Biology 5357

Chemistry & Physics of Biomolecules

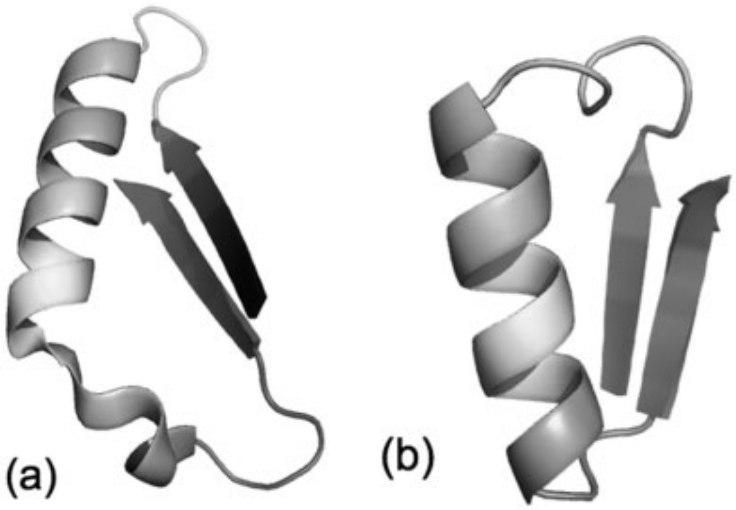
Examination #1

Proteins Module

September 29, 2017

Name: _____

Question 1. (10 points) The structures below are **(a)** residues 123-165 of SurE, a metal ion-dependent phosphatase (PDB Code: 1J9L), and **(b)** residues 57-87 of a phospholipase D family member (PDB Code: 1BYS).



(A) Characterize each of these β-α-β motifs as having either left- or right-handed crossover connections. One of the above structures occurs about 65 times more often than the other in a recent study of the Protein Data Bank. Which structure is the one more observed, and why?

(B) In a set of 431 three-helix bundles contained in all-α proteins, 61.5% were found have right-handed connections and 38.5% left-handed. Compare this to the results for the β-α-β motif, and suggest an explanation for the milder structural preference found in helical bundles.

Question 2. (10 points) Reduced glutathione is a "tripeptide" with the sequence Glu-Cys-Gly, but where the peptide bond between Glu and Cys utilizes the side chain carboxylate instead of the backbone carboxylate.

(A) Draw the structure of reduced glutathione at pH 7, paying attention to chirality and protonation state.

(B) Indicate how the structure changes upon going from the reduced form of glutathione to the oxidized form?

(C) Explain why glutathione is often a component of *in vitro* protein refolding buffers.

Question 3. (15 points) Pseudoazurin is a monomeric 123-residue protein containing mostly β-sheet and having the following primary sequence:

