BCHM465 **Your Name:** Key

Biochemistry III, Molecular Genetics **Prof. Jason Kahn**

Exam I **March 6, 2001**

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.

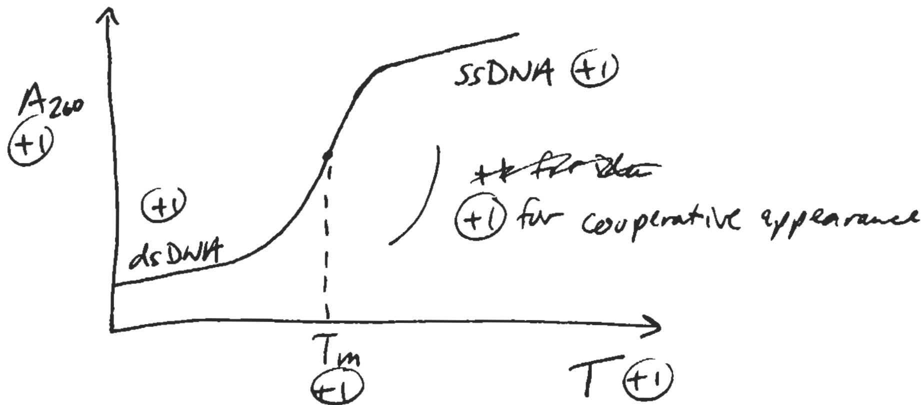
You may need a calculator for this exam. No other study aids or materials are permitted. N=24

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

1. Secondary Structure and Thermodynamics (20 pts):

We discussed using thermal melting curves to analyze oligonucleotide thermodynamics.

(a; 8 pts) Sketch a graph representing an absorbance thermal melting curve below, identifying the axes, the portions of the curve corresponding to single-stranded and double-stranded DNA, and the T_m. What physical change does the melting curve monitor?



(+2)

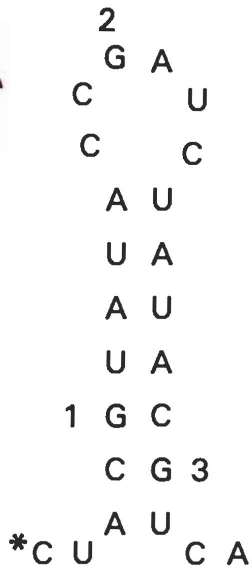
The hyperchromism upon melting is due to loss of stacking upon strand separation.

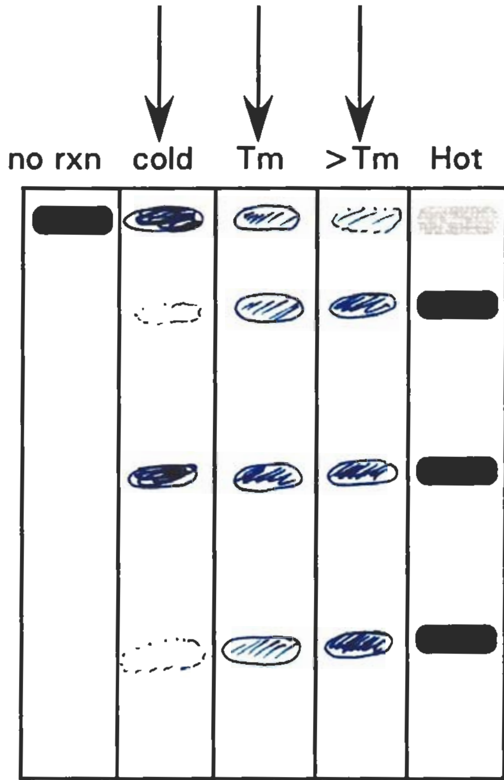
↳ +1 if missed stacking but there is a suggestion of disorder

(b; 7 pts) Anything that changes when a nucleic acid goes from ds to ss can be used as the basis of a melting curve experiment. One example is the accessibility of an RNA nucleotide to a single-strand specific enzyme like ribonuclease T_1, which cuts at G. You are given the RNA oligonucleotide below, labeled at the 5' end. Sketch the appearance of a polyacrylamide gel run on samples reacted with ribonuclease T_1 as you increase the temperature from below T_m to above T_m as indicated.

