Biology 5357

Chemistry & Physics of Biomolecules

Examination #1

Proteins Module

September 27, 2019

Name: _____

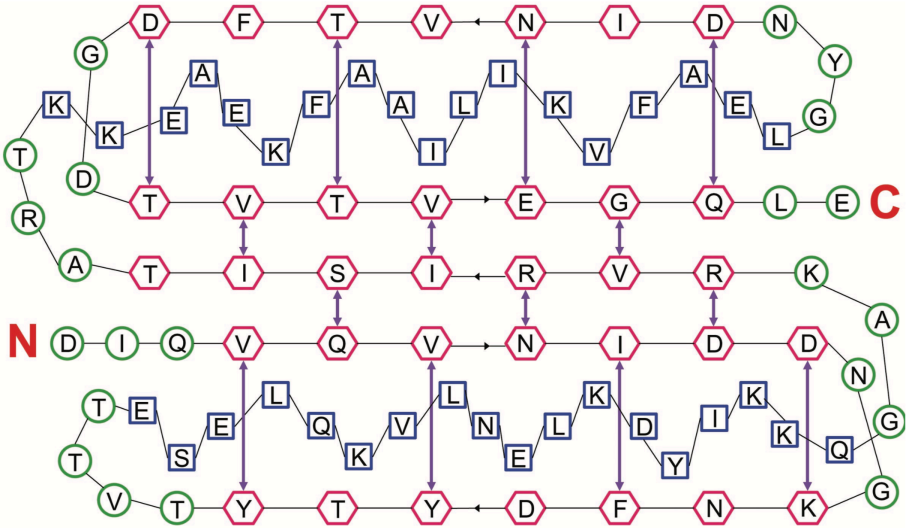
Question 1 (10 points)

(A) The widely-used non-nutritive sweetener aspartame (a.k.a. "NutraSweet"), is the C-terminal methyl ester of L-Asp-L-Phe. Draw the chemical structure of aspartame in neutral aqueous solution, paying attention to protonation state and chirality. (Remember an ester is a carboxylic acid derivative, and a methyl ester has the formula R-$COOCH_3$.)

(B) In the protease subtilisin, removal of a negative charge via mutagenesis changes the pK_a of a His residue located some 15 Å away by about 0.4 units. Use this information to estimate an "effective dielectric constant" which measures the shielding between these charges located at the protein surface.

Question 2 (15 points)

Shown below is a schematic diagram of the amino acid sequence of the TOP7 protein designed by the David Baker lab at the University of Washington using their ROSETTA software.



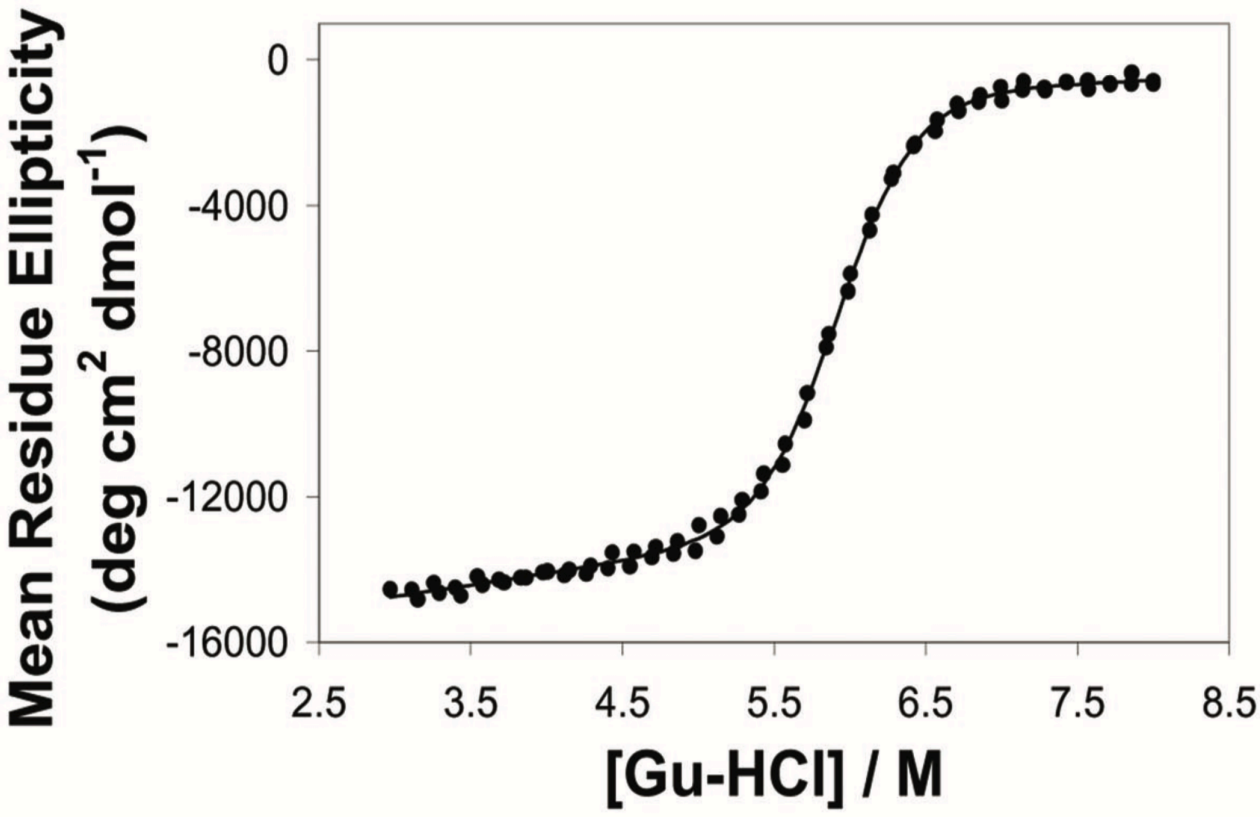
(A) Is the TOP7 β-sheet structure parallel, antiparallel, or a mixture of the two?

