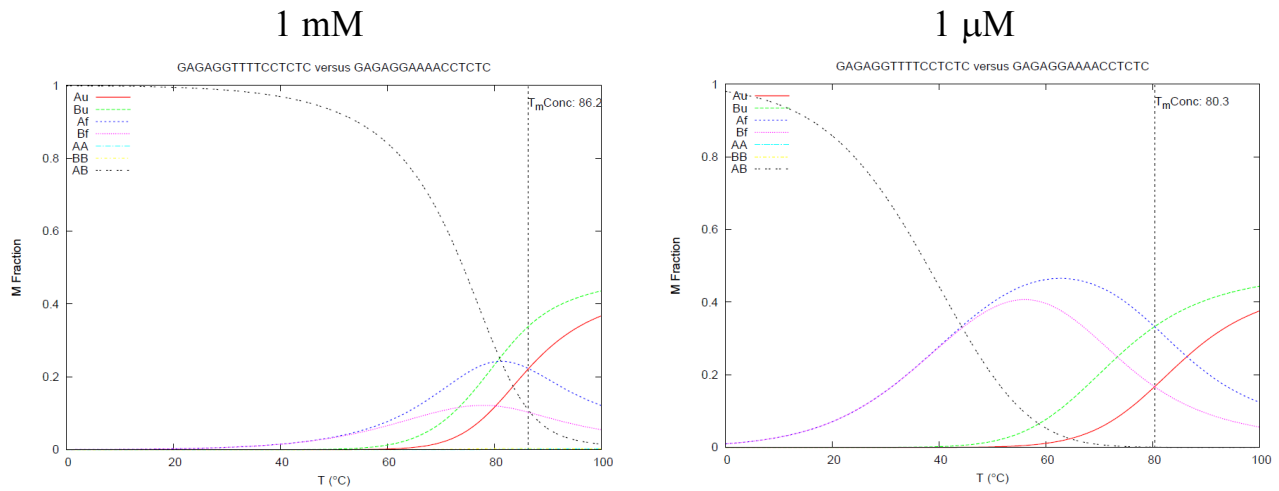# Biology 5357

Chemistry & Physics of Biomolecules

Examination #2

Nucleic Acids Module

November 4, 2019

Name: _____

Question 1. (9 points) Below are melting temperature curves for the sequence shown at 1 mM and 1 μM concentration.

1 mM

GAGAGGTTTTCCTCTC versus GAGAGGAAAACCTCTC

1 μM

GAGAGGTTTTCCTCTC versus GAGAGGAAAACCTCTC

(A) Propose structures for Af (A folded) and Bf (B folded).

(B) Explain why Af and Bf predominate at 60°C at 1 μM concentration whereas at 1 mM concentration A-B (duplex form) predominates.

(C) What do you conclude from the observation that the curves for Af and Bf are different?

Question 2. (9 points) The nucleotide shown below in two views is G21 that was found to be bound to a guanine specific pocket in the ReqQ helicase bound to a G-quadruplex forming sequence. Strangely, the authors of the discussion paper you read for class did not comment on the conformation of this nucleotide in spite of it being a central focus of their paper.
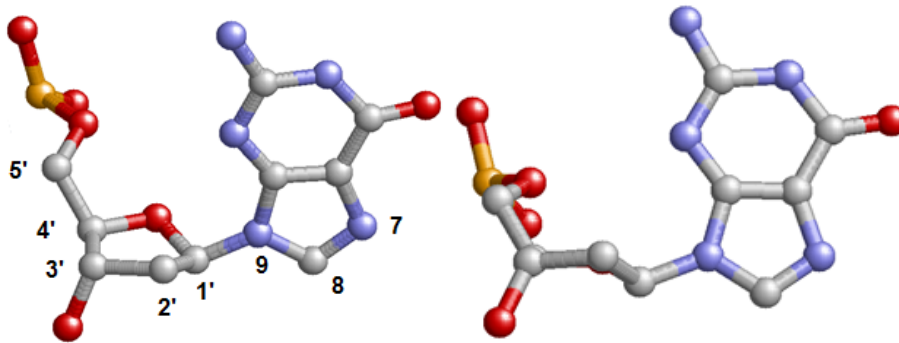
(A) What is different about the conformation of G21 than for of a nucleotide in B DNA? Be specific as to the conformational feature. How might this be relevant to the purported function of the G binding pocket in RecQ helicase?

(B) What type of pucker is the sugar subunit adopting (using endo, exo, twist nomenclature. How is this different than for B DNA? How might this sugar pucker facilitate the nucleotide to adopt the particular conformational feature discussed in (A).

(C) The two amino acids, Asp312, and Ser 245, were each found to recognize G21 using two H-bonds. For Asp312, these involved the carboxylate side chain, and for Ser 245 it involved the amide NH and the alcohol group. Sketch the guanine base showing one of these interactions (show all relevant protons and charges).

Question 3. (14 points) Consider the proposed folding scheme of single strand DNA of the form 5'-d(GGGNNN)₃(GGG) into various folded structures A-D, including two G-quartet type structures.


