

Biochemistry 674: Nucleic Acids**Prof. Jason Kahn****Exam I****October 8, 1996**

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.

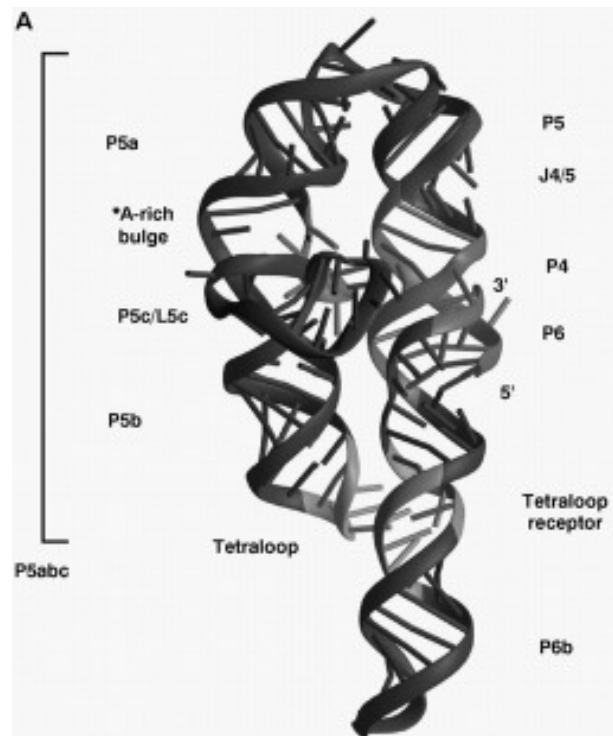
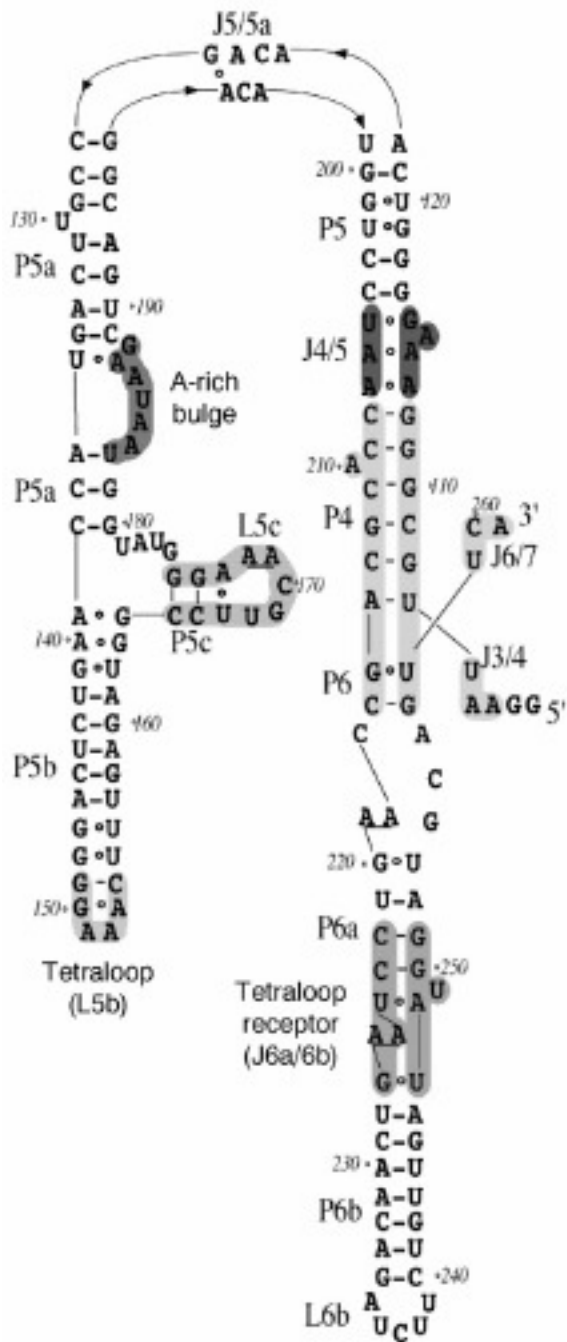
Generous partial credit will be given, i.e., if you don't know, guess.

1. RNA and DNA chemistry. RNA and DNA have interesting similarities and differences.
 - a. (6) What are the two chemical differences between RNA and DNA? (Draw structures to illustrate).

- b. (3) In the table below, indicate which observations are characteristic of A, B, and Z form helices. Put one and only one X in each row.