(B) There are two α-helices, one closer to the N-terminus and another closer to the C-terminus. For each helix, indicate whether it lies above or below the plane of the page, assuming the β-sheet lies roughly in the plane of the page.

(C) What is the definition of a β-turn? Circle the residues comprising one of the β-turns in the TOP7 structure.

Question 3 (15 points)

Provided below is the equilibrium folding-unfolding curve for the TOP7 protein from the previous question as measured at room temperature (25°C = 298K). The plot shows circular dichroism (CD) signal at 222 nm as a function of guanidinium chloride (Gu-HCl) concentration. Use this data to estimate the ΔG between the folded and unfolded states of TOP7. (Note R = 1.987 cal/mol/K)



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 the Hα protons as a function of temperature in both pure water and 50% methanol. Analysis of the data gives the thermodynamic values shown below for the folding process. All values are corrected to 25°C (298K), and are given in kJ/mol for ΔH, and J/mol/K for ΔS and ΔC_p (1 cal = 4.184 J, R = 1.987 cal/mol/K).

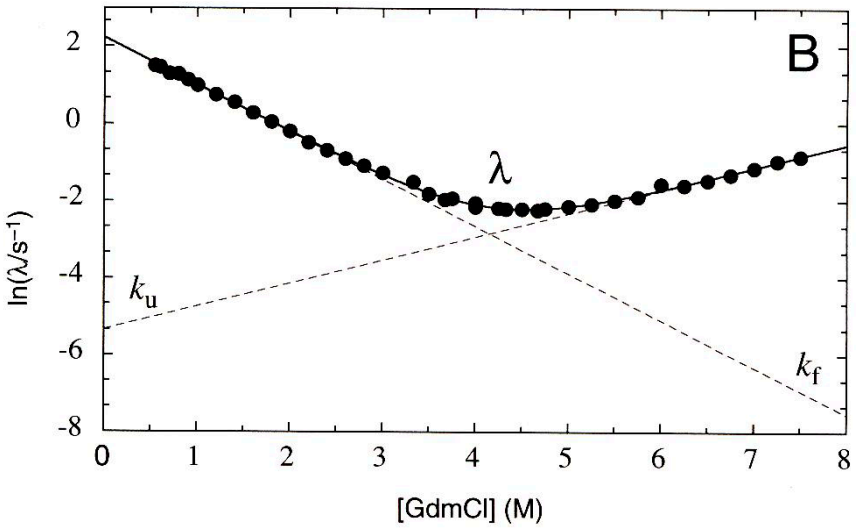
Solvent	**ΔH^o**	**ΔS^o**	**ΔC_p^o**
Pure H_2O	+7.2	+23	-1400
50% CH_3OH	-38.5	-114	-11

(A) Briefly explain the data 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 reason for the observed shift in each value.

