Biochemistry 661 **Your Name:** Key

Nucleic Acids, Module I **Prof. Jason Kahn**

Exam I (100 points total) **September 29, 2011**

You have 60 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. There is a extra space on the last page if you need it.

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.

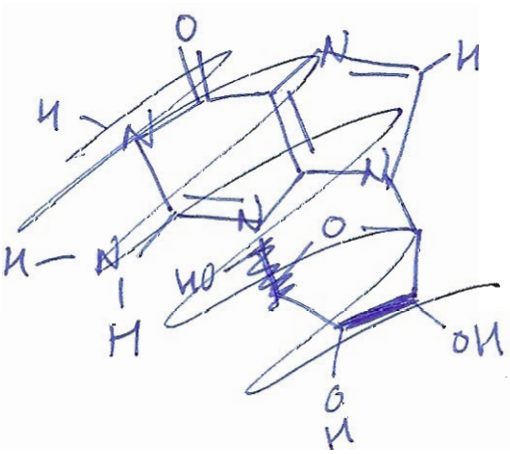
Honor Pledge: At the end of the examination time , please write out the following sentence and sign it, or talk to me about it:

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

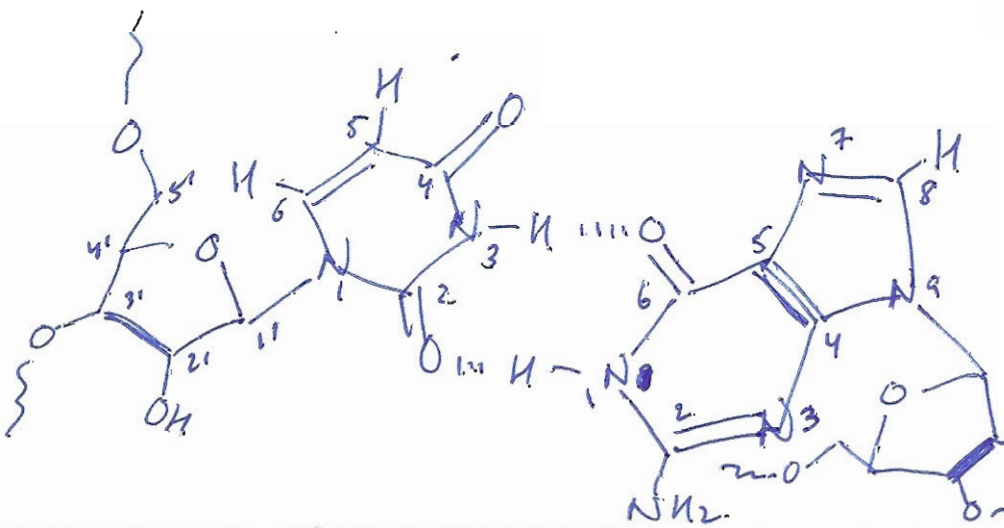
1. DNA Structure, Stability, and Flexibility (48 pts):

(a; 12 pts) Draw a plausible G:U pair in RNA, with the G being in the *syn* conformation. Draw the sugars and include the numbering on one sugar and both bases. Are the backbones locally parallel or antiparallel?

+2 for U
+2 for G
+2 for sugar
+2 for syn
+2 for numbers



(+2) – Backbones are locally parallel – 5' to 3' backbone both sides



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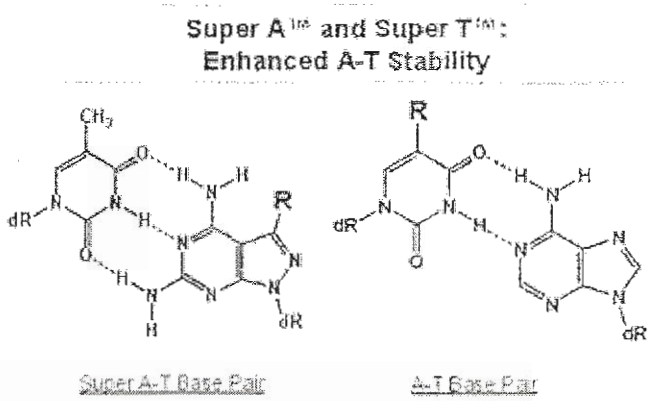
(b; 6 pts) The "Super-A" base makes more stable base pairs with thymine than A does. It can form three hydrogen bonds with thymine, as shown.