Property	A-form	B-form	Z-form
<u>Left-handed helix</u>			
<u>Bases are not $\perp$ to helix axis</u>			
<u>Most common DNA form</u>			
<u>Syn purines</u>			
<u>Most common RNA form</u>			
<u>Has spine of hydration</u>			
<u>2-base pair repeat unit</u>			
<u>C3'endo sugar pucker</u>			

The three-dimensional structure of a portion of a self-splicing intron RNA has recently been determined by X-ray crystallography (Cate, J. H., Gooding, A. R., Podell, E., Zhou, K., Golden, B. L., Kundrot, C. E., Cech, T. R., and Doudna, J. A. (1996), *Science* **273**, 1678-1685). The secondary and tertiary structures of the molecule are shown on the next page.



↑
← Note interaction between Tetraloop and Tetraloop Receptor.

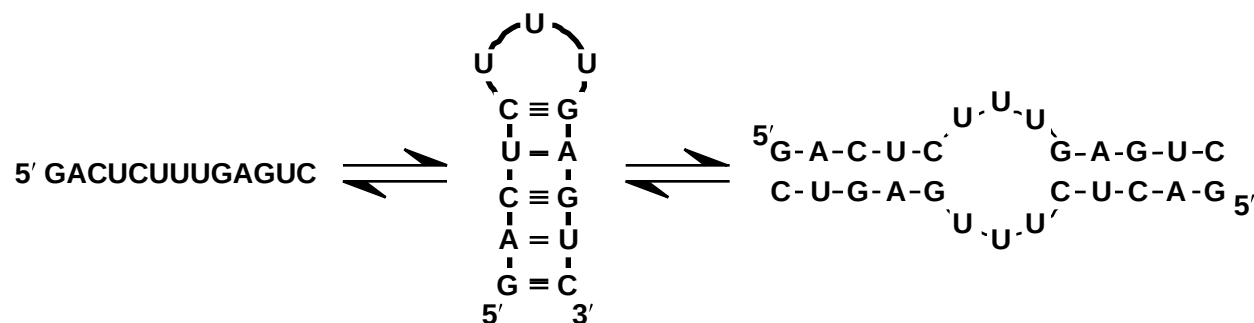
- c. (4) Is the “Tetraloop” (L5b) – “Tetraloop receptor” (J6a/J6b) interaction a secondary structure element or a tertiary structure element? Why?

- d. (5) Why is the tertiary folding of this RNA dependent upon the presence of divalent metal ions like Mg^{++} ? On the figure on the right above, indicate generally where you would expect to find specifically-coordinated divalent metal ions, and give a one-sentence explanation for your choice.
- e. (6) Briefly describe the process of dye-terminated automated fluorescent DNA sequencing. How many gel lanes are used per template molecule? What are three advantages of this method over traditional radiolabeled dideoxy sequencing?

2. “Tinoco rules” for RNA (from the Freier paper) are given in the table at the right. Values refer to base-pair formation. In addition, we have $DS^{\circ}_{initiation} = -10.8$ cal/mole $^{\circ}K$ (or eu, entropy units), and for self-complementary oligonucleotides $DS^{\circ}_{symmetry} = -1.4$ eu.

Any oligonucleotide with regions of self-complementarity may form a unimolecular hairpin (see the figure below). We are interested in the thermodynamics of single strand (ss) vs. hairpin (hp) vs. duplex (ds) formation.

Dinucleotide	DH° (kcal/mole)	DS° in eu (= cal/mole K)
5'AA/3'UU	-6.6	-18.4
AU/UA	-5.7	-15.5
UA/AU	-8.1	-22.6
CA/GU	-10.5	-27.8
AC/UG	-10.2	-26.2
CU/GA	-7.6	-19.2
GA/CU	-13.3	-35.5
CG/GC	-8.0	-19.4
GC/CG	-14.2	-34.9
GG/CC	-12.2	-29.7

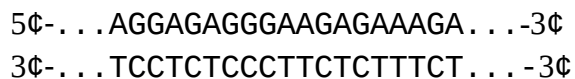


- a. (5) The equation for the equilibrium constant for hairpin formation is just $K = [hp]/[ss]$. Using the relationships $DG^{\circ} = -RT \ln K_{eq}$ and $DG^{\circ} = DH^{\circ} - T DS^{\circ}$, and defining T_m as the temperature at which the half of the molecules are in the hairpin form, derive an equation for T_m as a function of DH° and DS° for hairpin formation. How does T_m depend on total strand concentration C_T ?
- b. (5) Calculate DH° and DS° for the formation of the hairpin DNA oligonucleotide from its single stranded components. Assume DH° for loop closure = 0 and $DS^{\circ} = -23.9$ eu (derived from Freier assuming DG° is all entropic). Note that there is no DS° for initiation or symmetry in this case; you should be able to figure out why but you don't have to for the exam.