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  1  ENIEVHMLNKGAEGAMVFEPAYIKANPGDTVTFIPVDKGHVNESIKDMIP
 51  EGAEKFKSKINENYVLTVTQPGAYLVKCTPHYAMGMIALIAVGDSPANLD
101  QIVSAKKPKIVQERLEKVIASAK
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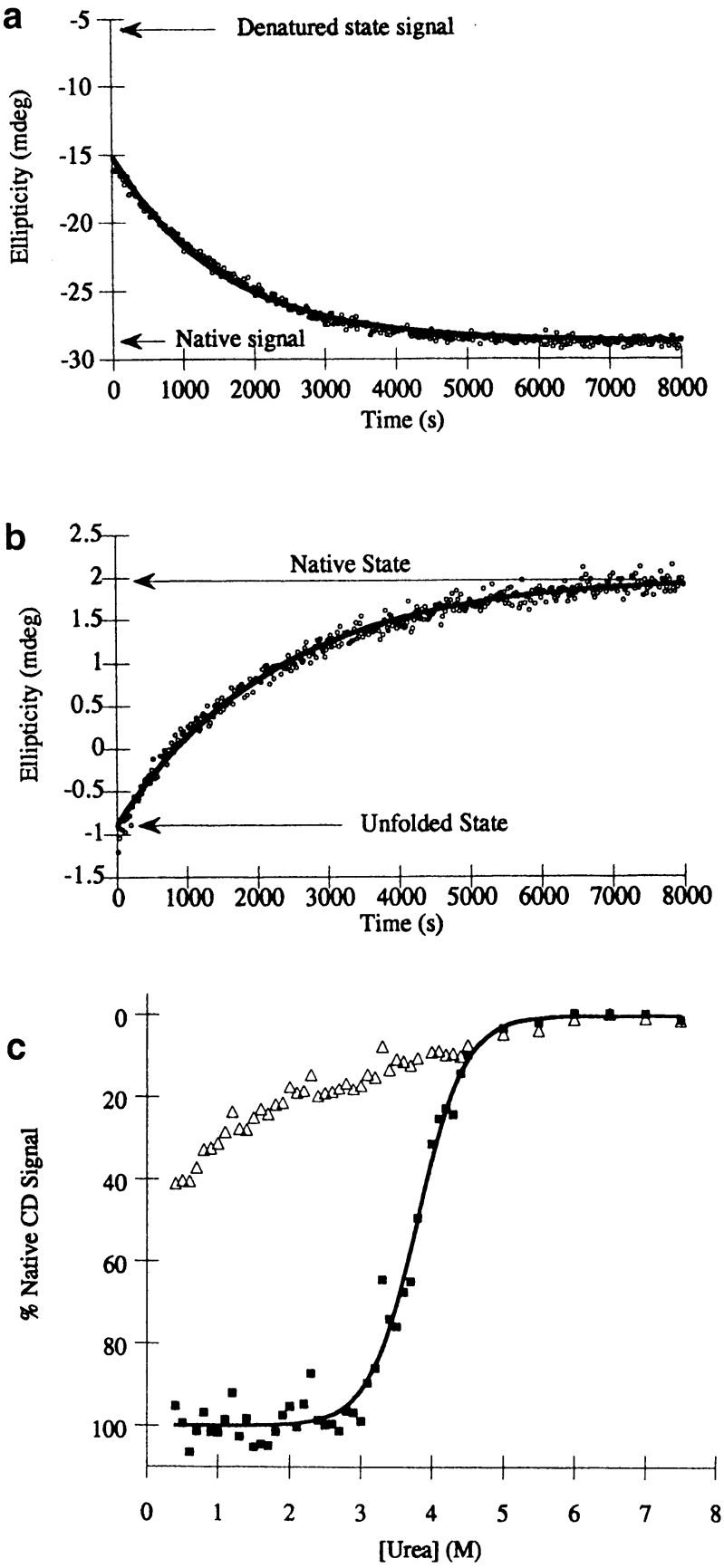
It binds a single Cu^{+2} ion and contains a complex "double wound" Greek key motif. The *apo*- form of the protein (without the ion) is stable and its folding is fully reversible. A sequential mixing stopped flow CD apparatus with a minimum dead time of 15 sec. was used to monitor the refolding of *apo*-pseudoazurin. Data from the far-UV CD at 220 nm and from the near-UV CD at 261 nm are shown on the following page in panels (a) and (b), respectively. The data shown is well modeled by a single exponential process, and in both cases the rate constant at 15°C is about 0.035 min^{-1}. In addition, the refolding rate was measured at several temperatures, and the rate was found to increase 14-fold between 5°C and 25°C. Finally, in panel (c), *apo*-pseudoazurin was refolded into buffer samples containing various concentrations of urea. After waiting 15 sec., the CD at 220 nm was recorded over an acquisition time of 120 sec (△). The samples were then incubated overnight at 15°C, and the CD was rmeasured again at 220 nm (■).

(A) Estimate the stability (native *vs*. denatured) of *apo*-pseudoazurin. What assumptions have you made in arriving at your answer?

(B) The Arrhenius equation describes the temperature dependence of reaction rates by $k = A \exp(-E_a/RT)$, where A is a constant, E_a is the activation enthalpy, T is the Kelvin temperature, and R = 1.987 cal/mol/deg is the ideal gas constant. Differentiation with respect to temperature gives the relation $d(\ln k) / d T = E_a / RT^2$. This last equation can then be integrated between two temperatures, T_1 and T_2, resulting in $\ln (k_{T2}/k_{T1}) = (E_a/R) (T_2-T_1) / (T_1T_2)$. Using this derivation, compute the activation enthalpy for the refolding of *apo*-pseudoazurin.

