Biochemistry 465 **Your Name:** Key

Biological Information Processing **Prof. Jason Kahn**

Exam I (100 points total) **March 7, 2011**

You have 50 minutes for this exam. N=62

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 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.

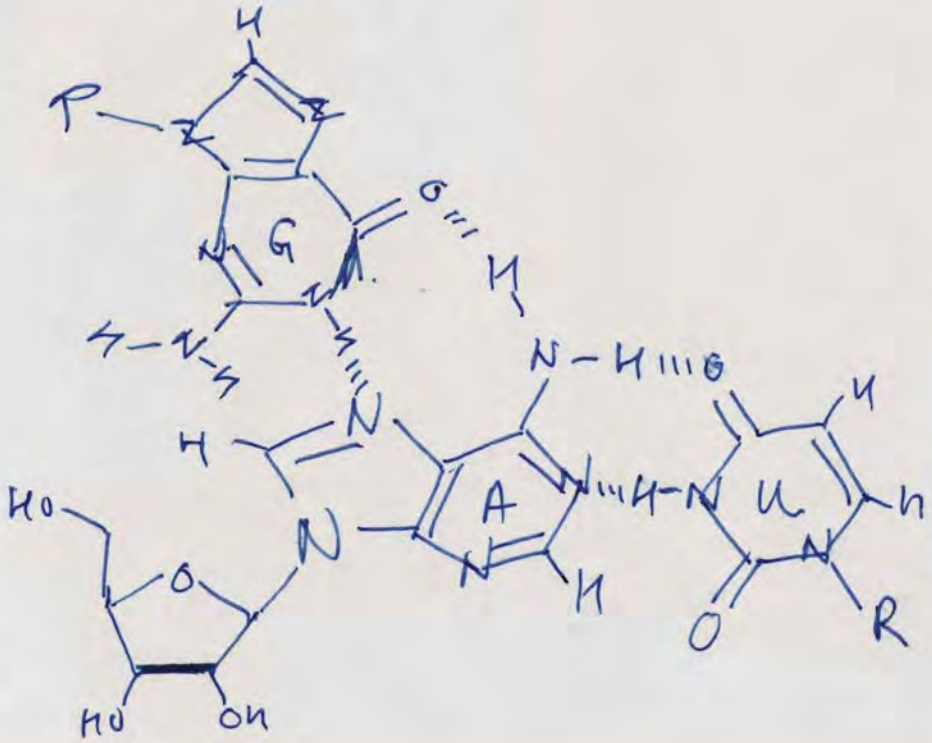
Honor Pledge: At the end of the exam time, please write out the following sentence and sign it, or talk to me about it:

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

(+1 pt)

1. DNA Structure and Base Pairing (25 pts):

(a; 15 pts) Draw a plausible G•A-U triple base pair with at least two H-bonds between the G and the Watson-Crick A-U pair.



+3 for A
+3 for U
+3 for G
+1 for ribose
+3 for reasonable triple
~~+2 for~~
+1 for W-C H-bonds
+1 for neatness

Score for the page_____

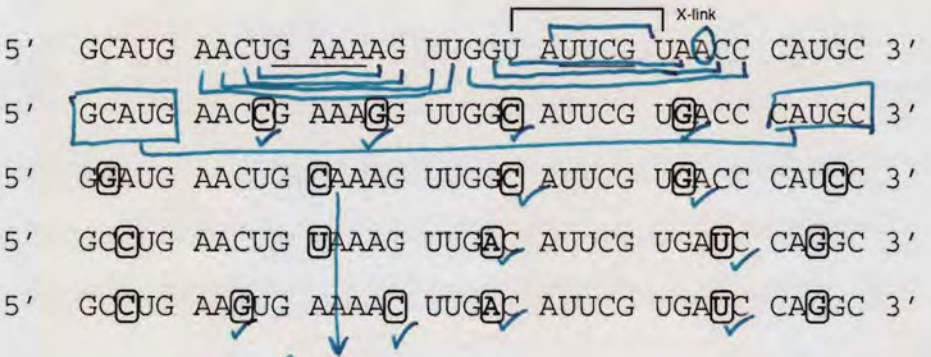
(b; 10 pts) Enter "A" for A-form, "B" for B-form. or "Neither" or "Both" in the in the table below.

