BCHM465 **Your Name:** _____

Biochemistry III, Molecular Genetics **Prof. Jason Kahn**

Exam I **March 6, 2001**

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.

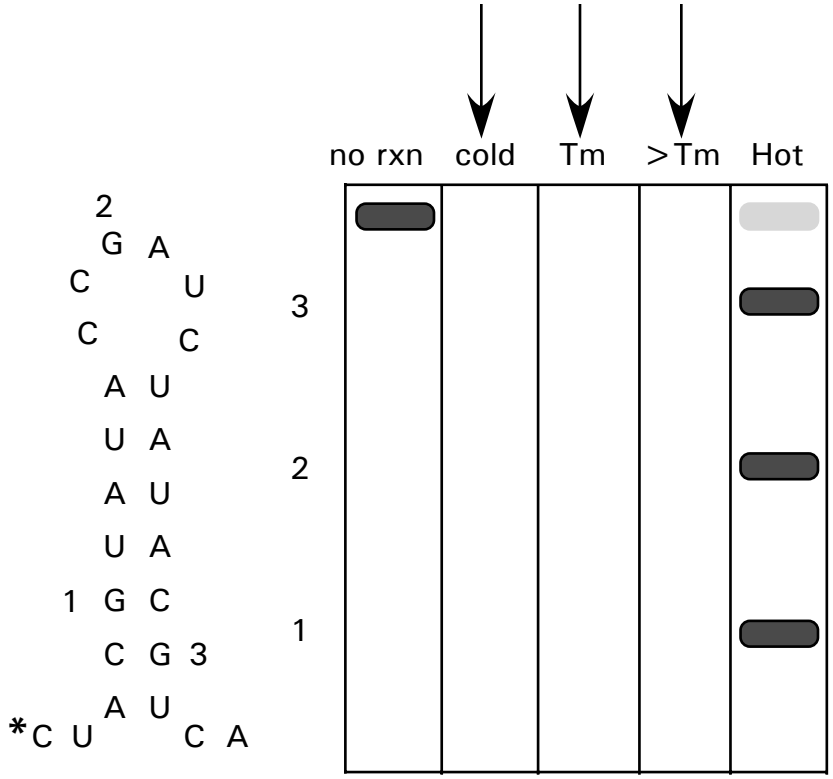
Generous partial credit will be given, *i.e.*, if you don't know, guess.

1. Secondary Structure and Thermodynamics (20 pts):

We discussed using thermal melting curves to analyze oligonucleotide thermodynamics.

(a; 8 pts) Sketch a graph representing an absorbance thermal melting curve below, identifying the axes, the portions of the curve corresponding to single-stranded and double-stranded DNA, and the T_m. What physical change does the melting curve monitor?