Would you expect the main effect of adding a hydrogen bond into an otherwise rigid structure to be (circle one) enthalpic or entropic?

(+2) more binding, same ΔS

"Super-A" is not the same as 2,6-diaminopurine, though they share the same three WC H-bonds. It turns out that 2,6-diaminopurine has context-dependent effects on the thermodynamics, and it doesn't always stabilize base pairing. Reality is complicated. Also, note that the "R" group in Super-A is proprietary, i.e. we don't know the structure. What do you think the R group might be doing?

(+4 for anything reasonable) Maybe R stacks on neighboring bases, or ~~[illegible]~~ perhaps it H-bonds to the $-NH_2$ to lock it down. One could probably figure ~~out~~ it out by looking at mismatch thermodynamics.

(c; 5 pts) We have emphasized over and over what it is that makes the Watson-Crick base pairs special. What is it? Why did we similarly emphasize the particular triple base pairs seen in the Moser and Dervan triplex paper?

(+2) – All four W-C base pairs fit in the same helical geometry

(+3) – The T•A-T and C+•G-C triples similarly are isosteric, so they can stack up into a regular triple helix

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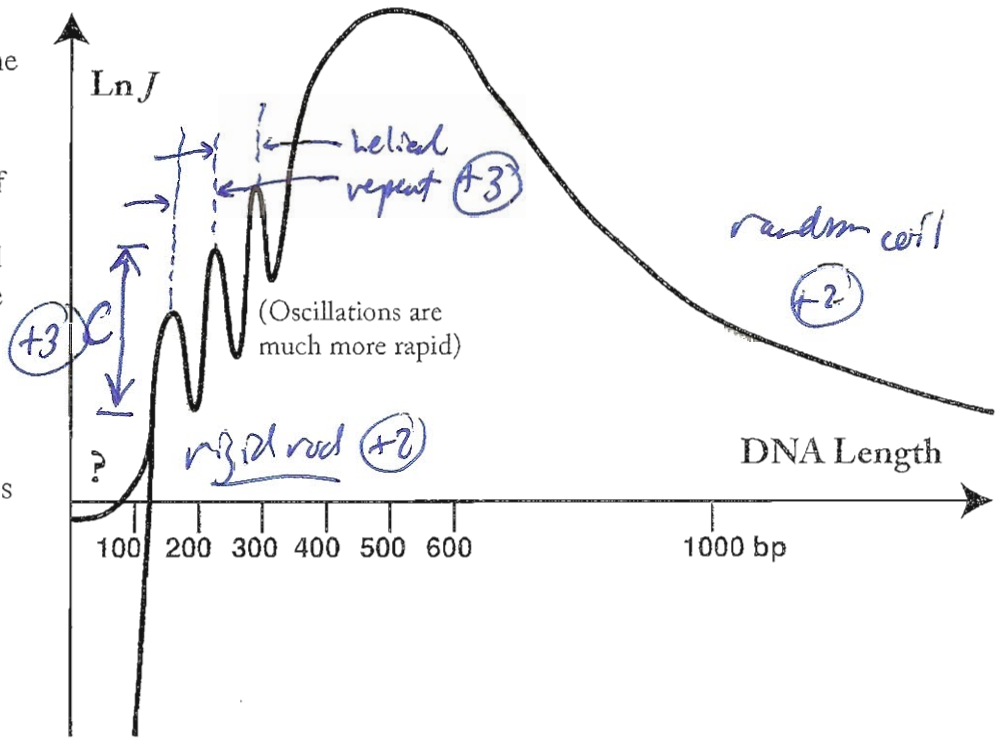
The Shore and Baldwin curve is sketched to the right.



(d; 4 pts) Label the part of the curve that shows rigid rod behavior and the part that looks like random coil.

(e; 3 pts) Sketch on the figure how we can measure the DNA helical repeat from this curve.

