Biochemistry 661 **Your Name:** Key

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

Exam I (100 points total) **September 25, 2012**

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. (16 pts) You have sequenced an RNA isolated from an extract that helps carry out splicing. You would like to know its secondary structure. Describe how you would proceed. Additionally, you would like to identify base pairing interactions between your new RNA and potential splicing partners. What technique(s) could allow you to catch them? (More space on next page if you need it.)

1 - Predict 2°S using mfold or similar program - based on calculating minimum free energy structure from nearest neighbors (+4)

2 - Make variant RNA's that have correlating mutants to preserve predicted base pairing - see if TE melting profile changes (+4)

3 - Use ss-specific or ds-specific nucleases or perform SHAPE analysis to analyze 2°S experimentally, directly (+4)

- To find partners -

1.- Attempt psoralen crosslinking and map with reverse transcriptase (+4)

2 - Make variants that disrupt or preserve putative contacts, see if function changes

Score for the page 16

2. (6 pts) Why don't sequence-specific RNA binding proteins recognize a fully double-stranded A-form RNA helix?

(+3)

- The deep major groove is not accessible to protein, so the sequence-differentiating H-bond surface of the major Hoogsteen face cannot be read out to distinguish different sequences. (+3)

3. (8 pts) The MAGE paper described a technique that allows rapid genome engineering/evolution. Briefly describe the advantages of this technique as opposed to either traditional cloning or random mutagenesis in generating new or enhanced phenotypes.

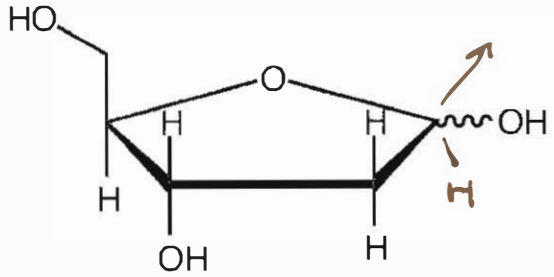
(+3)

- The technique allows creation of combinatorial genetic diversity - For n target sites there are 2^n combinations of +/- mutations. For n=1 traditional cloning is fine, but for n=20 it is impossible → explore many more variants (+2)
- But after all we are limited by the # of cells to screen, and random mutagenesis could make an essentially infinite number of different cells → but the vast majority of the mutants are at places we don't care about. MAGE makes changes at targeted locations so new phenotypes are much more likely to appear and be understandable if they do. (+3)

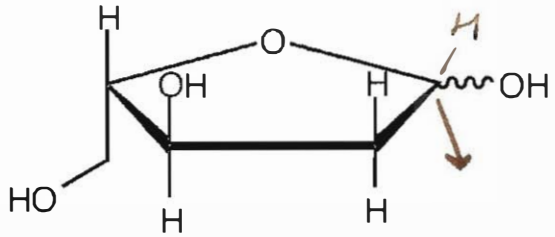
Score for the page 14/

4. (18 pts) Here is the structure of L-2′-deoxyribose vs. the usual D-2′ -deoxyribose.

