Biochemistry 674 **Your Name: Key**

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.

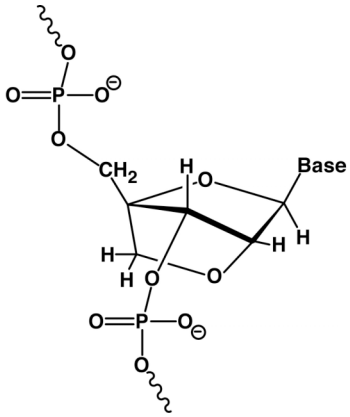
Generous partial credit will be given, *i.e.*, if you don't know, guess.

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). ΔH° and ΔS° are both negative for duplex formation.



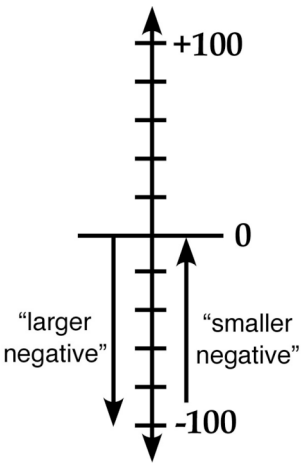
(a; 10 pts) Rationalize the observation that the effect of LNA is generally to reduce the unfavorable (negative) entropic cost of hybridization. What kind of experiment would have led to this conclusion?

+4: The Locked Nucleic Acid has the effect of decreasing the entropy of the single-strand starting material.

+3: Therefore the entropic cost of forming ordered double-stranded DNA is decreased

+2: Melting curves or calorimetry

+2 comparing LNA-substituted oligos to all-DNA or all-RNA oligos would tell us whether stabilization is entropic or enthalpic.



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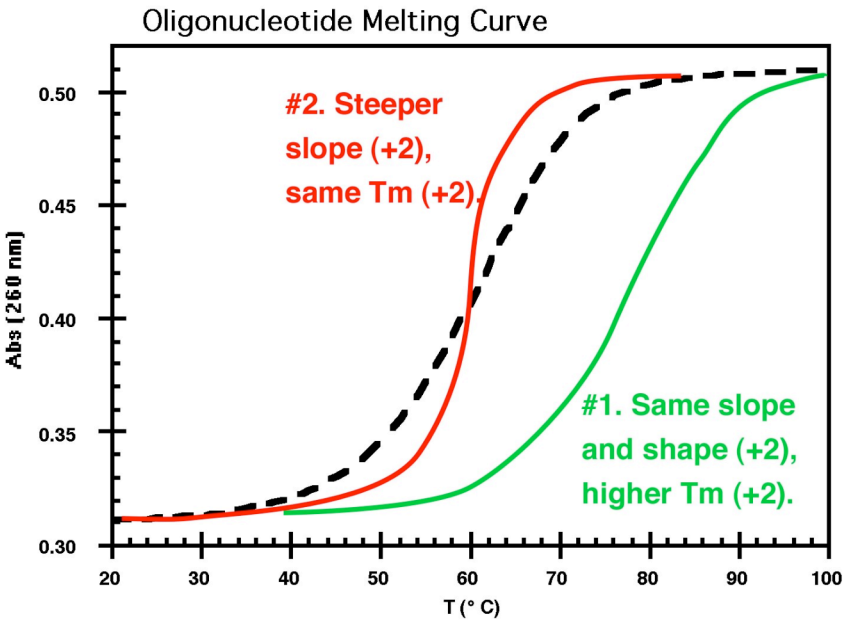
(b; 8 pts) On the graph at the right, sketch (1) the melting curve for an oligonucleotide that has the same enthalpy of hybridization (ΔH°) as the curve shown but a smaller negative entropy of hybridization (ΔS°). Also, sketch (2) the curve for an oligo with the same T_M but a larger negative hybridization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

+2 LNA should stabilize RNA more than DNA.

+2 This is because LNA embedded in RNA should presumably maintain the A form, which is the thermodynamic minimum for RNA (otherwise it wouldn't be observed!).

+2 In contrast, LNA embedded in DNA presumably must force the dsDNA into a conformation different from the optimal B-form that would otherwise be observed, and this conformational change must cost free energy.

(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?)

+2 The 2' oxygen in LNA is not nucleophilic because it cannot be deprotonated like the 2' hydroxyl in RNA, so LNA should be just as stable to base as DNA.

+2 LNA is stable to nucleases because as a xenobiotic it is much less likely to fit their active sites. Presumably biology could have evolved an LNase if there had been selection for it.

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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?

