Biochemistry 674 **Your Name:** _____

Nucleic Acids **Prof. Jason Kahn**

Exam I (100 points total) **October 6, 2005**

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. I have given you more space than you should need.

You do not need a calculator for this exam, and no other study aids or materials are permitted.

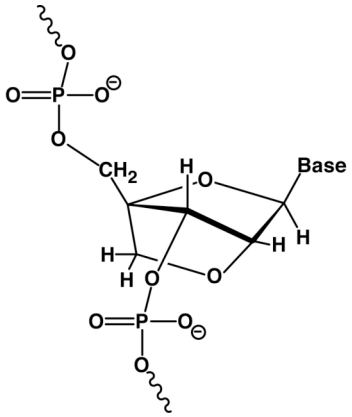
Generous partial credit will be given, *i.e.*, if you don't know, guess.

Honor Pledge: At the end of the exam, please write out the following sentence and sign it, or talk to me about it:

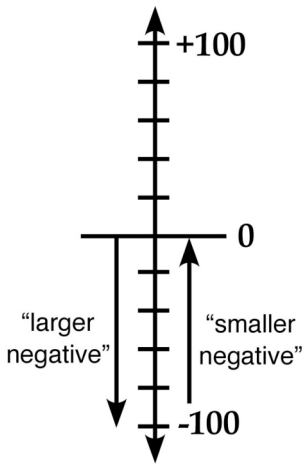
"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

1. Conformation and Thermodynamics (28 pts):

The bicyclic modified RNA nucleotide LNA shown at the right locks the sugar conformation into a C3′-endo conformation resembling that found in A-form RNA. Substitution of LNA into DNA or RNA stabilizes duplex formation (hybridization). $\Delta H°$ and $\Delta S°$ are both negative for duplex formation.



(a; 10 pts) Rationalize the observation that the effect of LNA is generally to reduce the unfavorable (negative) entropic cost of hybridization. What kind of experiment would have led to this conclusion?



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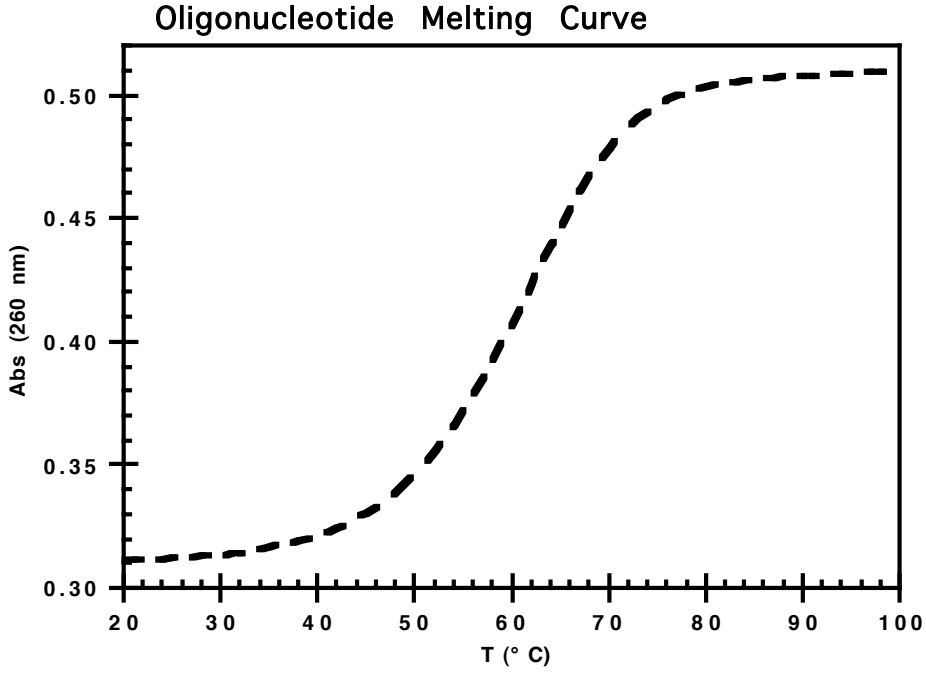
(b; 8 pts) On the graph at the right, sketch (1) the melting curve for an oligonucleotide that has the same enthalpy of hybridization (ΔH°) as the curve shown but a smaller negative entropy of hybridization (ΔS°). Also, sketch (2) the curve for an oligo with the same T_M but a larger negative hybridization enthalpy. [This part of the problem is LNA-independent.]