D-2′-deoxyribose

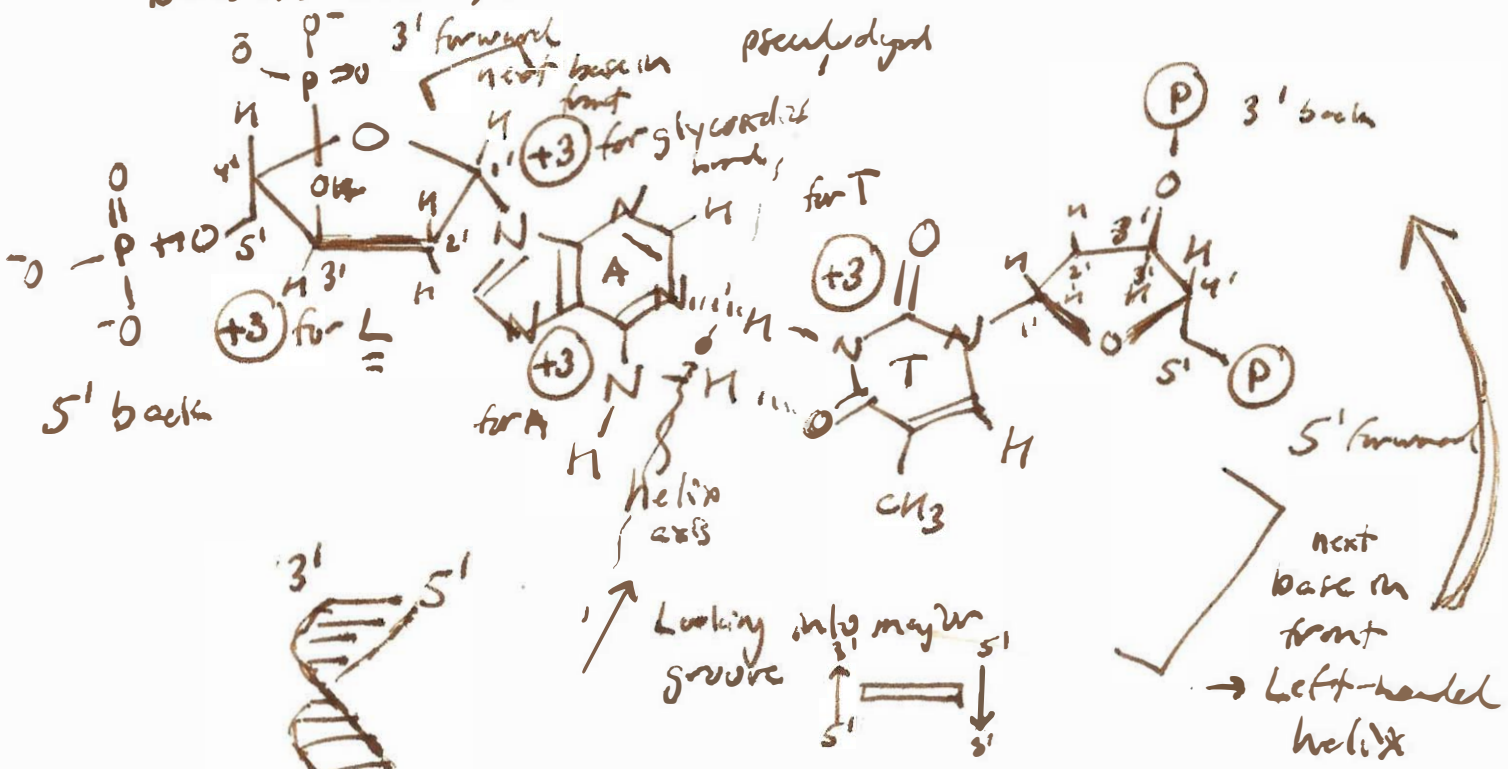


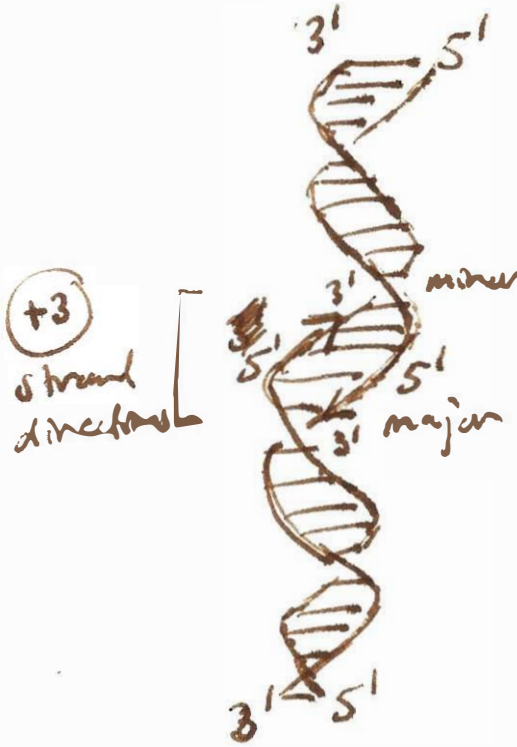
L-2′-deoxyribose



Draw an A-T base pair with L- instead of D-deoxyribose, including both sugars and the four pendant phosphates (three of them just as P's with a circles around them). Sketch the B-form double helix that would result, with strand directions indicated.

