Biology 5357

Chemistry & Physics of Biomolecules

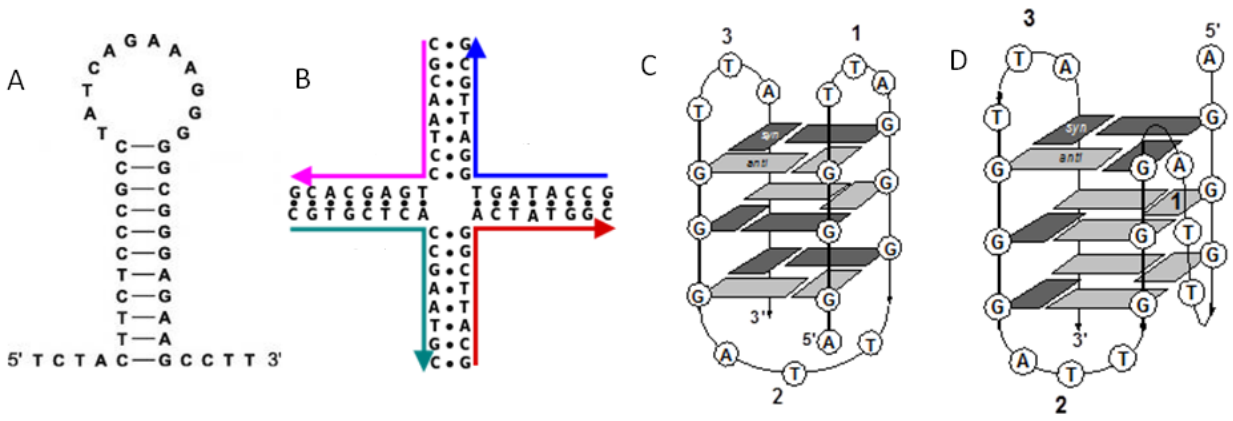
Examination #2

Nucleic Acids Module

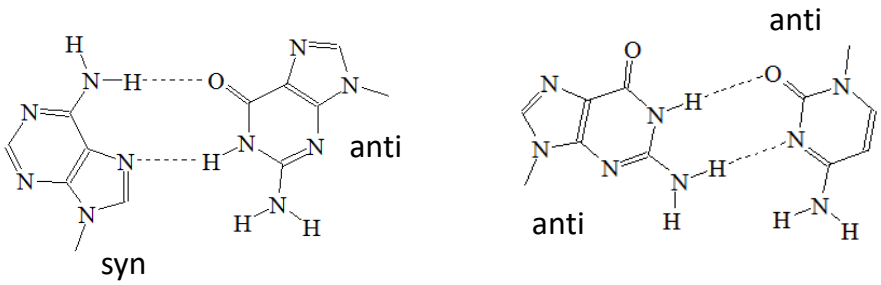
November 2, 2018

Name: _____

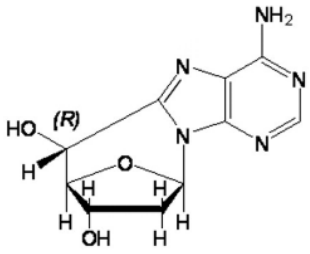
Question 1. (8 points; A-D, 2 pts each) Name the following types of DNA topologies.

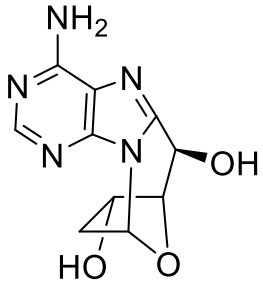


Question 2. (4 points) Predict whether or not the following base pairing with the indicated glycosyl conformations would lead to parallel or antiparallel helices. Briefly explain your answer.



Question 3. (6 points; A-B, 3 pts each) CyclodA is a product produced by ionizing radiation and results from attack of a radical at C5 onto the C8 position of the adenine. It is often presented in journal articles and textbooks as the structure on the left with a planar deoxyribose ring, which gives no indication of the conformation of the ring. The structure on the right is redrawn to show more accurately what its conformation is expected to look like.





(A) Based on the conformation depicted assign the sugar pucker adopted by this damaged nucleoside (using the *endo*, *exo*, or twist convention).

(B) Comment on how this sugar pucker in undamaged DNA would compare energetically to the puckers found in A- or B-form DNA, and give a reason why. You may use drawings to help explain your answer.

Question 4. (9 points; A-C, 3 pts each) Friedreich's ataxia (FRDA), is an autosomal recessive degenerative disorder of nervous and muscle tissue caused by the expansion of d(GAA) repeats that occur in the first intron of Frataxin gene. The highly repetitive DNA has been found to adopt altered structures among which are the H DNA structure that results from intramolecular triplex formation.

Draw the two general H DNA topologies that $d(GAA)_n$ repeats could adopt that use either the purine strand or the pyrimidine strand as the triplex forming deoxyoligonucleotide, as instructed below. Assume all nucleotides are in the *anti* glycosyl conformation. Be careful to consider the orientation of the strands.

