Biology 5357

Chemistry & Physics of Biomolecules

Examination #2

Nucleic Acids Module

November 4, 2019

Answer Key

Question 1

(A) (3 points)

Both sequences adopt hairpin structures:

$GAGAGGT_4$	$GAGAGGA_4$
CTCTCC	CTCTCC

(B) (3 points)

Af and Bf form intramolecularly, and hence will be favored at low concentrations over the bimolecular A-B duplex

(C) (3 points)

The Af and Bf curves are different since the stability of the hairpin loops shown above are sequence dependent.

Question 2

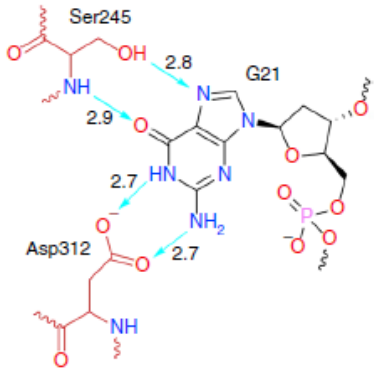
(A) (3 points)

The nucleotide is in a syn-glycosyl conformation. G-quadruplexes with antiparallel strands contain syn-glycosyl conformations.

(B) (3 points)
The sugar pucker is C1′-exo, in contrast to the C2′-endo that predominates in B DNA. Such a conformation would minimize bad steric interactions between the guanine in a syn-glycosyl conformation and the rest of the sugar ring, in particular the C5′-group.

(C) (3 points)

The answer should also include the protons pointing at the H-bond acceptors (not the case for the amino group and carboxylate as shown in the paper.



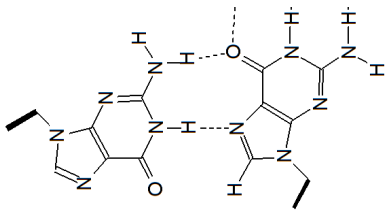
Question 3

(A) (2 points)

A: hairpin B: chair form G-quadruplex C: triplex D: hybrid G-quadruplex

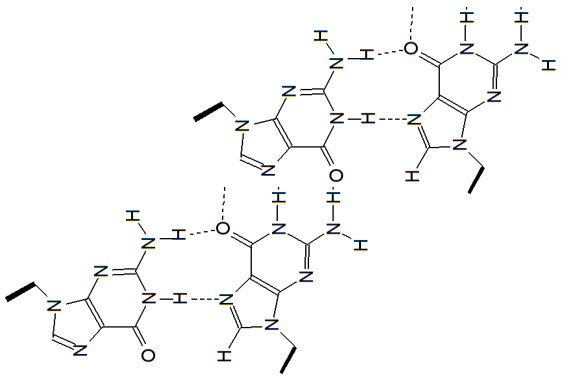
(B) (2 points)

Because the sugars are in a B-type conformation, the glycosyl bonds are in an anti-conformation. In such a case reverse Hoogsteen base pairing is required to make the antiparallel helix in the hairpin.



(C) (2 points)

No, because this structure cannot lead to a cyclic array of G's. It can only make an extended sheet-like structure.

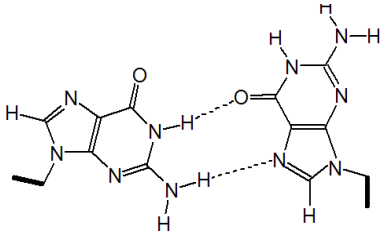


(D) (2 points)

If nucleotides 1-3 are in an anti-conformation, then any base paired strand that is antiparallel to it, must have the syn-glysocyl conformations, and vice versa. Then we have: 4-6 syn, 7-9 anti, and 10-12 syn.

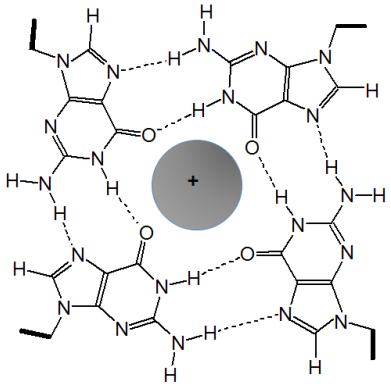
(E) (2 points)

A Hoogstein base pair.



(F) (2 points)

Potassium is needed to stabilize the quartet by electrostatic/dipole interaction with the carbonyl groups.



