Biochemistry 674: Nucleic Acids **Prof. Jason Kahn**

Exam I **October 14, 1999**

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. Supercoiling (20 pts):

This question concerns a 2500 base pair closed-circular plasmid. Assume a DNA helical repeat of 10.4 bp/turn.

(a; 5 pts) Calculate Lk°. for $\sigma = (Lk - Lk°)/Lk° = -0.05$, calculate ΔLk. If the linking number deficit partitions 80% into writhe and 20% into twist, calculate ΔTw and ΔWr.

The plasmid contains the 22 base pair sequence below , which can form a Z-DNA structure.

5′...CGCGCGCGCGCGCGCGCGCGCG...3′
3′...GCGCGCGCGCGCGCGCGCGCGC...5′

(b; 6 pts) If the segment of DNA above is converted into the left-handed Z form, with a helical repeat of 11 bp/turn, calculate the new partitioning of ΔTw and ΔWr for the plasmid. (Hint: what did the initially B-form DNA contribute to ΔTw, and what does the Z form contribute?)

(c; 4 pts) If a protein were to bind and stabilize the Z-DNA, would that contribute to restrained or unrestrained supercoiling? Making the doubtful assumption that the protein could maintain Z-DNA in the face of topoisomerase relaxation of the plasmid, what would the final ΔLk be upon relaxation?

(d; 5 pts) What are the two forms of superhelical writhe? Sketch a solenoidal superhelix with Wr = +3 and indicate the superhelical nodes.

2. RNA and DNA Geometry and Chemistry (18 pts).

(a; 4 pts) In the space below, draw rGTP and indicate which phosphates are (i) removed by GTPases or (ii) incorporated into the RNA product by RNA polymerases.

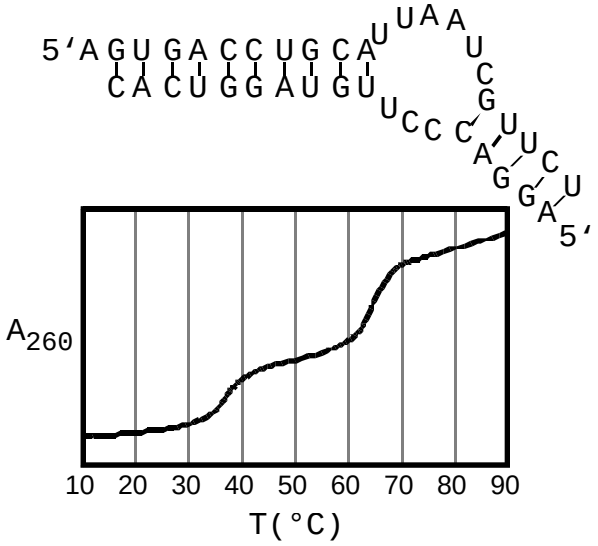
(b; 2 pts) Indicate the major and minor grooves and the 5′ and 3′ ends on the duplex DNA at the right.

(c; 12 pts) Draw the structure of a plausible T-T base pair. If your base pair were part of an extended paired structure, predict whether the two strands are parallel or antiparallel, and why (either can be right depending on what you've drawn). Now, draw a C-C base pair corresponding to the other polarity, i.e. if your T-T turned out parallel, draw a C-C which would support an antiparallel structure. Assume all anti glycosidic angles. You need not draw sugars but thinking about them may be helpful.

3. Structure and stability (14 pts)

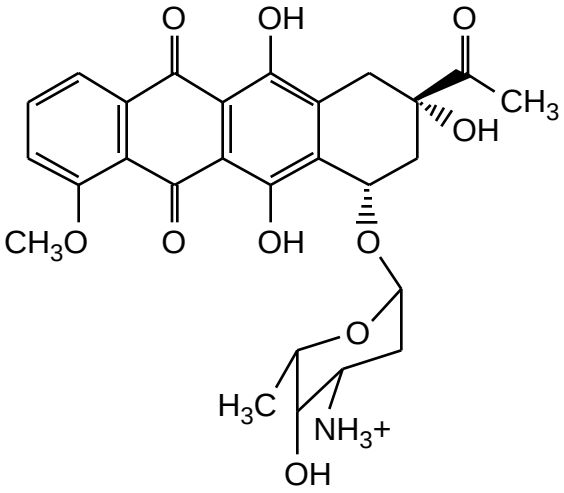
(a; 6 pts) How are RNA folding and protein folding fundamentally different from each other in terms of the relationship between secondary structure and tertiary structure? On the other hand, what is similar about RNA and protein tertiary folding?

