Biology 5325

Protein Structure & Function

Examination #1

Structure & Folding
Optical Spectroscopy
Protein Design

October 25, 2005

1. (10 points) Let T_m be the temperature at the midpoint of a protein unfolding curve. Results from the analysis of thermal unfolding curves for a wild-type protein and two mutants are given below. Use this data to compute the T_m value for each protein, and then to estimate the $\Delta\Delta G$ of the unfolding reaction for each mutant relative to the wild-type. Explain your work if you wish to receive partial credit.

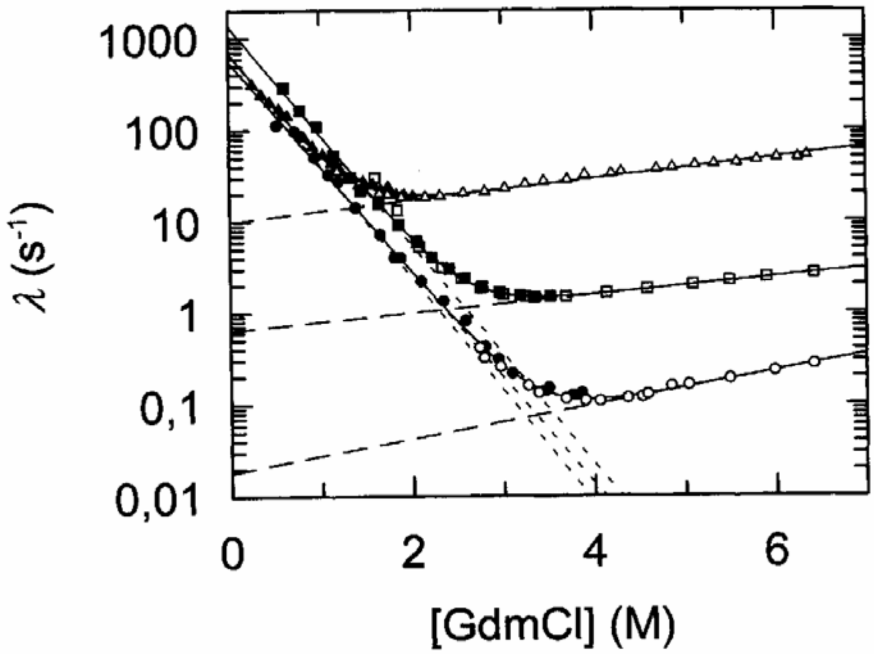
Protein	**ΔH_m** (kcal/mol)	**ΔS_m** (cal/mol/deg)
Wild-type	95	295
Mutant 1	110	335
Mutant 2	100	315

2. (8 points) Draw the full chemical structure of acetyl-L-arginine-*N*-methylamide, a "capped" Arg residue, as it would exist at physiological pH. Show correct stereochemistry at chiral centers. Label on your structure each of the phi, psi and chi angles used to specify the conformation of this molecule.

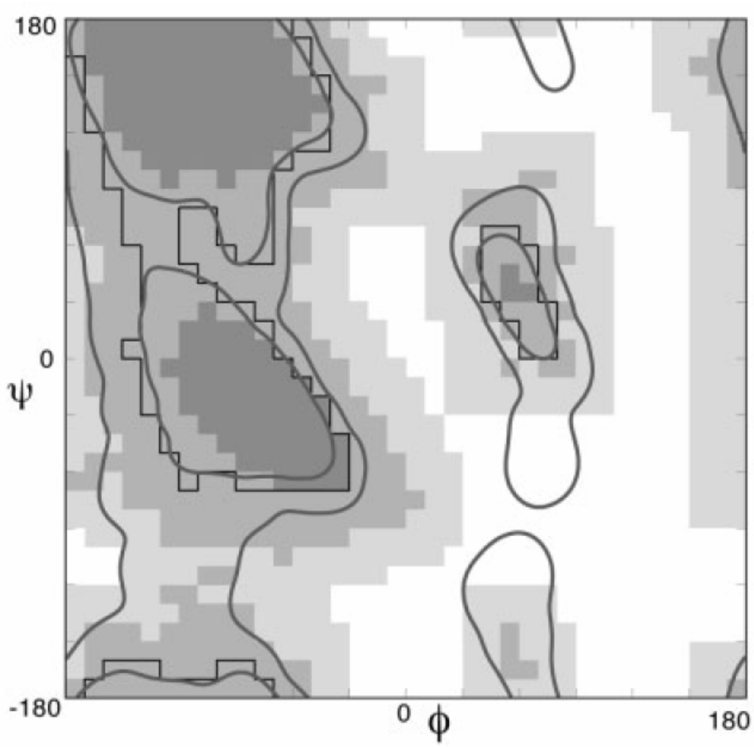
3. (12 points) Shown below are data from guanidinium chloride-induced kinetic unfolding/refolding experiments on homologous cold-shock proteins from *B. subtilis* (▲/Δ), the thermophile *B. caldolyticus* (■/□), and the hyperthermophile *T. maritime* (●/○). The apparent rate constant, λ, is plotted as a function of denaturant concentration.

