

Exam I

October 12, 2000

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 may 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):

At first glance it is paradoxical that a negative Lk gives a right-handed plectonemic superhelix rather than a left-handed one.

(a; 4 pts) Draw a right-handed plectonemic superhelix with three writhe nodes, using the familiar convention that a single line represents duplex DNA.

(b; 3 pts) Now “zoom in” on one node above and illustrate how the writhe node is composed of single-strand crossovers. You may ignore the DNA twist.

(c; 3 pts) Now draw one turn of a B-form DNA helix, with twist, and assign node signs.

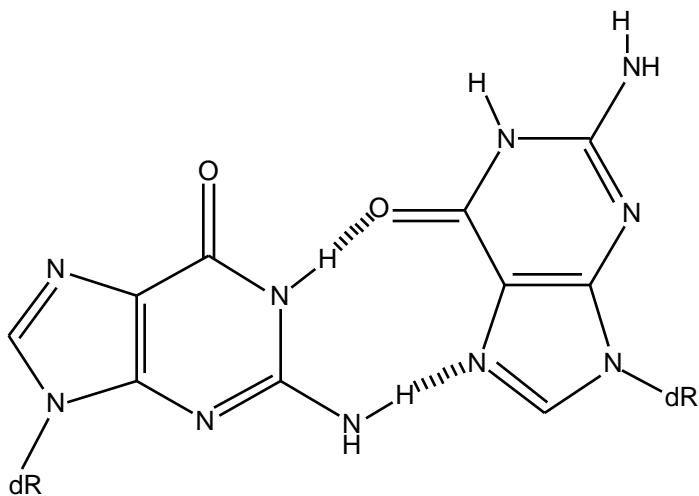
(d; 3 pts) What is the resolution of the apparent paradox mentioned at the beginning of this question?

(e; 7 pts) Briefly describe how you could use ethidium bromide and a topoisomerase to prepare negatively supercoiled plasmid DNA starting with a relaxed molecule. At each step, indicate the Lk, the Tw, and the Wr.

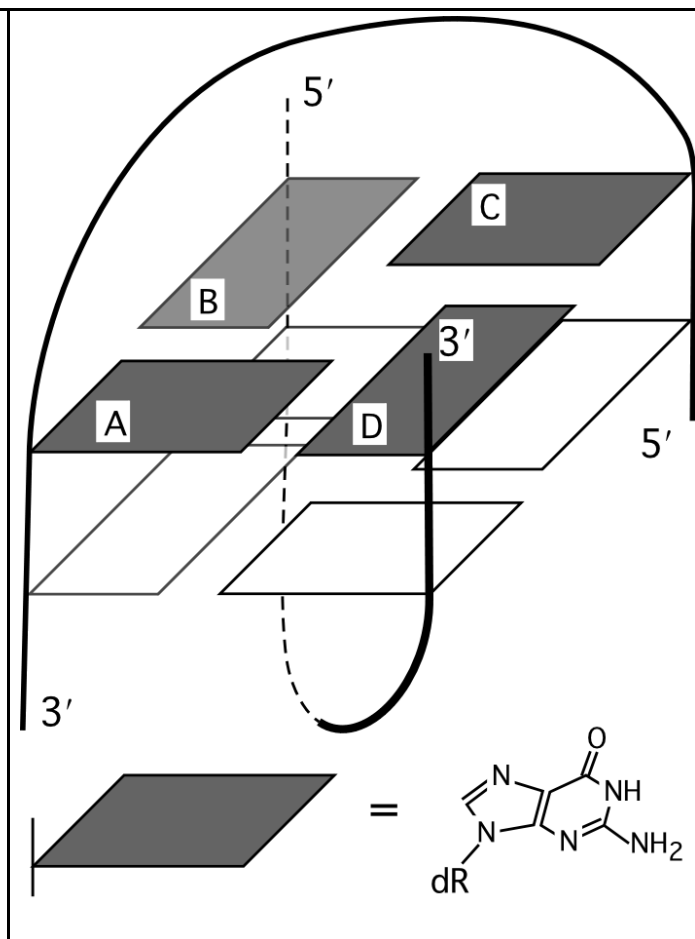
2. Base Pairing and Geometry (20 pts).