(f; 3 pts) Sketch on the figure what aspect of the curve reflects the torsional flexibility of the DNA. = "C"

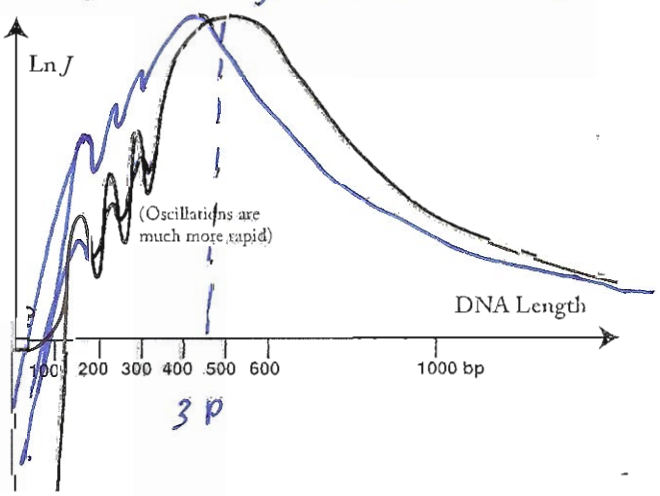
(g; 6 pts) What is being measured, i.e. what is the definition of *J*?

(+4) J is the effective concentration of one end in the neighborhood of the other, considering ~~lateral alignment~~ helix axis alignment and torsion. (+1) $J = K_c/K_a$ (+1)

(h; 3 pts) Why does *J* decrease as DNA length increases beyond 600 bp or so?

(+3) The ends occupy a larger and larger volume so the entropy cost of bringing them together increases.
Or - the concentration of one end ~~was~~ about the other is as they explore more volume. (same thing, different words)

(i; 6 pts) We have mentioned that the persistence length is not trivially determined from this data; it emerges from fitting of the wormlike coil model parameters. Sketch what the curve would look like if the DNA were significantly more flexible (in terms of both bending and torsion), for example if we did the experiment at higher temperature.



(+3) P ↓ so curve shifts left

(+3) Amplitude of oscillations ↓

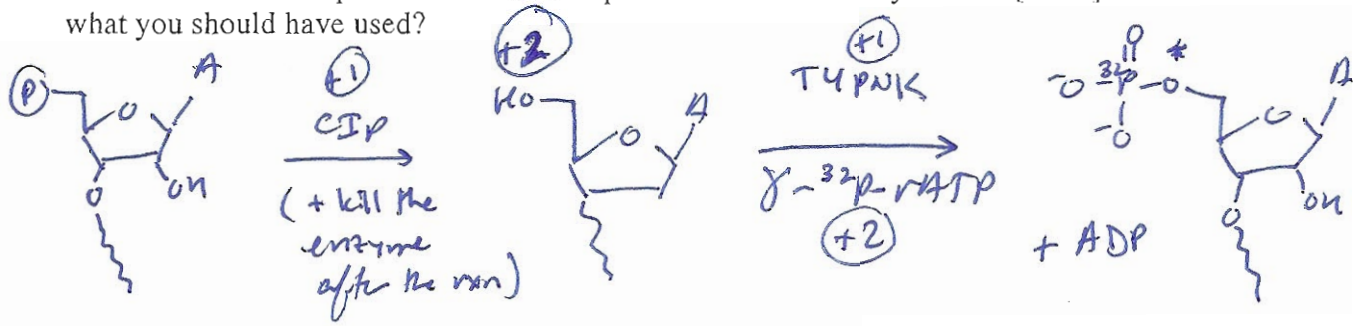
2. Molecular Biology Techniques (18 pts):

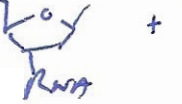
(a; 6 pts) Why is it important for your productivity to do all of the necessary controls in a plasmid cloning experiment? Why should you endeavor to make all the clones you need in parallel rather than one at a time? (Give one reason that covers both). What control would you run that would address whether or not your expensive competent cells survived the latest freezer mishap?

(+4) – When something goes wrong you want to know why immediately rather than needing to do a separate 3-day experiment. Similarly it takes the same time to do 5 transformations as it does to do 1.

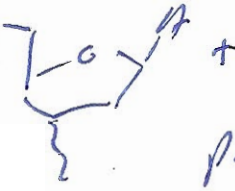
(+2) – Transform the competent cells with plasmid DNA, or just plate them on media without antibiotics to see if they are alive.

(b; 12 pts) Sketch and briefly describe how you would use CIP and T4 polynucleotide kinase and maybe some other stuff to radiolabel the 5' end of an RNA that starts with a 5' phosphate group. What would you get if you forgot to remove CIP at the appropriate time? Why is a phosphatase from Antarctic shrimp sometimes used in place of CIP? What if you used [α-^{32}P]rATP instead of what you should have used?