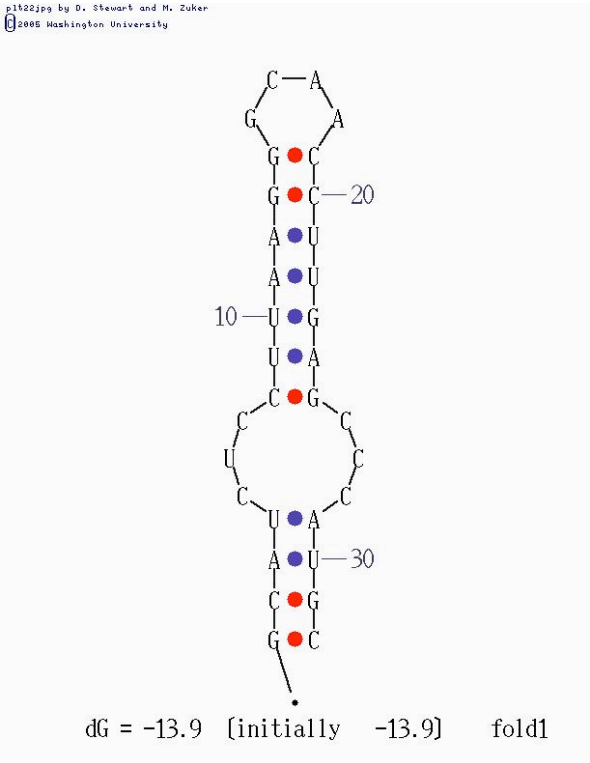
+3 Divalent (or higher-valent) metal ions to neutralize phosphate repulsion in structured regions

+3 Non-Watson Crick hydrogen bonds confer specificity and stability

We need to imagine a rapid mixing experiment going from conditions where tertiary structure is not stable to conditions where it is stable. The most obvious answer (+3) is to dilute the RNA from a solution containing only monocations into a solution containing Mg++.

(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′



Structure is from
http://www.bioinfo.rpi.edu/~zukerm/rna/

+3 for the basic idea
+3 for each dsRNA region
+2 for identifying a tetraloop at the top
+2 for identifying internal loop at the bottom

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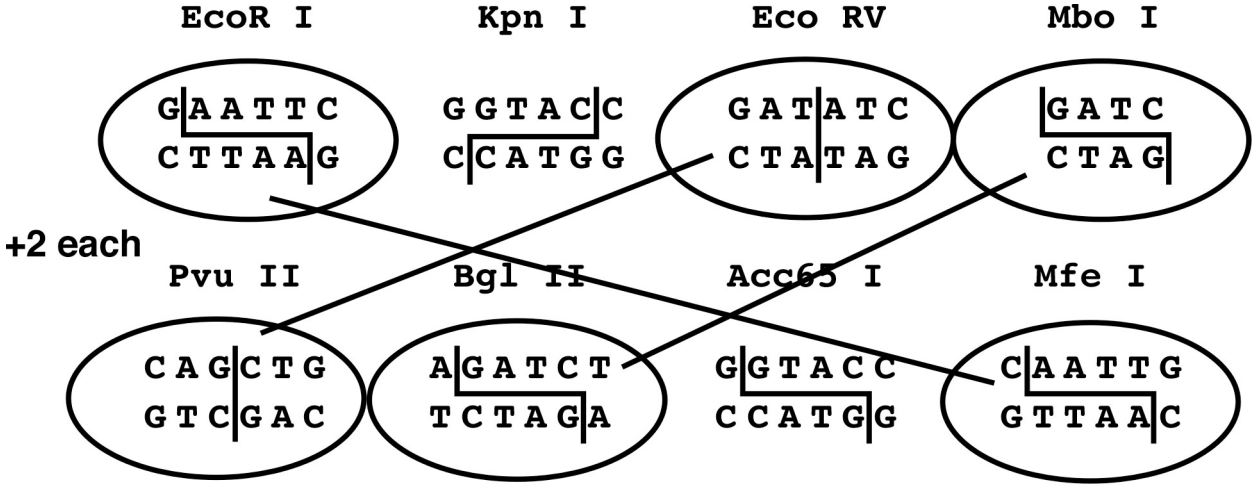
(c; 3 pts) What cellular structure has been a gold mine for studies of RNA tertiary structure?

+3 The ribosome
(+2 for any other big RNA whose structure has been solved)

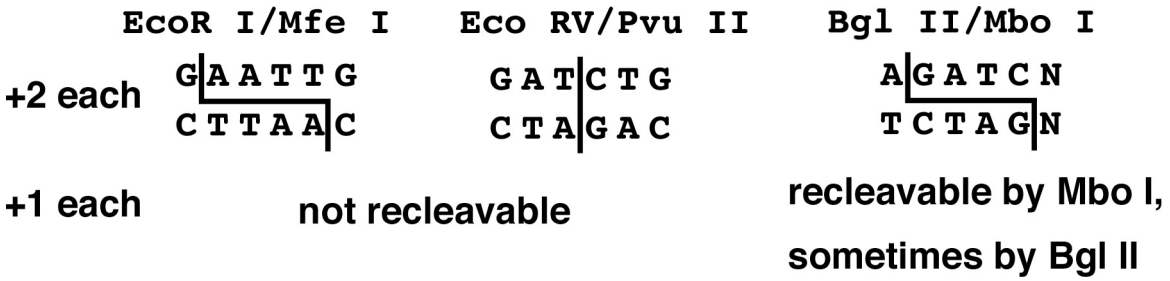
3. Genetic Engineering Methods (25 pts):

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.



(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?

+2 Restriction enzymes are usually dimers
+2 And each monomer makes the same interactions with DNA (this does not mean that each monomer recognizes one strand!)

