

## Biochemistry 674: Nucleic Acids

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### Exam I

October 9, 1997

You have 80 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

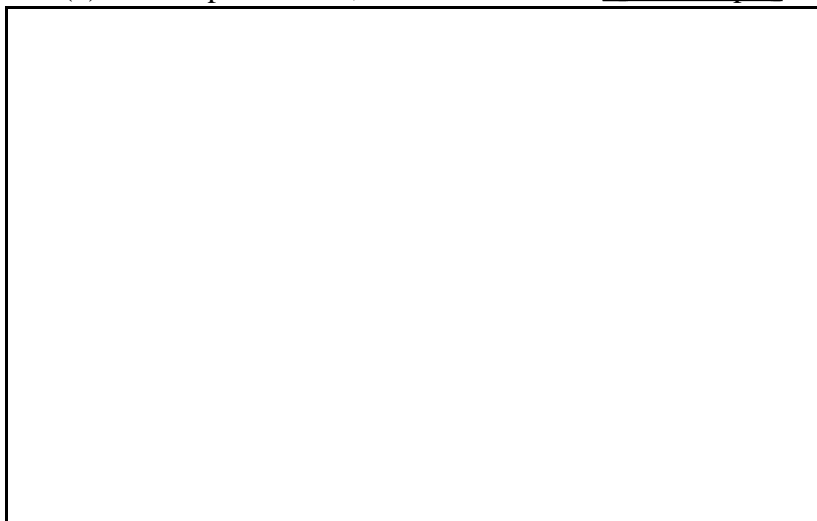
Explanations should be concise and clear.

You will need a calculator for this exam. No other study aids or materials are permitted.

Generous partial credit will be given, i.e., if you don't know, guess.

1. RNA and DNA geometry and chemistry (20 pts).
  - a. (8) Draw the structure of the T•A-T triple base pair found in the Moser and Dervan style DNA triple helix. Draw the sugar only of the adenosine.

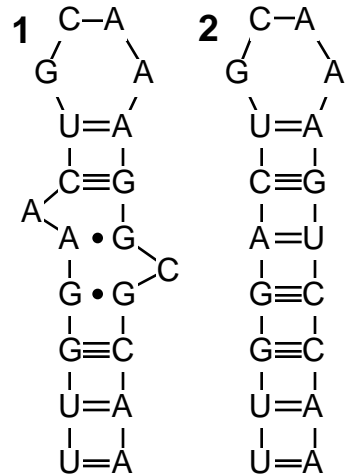
- b. (4) In the space below, draw a reasonable G•G base pair.



- c. (2) Indicate major and minor grooves and 5' and 3' ends on the duplex DNA at the right.



- d. (6) Which of the RNA structures **1** or **2** shown at the right is more likely to be a target for a protein which binds RNA in a sequence-specific fashion, and why?



2. Supercoiling (20 pts):

This question concerns a 3000 base pair closed-circular plasmid. Assume a DNA helical repeat of 10.5 bp/turn.

- (a) (4) Calculate  $Lk^\circ$ . for  $\Delta Lk = (Lk - Lk^\circ)/Lk^\circ = -0.06$ , calculate  $Lk$ . If the linking number deficit partitions 80% into writhe and 20% into twist, calculate  $Tw$  and  $Wr$ .