→ should have specified denaturing gel, not melting in the gel (+2) for general idea

+4 for general increase w/ T.





(+1) for constant cleavage

(+2) for ↑ cleavage

(+1) for 50:50 cleavage at Tm

(+1) for levels

(c; 5 pts) You find experimentally that the melting temperatures you determine on the same oligonucleotide using the two methods are different. Why might this be?

[fuzzy question]

(+1) [absorbance measures stacking, T_1 measures accessibility of backbone

- RNAse T_1 is a ss RNA binding protein, therefore can shift the equilibrium toward ss form

- Melting might not be two-state - A/T-rich parts could melt first, detect melting of G-C later

- If there are for example 3° structure changes that make backbone accessible, T_1 could cut even if 2°s is still there

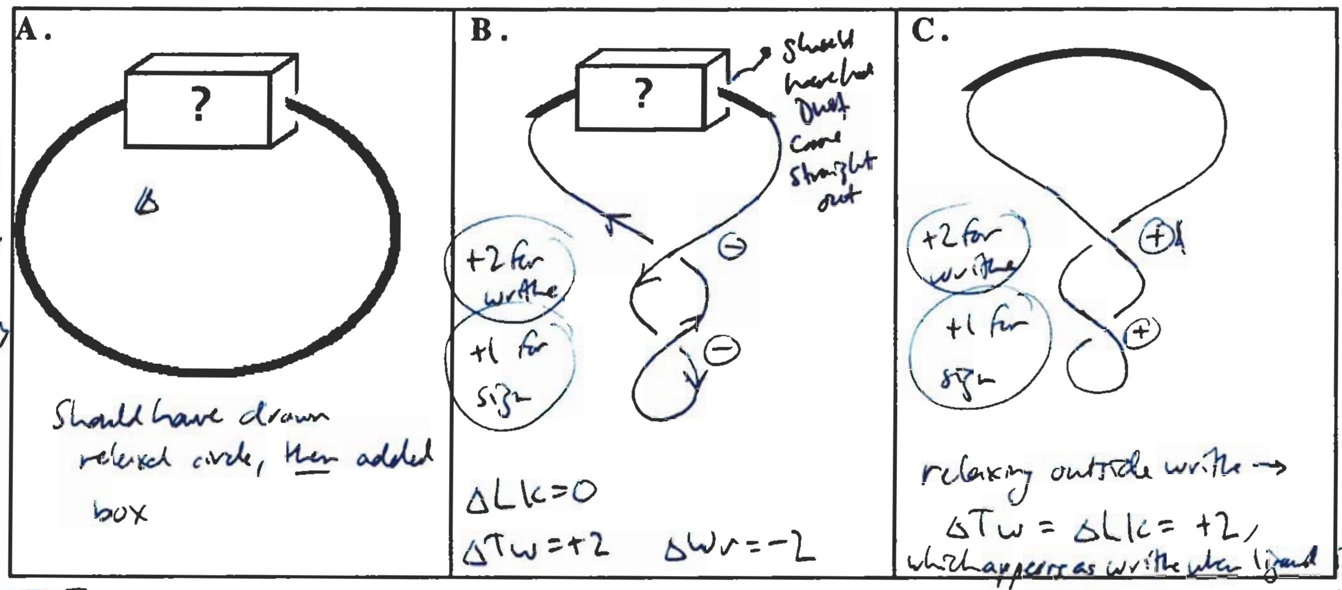
- (+3) +2 for anything reasonable
(+1) +2 for clarity

2. DNA Flexibility and Topology (20 pts).

(a; 9 pts) DNA topology is a useful probe for ligand-induced structural changes. Imagine that within the white box below, a ligand binds that induces a ΔTw of +2 on an initially relaxed DNA. Draw the resulting shape of the DNA in Box B. Then we add a topoisomerase which relaxes away all writhe outside the box, and then we remove the topoisomerase and the ligand. Draw the final result in box C. From topology alone, do we have any way of telling whether the ligand in the box introduced twist or writhe? Why or why not?

