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 Tm. **What physical change does the melting curve monitor?**

![Graph](image)

The hyperchromism upon melting is due to **loss of stacking**.  

1. It indicates 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 T₁, 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 T₁, as you increase the temperature from below Tₘ to above Tₘ as indicated.

(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?

- Absorbance measures stacking, Tₘ measures accessibility, which means Tₘ is a measure of the stability of the double helix. Hence, the two methods could give different results.
- Melting might not be two-stage; Adenine-rich parts could melt first, leaving the A-G base pair interactions accessible, which could shift the equilibrium toward ss form.
- If the RNA were, for example, in a hydrophobic environment, the accessibility of the RNA could be different, affecting the melting temperature.

For further clarification:
- A for anything reasonable
- C 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 writhes 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 increased twist or writhe? Why or why not?

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?
(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 DNA. Typically we observe average properties.

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 H•H•H•H•H•H.

[Diagram]

Anti A gives 2 or 3 H-bonds with G's

+2 for correct structure correct
+2 for reasonable H-bonding
+1 for overall reasonable
+1 for parallel/antiparallel correct

A common answer was

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

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

1. Stimulate cells
2. Prepare mRNA
3. Make fluorescent probes by PCR to get oligos
4. Hybridize to arrays
5. Compare levels
6. Read fluorescent signals from arrays
7. If all correct but don't specifically mean that a change in 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 failed. When there's fierce competition, no one company or even the whole industry (e.g. airlines) is competition guaranteed to make money.
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.

```
A A 10
G A
U A
G C 15
C G
U A
G C
5' C G A C A C U G 3'
20 25
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(a; 4 pts) Explain the notion of correlated invariants in phylogenetic studies of RNA structure.

"We look for pairs of nucleotides that change together to maintain base pairing. This confirms the existence of a base pair

A-U pairing is invariant 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 special function, and in and of itself that doesn't say anything about 25. Other correlated invariants."

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

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AGUCGUGAAACGACUCGACGACUG
AGUCUGAAACAAACUCGAGGACUG
AGUACUGAAACACCACUCGAGUACUG
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If the stem is 

[c] with C -- C, in (c); which expect G5-C to be accompanied by C15-G, which is not seen, and C4-U should give G5-U also not seen.

In fact, G11 is variable, suggesting not base pair.

In fact, a stem with base to 1-6 paired to 19-24 is consistent with the nucleotide change.
(d; 5 pts) Draw an alternative secondary structure that is more consistent with the phylogenetic data.

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?

(b; 6 pts) Draw the structure of dCTP, with the numbering and identifying the α, β, and γ phosphates.
(c; 6 pts) Why is Mg\(^{2+}\) or another divalent metal essential for RNA tertiary structure? Can RNA secondary and tertiary structure be studied independently, if so how?

- Divalent ions are required to permit close approach of 3\(^{\circ}\) helix due to compact 3\(^{\circ}\) - shield electrostatic repulsion.
- Yes
- In the absence of Mg\(^{2+}\), 3\(^{\circ}\) is still stable but 3\(^{\circ}\) disappears can form like at 3\(^{\circ}\) structures changes by adding back Mg\(^{2+}\).

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

mainly the minor groove and major groove edges of base pairs and stripes of \(\Theta\) charge.

Score: Question 1: ____ out of 20: 2\(^{\circ}\) 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