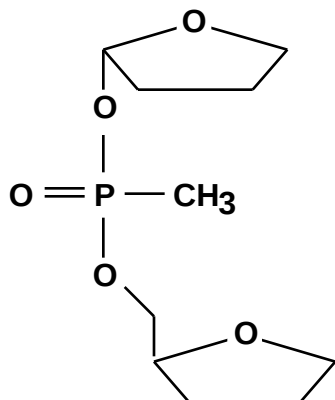
- c. (5) Calculate the ΔH° and ΔS° for the formation of the ds oligonucleotide with an internal loop. Here, we do include ΔS° for initiation and symmetry, as well as $\Delta S^\circ = -4.2$ eu for the internal loop.
- d. (6) Calculate T_m for hairpin formation. If you can't do this, use 50°C for the rest of this part.
- For the duplex formation, $T_m(\text{K}) = \frac{\Delta H^\circ}{\Delta S^\circ + R \ln(C_T/4)}$. Use this equation and the hairpin T_m to determine the strand concentration at which the T_m for duplex and hairpin are equal. At lower strand concentration, which form will be preferred?

3. Base triples are important in stabilizing tertiary structure of nucleic acids. We have focused on the Py•Pu-Py base triples found in triple helical DNA (and RNA). There are other possibilities, though they are not as stable.
- a. (8) Beal and Dervan have proposed the existence of G•G-C and A•A-T base triples, where the • indicates the interaction between the third strand and the base pair, indicated by the -. The third-strand purine of the R•R-Y triple binds in the major groove, like the Moser and Dervan triple. Draw a plausible structure for the G•G-C triplet, including the third strand sugar in the anti conformation.
- b. (4) Assuming the anti sugar conformation, would the geometry you drew above predict a parallel or an antiparallel orientation of the third strand with respect to the purine strand of the W-C duplex? Why?

- c. (5) How could you test the polarity (parallel or antiparallel) using a 3rd-strand purine oligonucleotide with a 5' terminal cleaving moiety, targeted to the sequence below? In other words, what oligonucleotides would you synthesize and what cleavage patterns would you expect?

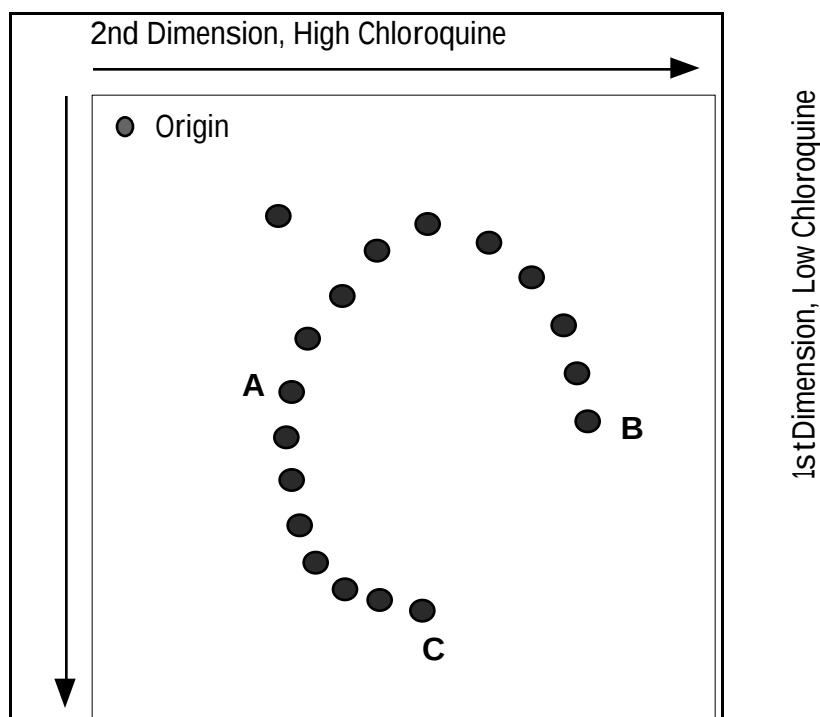


- a. (4) Chemists have synthesized a number of variations on the phosphodiester backbone. Identify the modification shown below and discuss two reasons why it might have advantages over natural DNA as an antisense or other therapeutic agent.