(G) (2 points)

They would have to flip fro whatever glycosyl conformation they were in to the other (anti <=> syn).

Question 4 (1 point each part)

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

False

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

(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

(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. False

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

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

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

Question 5 (2 points each part)

(A) other: 25.46 nm (lc = # amino acids * Ca-Ca bond length = 25.46 nm)

(B) 4.90 nm (sqrt<ree^2> = sqrt<rg^2> * 6^0.5 = 4.90 nm)

(C) 7.09 nm (sqrt<ree^2> / sqrt(67) * 67^0.588 = 7.09 nm)

(D) 3.28 nm (sqrt<ree^2> = sqrt<67> * sqrt(30) = 3.28 nm)

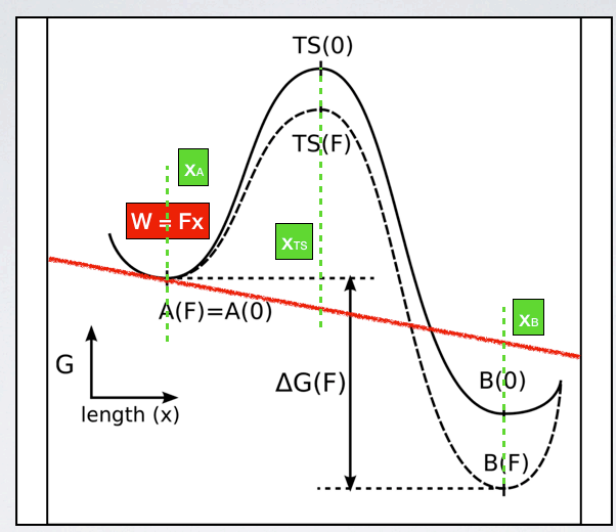
(E) N_K=27, b_K 0.94 nm (r^2 / lc = bk = 0.94 nm)

Question 6

(6 points) The equation for Twist, Writhe and Linking number is Lk = Tw + Wr. At low force, the rotation-extension curve is symmetric as both negative and positive links introduced by magnet rotation are absorbed by the system in the form of writhes (Wr) which reduce the end-to-end extension of the DNA. At high force, the curve flattens out in the presence of negative links. This is due to the system preferentially absorbing negative links as negative twist (Tw, *i.e.*, DNA melting).

Question 7

(A) (5 points)



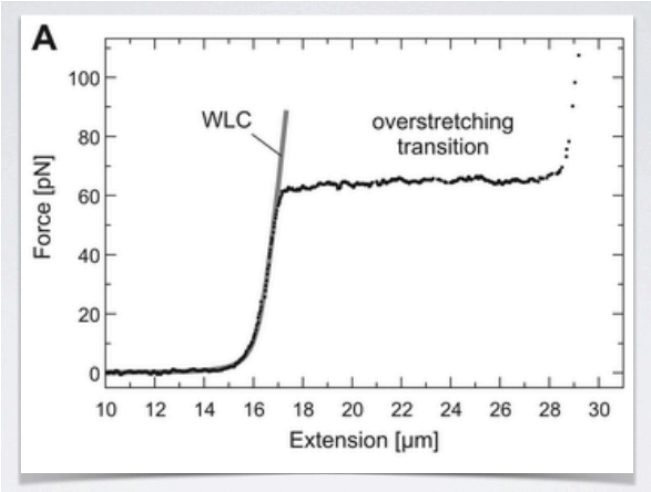
(B) (2 points)

Force = F = $\Delta G(0) / x$, where $x = x_A + x_B$.

Question 8

(3 points) The low force regime is dominated by *entropic elasticity* (i.e., limitation of number of states, persistence length). The high force regime is dominated by enthalpic elasticity (*i.e.*, deformation of DNA structure, stretch modulus).

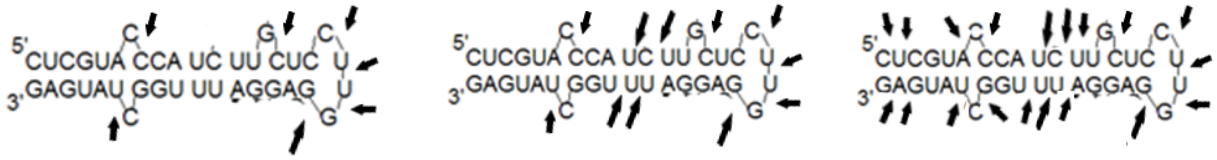
(1 extra credit point) Below is a DNA force extension curve from 0 to 100 pN.