If answer is wrong, +4 for anything that demonstrates an understanding of $\Delta Lk = \Delta Tw + \Delta Wr$

+2 for reasonable changes



(+3) [We can't tell - all we know is what the overall perturbation is - $\Delta Wr = +2$ would have the same effect
[Strictly, this is true only if the "exit angles" from the box do not change and the DNA [illegible] is colinear on each side of the box]

The wormlike coil model describes the length dependence of apparent DNA flexibility.

(b; 8 pts) Give a very brief explanation of the essence of the wormlike coil model, in terms of how DNA behaves at short and long lengths. What quantity parametrizes the changeover?

(+2) at short lengths DNA behaves like a rigid rod - direction is predictable based on initial direction (+1) (Equivalently, displacement is in the direction of initial tangent vector.)

at long lengths, DNA behaves like a random coil (+2) - chain directions are uncorrelated, Radius $\propto N^{1/2}$, displacement is constant at a = persistence length. (+1)

The persistence length (+2) - basically, DNA < 1 persistence length is quite stiff.

(c; 3 pts) We used the analogy of 100 pots of boiling water, each with a strand of spaghetti. What did that have to do with DNA?

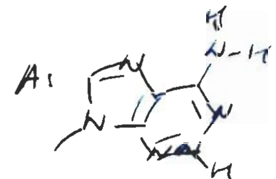
Each strand represents one possible conformation of a DNA molecule in a thermal bath. Typically we observe average properties. (+3)

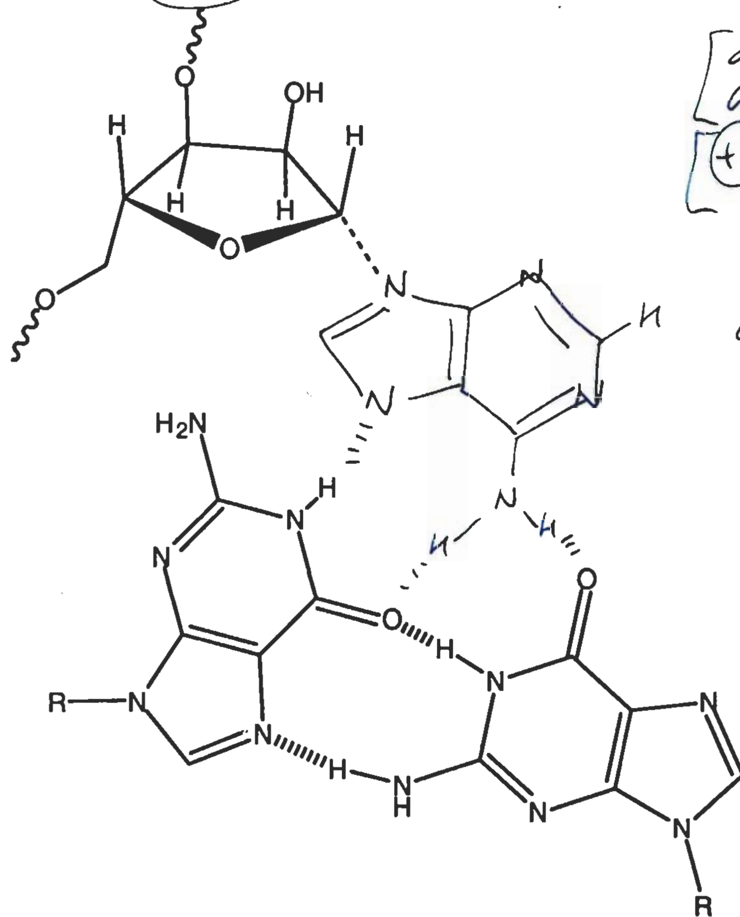
should have specified A, C, G, ...