(a; 6 pts) In the space below, draw a G•U wobble base pair and indicate the two imino protons.
You need not draw out the sugar protons.

(b; 8 pts) The diagram below shows the bottom half of one plane of a “G quartet,” which is an extremely stable base pairing arrangement with four equivalent guanine bases. Draw the top half of the G quartet.



While the guanine bases in the quartet are all essentially equivalent, the same is not true of the guanosine nucleosides. The three-dimensional structure of oligonucleotides forming a stack of G quartets at the right shows why. The sketch that you started with above represents bases "A" and "D" in the sketch to the right. The heavy lines represent the phosphate backbone, and the outlined parallelograms are a G quartet underneath the one we're interested in, which is gray.



(c; 6 pts) Based on the above, identify whether each of the guanines A, B, C, and D above is in the syn or anti conformation. Explain your reasoning.

3. Secondary structure (15 pts)

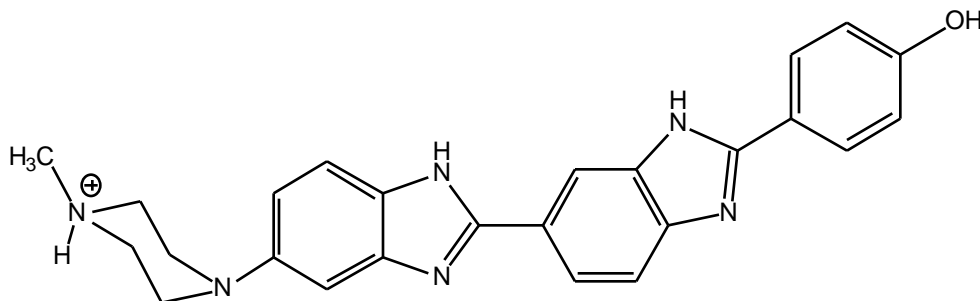
(a; 9 pts) The following sequence can potentially fold into at least three reasonable secondary structures. Draw them (3). The underlines hint at possible helices. Ignore any potential tertiary structure.

1	5	10	15	20	25	30
<u>GAUGGCUAGAAA</u> <u>UAGCUAUAGUAGCCAUCUJUC</u>						

(b; 6 pts) Briefly describe two experiments or approaches that could be used to determine which of the three possible structures above is the real one.

4. Interactions of Nucleic Acids with Small Molecules (15 pts).

The DNA-binding drug Hoechst 33258:



(a; 4 pts) What features of this molecule suggest that it might either (a) intercalate or (b) bind in a groove?

(b; 4 pts) Describe an experiment which could differentiate between these possibilities.