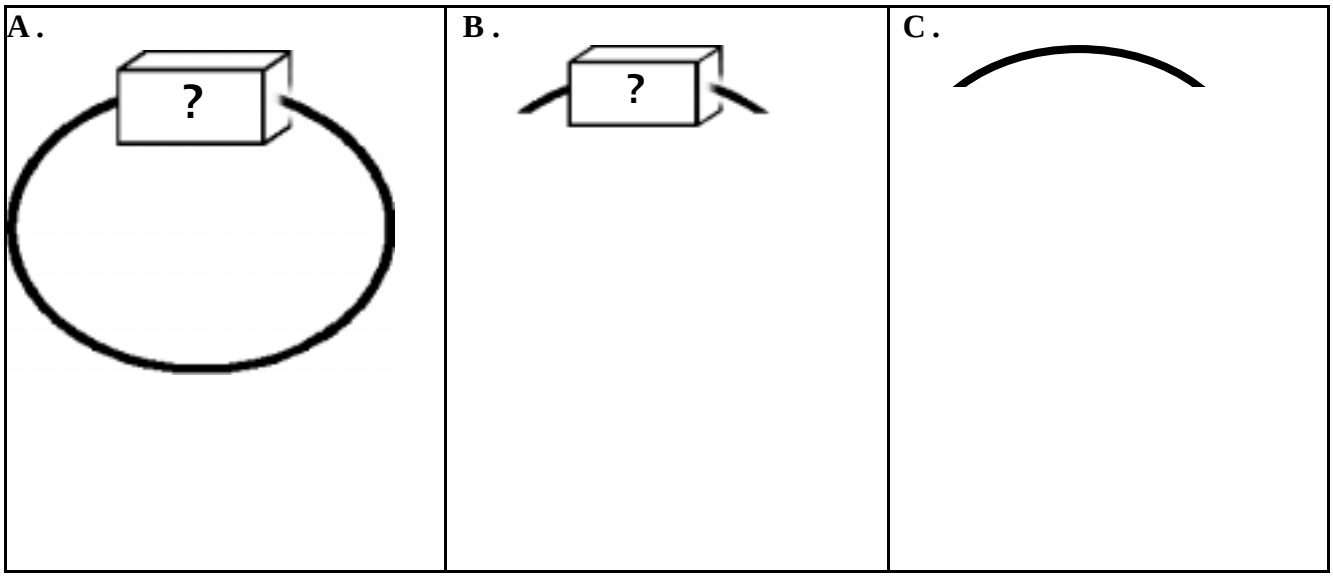
(b; 7 pts) Anything that changes when a nucleic acid goes from ds to ss can be used as the basis of a melting curve experiment. One example is the accessibility of an RNA nucleotide to a single-strand specific enzyme like ribonuclease T_1, which cuts at G. You are given the RNA oligonucleotide below, labeled at the 5' end. Sketch the appearance of a polyacrylamide gel run on samples reacted with ribonuclease T_1 as you increase the temperature from below T_m to above T_m as indicated.



(c; 5 pts) You find experimentally that the melting temperatures you determine on the same oligonucleotide using the two methods are different. Why might this be?

2. DNA Flexibility and Topology (20 pts).

(a; 9 pts) DNA topology is a useful probe for ligand-induced structural changes. Imagine that within the white box below, a ligand binds that induces a ΔTw of +2 on an initially relaxed DNA. Draw the resulting shape of the DNA in Box B. Then we add a topoisomerase which relaxes away all writhe outside the box, and then we remove the topoisomerase and the ligand. Draw the final result in box C. From topology alone, do we have any way of telling whether the ligand in the box introduced twist or writhe? Why or why not?



[Note added in proof: This is a clumsy question that no one got right. The picture in A should have had the white box ligand off the DNA.]]

The wormlike coil model describes the length dependence of apparent DNA flexibility.

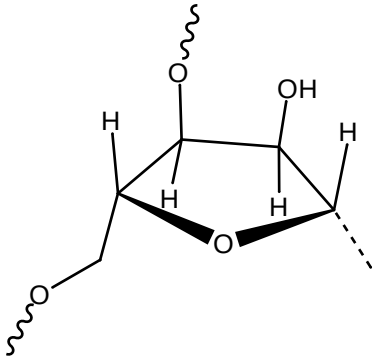
[Note that in F2001 I didn't use the words wormlike coil but the ideas are the same]

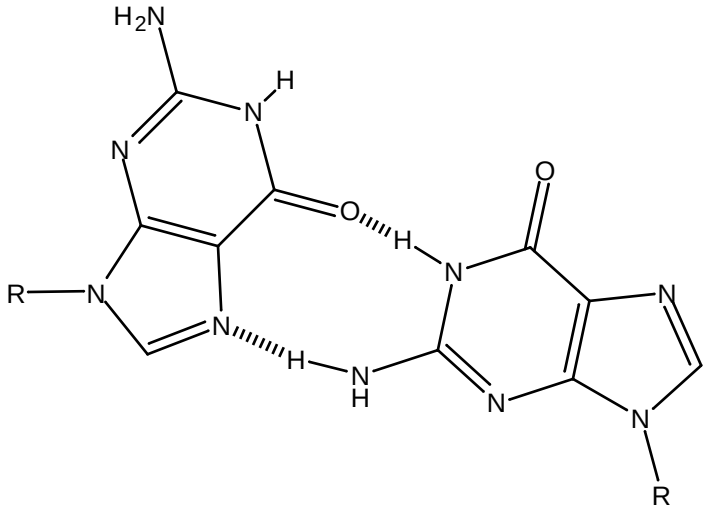
(b; 8 pts) Give a very brief explanation of the essence of the wormlike coil model, in terms of how DNA behaves at short and long lengths. What quantity parametrizes the changeover?