3. Base Pairing and Hybridization (20 pts).

(a; 7 pts) In the space below, attached to the sugar given, draw a reasonable triple-base partner for the G•G pair below, forming at least two hydrogen bonds. Does the third-strand backbone run parallel or antiparallel to the G at the bottom left (circle one answer)?

[assuming both G's are anti - oops] [(+1) for recognizing this]





anti A forming 2 or 3 H-bonds with G's

no one gave this answer

(+2) for covalent structure correct

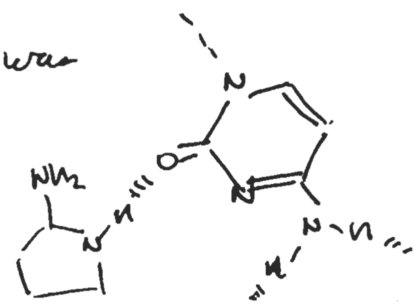
(+2) for reasonable H-bonding (+1 for each of at least 2)

(+1) for overall reasonable, syn-anti

(+1) for parallel/antiparallel correct

(many possible answers)

A common answer was

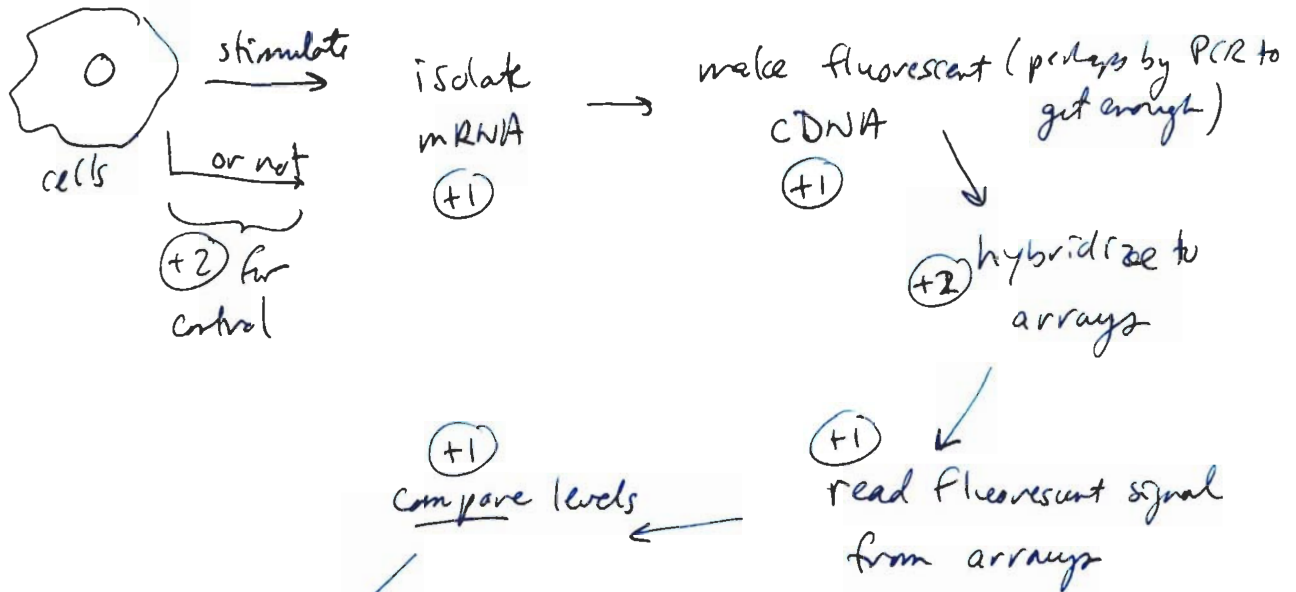


but this requires a syn C

or also a syn G was common

(b; 10 pts) DNA microarrays or "gene chips" are a transforming technology. Describe how you would use a an expression profiling experiment to identify changes in gene expression due to insulin or other hormone stimulation of cultured cells.