Question 9

(9 points; 3 pts each cleavage pattern)

10º C 30º C 60º C



(1 point) Mg^{2+}-mediated cleavage relies on flexibility of the RNA strand that allows the correct geometry to form that leads to cleavage. This geometry is not possible in a stable A-form duplex, but it can occur transiently where nucleotides are 'single-stranded' such as in a loop or internal bulge. The transient nature of conformational arrangements is why the incubation is for 2 hours – to capture those events.

(2 points) At 10° C, the duplex will be stable so no cleavage. However, the loop does not have a stable structure, so it can be cleaved (probably with low efficiency). If the bulged bases are extruded or are flipping in and out of the duplex, cleavage could occur to their 3′ side.

(2 points) At 30° C, the loop is floppy, the bulged bases are floppy, and the U:U and U:C mismatches are not stable in the duplex. These sites can all be cleaved, some with higher efficiency than others.

(2 points) At 60° C, the terminus of the hairpin is fraying, and those bases become susceptible to cleavage. Nucleotides flanking the bulged nucleotides can also move, and the mismatches have extended the duplex instability to flanking base pairs.

Question 10

(10 points) This construct contained a linker, the tetraloop, and the receptor. We can speculate that the ion is in the receptor, and that it becomes "bound" when the tetraloop is docked.

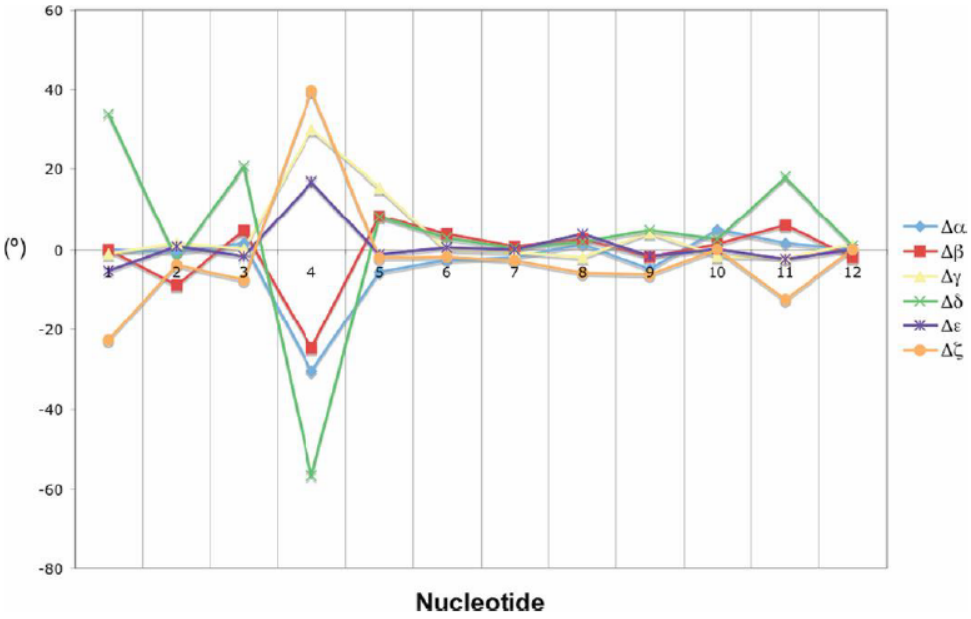
The GAAA tetraloop can adopt different conformations in solution. Assuming that there is an ensemble of structures that are interconverting, some of them will be conformationally poised to dock while others are not. The ensemble might be sensitive to divalent ions, but might not. The linker will use the sea of divalent ions

to nonspecifically shield its phosphates to allow close approach to the duplex, but it will need more than one ion. The receptor has mismatches and unpaired nucleotides that need to be stabilized in the docked conformation. Therefore, we can speculate that a Mg^{2+} ion is required to stabilize the docked complex.

Since we don't know where the ion is, if you suggest that it is within the tetraloop and provide a cogent rationale, you will be given credit.

Question 11

(10 points) The backbone will be severely deformed around the insertion, as seen in this NMR structure of the Dickerson dodecamer that contains a single rG. The enzyme RNase H2, which is present in all branches of life, has a specialized function in the cleavage of single ribonucleotides embedded in the DNA.



d(C1GC)rG4d(AATT C GCG12)
d(GCG C TTAA)rG4d(CGC)