Biology 5357

Chemistry & Physics of Biomolecules

Examination #1

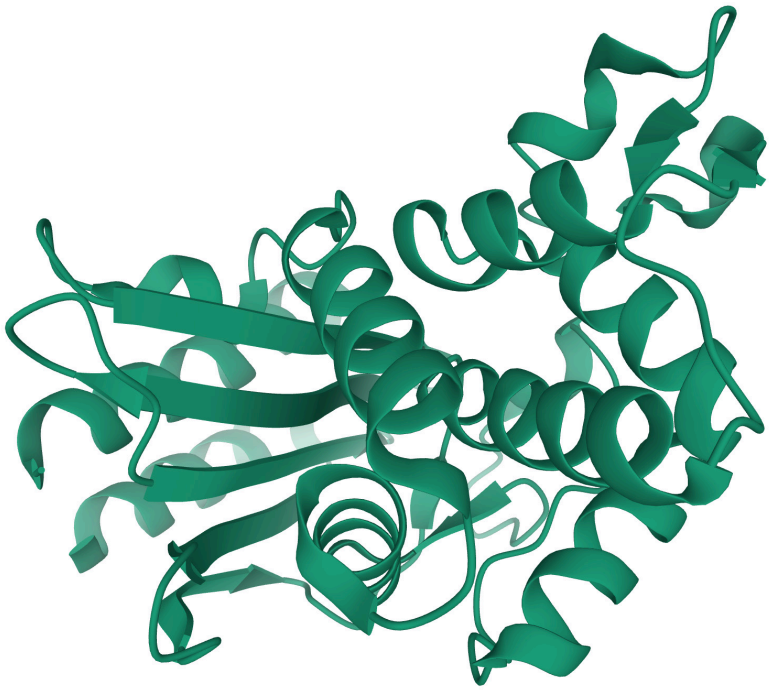
Proteins Module

September 30, 2022

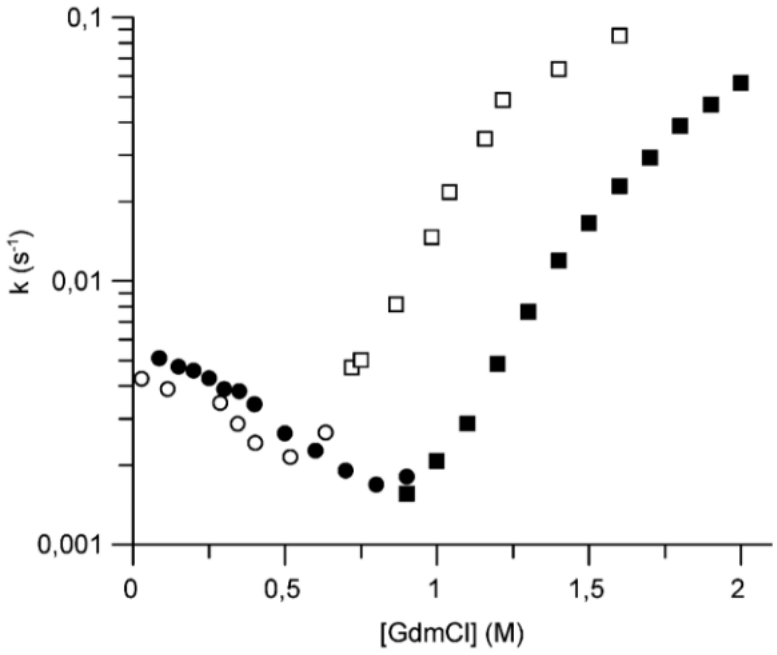
Name: _____

Question 1 (30 points; A-C = 10 pts each)

(A) Analyze the structure shown below of the TEM-1 β-lactamase protein, which is derived from the PDB entry 5HVI. For example, what kinds of secondary structural elements and what motifs does it contain. Comment on packing, twist, *etc*. of the various elements. What top-level class of protein fold does the structure belong to?



