Biology 5357

Chemistry & Physics of Biomolecules

Examination #1

Proteins Module

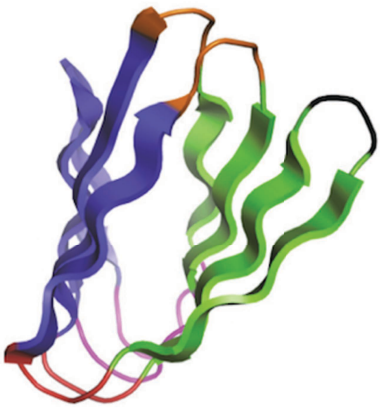
October 1, 2021

Name: _____

Question 1 (10 points; A-B, 5 points each)

(A) Tuftsin is an immunostimulatory tetrapeptide of sequence Thr-Lys-Pro-Arg that is released via cleavage of residues 289-292 from immunoglobulin G. It is named for Tufts University, where it was first discovered in 1983. Draw the chemical structure of tuftsin in aqueous solution at pH = 7, paying attention to protonation state and chirality.

(B) The Baker lab at the University of Washington designed and produced a novel jellyroll fold. Which of the two structures shown below, ***1*** or ***2***, is the correct protein. Explain. What are the two main sheet-sheet packing motifs mentioned in class, and which one is exhibited by this protein?



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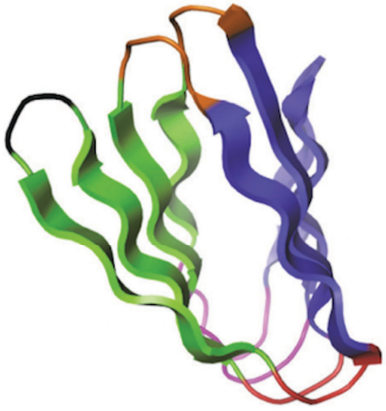

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Question 2 (15 points; A-C, 5 points each)

The urea equilibrium folding-unfolding curve for a protein that follows 2-state behavior can be characterized by the urea concentration at the midpoint of the transition region, $[urea]_{50\%}$, and by the slope of the curve at the midpoint of the transition, the "*m*" value.

(A) Find the $\Delta\Delta G$ between folded and unfolded protein in 1M urea for a protein with $[urea]_{50\%} = 3.5M$ and $m = 1.80$ kcal/mol/M.

(B) How does the relative stability of the folded and unfolded protein states in pure water change if the $[urea]_{50\%}$ concentration increases? What is the change in relative stability as the *m* value increases?

(C) What kinds of physical interactions in the protein folding process are associated with changes in the *m* value of a folding-unfolding curve?

Question 3 (15 points; A-C, 5 points each)

In a Metropolis Monte Carlo calculation, a move from the current state to a trial state is accepted if the energy of the system is lower in the trial state. If the trial state is higher in energy, then the move is accepted only if a uniform random number between 0 and 1 is less than $e^{-\Delta E/kT}$, where ΔE is the energy of the trial state minus the energy of the current state, k is Boltzmann's constant, and T is the temperature.

(A) Why is Metropolis Monte Carlo typically preferred for biomolecular sampling over the simpler Monte Carlo protocol making a series of trial moves and then accepting all of the moves.

(B) Explain the importance of the factor of $e^{-\Delta E/kT}$ in the Metropolis method. As part of your answer, justify the use of that precise value in the acceptance criterion.

(C) Hybrid Monte Carlo-Molecular Dynamics methods can help to increase the sampling of conformational or configurational space during protein simulations. One such method mentioned in class is Replica Exchange, also known as Simulated Tempering. Briefly describe how this method works.

Question 4 (15 points; A-C, 5 points each)

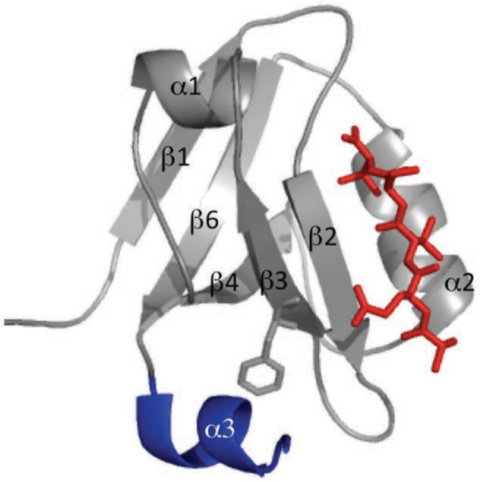
(A) Many commonly used drugs bind tightly to their target proteins with a dissociation constant (K_d) in the nanomolar range, where the K_d for a drug (D) binding to a protein (P) is defined as $K_d = [P][D] / [P \cdot D]$. What is the binding energy for a drug having $K_d = 1 \times 10^{-8}$ M? Is this binding value a free energy, an enthalpy, or an entropy?