(c; 3 pts) We used the analogy of 100 pots of boiling water, each with a strand of spaghetti. What did that have to do with DNA?

3. Base Pairing and Hybridization (20 pts).

(a; 7 pts) In the space below, attached to the sugar given, draw a reasonable triple-base partner for the G•G pair below, forming at least two hydrogen bonds. Does the third-strand backbone run parallel or antiparallel to the G at the bottom left (circle one answer)?



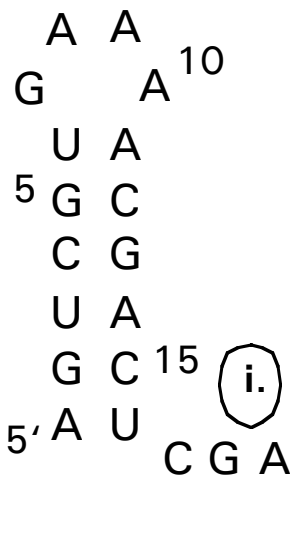


(b; 10 pts) DNA microarrays or "gene chips" are a transforming technology. Describe how you would use a an expression profiling experiment to identify changes in gene expression due to insulin or other hormone stimulation of cultured cells.

(c; 3 pts) Based on (b) above, you might think that putting your college fund into Affymetrix stock would be a good idea. Drawing on the experience of people who invented in most of the hundreds of automobile companies that were around in 1910, why is this not necessarily the case?

4. Secondary structure prediction (18 pts).

The essential RNA (i) below was proposed to form the structure shown based on computer modeling. Then homologous sequences (ii) and (iii) were discovered. The bases that differ from RNA (i) are indicated in bold. The underlines are hints.



i. 5' AGU**CG**UGAAAAC**G**ACUCGA**CG**ACUG 3'

ii. AGUC**C**UGAAAAC**A**ACUCGA**G**GACUG

iii. AGU**A**CUGAAAAC**C**ACUCGAG**U**ACUG

5 10 15 20 25

(a; 4 pts) Explain the notion of correlated invariants in phylogenetic studies of RNA structure.

(b; 3 pts) Why don't the invariant bases in the sequences tell us as much about secondary structure as the ones that do vary?

(c; 6 pts) Do the sequences (ii) and (iii) support the structure shown? Why or why not?

(d; 5 pts) Draw an alternative secondary structure that is more consistent with the phylogenetic data.

5. Miscellaneous (22 pts).

(a; 6 pts) What are the two chemical differences between RNA and DNA? Why are there no organisms with large RNA genomes?

(b; 6 pts) Draw the structure of dCTP, with the numbering and identifying the α, β, and γ phosphates.

(c; 6 pts) Why is Mg^{++} or another divalent metal essential for RNA tertiary structure? Can RNA secondary and tertiary structure be studied independently, if so how?

(d; 4 pts) What do proteins see when they approach duplex DNA?

Score:	Question 1: _____	out of 20: 2° Structure and Thermo
	Question 2: _____	out of 20: DNA Flexibility and Topology
	Question 3: _____	out of 20: Base Pairing and Hybridization
	Question 4: _____	out of 18: Phylogeny
	Question 5: _____	out of 22: Miscellaneous
	Total: _____	**out of 100**