A   B   C   D
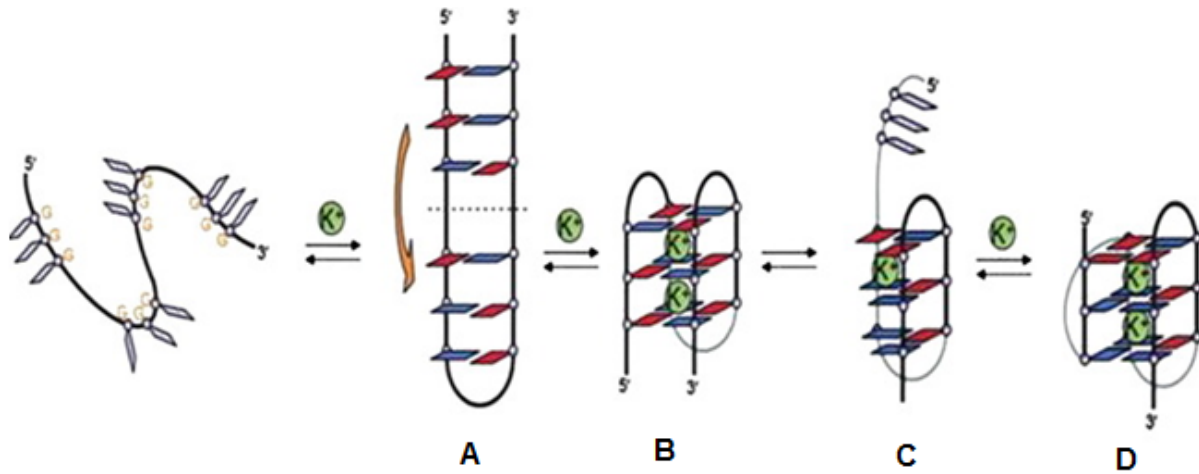
(A) Give the name by which these structures are commonly referred to.

(B) If a single stranded DNA containing all the nucleotides in a normal B-DNA conformation, were to simply fold in half, what type of base pairing would take place between the G's? Draw the base pair below. What is it called?

(C) Could the folded structure discussed in part (B) simply fold in half again into a G-quadruplex, as is shown for A going to B? Why?

(D) Assign the key conformational feature for nucleotides 4-12 in the G-quadruplex shown below, given that nucleotides 1-3 are all in a B DNA-like conformation.
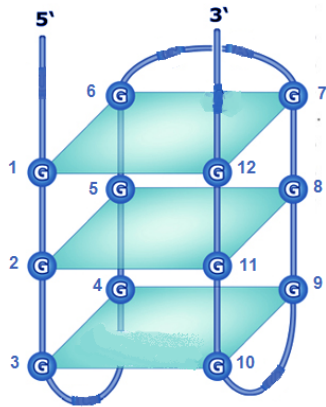
4		7		10	
5		8		11	
6		9		12	

(E) Draw the base pairing involved between the G's in the structure in d). What is it called?

(F) Why is potassium needed for the folding?

(G) What would have to happen to the conformation of nucleotides 1-3 when going from structure B to structure D?

Question 4. (7 points) Choose whether the following statements are true or false.

(A) End-to-end distance and the radius of gyration distribution can be described by the same mathematical expression. ☐ True ☐ False

(B) The mean end-to-end vector distance is different in poor and good solvent ☐ True ☐ False

(C) The ratio between the mean-square radii of gyration of two ideal polymers with identical monomers is equal to the ratio of their degrees of polymerization. ☐ True ☐ False

(D) The ratio between the root-mean-square end-to-end distance and the root-mean-square-radius of gyration for an ideal chain is equal to 1/6. ☐ True ☐ False

(E) The contour length of a polymer in good solvent is larger than the one in poor solvent. ☐ True ☐ False

(F) At the theta temperature, the two-body interactions cancel out and the polymer follows the statistics of an ideal chain. ☐ True ☐ False

(G) In a phase-separated polymer solution, the chemical potentials in the light phase and dense phase are identical ☐ True ☐ False

Question 5. (10 points) The sequence of a Cold Shock protein from *Thermotoga maritima* contains 67 amino acids.

(A) If the distance $C\alpha$-$C\alpha$ is equal to 0.38 nm, what is the contour length of the protein?

☐ 1705.82 nm   ☐ 9.6748 nm   ☐ 25.46 nm²   ☐ other:_____

(B) Assuming that an aqueous buffer is a theta-solvent for the unfolded state of the protein and that the root-mean-square radius of gyration is equal to 2 nm, which is the expected root-mean-square end-to-end distance of the protein?

☐ 4.90 nm   ☐ 12 nm   ☐ 2 nm   ☐ other:_____

(C) Assuming the same number and length of Kuhn segments, what would be the root-mean-square end-to-end distance of the protein in an athermal solvent?

 ☐ 2.40 nm   ☐ 7.09 nm   ☐ 5.56 nm   ☐ other:_____

(D) Assuming the same number and length of Kuhn segments and ideal chain behavior, what would be the root-mean-square internal distance for a segment of the protein of 30 amino acids?