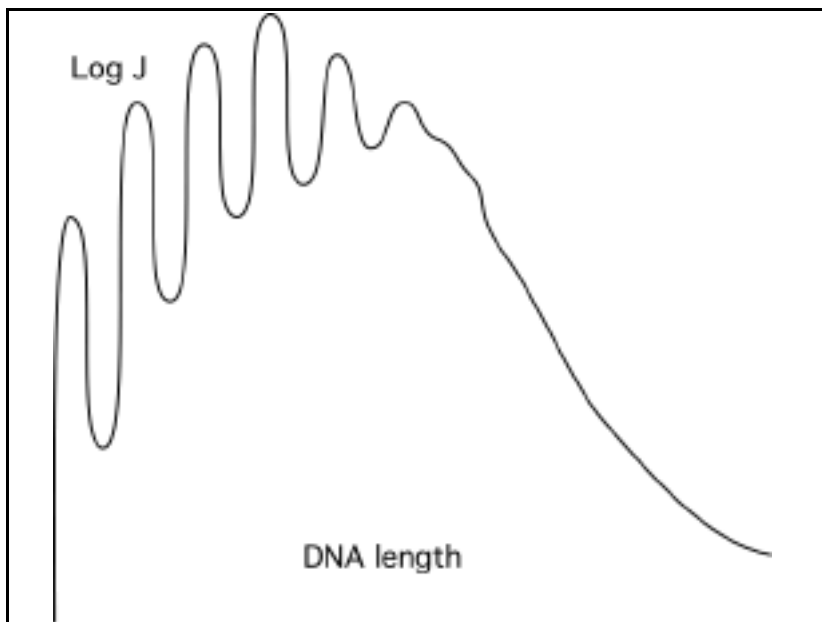
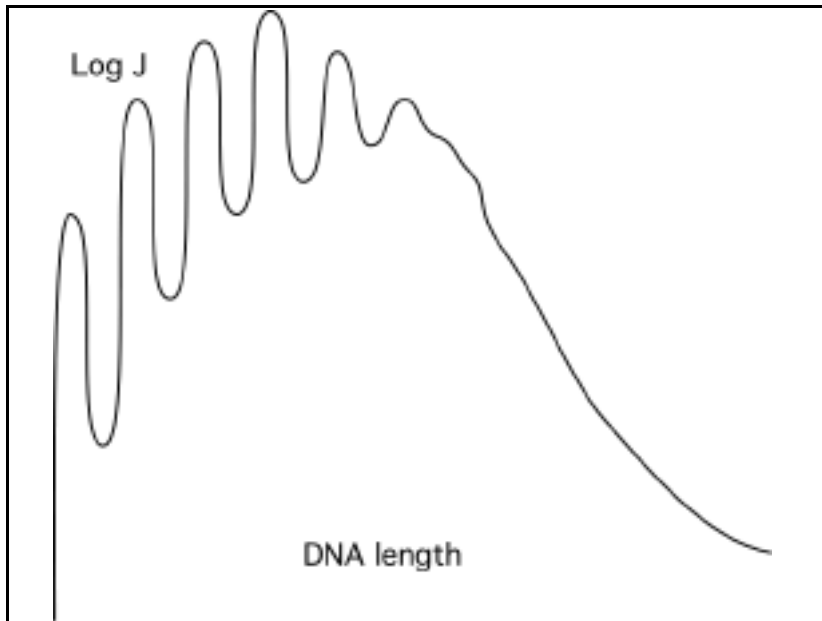
(c; 3 pts) In fact, Hoechst 33258 is quite sequence-selective, preferring to bind 5' AATT. How does this give us a strong suggestion as to the kind of binding mode it prefers?

(d; 4 pts) If Hoechst 33528 is a groove binder, which groove would you expect it to occupy? Why does its fluorescence quantum yield increase dramatically upon DNA binding?

5. DNA Bending and Flexibility (15 pts)

(a; 7 pts.) Briefly sketch the circular permutation assay for DNA bending. What does it tell us about bend existence, location, and direction?

(b; 8 pts) The curve for the length dependence of the J factor for DNA cyclization (the effective concentration of one DNA end about the other) is sketched below. The period of the torsional oscillation is greatly exaggerated. On the same graph, sketch the shape of the curve if some ethidium bromide was added, at a binding ratio averaging 1 molecule of ethidium per 10 bp. Recall that intercalation lengthens, unwinds, and stiffens DNA. The graph is given twice in case you make a mistake the first time. Briefly explain your reasoning. I am looking for qualitative features.



6. Sequencing and Bioinformatics (15 pts)

(a; 6 pts) Briefly describe how one (or many) goes about sequencing a bacterial genome. Do not discuss any details about the individual dideoxy sequencing reactions.

(b; 9 pts) Usually, sequence comparisons of YFG (your favorite gene) to the sequence databases are done on the protein sequence, not the nucleic acid sequence. Why? What kind of pre-existing model or knowledge is necessary to perform protein sequence comparisons? What kind of gene or field of study might emphasize nucleic acid comparisons instead?

Question	Score
1	/20
2	/20
3	/15
4	/15
5	/15
6	/15
Total	/100