(C) Based your computed value for E_a, suggest a mechanism that is consistent with all of the refolding data. Explain the structural phenomenon that underlies your proposed mechanism. How might you test your hypothesis?


a
Denatured state signal
Native signal
Ellipticity (mdeg)
-5
-10
-15
-20
-25
-30
0
1000
2000
3000
4000
5000
6000
7000
8000
Time (s)
b
Native State
Unfolded State
Ellipticity (mdeg)
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
0
1000
2000
3000
4000
5000
6000
7000
8000
Time (s)
c
% Native CD Signal
0
20
40
60
80
100
0
1
2
3
4
5
6
7
8
[Urea] (M)

Question 4. (15 points) A synthetic 16-residue peptide capped with *N*-acetyl at the *N*-terminus, Ac-KKYTVSINGKKITVSI, has been shown to reversibly fold into a β-hairpin structure. NMR experiments monitored the chemical shift of H_α protons as a function of temperature in both pure water and 50% methanol. Analysis of the data gave the thermodynamic values shown below for the folding process. All values are corrected to 298K, and are given in kJ/mol for ΔH, and J/mol/K for ΔS and ΔC_p (Note: 1 cal = 4.184 J).

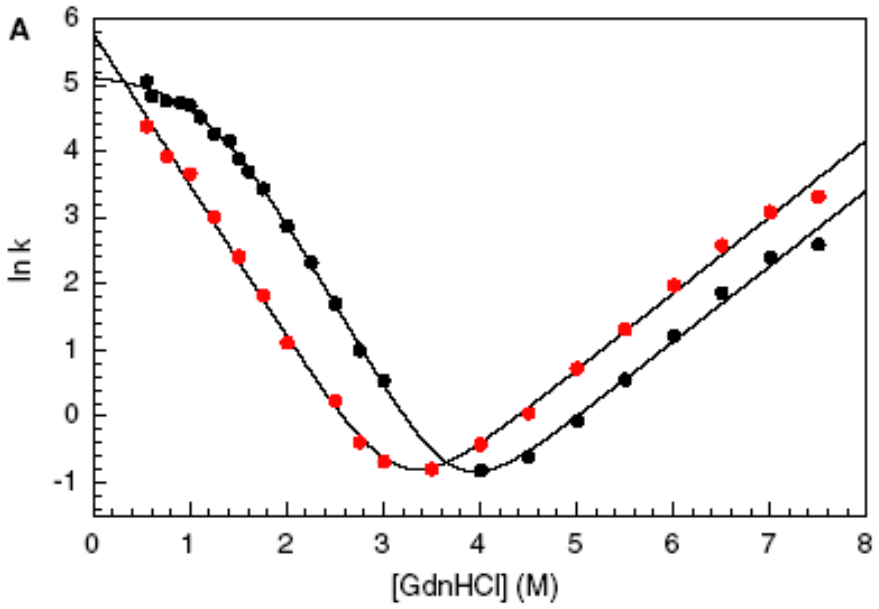
Solvent	**ΔH^o**	**ΔS^o**	**$\Delta C_p{}^o$**
Pure H_2O	+7.2	+23	-1400
50% CH_3OH	-38.5	-114	-11

(A) Briefly explain the analysis procedures used to derive these thermodynamic values from the experimental data.

(B) What percentage of the time is the peptide in the β-hairpin conformation in each of these two solvents?

(C) All three of the thermodynamic parameters undergo a change with solvent composition. Provide a physical rationalization for the observed shift in each value.

Question 5. (15 points) Shown below are chevron plots for two variants of the 76-residue protein ubiquitin at pH 5.0. The black circles are data for the F45W mutant of ubiquitin, where the mutation was made to increase fluorescence and aid kinetic measurements. The red circles are for ubiquitin with the F45W mutation and a few additional residues of an affinity tag sequence (GLVPRGS-) attached at the N-terminus. The tag happens to significantly increase the solubility of the protein.



(A) Estimate the stability of the folded form of the affinity tagged protein relative to its unfolded form.

(B) Nonlinearity of chevron plots at low denaturant concentration has been used to argue for non-2-state folding behavior, *i.e.*, for the presence of folding intermediates. Explain.