- Bases are achiral, so L- and D- will be enantiomers





Imaginary Left-handed B-DNA based on L-deoxyribose

Score for the page 18/18

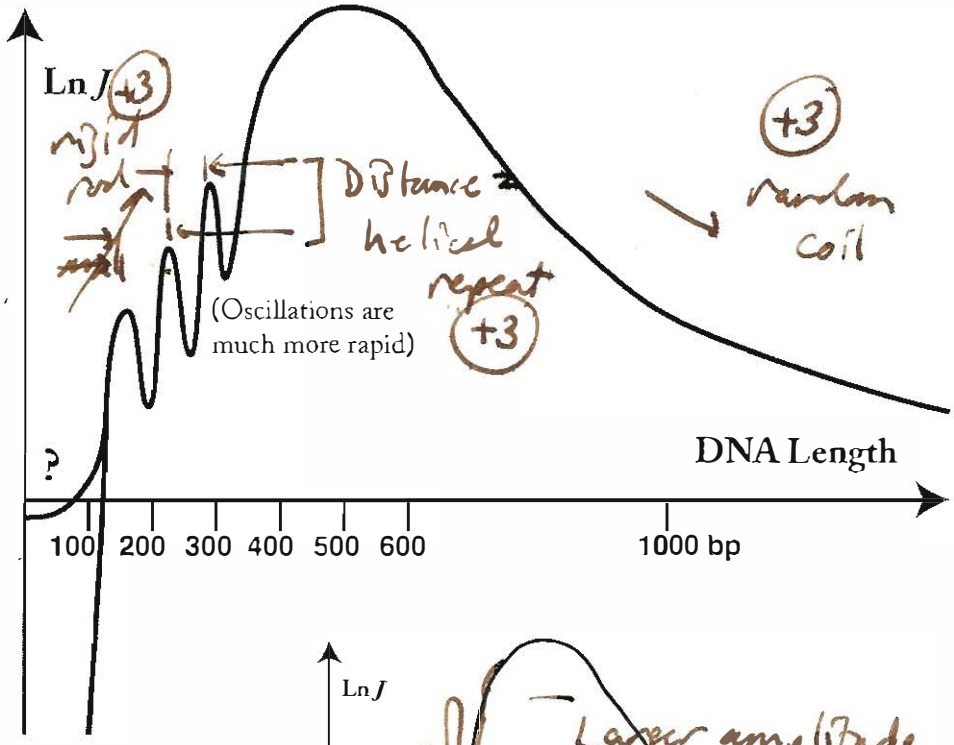
5. (7 pts) When we talked about DNA flexibility I said, "There is no 'the structure' there." What does this mean? Define a "persistence length."

- DNA structure is statistical – for a long DNA we think about an ensemble of possible structures, not one unique structure (+3)
- Persistence length = a measure of how long a segment is needed to "forget" initial direction based on $\langle \vec{\mu}_0 \cdot \vec{\mu}_L \rangle = e^{-L/P}$ (+4)
  or distance, on average, that the end is displaced in the initial direction. $\langle \rho \rangle = P(1 - e^{-L/P})$

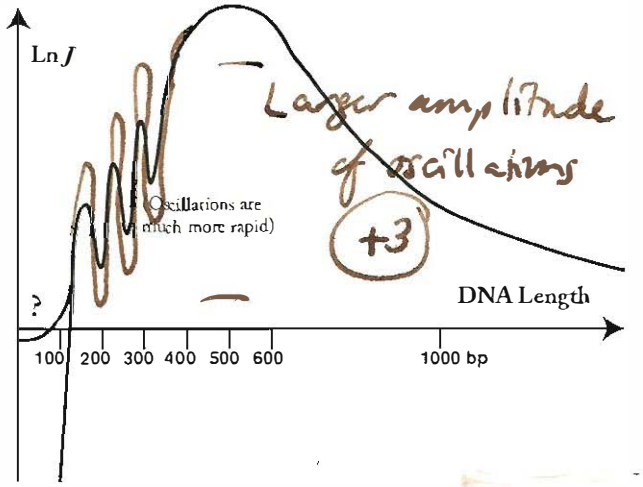
6. (12 pts) The T4 ligase mediated cyclization assay provided a conclusive demonstration that DNA is a double helix in solution. The Shore and Baldwin curve of cyclization probability vs. DNA length is sketched here.