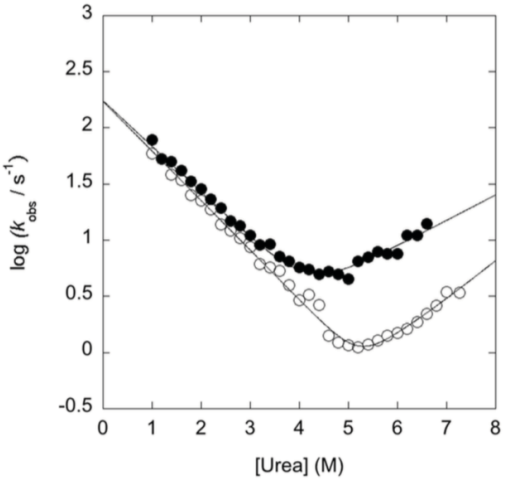
(B) Two ways to improve the binding enthalpy of a drug are by adding groups to the drug that can hydrogen bond to the target protein, or adding hydrophobic groups that can pack into nonpolar pockets of the protein. Both methods are used, but one is more generally effective. Suggest an explanation.

(C) It is often observed that improving the binding enthalpy of a drug to its target protein results in a corresponding unfavorable change in the entropy of binding, a so-called "enthalpy-entropy compensation". Provide a physical rationale for this effect.

Question 5 (15 points; A=10 points, B=5 points)

At left below is the structure of a typical PDZ domain (PDB:1BE9). There are over 200 PDZ domains in the human genome, and they modulate protein interactions. Shown on the structure as grey sticks is residue 337, which was mutated to Trp and used as a fluorescence probe during rate measurements. In red is a peptide bound in the PDB structure, but not present during the kinetic experiments. The chevron plot shows rate data for the full PDZ domain (empty circles), and the same domain truncated to delete the α3 helix shown on the structure in blue (filled circles).





(A) From the data in the chevron plot, estimate the ΔΔG between the folded and unfolded states for the full PDZ domain and the Δα3 truncated domain.

(B) What is the effect of the helix truncation on the folding of this PDZ domain? Is the effect primarily on the stability of the unfolded state, the folded state, or the transition state for folding? Explain using a "reaction coordinate" diagram.

Question 6 (10 points; A-B, 5 points each)

(A) What features of a protein sequence might influence or determine if it is folded or disordered?

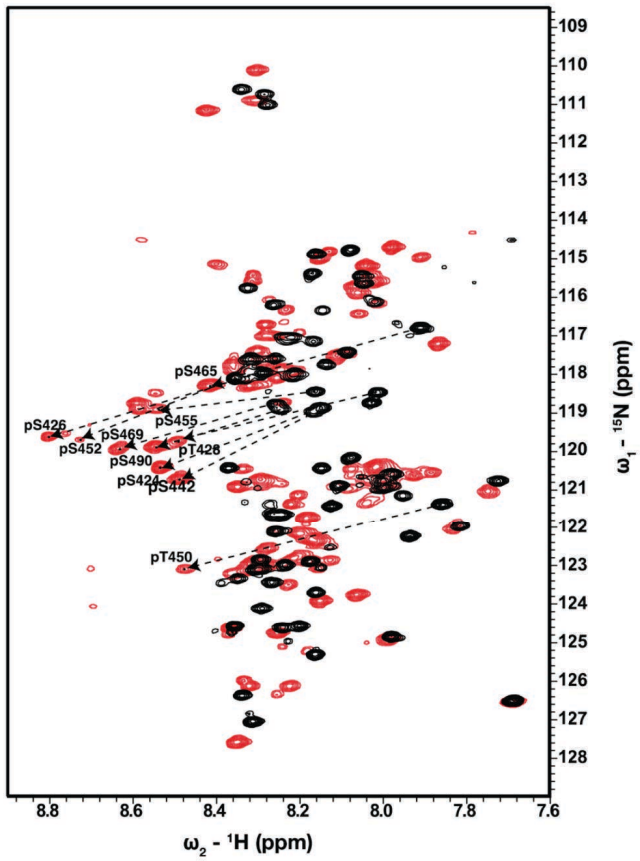
(B) What are some of the challenges in studying disordered proteins experimentally?

Question 7 (5 points)

Nick prefers to use molecular dynamics to study his disordered proteins, while Sonia prefers to use Monte Carlo simulations. What reasons might Nick and Sonia have to prefer one over the other?

Question 8 (5 points)

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 9 (10 points; A-B, 5 points each)

(A) 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 can disordered regions confer specificity?

(B) Describe one or two functions of disordered regions, explaining why disordered is relevant/important in that function?