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

+3 Many possible variations…basic idea is that purification or identification of the tagged protein is done based on the properties of the tag or fusion partner, which can be worked out beforehand by someone else! Examples: His tags, FLAG tags for antibody recognition, GST fusions.

+3 There's a sequence coding for a specific protease cleavage site built in to the expression vector.

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

+1 Its substrate is a nick in a double helix…
+2 which is much more stable when it's held together by base pairs rather than just coaxial stacking.

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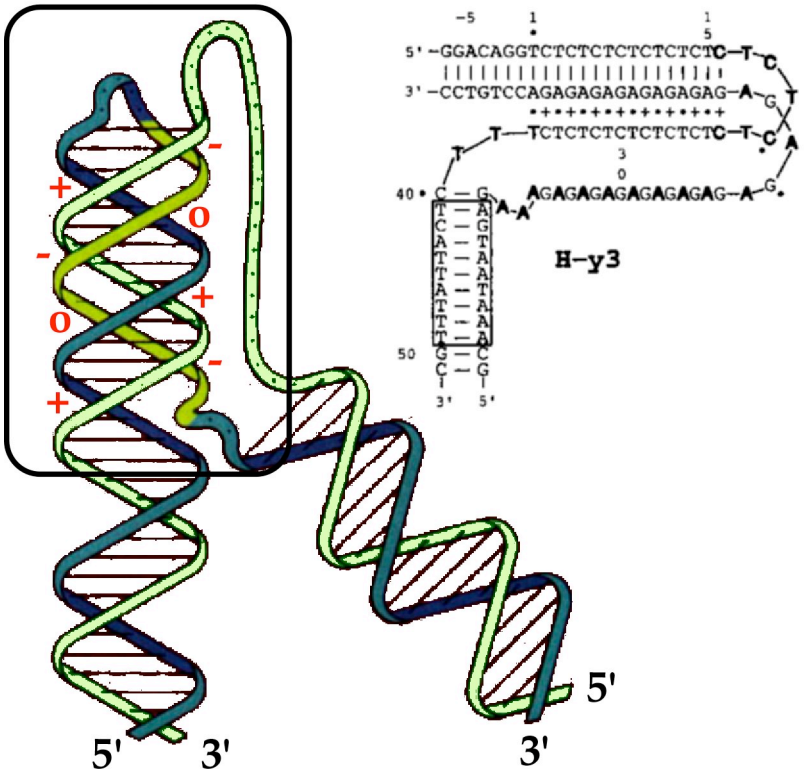
4. DNA Topology (22 pts):

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?



+1/2 for each correct node for total of 4 pts.

+1 for recognition that contribution to total twist is the sum over nodes

+2 for calculation: sum = 0 (or consistent answer). Note that this is true even though only about 25 bp of duplex is disrupted, because the third strand of the triplex makes a parallel, hence negative wrap around the polypurine strand

+2 for 40 bp contributing +4 in twist

+3 For ΔTw caused by H-DNA formation = -4 (full credit for consistent answer)

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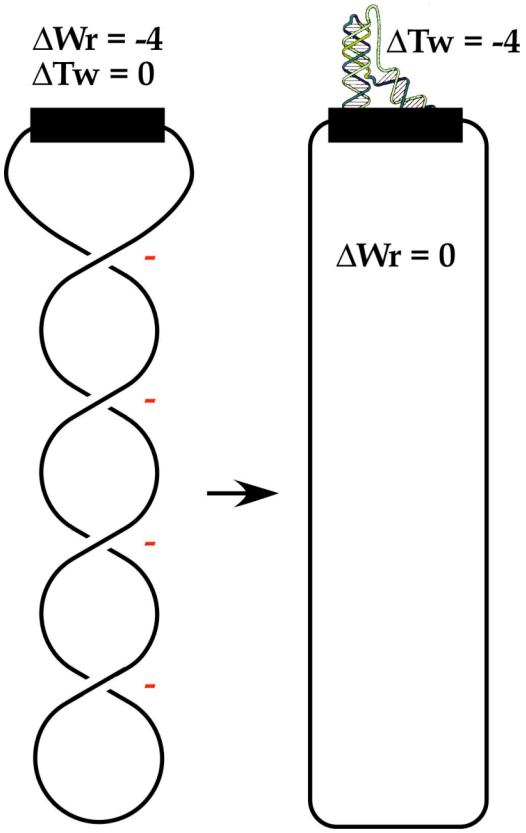
(b; 6 pts) If one started with the plasmid DNA below and caused/allowed the 40 bp segment in the black box to assume the H-DNA form as above, what would the result look like? Assume that ΔTw of the starting DNA and the DNA outside the final H-DNA regions are zero. The H-DNA is in the black box: there 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.



+3 for any answer with a conserved linking number
+3 for the relaxed circle or the appropriate plectoneme for your ΔTw.
(I should have had 5 nodes in the starting DNA to make you draw one!)

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

+3 The removal of writhe in the rest of the circle is thermodynamically favorable
+1 because bending energy and electrostatic repulsion is reduced.

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