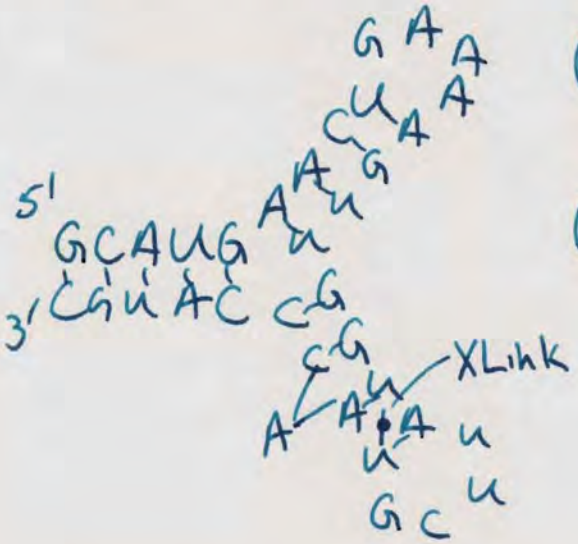
Original Watson-Crick Model	B
Helical rise and base pair spacing are the same	B
Helix axis runs through "empty space"	A
Stabilized primarily by base stacking	Both
Large base pair inclination	A
C2'-endo sugar pucker	B
Characteristic of dsRNA	A
Each base pair has a pseudodyad axis that intersects the helix axis	Both
The helix axis is nearly perpendicular to the base pair plane	B
"Cavernous" major groove	A
Looking into the minor groove, left strand runs 5'->3' bottom to top	both

+1 each

2. Secondary and tertiary structure (20 pts):

(a; 16 pts) Propose a secondary structure for the RNA below. Each line is a sequence from a homologous RNA, with changes boxed and in bold. The underlined regions are single-stranded by nuclease mapping, and X-link indicates a psoralen crosslink.





+2 for idea
+4 for each helix = 12
+2 for extrahelical A

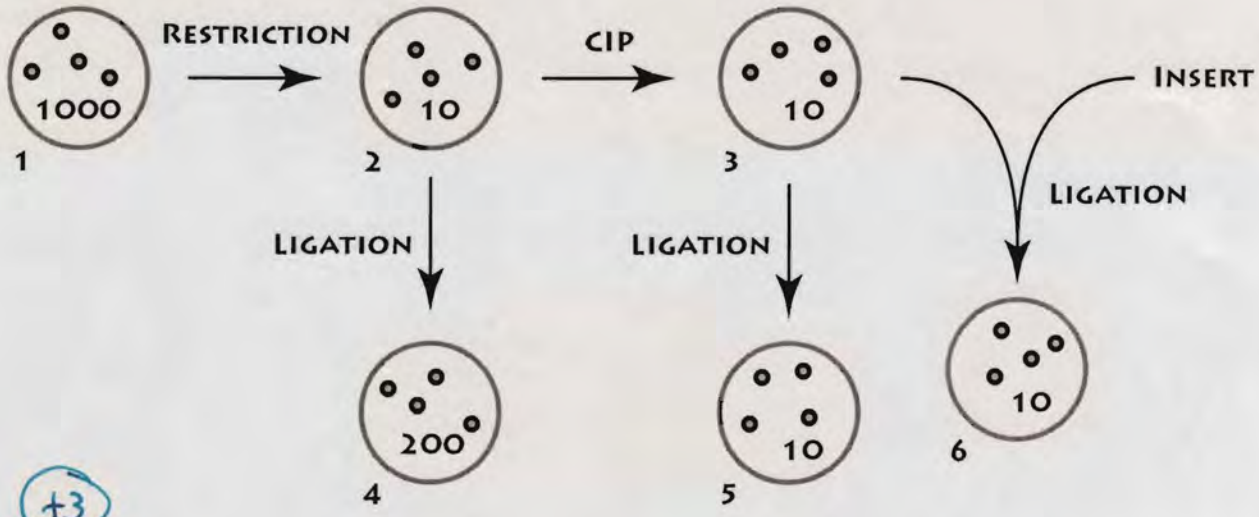
Score for the page_____

(b; 4 pts) Why is a divalent cation like Mg^{++} typically required for the formation of RNA tertiary structure?

(+2) Neutralizes charge, which is necessary to allow close approach of (P) backbone to itself. (+2)

3. Basic Cloning (19 pts)

(a; 15 pts) The cloning experiment below did not work. Each circle indicates a plate of bacteria, with the number inside the plate being the number of colonies. All colonies are blue. Which plate gives an undesirable answer, and what should it have looked like? There are at least two possibilities for what went wrong. What are they, and how could you distinguish them or solve them in the next cloning attempt? [Hint: if your research director had been willing to spring for a phosphatase from an Antarctic shrimp instead of a cow, one of the possibilities could have been prevented.]



(+3) → Plate #6 should have lots of white colonies in addition to (+3) the blue ones. The others look okay given 99% restriction

What went wrong?

(+3) 1. CIP activity was not destroyed before ligation

(+3) 2. Insert was bad (degraded, wrong ends, etc.)

(+3) → Do an experiment with a known-good (+) control insert -or- omit the CIP and accept higher background

Score for the page_____

(b; 4 pts) Give a reason that 5' overhangs are generally more useful in cloning or manipulation of restriction fragments than 3' overhangs.

- They can be filled by polymerases for labeling or blunt-end cloning
- They are more readily ligated or phosphatased.