Should have made it more clear that I wanted to know how it works.



thereby identifying genes which are activated or repressed by the change in conditions +2

+8 if all correct but don't specifically mention that we characterize relative changes

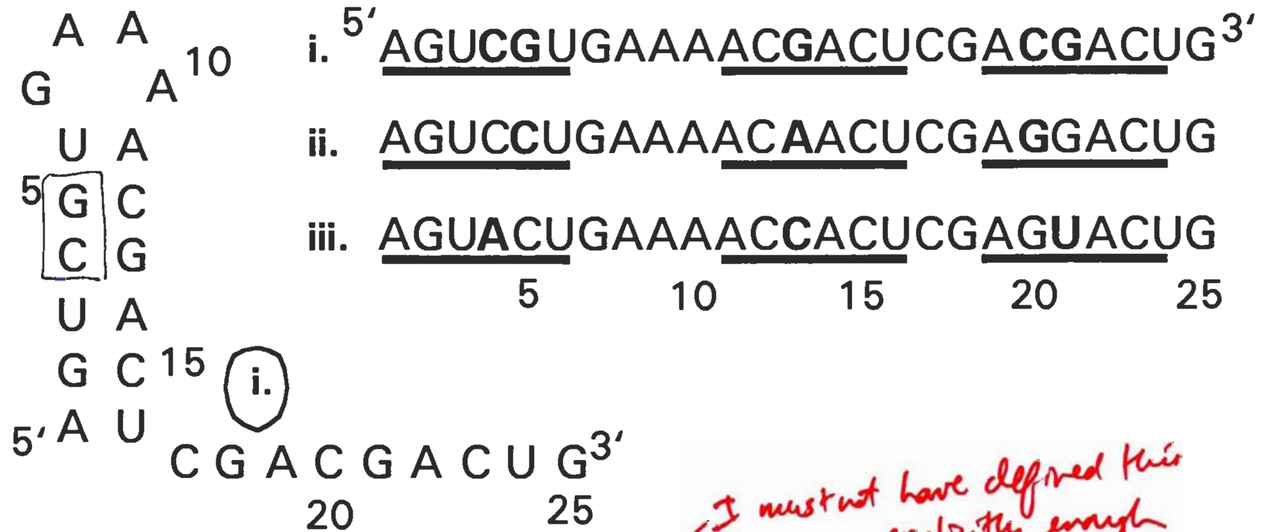
(c; 3 pts) Based on (b) above, you might think that putting your college fund into Affymetrix stock would be a good idea. Drawing on the experience of people who invented in most of the hundreds of automobile companies that were around in 1910, why is this not necessarily the case?

The automobile was a transforming technology but 95% of the companies folded. When there's fierce competition, no one company or even the whole industry (e.g. airlines) is guaranteed to make money.

+3 for idea of competition

4. Secondary structure prediction (18 pts).

The essential RNA (i) below was proposed to form the structure shown based on computer modeling. Then homologous sequences (ii) and (iii) were discovered. The bases that differ from RNA (i) are indicated in bold. The underlines are hints.



I must not have defined this explicitly enough

(a; 4 pts) Explain the notion of correlated invariants in phylogenetic studies of RNA structure.

we look for pairs of residues that change so as to maintain base pairing. This confirms the existence of a ~~base~~ base pairing interaction in the family of species.

(b; 3 pts) Why don't the invariant bases in the sequences tell us as much about secondary structure as the ones that do vary?

They could have some essential function, and in and of itself that doesn't say anything about 2°S. Also - no correlated invariants.

(c; 6 pts) Do the sequences (ii) and (iii) support the structure shown? Why or why not?

If the stem is U6A11 in (i), (+2) would expect $G_5 \rightarrow C$ to be accompanied by
ii, iii C ← G C
iii A ← C G
U A
G C
A, U16

$C_{12} \rightarrow G$, which is not seen. And $C_4 \rightarrow A$ should give $G_{13} \rightarrow U$, also not seen.