(+2) – If CIP is still present during kinase rxn, it will simply remove the labeled phosphate, leaving  + $^{32}P_i$ + ADP

(+2) – The antarctic shrimp phosphatase can be inactivated easily at 37°C so it doesn't interfere with labeling

(+2) – use α-^{32}P-rATP would give  + α-^{32}P-rADP, probably not what you wanted!

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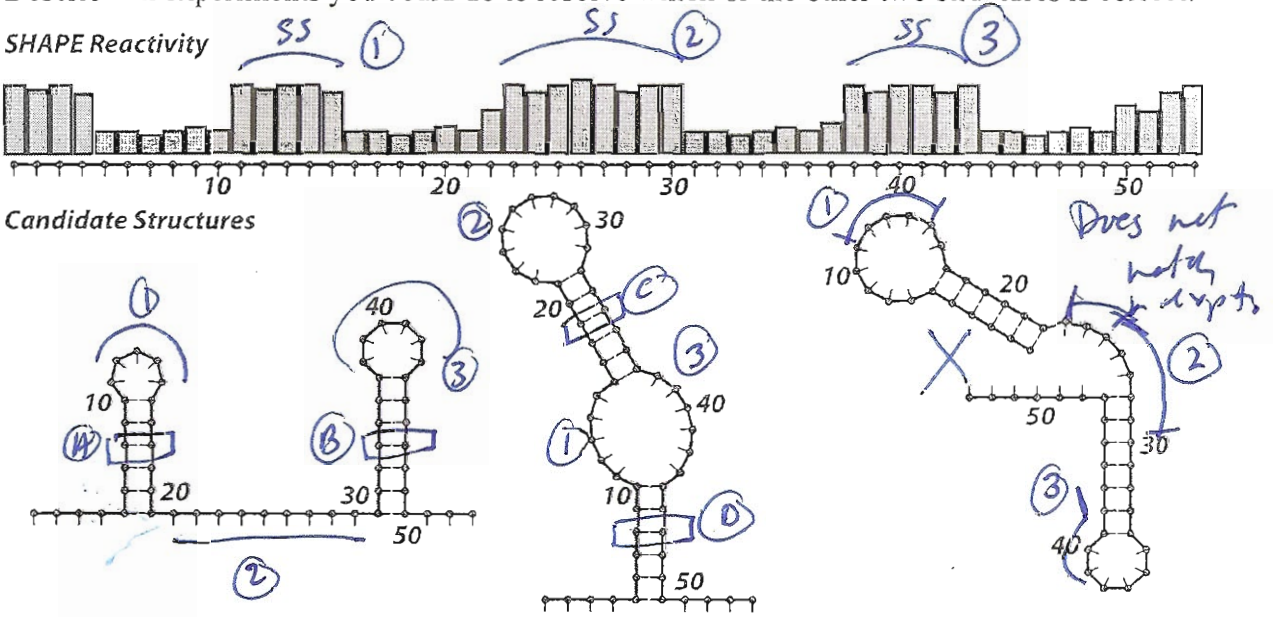
3. RNA Structure (16 pts):

(a; 8 pts) Briefly describe how computations based on nearest neighbor thermodynamics and phylogenetic data can be used to predict the secondary structure of an RNA.

- the stability of any 2°S can be calculated from NN parameters and some information on loops and junctions - the stabilities of candidate structures can be compared.
- Phylogeny is used to set constraints on the possible RNA structures - it improves speed + accuracy. For example, forcing two bases to be paired to each other cuts the problem in two: 

(b; 8 pts) The sketch below shows experimental SHAPE analysis of an RNA. Describe how SHAPE works. Which of the three structures shown is obviously inconsistent with the SHAPE results? Describe an experiments you could do to resolve which of the other two structures is correct.



- In SHAPE, 2'-OH groups that are accessible at least part of the time are modified and the positions determined by primer extension
- Make correlated invariant mutants at positions A, B, C, D and see whether shape results or thermal stability changes.