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5′----GAAGAAGAAGAAGAAGAAGAA----3′
3′----CTTCTTCTTCTTCTTCTTCTT----5′
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(A) Draw the topology of the H DNA that favors lower pH on the left. Then on the right side draw the structure of the base triplet that explains the origin of this effect. Indicate the type of base pairing that is involved.

(B) Draw the other type of H DNA topology to the left, and one of the triplets involved on the right. Indicate the type of base pairing that is involved.

(C) How could one differentiate these two types of H DNA with chemical probes.

Question 5. (6 points; A-B, 3 pts each)

(A) Why does DNA produce a restoring force when its end-to-end distance in smaller than its contour length?

(B) Based on your answer to (A), and the equation for an extensible worm-like-chain below, how do you expect the spring constant of DNA to change with temperature?

$$x = L_0 \left(1 - \frac{1}{2}\left(\frac{k_B T}{FP}\right)^{1/2} + \frac{F}{K_0}\right)$$

Question 6. (4 points) How does the stiffness of DNA (*i.e.*, its spring constant) change as a function of force? Use a Force-Extension plot to illustrate this effect.

Question 7. (6 points) What are the physical principles/effects that lead to the production of a three-dimensional optical trap? You may use diagrams to help explain.

Question 8. (7 points; A-G, 1 pt each) Choose whether the following statements are true or false.

The root-mean-square radius of gyration of an ideal polymer…

(A) is smaller than the root-mean-square end-to-end distance	☐ True ☐ False
(B) is the radius of the average sphere that comprises each monomer	☐ True ☐ False
(C) can be measured with SAXS	☐ True ☐ False
(D) can be measured with FRET	☐ True ☐ False
(E) is the radius at which the Gaussian distribution of the radii of gyrations adopted by the polymer has maximum probability	☐ True ☐ False
(F) scales as the root-mean-square end-to-end distance	☐ True ☐ False
(G) is the radius of the largest sphere that comprises each monomer	☐ True ☐ False

Question 9. (15 points; A-E, 3 pts each) In Murphy, *et al.* (*Biophysical Journal*, 2004) ssDNA of 23 nucleotides is measured with FRET and analyzed in terms of a wormlike chain. The unit length of each base is estimated as 6.3 Å. The persistence length in buffer is 3 nm.

(A) What is the contour length of the ssDNA?

(B) Assuming we can describe the ssDNA as a flexible wormlike chain, what is the mean-square end-to-end distance?

(C) What is the length of the Kuhn segment if we interpret the data in terms of an ideal chain?

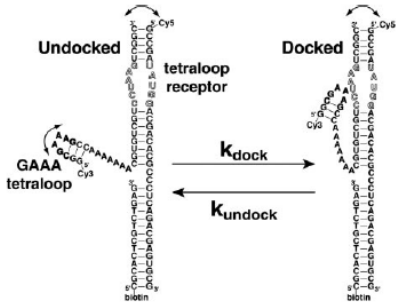
(D) If the scaling exponent of the ssDNA in buffer is 0.53 what type of solvent is the buffer for ssDNA?

(E) What is the mean-square end-to-end distance for a chain that is twice the number of nucleotides?

Question 10. (4 points) What does the Flory χ parameter describe?

Question 11. (7 points) Higher concentrations of monovalent salts [MX] increases the stability (melting temperature) of duplex nucleic acids. Higher concentrations of monovalent salts [MX] decrease the stability of a protein:nucleic acid complex. Explain the physical chemical basis of these phenomena.

Question 12. (24 points; A-C, 8 pts each) Consider the RNA shown below. The solution conditions are T = 22C, buffer with 100 mM KCl, 10 mM HEPES at pH = 7.0, and [RNA] << $[Mg^{+2}]$.



Rate constants and associated equilibrium for docking and undocking at various $[Mg^{2+}]$

$[Mg^{2+}]$, mM	k_{dock}, s^{-1}	k_{undock} s^{-1}	K_{dock} (k_{lock}/k_{undock})
≈ 0.0	5.1 ± 0.3	10.3 ± 0.4	0.49 ± 0.04
0.35	10.5 ± 0.2	7.7 ± 0.2	1.36 ± 0.05
0.5	17.7 ± 0.5	6.8 ± 0.2	2.6 ± 0.1
1.0	30.1 ± 1.3	7.2 ± 0.3	4.2 ± 0.2
2.0	38.6 ± 1.3	5.5 ± 0.1	7.0 ± 0.3
5.0	51.2 ± 1.1	4.2 ± 0.2	12.3 ± 0.6
10.0	63.1 ± 1.9	3.3 ± 0.1	19.1 ± 0.9

(A) How do you interpret the effect of Mg^{+2} on the rate constants (k_{dock}, k_{undock}) of the interaction?

(B) If the A_7 linker were longer, would you expect to see a difference in the rates of docking/undocking? Why or why not?

(C) If the linker was U_7, would you expect to see a difference in the rates of docking/undocking? Why or why not?