The plasmid contains the 42 base pair palindromic sequence below (i.e. the two 18-base inverted repeats in capital letters), which can form a cruciform structure.

```
5 ...agtGCTAGCTCCGTAGCAAGAtacaggTCTTGCTACGGAGCTAGCttcc...3
3 ...tcacCGATCGAGGCATCGTTCTatgtccAGAACGATGCCTCGATCGaagg...5
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- (b) (2) Sketch the cruciform secondary structure. You need not write the sequence.

- (c)(5) What does the segment of DNA in the cruciform contribute to the overall linking number of the plasmid, and thus how does it change the  $T_w$  and  $W_r$  of the plasmid when it “pops out”? (Hint: what did the initial duplex DNA contribute to  $T_w$ ?)

- (d) (5) It has been observed that the cruciform will “pop out” of a plasmid with  $Lk = -25$  but not  $Lk = -10$ , although the resulting change in  $Wr$  and  $Tw$  from extrusion is the same for each plasmid. What provides the thermodynamic driving force for extrusion? Qualitatively, explain why increased negative supercoiling is needed for pop-out.

- (e)(4) What are the two basic forms of superhelical writhe? Sketch an interwound plectonemic superhelix with negative writhe, indicating the superhelical nodes.

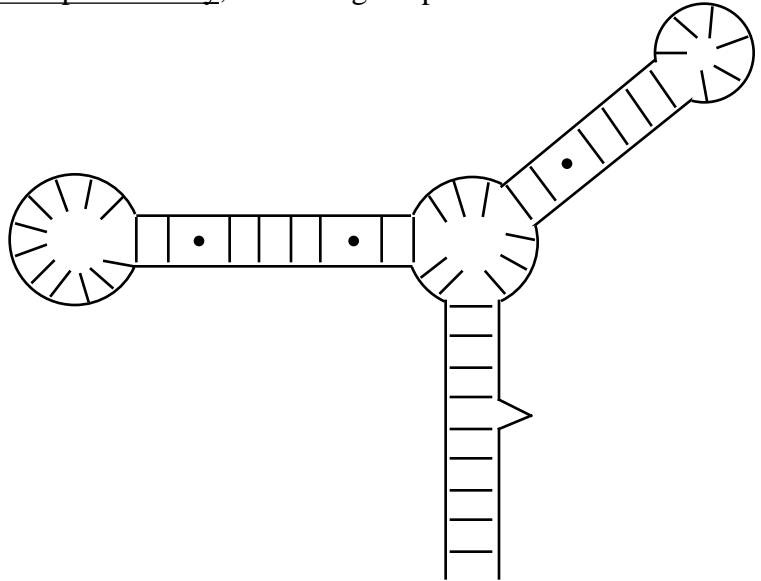
### 3. Structure and stability (20)

We have explained the thermodynamics of hybridization and the phenomenon of sequence-dependent microheterogeneity with empirical dinucleotide or nearest-neighbor models.

- (a) (4) What does the above statement mean in terms of how we go about predicting helix stability or structure? In principle, what other types of models could there be?

- (b) (4) The dinucleotide models for structure and thermodynamics originate from variations in the same physical interaction. What is this interaction? What spectroscopic signal is typically used to measure it, and what do we call the resulting graph?
- (c) (4) Why do we usually perform hybridizations (e.g. Southern) at just below the  $T_m$  of the hybridization probe, when it would hybridize much more stably at lower temperature?

- (d) (8) You have isolated an RNA and fed its sequence to the computer, which has come up with the model at the right based on dinucleotide thermodynamics. Briefly discuss two ways to test the predicted secondary structure experimentally, annotating the picture as needed.



4. Interactions with small molecules.

- (a) (6) Describe the phenomenon of counterion condensation around nucleic acids. What is the (somewhat surprising) thermodynamic driving force for the interaction of polycationic ligands with DNA?

- (b) (2) Why is  $Mg^{++}$  or other divalent metal required for tertiary folding of RNA?

- (c) (4) Draw a reasonable mechanism for the alkylation of G-N7 by dimethyl sulfate. Why is the glycosidic bond weakened by this reaction, and why do we care?
- (d) (4) We can use the intercalator ethidium bromide to make negatively supercoiled DNA by using a topoisomerase to relax DNA in the presence of ethidium. Why is it difficult to make positively supercoiled DNA by an analogous method?

- (e) (4) Why do groove-binding drugs tend to bind the minor groove whereas proteins tend to bind the major groove of DNA? How do the imidazole/pyrrole groove-binding drugs distinguish G from the other three bases?

5. Miscellaneous (20)

- (a) (5) In the equation below, what do  $a$  and  $L_c$  represent? With reference to the limiting behavior of the equation as  $L_c \rightarrow 0$  or  $L_c \rightarrow \infty$ , briefly describe the wormlike coil model for large-scale DNA structure.

$$\rho = a \left( 1 - e^{-L_c / a} \right)$$

- (b) (5) Sketch the reptation model for DNA electrophoresis, and discuss how it is used to rationalize the retarded electrophoretic mobility of bent DNA.



(c) (5) Draw a picture of the basic idea of Sanger sequencing of DNA.

(d) (5) How is sequencing a genome like sequencing a protein (what are the basic steps involved)? How does the adage “the first 90% of the work takes 10% of the time, and the last 10% of the work takes 90% of the time” apply to genome sequencing?

Question	Score (/20)
1	
2	
3	
4	
5	
Total	