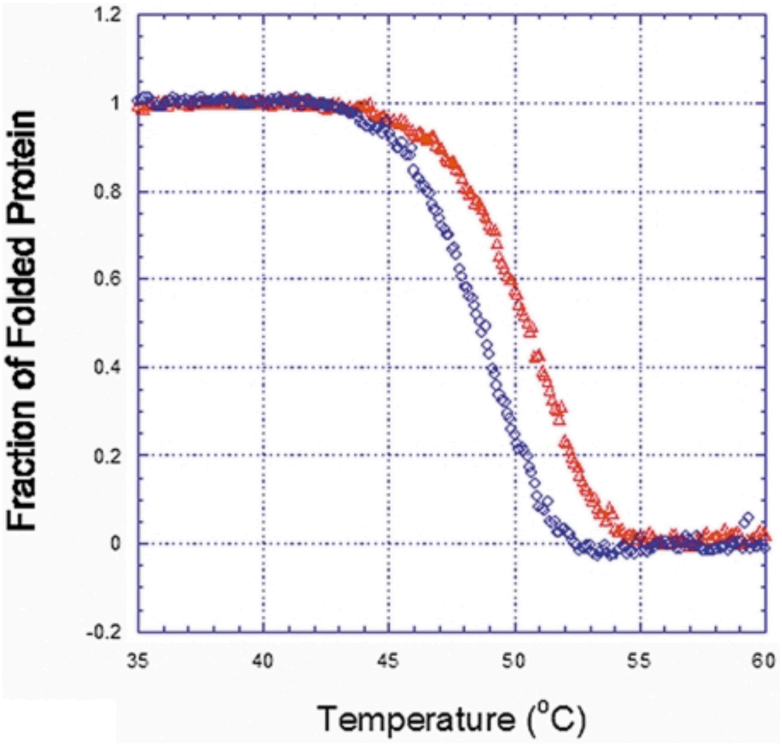
(B) The plot below shows data from kinetic folding-unfolding experiments on wild-type (filled symbols) and the W290F mutant (empty symbols) of β-lactamase. Explain the difference between points represented by circles and those represented by squares. What do the plots indicate about relative rates of folding and unfolding for the wild-type and mutant? What does the data suggest about involvement of residue 290 in the folding path of β-lactamase?



(C) "Chevron plots" demonstrate that rates of folding (k_f) and unfolding (k_u) vary with denaturant concentration, [d]. Let m_f and m_u represent the slope of the folding and unfolding arms of a typical 2-state chevron plot. Suggest an equation that gives the equilibrium folding-unfolding free energy (ΔG) as a function of denaturant concentration [d] and the two slopes, m_f and m_u.

Question 2 (20 points; A-B = 10 pts each)

The plot below shows equilibrium thermal unfolding data for TEM-1 β-lactamase. The figure is taken from a paper by Horn and Shoichet (*Journal of Molecular Biology*, ***336***, 1283-1291, 2003). The red curve corresponds to the *apo* β-lactamase protein, while the blue curve is with a non-competitive inhibitor bound.



(A) Analyze the data to estimate the ΔG value between folded and unfolded forms for both the *apo* and ligated protein, assuming for the moment that ΔC_P is zero. Additionally, find the ΔS associated with the folding-unfolding process.

(B) The heat capacity change, ΔC_P, associated with protein unfolding is not zero, but can be estimated from an approximate formula provided by Pace and Scholtz in their review *Measuring the Conformational Stability of a Protein*:

$$\Delta C_P = 172 + (17.6 \times N) - (164 \times SS)$$

In this equation, ΔC_P is the heat capacity change in cal/mol/K, N is the number of residues in the protein, and SS is the number of disulfide bonds in the structure. Note that β-lactamase has 263 residues, and contains a single disulfide bond between residues 77 and 123. What is the estimated ΔC_P value for β-lactamase? Why might the equation contain a term involving the number of disulfide bonds?

The ΔC_P value can then be used to compute ΔG at different temperatures, T, using another formula given in the Pace article:

$$\Delta G(T) = \Delta H_m (1 - T/T_m) - \Delta C_P [(T_m - T) + T \ln (T / T_m)]$$

How could you use this approach to find the stability of *apo* β-lactamase at room temperature (25°C) and at physiological temperature (37°C)? *You do not need to actually perform the calculations. Simply describe in words the steps you would follow to estimate the stabilities.*

Question 3 (12 points; A-E=4 pts each)

Briefly discuss and explain the following statements of about protein folding.

