

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. I have given you more space than you should need.

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.

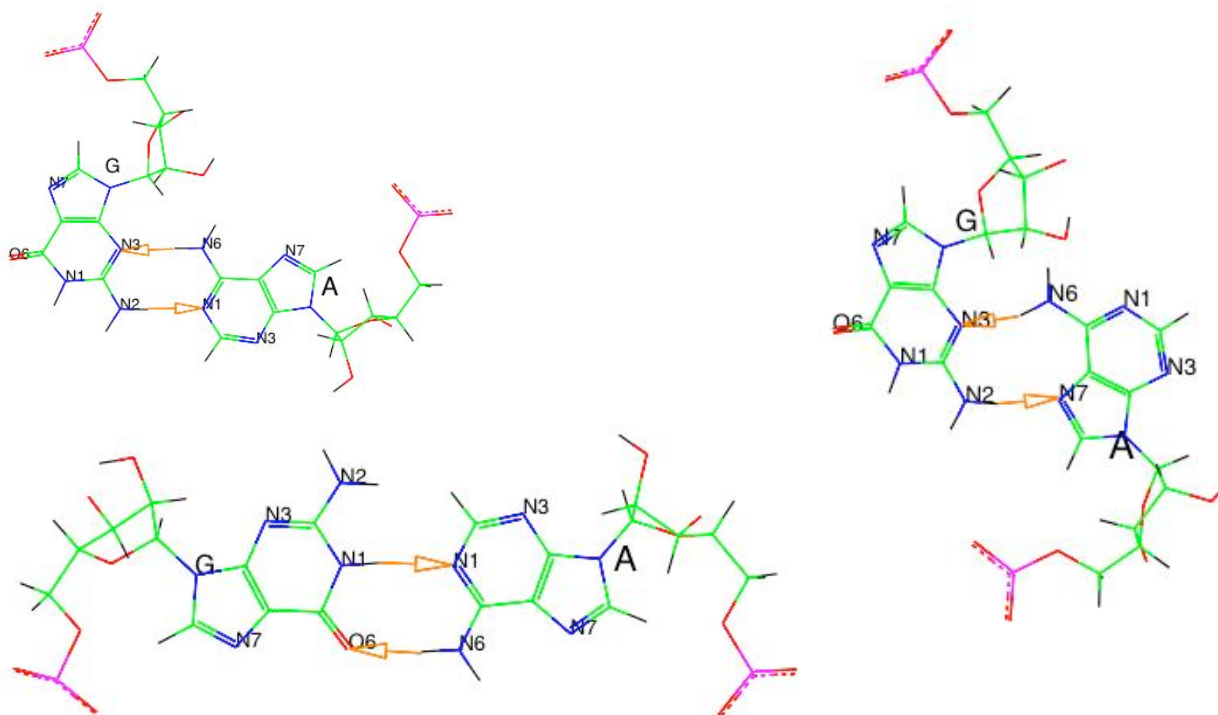
Honor Pledge: At the end of the exam time, please write out the following sentence and sign it, or talk to me about it:

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

1. DNA Structure and Base Pairing (30 pts):

(a; 9 pts) Draw a possible non-Watson-Crick guanosine-adenosine base pair. You need not draw out the sugars.

There are several possible answers. These are from <http://www.imb-jena.de/ImgLibDoc/bp/ga.html>.
+2 for G, +2 for A, +2 for each reasonable H-bond, +1 for accuracy



(b; 9 pts) Sketch examples of each of the following moieties in the context of nucleic acid biochemistry and rank them in order of increasing negative free energy of hydrolysis: phosphoanhydride, phosphodiester, phosphomonoester.

Many possible answers.

+1 for each moiety

+1 for any reasonable context

+3 for ranking

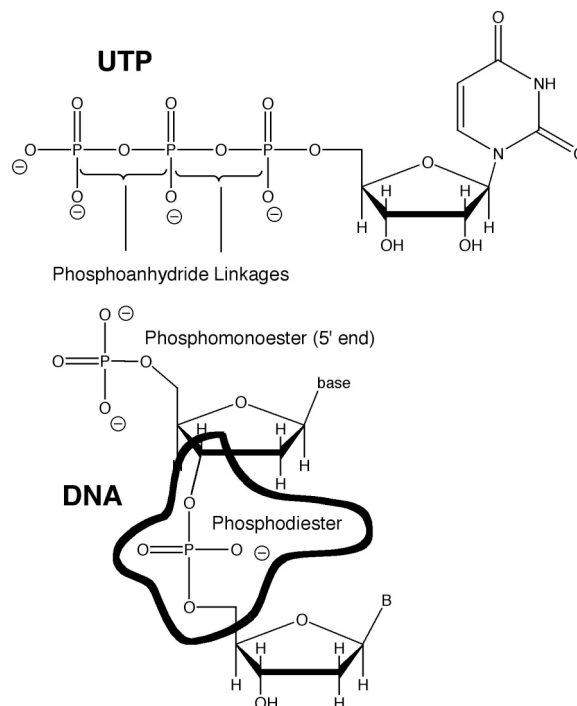
$-\Delta G^\circ$ (phosphoanhydride) > diester > monoester