4. Supercoiling and Intercalators.

The autoradiogram at the right shows a two-dimensional agarose gel separation of plasmid topoisomers generated under interesting conditions. The intercalating drug chloroquine is used at two different concentrations in the first and second dimensions.



- a. (9) The topoisomers with the following sets of (DLk, DTw, DWr), under the conditions of the second dimension, are labeled A, B, and C on the gel above. In the table below, indicate which is which and estimate (DLk, DTw, DWr) for these molecules under the conditions of the first dimension. Give a very brief explanation of your reasoning.

2nd dimension (DLk, DTw, DWr):	(+3, -4, +7)	(-13, -9, -4)	(-6, -6, 0)
Identity (A, B, or C):			
First dimension (DLk, DTw, DWr):			

- b. (3) Where does nicked DNA migrate (write an N on the gel above) and why?

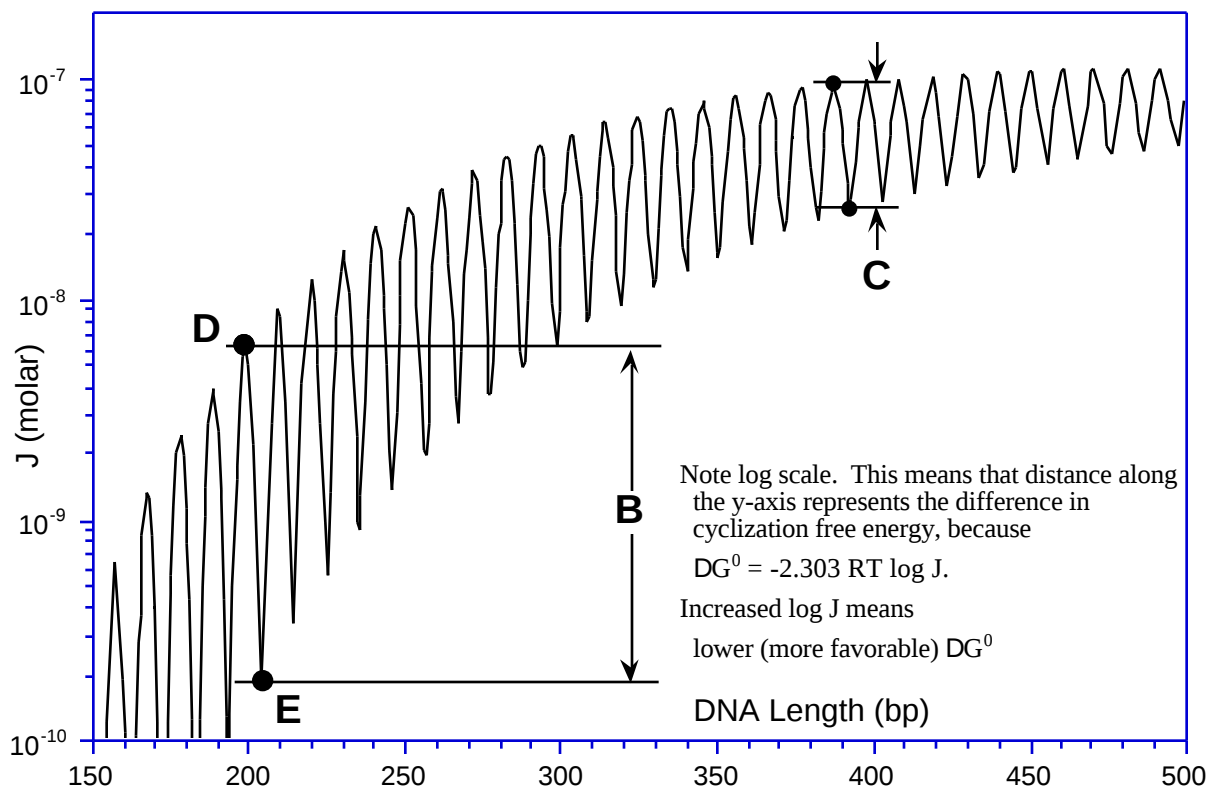
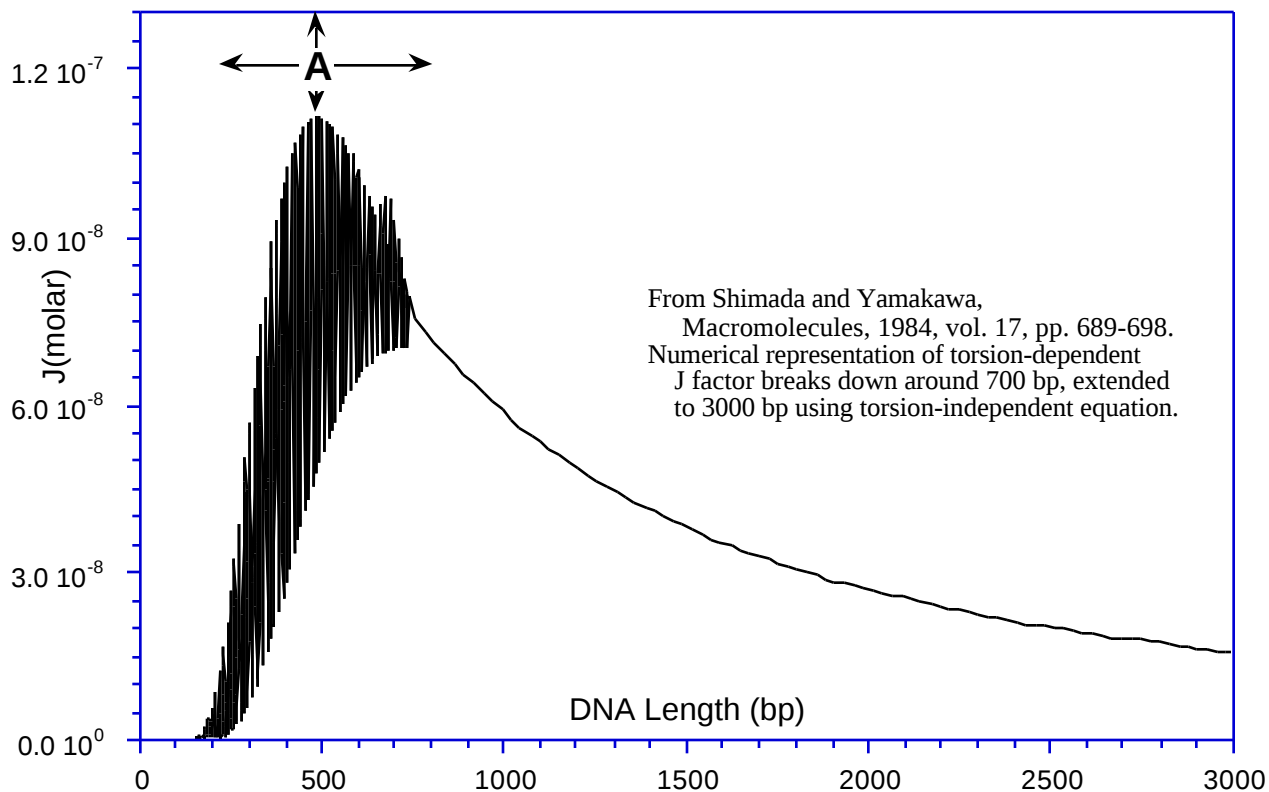
- c. (6) In the boxes below, draw the DNA structures corresponding to (+3, -4,+7), (-6, -6, 0), and (-13, -9, -4). Where appropriate, draw two superhelical nodes for each, indicate how many nodes there really are, and be careful of the node signs. Do not draw twist decreases. Assume plectonemic rather than toroidal supercoiling. Indicate with +, ++, and +++ the relative amounts of chloroquine bound by each topoisomer.

(DLk, DTw, DWr) = (+3,-4,+7)	(-6,-6,0)	(-13,-9,+4)
Chloroquine Binding:		

- d. (3) The experiments leading to such gels can be done using labeled plasmid but usually are not. How, then do you think an autoradiogram is obtained in the end?

5. DNA bending, flexibility, and cyclization. The graphs on the next page show the DNA length-dependence of the J factor for cyclization, that is, the efficiency of cyclization.
- a. (4) Qualitatively, why is J small at short lengths and at long lengths?
 - b. (2) On the top graph of the next page, indicate how the maximum of the curve (position A) would shift if the DNA became stiffer, i.e. if the persistence length became longer.
 - c. (5) How would the position of A and the overall shape of the curve change if two in-phase 50-bp sequence-directed A-tract bends were introduced into the molecule? Give a short explanation.
 - d. (4) What causes the pronounced oscillations in J in the bottom graph? Why does the peak-to-trough separation decrease as the length increases (cf. distance B vs. distance C).

J Factor vs. DNA Length



- e. (6) Draw in-phase and out-of-phase bending constructs designed to determine bend direction, just as in lecture. Looking at dots D and E on the bottom graph above, explain why we need two phasing linkers in cyclization experiments on phasing constructs.

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