(c; 6 pts) Would you expect LNA to stabilize RNA hybridization to a greater or a lesser extent than it stabilizes DNA? Explain your reasoning briefly.

(d; 4 pts) Even though LNA is a modified RNA, it is much more stable to base (hydroxide) or nucleases than RNA. Why? (Hint: why did DNA evolve?)

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2. RNA structure and base pairing (25 pts):

In protein folding, we generally believe that secondary and tertiary structure fold and unfold in concert. In contrast, secondary and tertiary structure are independent in RNA folding.

(a; 9 pts) What are the two main components that hold RNA tertiary structure together? We study protein folding by rapid dilution of proteins from high to low concentration of denaturants. What would the analogous RNA folding experiment be?

(b; 13 pts) Sketch a likely unimolecular secondary structure for the RNA sequence below, and identify two secondary structure elements. Hint: U_{10} crosslinks to U_{22}. [Note: if you didn't appreciate it before, this problem should help you see that computers are essential tools for finding folds!]

5′- GCAUCUCCUU$_{10}$AAGGGCAACC$_{20}$UUGAGCCCAU$_{30}$GC-3′

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(c; 3 pts) What cellular structure has been a gold mine for studies of RNA tertiary structure?

3. Genetic Engineering Methods (25 pts):

Two restriction enzymes are said to give compatible cohesive ends if their digestion products can be ligated to each other.

(a; 6 pts) From the list of 8 enzymes and their recognition sites below, identify the three pairs of different enzymes that give compatible cohesive ends.

EcoR I	Kpn I	Eco RV	Mbo I
G|AATTC CTTAA|G	GGTAC|C C|CATGG	GAT|ATC CTA|TAG	|GATC CTAG|

Pvu II	Bgl II	Acc65 I	Mfe I
CAG|CTG GTC|GAC	A|GATCT TCTAG|A	G|GTACC CCATG|G	C|AATTG GTTAA|C

(b; 6 pts) Sometimes cohesive end ligation gives "re-cleavable" ends, sometimes not. For two of the pairs you identified above, draw the sequences obtained upon ligation of the cohesive ends, and indicate whether the junction can be cut by either of the initial enzymes.

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(c; 4 pts) Why are most restriction enzyme recognition sites palindromic?

(d; 6 pts) In general, why are fusion proteins or tagged proteins so useful? How are the tags removed if necessary?

(e; 3 pts) Why does T4 DNA ligase work much more efficiently on cohesive ends than on blunt ends?