(b; 8 pts) The RNA structure at the right gives the "melting curve" shown. Estimate the T_m for each of the two cooperative transitions on the melting curve, and speculate on what is happening to the RNA as it goes through each transition (sketch structures). Ignore any possible tertiary structure.



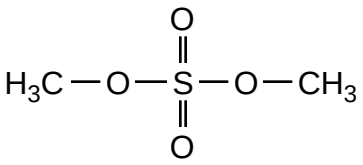
4. Interactions of Nucleic Acids with Small Molecules (14 pts).

(a; 8 pts) The drug daunomycin is shown at the right. How do you think it will interact with DNA? How could you test your hypothesis experimentally?



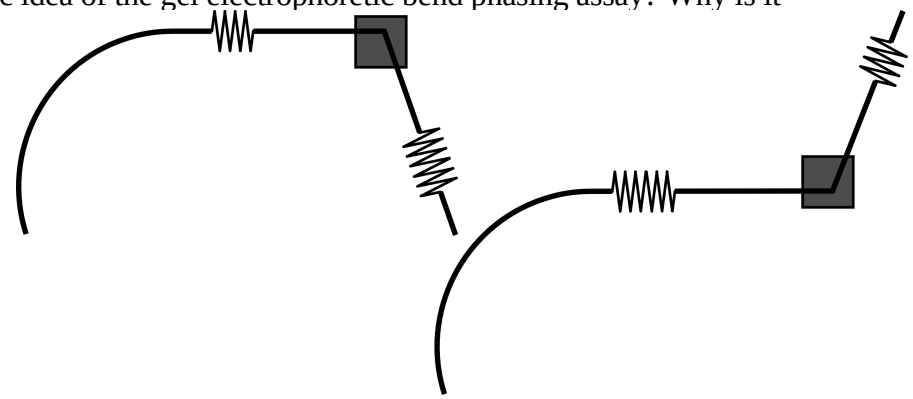
(b; 2 pts) Why is Mg^{++} or other divalent metal generally required for tertiary folding of RNA?

(c; 4 pts) Dimethyl sulfate can alkylate T at N-3, but only when the T is in a single-stranded state. Sketch the reaction mechanism and explain the dependence on secondary structure.



5. DNA Bending (12 pts)

(a; 8 pts.) The system sketched here has been used to characterize DNA bending in gels and in solution. What is the basic idea of the gel electrophoretic bend phasing assay? Why is it necessary to have two phasing adapters instead of just one for studying these molecules by DNA cyclization?



(b; 4 pts) "Macroscopic," i.e. big, curvature of DNA is observed for sequence (1) below but not sequence (2). Why?

(1) 5′ AAAAAACGGGCAAAAAACGGCAAAAAACGGGCAAAAAACGGCAAAAAAGCCG
TTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTTCGGC 5′

(2) 5′ AAAAAACGGCGCGGCAAAAAAGCGGCGCGGCAAAAAAGCCGCGCGGAAAAAA
TTTTTTGCCGCGCCGTTTTTTCGCCGCGCCGTTTTTTCGGCGCGCCTTTTTT 5′

Hint: Sequence (3) below is curved, albeit less than sequence (1).

(3) 5′ AAAAAACGGGCGATCGACGGCAAAAAACGGGCACGTTGCGGCAAAAAAGCCG
TTTTTTGCCCGCTAGCTGCCGTTTTTTGCCCGTGCAACGCCGTTTTTTCGGC 5′

6. Sequencing and Bioinformatics (22 pts)

(a; 6 pts) What is the difference between dye-primer and dye-terminator automated fluorescent sequencing? Why would one be more likely to use dye-terminator for chromosome "walking," where one sequences through a long stretch using the end of the previous sequenced segment?

(b; 3 pts) Why is it important that fragmentation in genome shotgun sequencing is random, i.e. why not just use an EcoR I digest?

(c; 5 pts) Briefly describe the process of colony/plaque hybridization and its purpose.

(d; 8 pts) Briefly discuss how "gene chips" and "genome sequencing" each make the other more useful. Briefly describe one application of gene chip technology.

Question	Score
1	/20
2	/18
3	/14
4	/14
5	/12
6	/22
Total	