(a) Estimate the rate constants for folding and unfolding of each of these proteins. What does the data suggest about the mechanism used by thermophilic organisms to achieve protein stability?

(b) Estimate the $\Delta G_{U \rightarrow N}$ for the cold shock protein from each of these three organisms. What assumptions are made in arriving at your answers?



4. (10 points) The consensus Ramachandran plot for L-alanine, as derived from a representative subset of the Protein Data Bank, is as shown below:



(a) Draw the expected Ramachandran map for the uncommon, but naturally occurring, amino acid Aib (*ie*, α-aminoisobutyric acid; an amino acid with two methyl groups at the α-carbon atom). What is the relationship between your answer and the alanine data shown above?

(b) What type of secondary structure do you think would be favored for a peptide sequence containing a mixture of several Aib residues with some of the 20 standard L-amino acids? Explain.

5. (10 points) A protein is labeled at one site with a donor fluorophore and at another site with an acceptor fluorophore. For this donor-acceptor pair R_0 is 2.0 nm.

(a) The quantum yields for donor fluorescence in the presence and absence of the acceptor are φ^{D+A} and φ^{D}, respectively. Suppose it is found that $\varphi^{D+A} = \varphi^{D}/2$. What is the efficiency of FRET, E?

(b) If τ^{D+A} and τ^{D} are lifetimes of the donor in the presence and absence of the acceptor, what is τ^{D+A}/τ^{D} under the conditions from Part (a)?

(c) Under one set of solution conditions the efficiency of FRET, E = 1.5 nm. What is the distance between the donor and acceptor?

(d) When the solution conditions are changed, E = 2.5. Also the Stern-Vollmer quenching constant for the donor increases and that of the acceptor diminishes. What has happened to the donor and acceptor fluorophores as a result of this conformational change?

6. (7 points) The molar ellipticities for α-helices and β-sheets at wavelengths 190 nm and 205 nm are as follows:

$\theta_\alpha(190) = 10^{-3}$ rad/cm $\qquad$ $\theta_\alpha(205) = 0$ rad/cm

$\theta_\beta(190) = 2\text{x}10^{-4}$ rad/cm $\qquad$ $\theta_\beta(205) = 1\text{x}10^{-4}$ rad/cm

For a certain protein, the values obtained are:

$$\theta(190) = 4.64\text{x}10^{-4} \text{ rad/cm} \qquad \theta(205) = 6.7\text{x}10^{-5} \text{ rad/cm}$$

What are the fractions of α-helix and β-sheet in this protein? Note that the reference ellipticities cited here are only approximate.

7. (10 points) As measured in a FCS experiment, a molecular complex composed of N identical subunits has a translational diffusion coefficient D_c.

(a) When the complex dissociates into monomers, the monomer translational diffusion coefficient is $D_m = 2D_c$. Supposing that both the complex and the monomer can be treated as spherical particles, what is the value of N?

(b) Supposing FCS measurements are carried out on both solutions, what is the ratio $G_m(0)/G_c(0)$ where $G_m(0)$ and $G_c(0)$ are the normalized autocorrelation function amplitudes for monomer and complex solutions, respectively?

(c) What would be the ratio of the rotational diffusion coefficients, D_R, for monomer and complex, D_R(complex)/D_R(monomer)?

(d) How would you measure the rotational diffusion coefficients?

8. You are entering a new lab for a rotation and your new boss (who knows you've taken our course) asks you to model a structure for a novel X protein they have found in the lab (which is not suitable for NMR or X-ray studies!). It has a sequence of 79 residues and some initial studies clearly indicate that it has at least one large coil (7-11 residues).

(a) (10 points) Design a strategy for getting the structure. The strategy should include reasoning and alternatives for each different procedure. Your boss wants to know the details, why and alternatives!

(b) (5 points) Across the lab there is a bright computer engineer who just invented a quantum computer (that is a computer with infinite power!). And even better, you get to use it. Design a method/procedure to obtain the structure knowing that now you have unlimited computer resources (hardware).

(c) (5 points) Your boss is obsessed with stabilizing mutants for the tumor suppressor p53 protein. So, she wants you to engineer this novel X protein for this purpose. Help her!