 ☐ 3.28 nm   ☐ 5.10 nm   ☐ 4.23 nm   ☐ other:_____

(E) Using the root-mean-square end-to-end distance and the contour length of the protein in aqueous buffer, can you estimate the size and number of the Kuhn segments?

 ☐ N_K=67, b_K 0.38 nm   ☐ N_K=27, b_K 0.94 nm
 ☐ N_K=132, b_K 0.19 nm   ☐ other:_____

Question 6. (5 points) Explain the plot below which was generated using magnetic tweezers. Remember that sigma represents a super-helical density which is linearly related to the number of turns introduced into the DNA using the magnets. Use the concepts of Twist, Writhe, and Linking number.
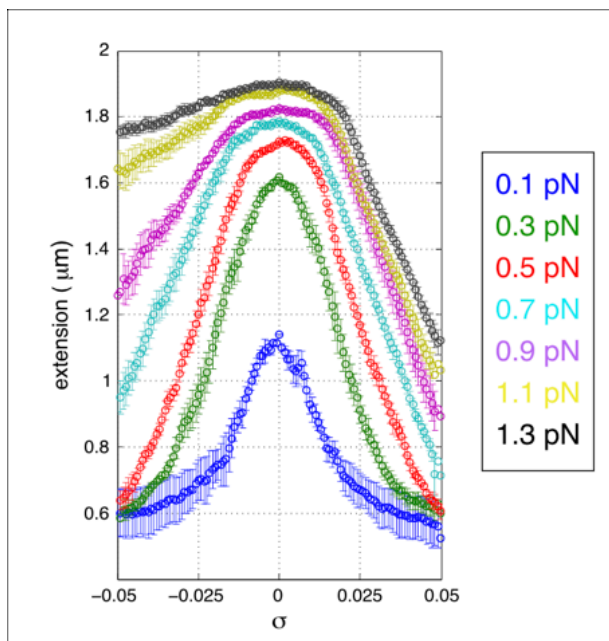
Question 7. (7 points; A=5 pts, B=2pts)

(A) Draw a free energy diagram that describes the effect of a force on the equilibrium between two states. Label all of the aspects of your diagram, and provide a detailed legend and caption to explain the figure.
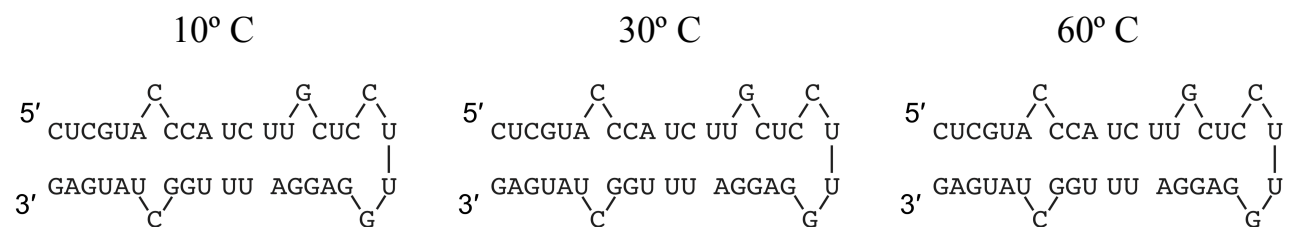
(B) Based on the figure you drew above and the quantities that describe the equilibrium in the absence of force (*i.e.*, the free energy difference between states), how would you calculate the amount of force required to make the probability of each state the same?

Question 8. (3 points) What energetics dictate the low-force and high-force regimes of double-stranded DNA stretching? What worm-like-chain parameters describe each regime?

💡 *For an extra point*, draw a force-extension curve of DNA from 0 to 100 pN and label the different regions of the plot.

Question 9. (16 points) Mg^{2+} ions use the phosphodiester and ribose in an RNA to facilitate cleavage of the backbone, as we described for the Group I intron chemistry. Now, use this chemistry to probe the solution structure of the RNA below as a function of temperature. Incubate the RNA in a solution of 100 mM KCl and 10 mM $MgCl_2$, buffered at pH 7.0, at 10° C, 30° C, and 60° C for two hours. Illustrate the predicted cleavage patterns for each temperature on the secondary structure models.

10° C	30° C	60° C
5′ CUCGUA CCA UC UU CUC U 3′ GAGUAU GGU UU AGGAG U	5′ CUCGUA CCA UC UU CUC U 3′ GAGUAU GGU UU AGGAG U	5′ CUCGUA CCA UC UU CUC U 3′ GAGUAU GGU UU AGGAG U

Provide a rationale for your predictions.

Question 10. (10 points) Investigations of the ion dependence of the GAAA tetraloop-receptor led to the conclusion that only divalent ion was 'bound' in the transition from undocked to docked conformations. Speculate where you think this ion could be physically located, and justify your speculation.

Question 11. (10 points) A single ribonucleotide (rN) is sometimes mistakenly incorporated into a DNA duplex by DNA polymerase. This is an unhappy event, and the rN must be excised. Fortunately, there is a specialized enzyme that can recognize a single inserted rN and remove it. What features of the rN in the duplex would allow the enzyme to identify it?