Biology 5357

Chemistry & Physics of Biomolecules

Examination #1

Proteins Module

September 29, 2023

Name: _____

Question 1 (16 points; A-D = 4 pts each)

Close to its melting temperature (T_m), lysozyme undergoes reversible thermal unfolding at pH 7 with $\Delta H° = 130$ kcal/mol and $\Delta S° = 373$ cal/mol/K. At room temperature of approximately 25°C, $\Delta H° = 60$ kcal/mol and $\Delta S° = 155$ cal/mol/K. Remember that 0°C = 273.15 K.

(A) Estimate the melting temperature of lysozyme.

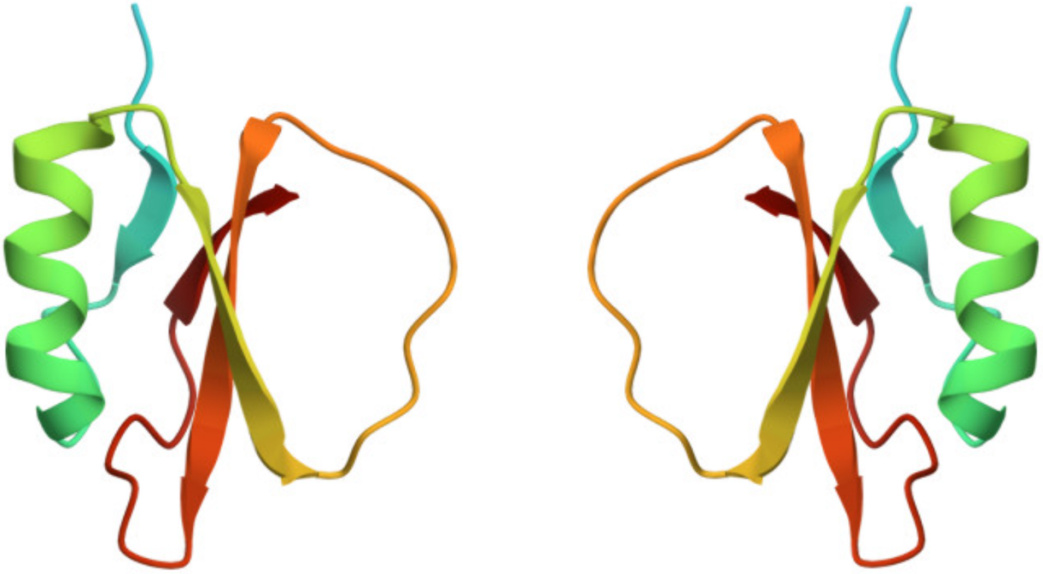
(B) What is the stability of folded lysozyme in kcal/mol, relative to its unfolded form, at room temperature?

(C) What percentage of lysozyme molecules are unfolded, on average, at room temperature?

(D) Explain the change in the enthalpy and entropy of unfolding between the two temperatures. For example, rationalize the large increase in enthalpy of unfolding from 60 to 130 kcal/mol upon shifting from 25°C to T_m.

Question 2 (15 points; A-C = 5 pts each)

Chymotrypsin inhibitor 2 (CI2) is a small globular protein of 83 residues, whose folding has been much studied by Alan Fersht's group and others in the biophysics community.



(A) Which of the diagrams above depicts the correct structure of CI2? Describe at least two features that justify your choice.

(B) Does the CI2 structure contain a β-α-β motif? Explain.

(C) What is the generally preferred arrangement for an α-helix packing against a β-sheet? Does the CI2 structure appear to follow this preference?

Question 3 (16 points; A-D = 4 pts each)

(A) Provide a rough sketch of the Ramachandran map for an Ala residue and for a Gly residue. Label the axes and indicate regions of the maps corresponding to α-helix and β-sheet structure. How would the map differ for a Pro residue?

(B) At some position in a folded globular protein of known structure you have made a Gly-to-Ala mutation. Assume the Ala mutant is fully compatible with the tertiary fold of the protein. Sketch a free energy diagram showing how the native and denatured states of the wild type and mutant proteins compare.

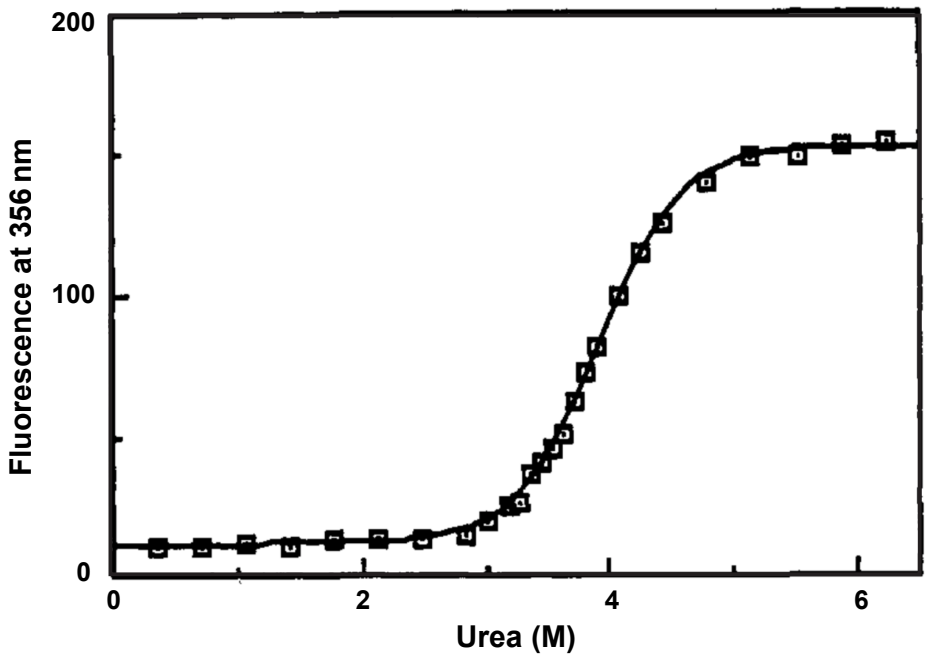
(C) Repeat part B above, only this time for a Pro-to-Ala mutation.