Label the part of the curve that shows rigid rod behavior and the part that looks like random coil.

Sketch on the figure how we can measure the DNA helical repeat from this curve.



On the curve to the right, sketch what the curve would look like if the DNA were significantly stiffer, in terms of torsion only.



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7. (15 pts) The portion of the table shown from Borer et al., 1974, provides evidence that a model for hybridization thermodynamics based solely on base composition is inadequate. What is the reasoning for this conclusion? They introduced a nearest-neighbor model for thermodynamics that has subsequently been elaborated by many groups. What is the basic idea of nearest-neighbor thermodynamics? Any scientific theory must be falsifiable (if a theory isn't falsifiable, we call it religion). How could the nearest-neighbor theory have been falsified?

Molecule†	T_m (°C) at‡ 10 μM	100 μM
$A_4CG + CGU_4$	−13·9	−1·3
U_2CGA_2	1·6	11.3
A_2CGU_2	10·8	22·1
$A_4G_2 + C_2U_4$	14·0	22·8
A_2CGU_2	19·6	28·3

GC-type in original

- U_2CGA_2 and A_2CGU_2 have the same composition of U's, C's, G's, and A's, but their T_m's differ by 10°C.

(+5) In fact all of these oligos have 4 A·U and 2 G·C pairs but their T_m's are very different. [I should have shown the ΔH°'s, and ΔS°'s – the latter two are the fundamental derived quantities.]

- The idea of the NN model is that nearest neighbors or dinucleotides make independent contributions to the overall ΔH° and ΔS° of hybridization.

(+5)
AACGUU
UUGCAA

- If the NN model had failed to predict the ΔH° and ΔS° of new sequences, we would know that longer-distance considerations were necessary. Or – if oligos of the same NN's but different sequence have different T_m's. In fact this does occur and requires a small correction.

(+5)

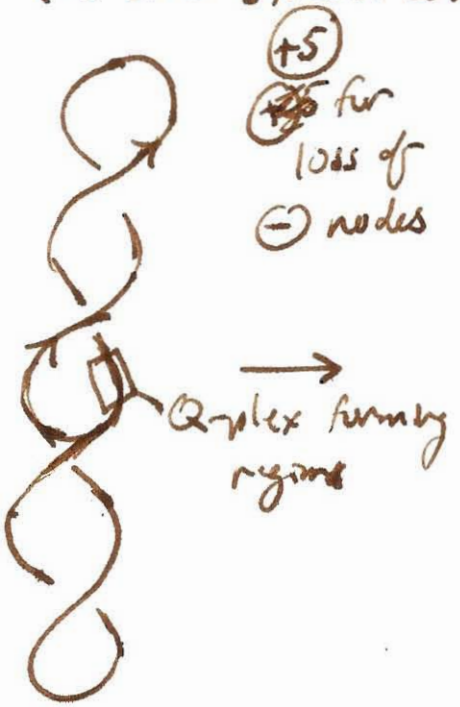
Score for the page 15

8. (18 pts) Negatively supercoiled DNA can extrude quadruplex segments of DNA. Give a sequence that could form a quadruplex. What structure can the other strand form? Sketch a plasmid with and without an extruded quadruplex to explain why negative supercoiling has this effect. Give estimates for before-and-after ΔTw and ΔWr.

—AAAGGGGATTTGGGGTTAGGGGATTGGGGACAT

(+4) 4 G segments separated by at least 3 nt ~28 nt ≈ 3 turns

– The other strand can form an i-motif (+3)



relieves bending strain in the supercoil

new ΔWr ≈ –12
new ΔTw ≈ –0.75
ΔLk does not change
(+3) for correct change.

$\Delta Wr \approx 0.8 \times -0.06 \times N/h$

for N = 3150 bp and h = 10.5 bp/turn

$\Delta Wr \approx -0.05 \times 300 = -15$

$\Delta Tw \approx -0.0125 \times 300 = -3.75$] ~4:1 ratio

$\Delta Lk = -0.06 \times 300 = -18$ turns

(+3) for reasonable estimates

Page	Score
1	
2	
3	
4	
5	
6	
Total	

Score for the page 18/18