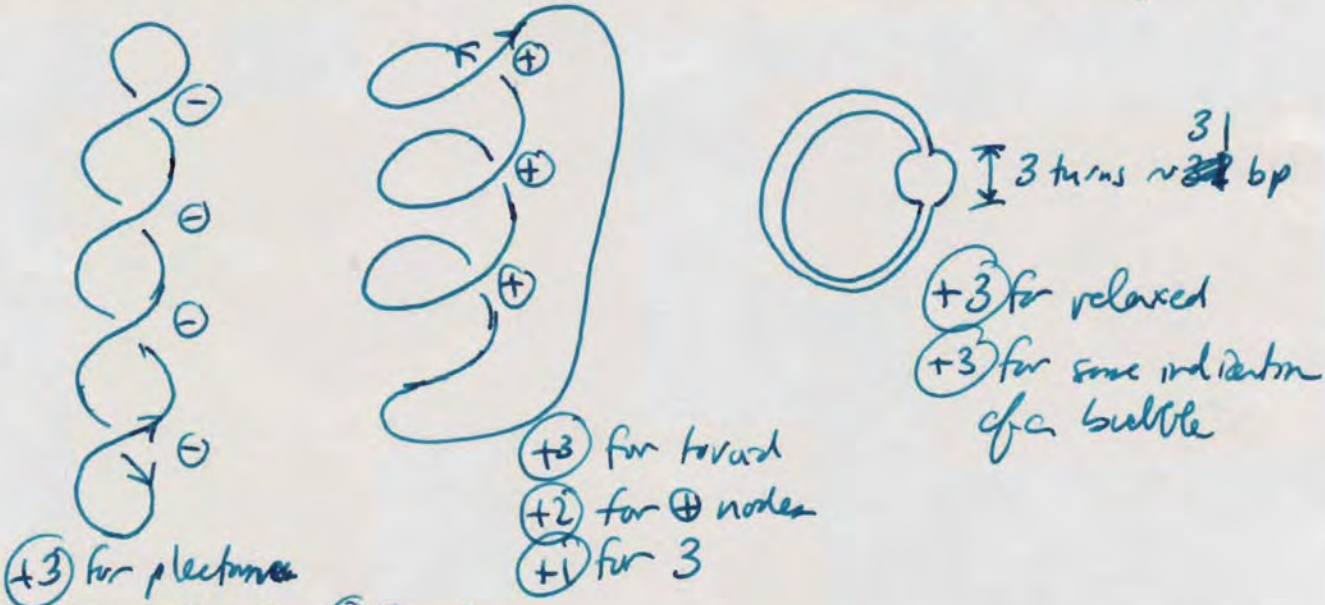
(+4) for anything that connects to an enzyme

3. DNA topology (24 pts):

(a; 18 pts) Draw three plasmids with the following properties:

1. ΔLk = -4, ΔTw = 0, plectonemic superhelix → Wr = -4
2. ΔLk = +3, ΔTw = 0, toroidal superhelix Wr = +3
3. ΔLk = -3, 3 turns of the helix unwound to make denaturation bubble. → ΔTw = -3, Wr = 0

1.



(+3) for plectonemic
(+2) for ⊖ nodes, (+1) for four

(+3) for toroid
(+2) for ⊕ nodes
(+1) for 3

(+3) for relaxed
(+3) for some indication of a bubble

(c; 6 pts) What are the linking number changes introduced by Type I and Type II topoisomerases? Topoisomerase poisons are useful anti-cancer agents. What makes a topoisomerase halted in the middle of its catalytic cycle particularly toxic to a cell (e.g. as opposed to a random metabolic enzyme that is inactivated by a drug)?

Type 1: ±1, Type 2: ±2 } (+3) all or none

(+3) The enzyme stuck to DNA makes repair difficult – can induce cell cycle arrest / apoptosis.

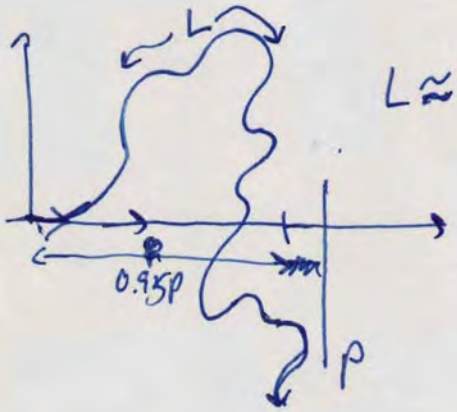
Score for the page_____

4. DNA Flexibility (12 pts):

(a; 12 pts) Define ρ, P, and L in the equation $\langle \rho \rangle = P\left(1 - e^{-L/P}\right)$. Which one do we control as experimentalists? Sketch an typical structure for a DNA with L = 3P that has the average value of ρ.

$\langle \rho \rangle = P(1 - e^{-3})$ & $\langle \rho \rangle = 0.95P$

$e^{-3} = 0.05$



$L \approx 3P$ by eye

(+2) for $\rho = 0.95P$

(+2) for reasonable L

ρ = projection in the initial direction (+2)

L = contour length = $N \cdot 3.4\text{Å}$ (+2) → we control L

ρ = persistence length (+2)

Page	Score
1	15
2	26
3	19
4	28
5	12
Total	

Score for the page_____

12