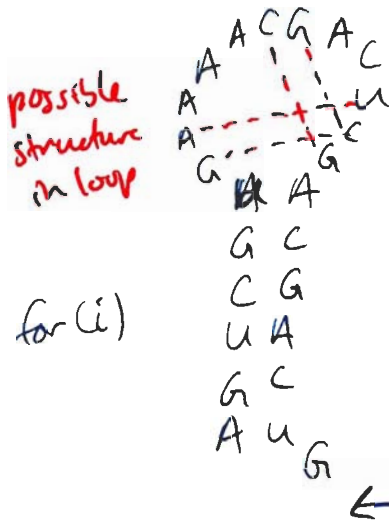
(+1) In fact G_{13} is variable, suggesting not basepaired.

7 points for max of six

↳ No. (+2) ~~Correlated~~ Lack of correlated invariants argues against it. (+1)

In fact a stem with bases ~~1-6~~ 1-6 paired to 19-24 is consistent (+1) with the nucleotide changes

(d; 5 pts) Draw an alternative secondary structure that is more consistent with the phylogenetic data.



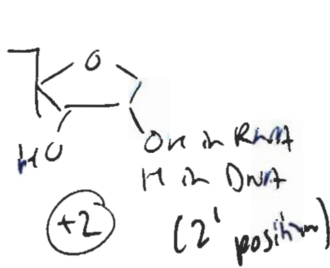
(+2) for anything reasonable

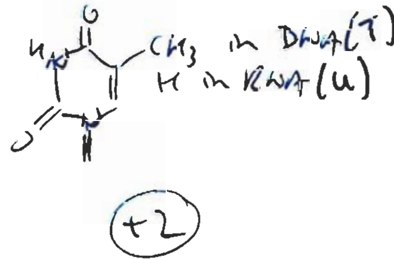
(+1) for mentioning that correlated invariants support it

or just (+5) for correct structure

5. Miscellaneous (22 pts).

(a; 6 pts) What are the two chemical differences between RNA and DNA? Why are there no organisms with large RNA genomes?

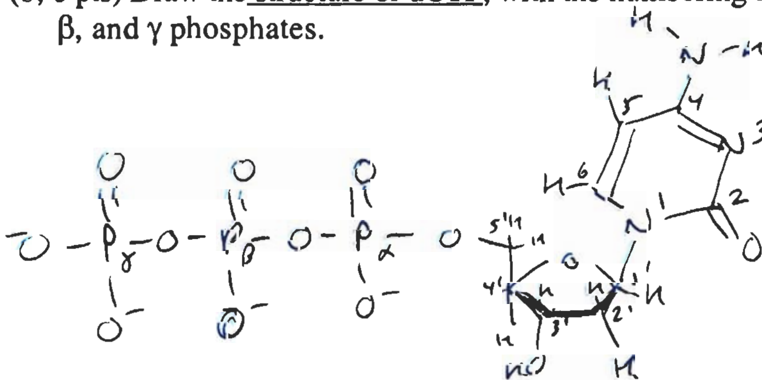




(+1) The RNA backbone is unstable — large

(+1) genomes would fall apart.

(b; 6 pts) Draw the structure of dCTP, with the numbering and identifying the α, β, and γ phosphates.



+1 for C

+1 for sugar

+1 for (P) backbone

+1 for α/β/γ

+1 for sugar #'s

+1 for base #'s

(c; 6 pts) Why is Mg^{++} or another divalent metal essential for RNA tertiary structure? Can RNA secondary and tertiary structure be studied independently, if so how?

(+2) — Divalents are required to permit close approach of (P) backbone in compact 3°S — shield electrostatic repulsion.

(+1) — Yes

(+3) — In the absence of Mg^{++}, 2°S is still stable but 3°S disappears can then look at 3°S ~~structures~~ changes by adding back Mg^{++}

(d; 4 pts) What do proteins see when they approach duplex DNA?

+2 mainly the minor groove and major groove edges of base pairs and stripes of ⊖ charge. +2

Score:			
	Question 1:	______	out of 20: 2° Structure and Thermo
	Question 2:	______	out of 20: DNA Flexibility and Topology
	Question 3:	______	out of 20: Base Pairing and Hybridization
	Question 4:	______	out of 18: Phylogeny
	Question 5:	______	out of 22: Miscellaneous
	Total:	______	**out of 100**