where ΔG° 's refers to the reactions

$UTP + H_2O \rightleftharpoons UDP + P_i$

$pTpA + H_2O \rightleftharpoons pT + pA$

$5' \text{ TMP (= pT) } + H_2O \rightleftharpoons T \text{ (nucleoside) } + P_i$



(c; 4 pts) Why can't proteins recognize double-stranded RNA in a sequence-specific manner?

The major groove is too narrow and deep (+2) for a protein α -helix (+2) to bind the hydrogen bond donors and acceptors on the edge of the W-C base pairs.

(d; 8 pts) Identify and fix the three errors in the picture of B-DNA at the right.

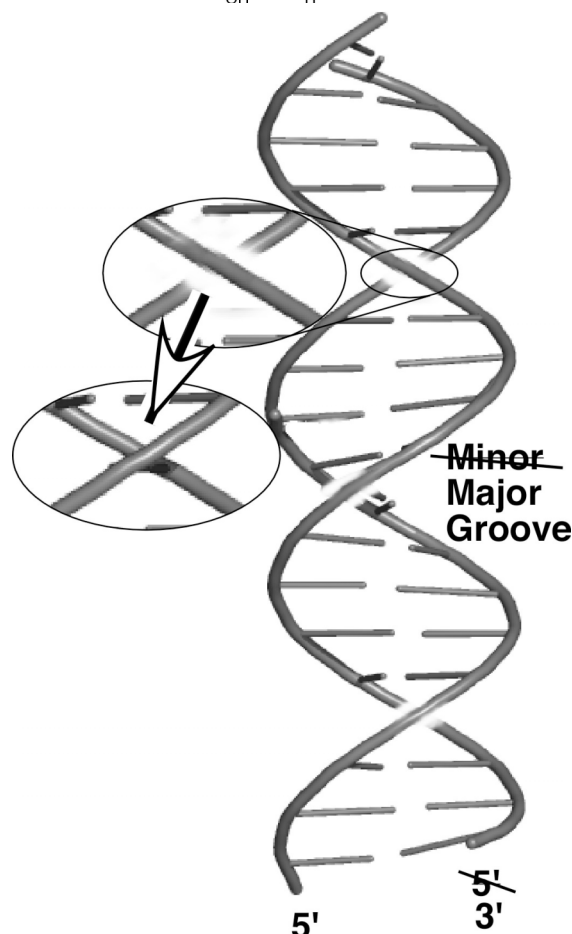
+1 for noticing two 5' ends

+2 for fixing the correct one

+2 for minor \rightarrow major groove

+1 for noticing non-helicity

+2 for correcting the sign of the top crossover.



2. Secondary and tertiary structure (22 pts):

(a; 8 pts) What are the two most important structural elements stabilizing tertiary structure in RNA?
How could you destroy tertiary structure in an RNA without disrupting secondary structure?

- +2 for specifically-bound Mg^{++} or other di/multivalent cations
- +3 for non-Watson Crick hydrogen bonding interactions
- +3 for chelation to remove bound metal ions or raising temperature to a point where tertiary structure melts but helices are still stable; the latter may not work if the tertiary structure is very stable or the base pairing is unstable. +2 for dilution or just "melting"

(b; 14 pts) Hydrogen bonding is one of the fundamental forces that stabilizes double-helical structure. What is the other fundamental force? One might suppose (incorrectly) that even though hydrogen bonds are strong interactions (as non-covalent bonds go) that they actually would not stabilize the hybridization of single strands to make a duplex. What is the argument for this non-effect, i.e. why did people think H-bonds weren't important? What is then the argument for the fact that they actually do stabilize duplex? What is the one-word reason for the reason that duplex structure melts out as temperature increases.

- +3 The other fundamental force is base stacking.
- +4 The argument for a non-effect is that H-bonds are simply exchanged with water, so duplex formation does not change the net number of bonds
- +4 The H-bonds in W-C pairs have near-optimal geometry, making them stronger than the random H-bonds in free solution
- +3 Entropy

Note: it is very difficult experimentally to approach this question, because any experiment that gets rid of either stacking or H-bonds is likely to affect the other. It is clear from isosteres that lack H-bonds that shape complementarity between bases is critical for recognition, but the isosteric base pairs are not very stable.