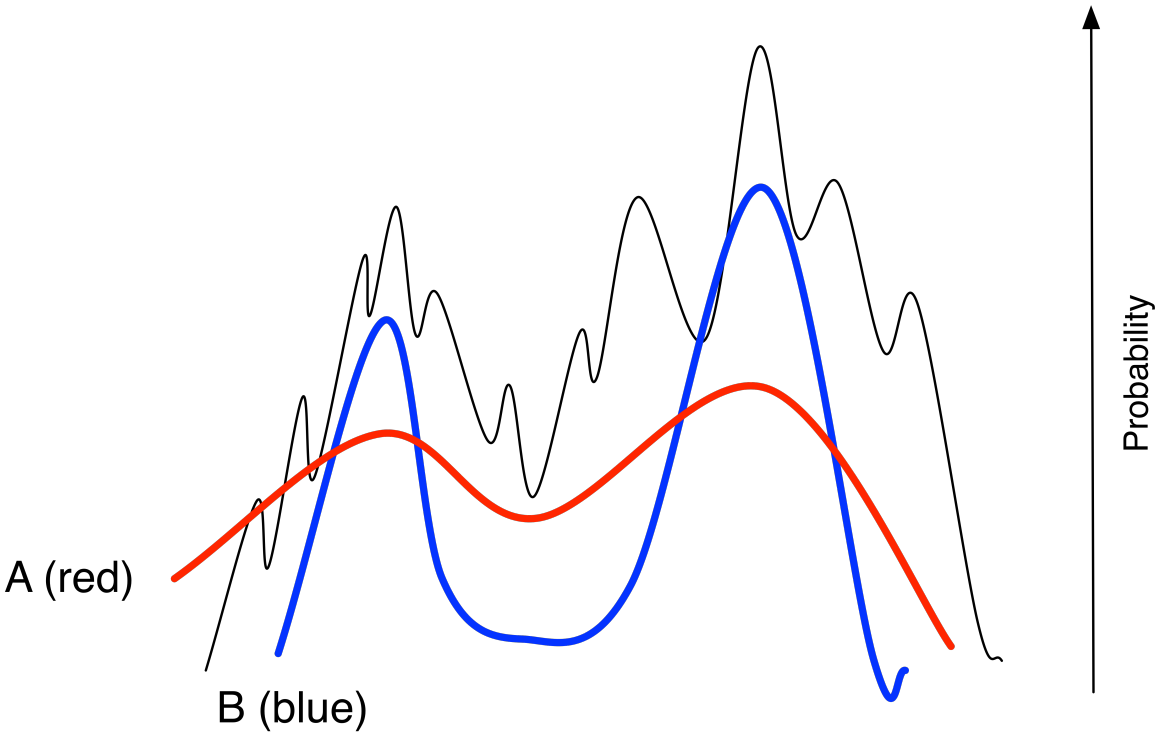
(C) Suggest another possible reason for the nonlinearity in this particular case, and propose an experimental test of your suggestion.

Question 6. (15 points)

(A) What is the equation for interconverting between probabilities and energies? Give the actual equation, not just its name.

$$\rho(x) =$$

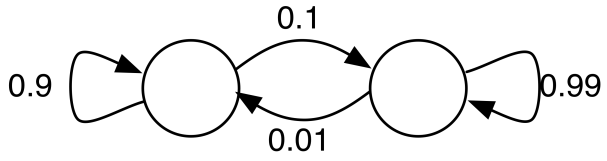
(B) The relative entropy measures the information loss between two probability distributions, such as a reference distribution and a coarse-graining of that distribution. Which of the following coarse-grainings (colored lines) of a reference probability distribution (in black) will minimize the relative entropy to the reference distribution and, therefore, is preferred over the other coarse-graining? Choose either A or B. Briefly explain your answer.



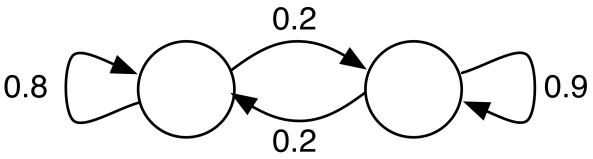
Question 7. (10 points)

(A) Which of the following two-state systems will reach a physically reasonable equilibrium? Choose either A, B, or C.

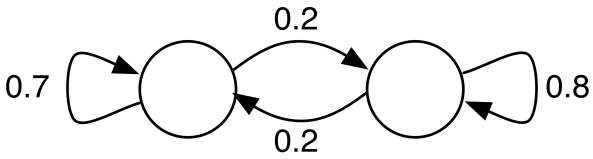
A



B



C



(B) What is wrong with the other two systems shown in part A?

Question 8 (10 points)

(A) What are two features that make it difficult to reach biologically relevant timescales in atomically detailed simulations?

(B) Briefly describe two enhanced sampling methods and how they are used to speed up molecular simulations.