Question 5 (10 points) Tendamistat is a 74-residue β-sheet protein that specifically inhibits mammalian α-amylases via formation of a 1:1 complex. Below is data from kinetic folding-unfolding experiments performed at room temperature and pH = 2.



(A) From the kinetic data, estimate the stability of the folded form of tendamistat relative to the unfolded form in the absence of denaturant.

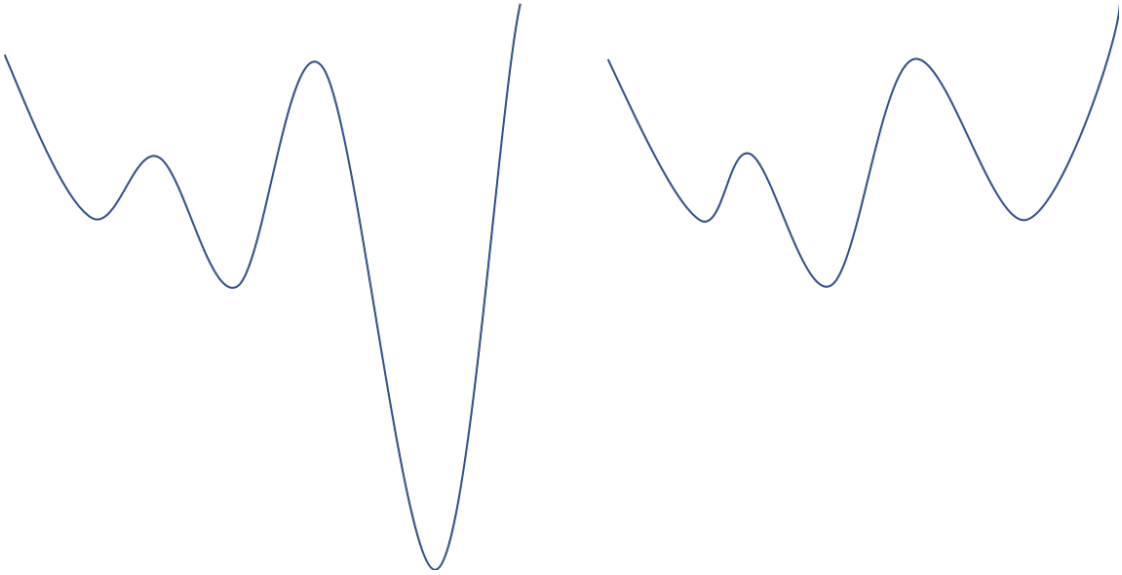
(B) Kinetic data is often treated via conventional transition state theory which connects the rate constant (k) to the free energy barrier ($\Delta G^{\pm}$) via the relation

$$k = \left(\frac{k_B T}{h}\right) \cdot e^{-\frac{\Delta G^{\pm}}{RT}}$$

where k_B is Boltzmann's constant (1.38×10^{-23} m^2 kg s^{-2} K^{-1}), h is Planck's constant (6.626×10^{-34} m^2 kg s^{-1}), and R is the gas constant (1.9872 cal/mol/deg). Draw a free energy *vs*. "reaction coordinate" diagram for the folding process. Find the energy of the folding transition state.

Question 6 (10 points)

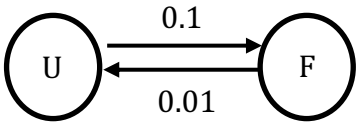
(A) Which landscape is more complex according to the Shannon entropy metric? Circle your answer.

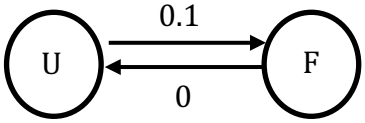


(B) Explain what it is that makes the landscape you chose in part (A) more complex?

Question 7 (10 points)

(A) The following Markov state models (MSMs) have an unfolded state (U), a folded state (F), and transition probabilities between them. Which one violates detailed balance? (circle one)





(B) What's "wrong" with the model that violates detailed balance?

Question 8 (5 points)

You are studying the folding of a protein that appears to have two-state behavior. The equilibrium probability of the folded state is 0.9 and the equilibrium probability of the unfolded state is 0.1. You've also determined that the probability of unfolding in a 100 ms time interval is 0.005. What is the probability of folding in a 100 ms time interval?

Question 9 (10 points)

Which coarse-graining is a better representation of the black energy landscape according to the relative entropy metric? (your choices are blue dotted or red dashed) Why is your choice a better representation?