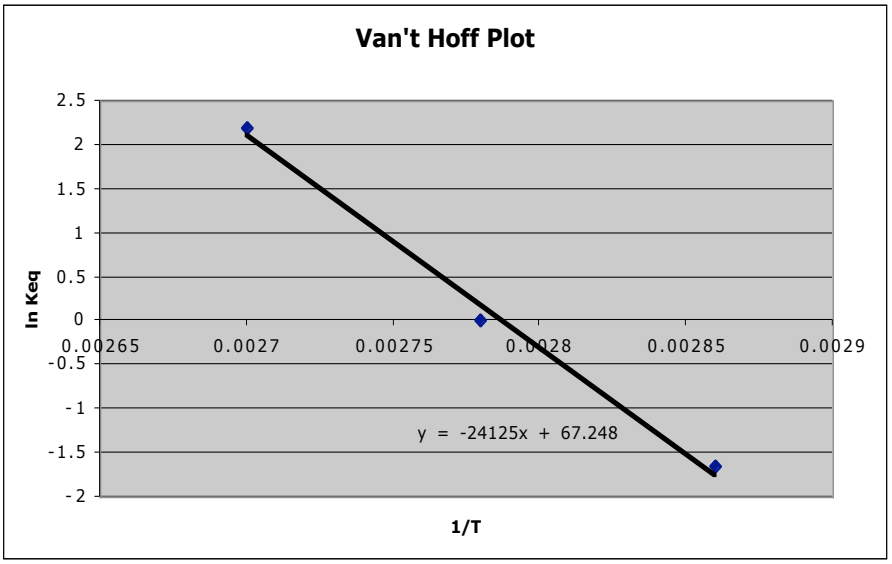
iii) At the Tm, $\Delta H = T_M \Delta S$ and $T_m = \frac{\Delta H}{\Delta S}$

Therefore, $T_m = \frac{\mathbf{110{,}000} J/mol}{\mathbf{401.7} J/mol \cdot K} = \mathbf{273.8\ K.}$ (2 pts)

6. (10 pts) i) The fraction of protein unfolded ($f_U$) at each of three temperatures is extracted
from the melting curve. From $f_U$, $f_N$ (1-$f_U$) and $K_{eq}$ (U/N) at the three temperatures can be derived. Plotting ln $K_{eq}$ against 1/T generates a line on a van't Hoff plot with a slope of of –24,125.

$\Delta H = \Delta R \cdot slope = (\mathbf{-8.31} J/mol \cdot K) \cdot (\mathbf{-24{,}125}) = \mathbf{200{,}479} J/mol = \mathbf{200.5} kJ/mol$ (7 pts)

| T (K) | 1/T | $f_U$ | $f_N$ | Keq=$f_U$/$f_N$ | ln $K_{eq}$ |
|---|---|---|---|---|---|
| 350 | .00286 | 0.16 | 0.84 | 0.19 | -1.66 |
| 360 | .00278 | 0.5 | 0.5 | 1 | 0 |
| 370 | .00270 | 0.9 | 0.1 | 9 | 2.2 |




ii) From $\Delta H$, $\Delta S$ can be calculated:

$$\Delta S = \frac{\Delta H}{T_m}$$

Since $T_m$ is 360 K,

$$\Delta S = \frac{\mathbf{200{,}479} J/m}{\mathbf{360} K} = \mathbf{557} J/m \cdot K$$ (3 pts)