(A) "The potential energy surface for a protein is funnel-shaped."

(B) "The hydrophobic effect is a driving force leading to globular structures."

(C) "Protein folding is a highly cooperative process."

Question 4 (8 points; A & B=3 pts each, C=2 pts)

(A) A typical drug inhibiting a target protein might have a dissociation constant, K_D, of 2.5 nM. Write the equation for the equilibrium represented by K_D.

(B) What is the binding free energy, ΔG, corresponding to K_D = 2.5 nM?

(C) For this value of K_D, at what drug concentration would the protein be half-saturated by drug?

Question 5 (10 points; A & B=3 pts each, C & D=2 pts)

(A) In general, flexible polymers are well-described by an equation that relates the chain dimension (*e.g.,* radius of gyration R_g) to some other parameters via the equation:

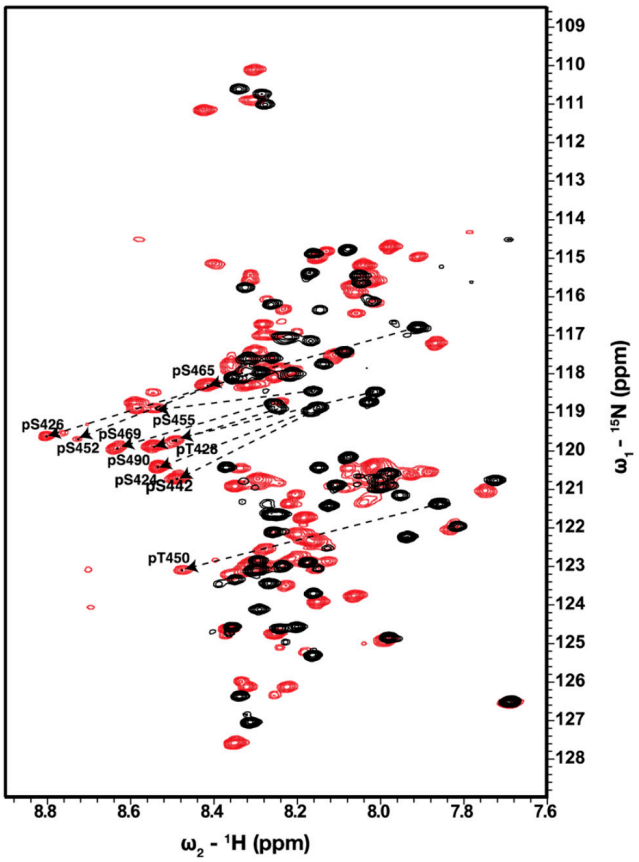
$$R_g = B_0 N^{\nu}$$

What are the meaning of B_0, N and ν? [For up to 3 bonus points, indicate the values ν can take in different situations?]

(B) Greg wants to study the binding of a disordered protein to a partner? Name a technique could Greg use and describe (very broadly) how it works. [You are free to name any technique you feel comfortable discussing.]

(C) Name one advantage and one disadvantage of single molecule FRET (smFRET) over SAXS.

(D) The HSQC spectrum below shows a disordered protein prior to (red) and upon (black) phosphorylation. What does each peak represent, and why do some of them move (black dashed lines) upon phosphorylation?



Question 6 (10 points; A=3 & B=3 pts each, C & D=2 pt each)

(A) Describe three possible functions of disordered regions, explaining how and/or why disorder could be relevant/important in that function?

(B) Disordered regions lack a fixed 3D structure. Despite this, they can engage in specific molecular interactions. In the absence of a fixed 3D structure, how might disordered regions confer specificity?

(C) Disordered regions are always more expanded that folded domains. Is this statement *True* or *False*. Briefly explain.

(D) What would be the best technique to measure local secondary structure in an IDR?

Question 7 (10 points; A=2 pt, B=4 pts, C=4 pts)

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

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

(C) What are some challenges in studying disordered proteins experimentally? Name two challenges and explain the molecular origin for this challenge and what kinds of problems it causes.