(D) For the above two cases, what happens to the stability of the mutant protein when compared to the stability of the wild type protein? Explain.

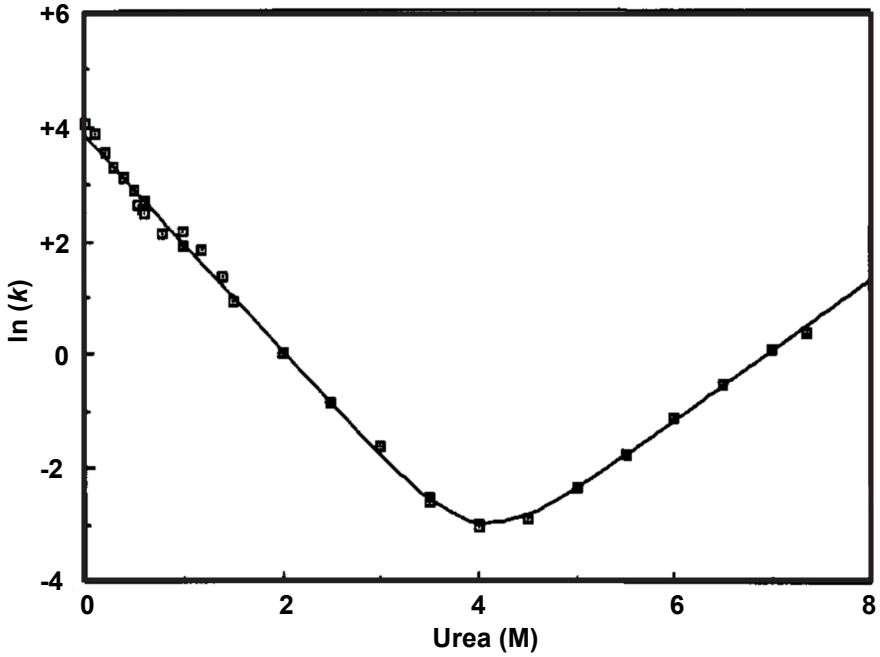
Question 4 (18 points; A-C = 5 pts each, D = 3 pts)

Shown below are an equilibrium folding-unfolding curve and a kinetic "chevron" plot for the CI2 protein. In both cases, urea was used as the denaturing agent, and experiments were performed at room temperature of 25°C.

(A) Analyze the equilibrium folding-unfolding curve to estimate a numerical value for the stability of folded CI2 relative to its unfolded form.



(B) Analyze the chevron plot to estimate a numerical value for the stability of folded CI2 relative to its unfolded form. How does your value compare to that determined in part A? Note "ln" is the logarithm in base *e*, not the logarithm in base 10 or "log", where ln(x) = 2.303 log(x).



(C) A simple Arrhenius model from transition state theory, given by the equation $k = D \exp(-\Delta G^{\#} / RT)$, often provides an excellent approximation of the protein folding rate. Here, k is the rate, $\Delta G^{\#}$ is the free energy of the transition state for the process, and R is the gas constant of 1.987 cal/mol/K. Note D is a prefactor set to 10^{10} s^{-1}, a typical rate for the elementary step of adding a residue at the end of a helix. Use this equation to estimate the barrier to be crossed when CI2 folds.

(D) What assumptions are made in the analysis you performed in parts A-C? How might these assumptions be tested or validated?

Question 5 (35 points total; individual point values given below)

On the following pages, provide a *brief* answer and discussion for each of the following questions:

(A) What is a disordered protein or protein region? (1 pt)

(B) When talking about disordered proteins, what is an ensemble? (2 pts)

(C) What features of a protein sequence might influence or determine if it is folded or disordered? Name two (2) features and briefly explain why each feature could influence if the protein is disordered or folded. (4 pts)

(D) What are some challenges in studying disordered proteins experimentally? Name two challenges, explain the molecular origin of each challenge, and indicate what kinds of problems it causes. (5 pts)

(E) How can charged residues influence conformational behavior in disordered regions? Name two effects and how they might influence IDR ensemble behavior. (4 pts)

(F) What are the three main ways that a disordered region can bind to a partner? (3 pts)

(G) Choosing one of the answers from part F, provide a molecular description of how this binding can occur? (2 pts)

(H) How can transient helices in a disordered region influence binding? (2 pts)

(I) What is conformational selection? (2 pts)

(J) Why might IDRs be sensitive to their solution environment? (2 pts)

(K) What is a Short Linear Motif (SLiM) and what do they do? (2 pts)

(L) What mechanisms allow IDRs to engage in specific molecular recognition in the absence of a 3D structure? (3 pts)

(M) How might post-translational modifications influence IDR conformational behavior? Feel free to choose whatever modifications you want. (3 pts)