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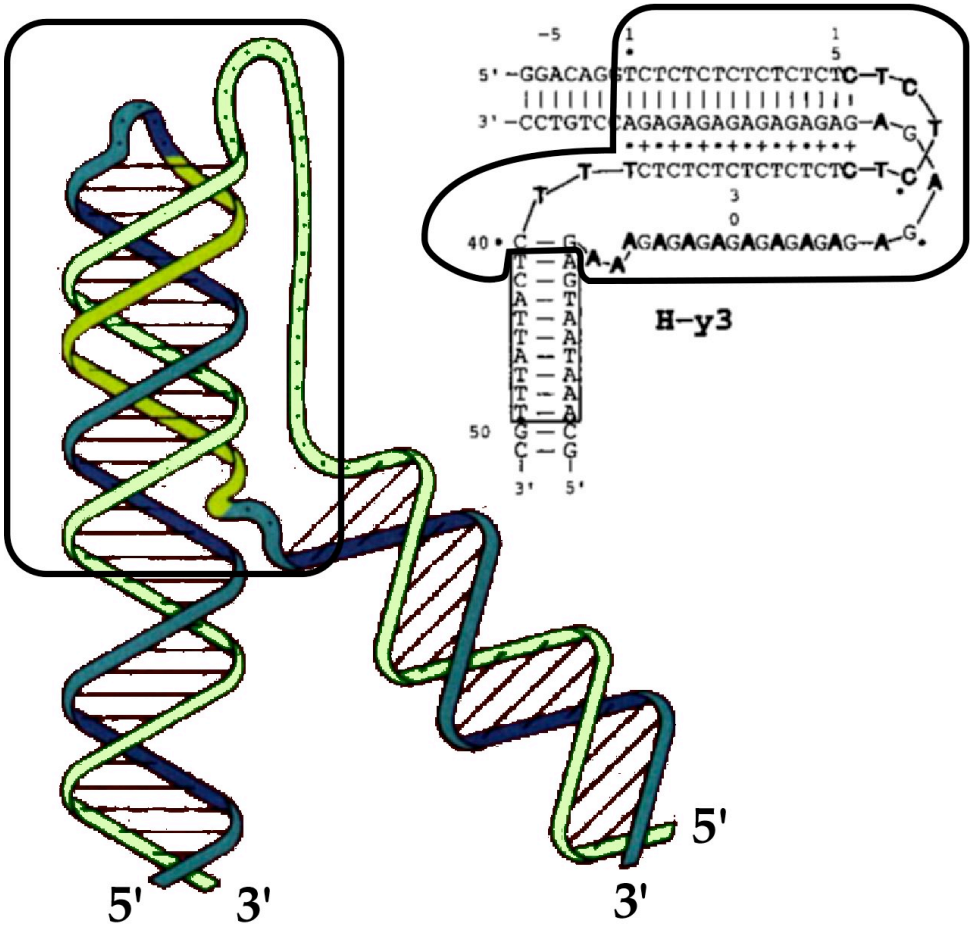
4. DNA Topology (22 pts):

The structure below is called "H-DNA" or "hinge DNA."

(a; 12 pts) Assign signs to the nodes that contribute to the twist in the boxed region at the left (it's the same region indicated in the sketch on the right). What is the contribution to the total twist from this region? What would the twist contribution be from the same 40 bp in good old B-form DNA? (Assume the helical repeat is 10 bp/turn for ease of computation.) So, what is the ΔTw caused by H-DNA formation from what was previously B-DNA in a plasmid?



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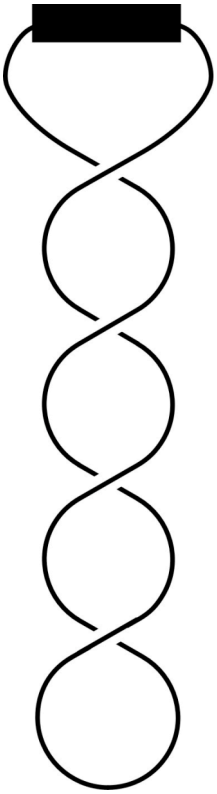
(b; 6 pts) If one started with the plasmid DNA below and caused/allowed the 40 bp segment in the black box to assume the H-DNA form as above, what would the result look like? Assume that ΔTw of the starting DNA and the DNA outside the final H-DNA regions are zero. The H-DNA is in the black box: there is no need to sketch it. All you need from the first part of this problem is the ΔLk and ΔTw induced by the region within the box. The ΔLk, of course, is zero. If you do not trust your answer for ΔTw from (a), assume it is -3 for this problem.



(c; 4 pts) Why is H-DNA formation potentiated by negative supercoiling?

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