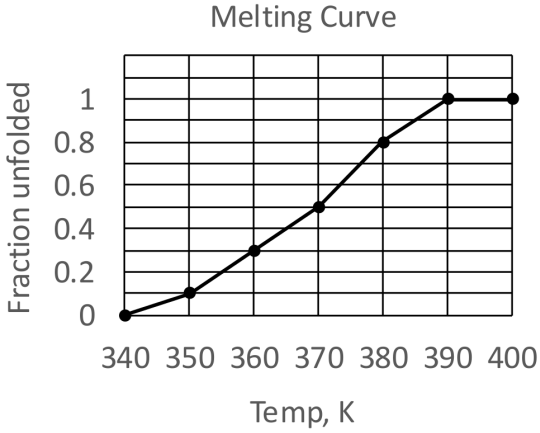
(50 pts total) Please show all your work.

1. (14 points, 20 min) The melting curve for a hypothetical protein of 40AA's is shown below.



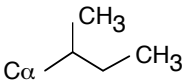
i) Use a van't Hoff analysis to determine the enthalpy associated with unfolding. Please include a screenshot of your Excel spread sheet. (6 pts)

ii) Once you have determined $\Delta H°$, calculate $\Delta S°$. (4 pts)

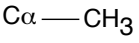
iii) Based on the value for ΔS° obtained above (ii) and a theoretical value of the configurational entropy change for unfolding a protein of 40AA's (as calculated in lecture 9), estimate the ***solvent*** entropy change for ***unfolding*** the protein. (4 pts)

2. (18 points, 20 min) T4 lysozyme is a phage enzyme that breaks down the bacterial cell wall. It is commonly used in the lab to break open bacteria. An altered version of the enzyme T4 lysozyme, with a single amino acid substitution of Ile to Ala at postion 27 (I27A), has been generated in the lab. In the wild type (wt) protein, I27 is buried in the hydrophobic core of the protein. The enthalphy and entropy of unfolding (reaction direction N→U) were measured for both proteins and the values obtained are shown below:

Protein variant	ΔH°	ΔS°
WT	472 kJ/mol	1452 J/mol-K
I27A	318 kJ/mol	1009 J/mol-K



Isoleucine



Alanine

i) Suggest an explanation for why the ΔH° value for unfolding the I27A variant might be *smaller* than for the wt protein. (4 pts)

ii) What is the melting temperature of *each* protein? (4 pts, 2 pts each)

iii) Which protein is more stable? Please justify your answer. (2 pts)

iv) What fraction of *each* protein is unfolded at 40°C? (6 pts, 3 pts each)

v) If you had to carry out cell wall lysis of bacteria using the altered I27A version of T4 lysozyme, is it OK to do it at 40°C? Please justify. (2 pts)

3. (18 pts, 20 min) You are asked to determine the affinity of a protein P for its ligand L. You carry out a series of binding assays using a fixed concentration of P

(5nM) bound to magnetic beads. In each assay, you vary the concentration of L added as indicated. The following data are obtained.

Assay #	Concentration of L added, μM	Concentration of L bound to P, nM
1	3	0.03
2	10	0.4
3	30	1.4
4	60	2.2
5	90	3.2
6	120	2.6

i) First, plot the data in Excel and estimate, just based on the graph you made, the K_D of the interaction as best as you can. (2 pts) Indicate how you arrived at your guess. (2 pts)

ii) Assuming that the interaction is a simple binding interaction, what equation should the data conform to? (2 pts)

iii) Next, use Solver and the equation above (ii) to determine the K_D of the binding interaction. Please submit your work showing the equation you used to fit your data, the solution by Solver, as well as the curve and K_D. (8 pts)

iv) Use your fitted curve to estimate the fraction of P bound to ligand at 200 μM ligand. (2 pts)

v) At approximately what concentration of ligand do you expect the protein will be 95% saturated with ligand? (2 pts)