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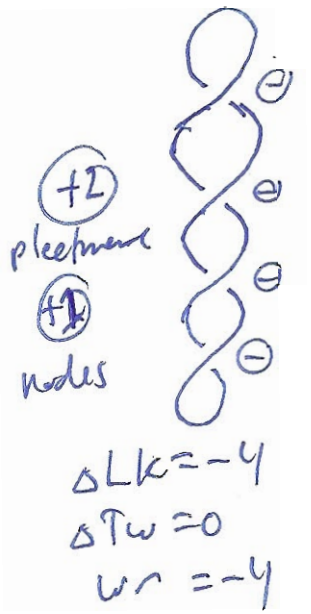
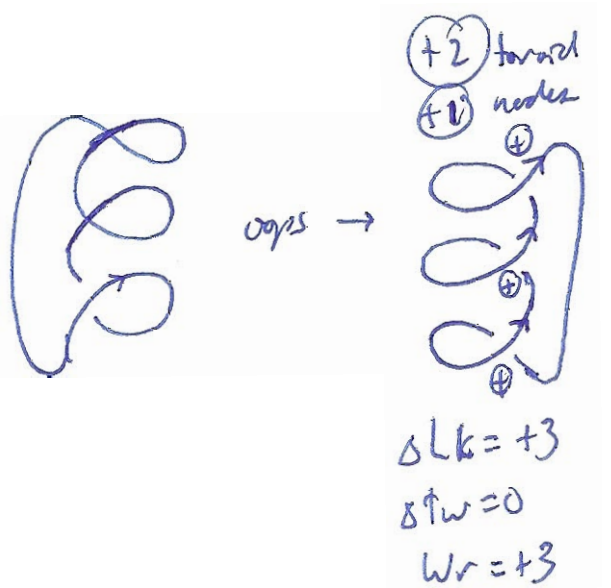
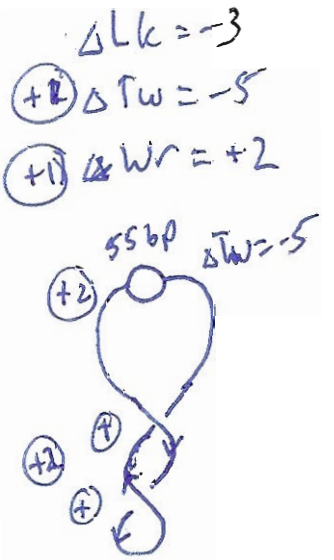
4. DNA Topology (18 pts):

(a; 12 pts) Draw three plasmids with the following properties:

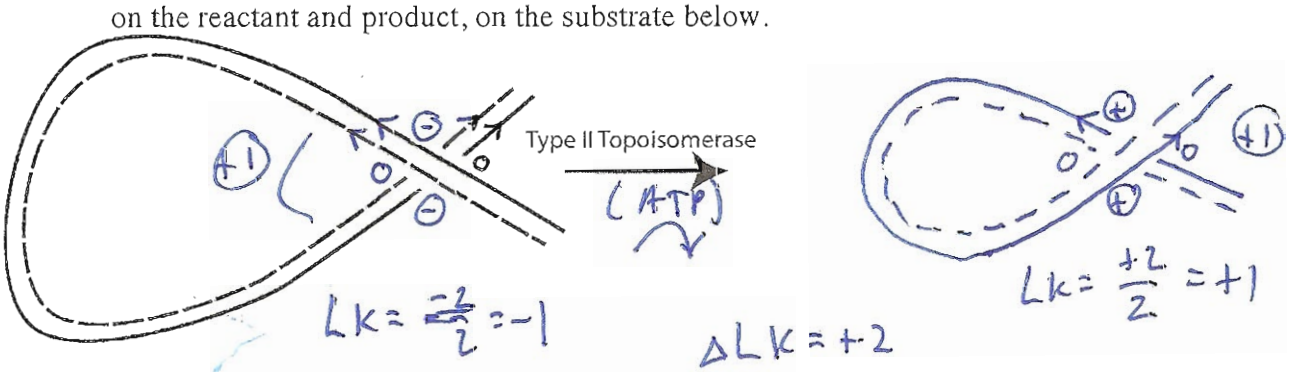
1. $\Delta Lk = -4$, $\Delta Tw = 0$, plectonemic superhelix
2. $\Delta Lk = +3$, $\Delta Tw = 0$, toroidal superhelix
3. $\Delta Lk = -3$, 5 turns of the helix unwound to make a denaturation bubble.







(b; 6 pts) Sketch the reaction catalyzed by a Type II topoisomerase, with appropriate labeling of nodes on the reactant and product, on the substrate below.



$Lk = \frac{-2}{2} = -1$ $\Delta Lk = +2$

+3 for correct product

+1 for Lk calculation

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Total	

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