9. (5 points) Given the *A* and *B* sequences *A*=HAGWAGEH and *B*=AGWEAH, produce the local and global alignment using the following scoring matrix and a gap penalty equal to -1.

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	6	-6	-3	-3	-6	-3	-2	-1	-6	-4	-5	-6	-4	-7	-1	0	0	-12	-7	-2
R	-6	8	-5	-9	-7	-1	-8	-8	-1	-5	-8	1	-3	-8	-3	-2	-5	-1	-9	-7
N	-3	-5	7	2	-9	-3	-1	-2	1	-4	-6	0	-7	-8	-5	0	-1	-7	-4	-7
D	-3	-9	2	7	-12	-2	3	-3	-3	-6	-11	-4	-9	-13	-7	-3	-4	-13	-10	-7
C	-6	-7	-9	-12	9	-12	-12	-8	-7	-5	-13	-12	-12	-11	-7	-2	-7	-14	-3	-5
Q	-3	-1	-3	-2	-12	8	2	-6	1	-7	-4	-2	-3	-11	-2	-4	-5	-11	-10	-6
E	-2	-8	-1	3	-12	2	7	-3	-4	-5	-8	-4	-6	-12	-5	-4	-5	-15	-8	-6
G	-1	-8	-2	-3	-8	-6	-3	6	-8	-9	-9	-6	-7	-8	-5	-1	-5	-13	-12	-5
H	-6	-1	1	-3	-7	1	-4	-8	9	-8	-5	-5	-9	-5	-3	-5	-6	-6	-3	-6
I	-4	-5	-4	-6	-5	-7	-5	-9	-8	8	-1	-5	0	-2	-7	-6	-2	-12	-5	2
L	-5	-8	-6	-11	-13	-4	-8	-9	-5	-1	7	-7	1	-2	-6	-7	-6	-5	-6	-2
K	-6	1	0	-4	-12	-2	-4	-6	-5	-5	-7	6	-1	-12	-6	-3	-2	-10	-8	-8
M	-4	-3	-7	-9	-12	-3	-6	-7	-9	0	1	-1	11	-3	-7	-5	-3	-11	-10	-1
F	-7	-8	-8	-13	-11	-11	-12	-8	-5	-2	-2	-12	-3	9	-9	-6	-8	-4	2	-7
P	-1	-3	-5	-7	-7	-2	-5	-5	-3	-7	-6	-6	-7	-9	8	-1	-3	-12	-12	-5
S	0	-2	0	-3	-2	-4	-4	-1	-5	-6	-7	-3	-5	-6	-1	6	1	-4	-6	-5
T	0	-5	-1	-4	-7	-5	-5	-5	-6	-2	-6	-2	-3	-8	-3	1	7	-11	-6	-2
W	-12	-1	-7	-13	-14	-11	-15	-13	-6	-12	-5	-10	-11	-4	-12	-4	-11	13	-4	-14
Y	-7	-9	-4	-10	-3	-10	-8	-12	-3	-5	-6	-8	-10	2	-12	-6	-6	-4	10	-6
V	-2	-7	-7	-7	-5	-6	-6	-5	-6	2	-2	-8	-1	-7	-5	-5	-2	-14	-6	7

10. (8 points) Suppose you have two ions with charges q_1 and q_2 and radii a_1 and a_2. The following questions consider solvation energies $\Delta_{\text{solv}} G_i[\varepsilon]$; *i.e.*, the energies of ion transfer from vacuum to a solvent of dielectric constant ε. Be sure to justify your answer using the Born model of ion solvation, a physical description of solvent interactions with ions.

(a) Assume that $q_1 = q_2$ and $a_1 > a_2$. Let $\Delta_{\text{solv}} G_1[\varepsilon]$ and $\Delta_{\text{solv}} G_2[\varepsilon]$ be the solvation energies of the two ions in the same solvent of dielectric ε. Which ion has the more favorable solvation energy?

(b) Assume that $|q_1| > |q_2|$ and $a_1 \approx a_2$. Let $\Delta_{\text{solv}} G_1[\varepsilon]$ and $\Delta_{\text{solv}} G_2[\varepsilon]$ be the solvation energies of the two ions in the same solvent of dielectric ε. Which ion has the more favorable solvation energy?

(c) Consider a single ion and two solvents with dielectric coefficients ε_A and ε_B. First, define the free energy $\Delta_{A \to B} G$ of transferring the ion from solvent *A* to solvent *B* in terms of the solvation energies $\Delta_{\text{solv}} G[\varepsilon_A]$ and $\Delta_{\text{solv}} G[\varepsilon_B]$. Now suppose $1 < \varepsilon_A < \varepsilon_B$; what is the sign of $\Delta_{A \to B} G$?