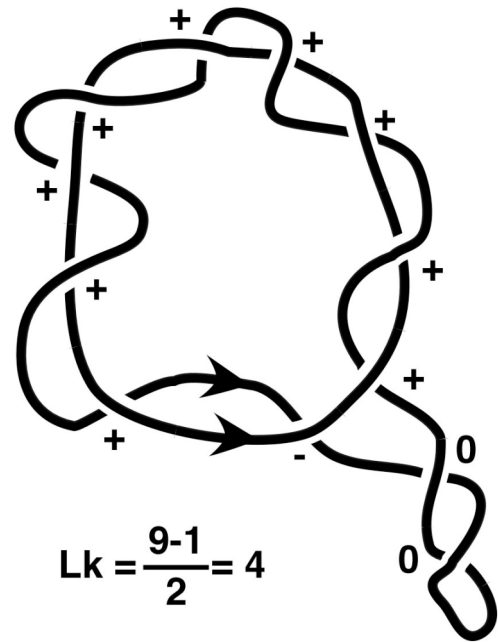
3. DNA tertiary structure (30 pts):

(a; 9 pts) At the right, draw in the remainder of the broken strand and fill in blanks in the dashed strand as necessary to end up with a drawing where the linking number Lk between the two strands is **+4**. Why are the nodes indicated with "0" irrelevant to the linking number?

+2 Self-crossings do not affect linking of one strand about the other.

+3 for the idea of completing the diagram and counting nodes to compute Lk

+2 for each correct crossover



(b; 4 pts) For the purposes of doing topology, why do we define the two antiparallel strands of B-DNA as running in the same direction?

The mathematicians who developed topology had the strands running in the same direction. We could have tried to keep track of which signs to change, but it was easier to make one big adjustment to our thinking up front.

(c; 4 pts) In the following list of terms, circle the ones which are always integers:

Helical repeat, linking number, writhe, number of base pairs, twist. (+2 each)

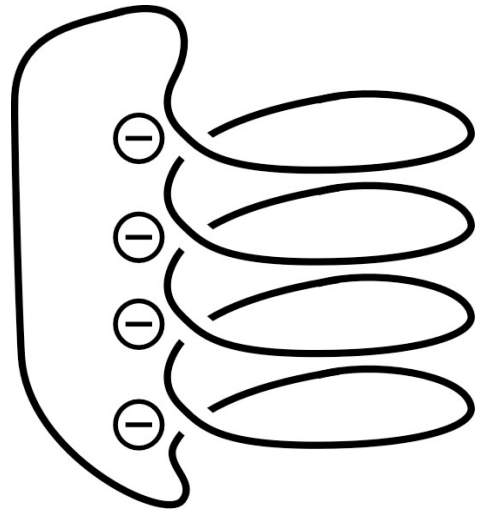
(d; 8 pts) Briefly describe some of the evidence that the helical repeat of DNA in solution is about 10.5, rather than the 10.0 seen in crystal structures.

The peaks and troughs (+2) observed in the graph of cyclization probability (J factor) vs. DNA length (+3) confirmed that DNA is helical, and the separation between peaks gives a helical repeat of about 10.5 rather than 10.0, which is imposed by crystal packing (+3).

Bend phasing experiments also have shown a helical repeat of 10.3-10.5, and also measurements of topoisomer distributions and mobilities in plasmid DNA of various lengths.

(e; 5 pts) Draw a toroidal superhelix with $W_r = -4$.

- +2 for a toroidal superhelix
- +2 for correct node signs
- +1 for correct number of nodes

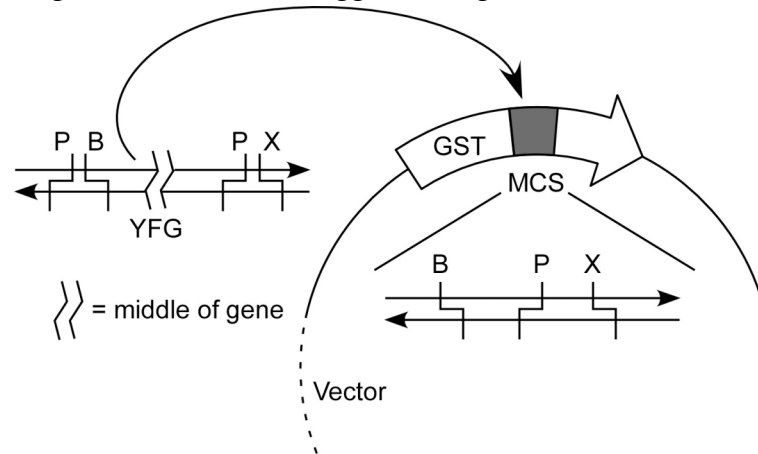


4. Methods (18 pts):

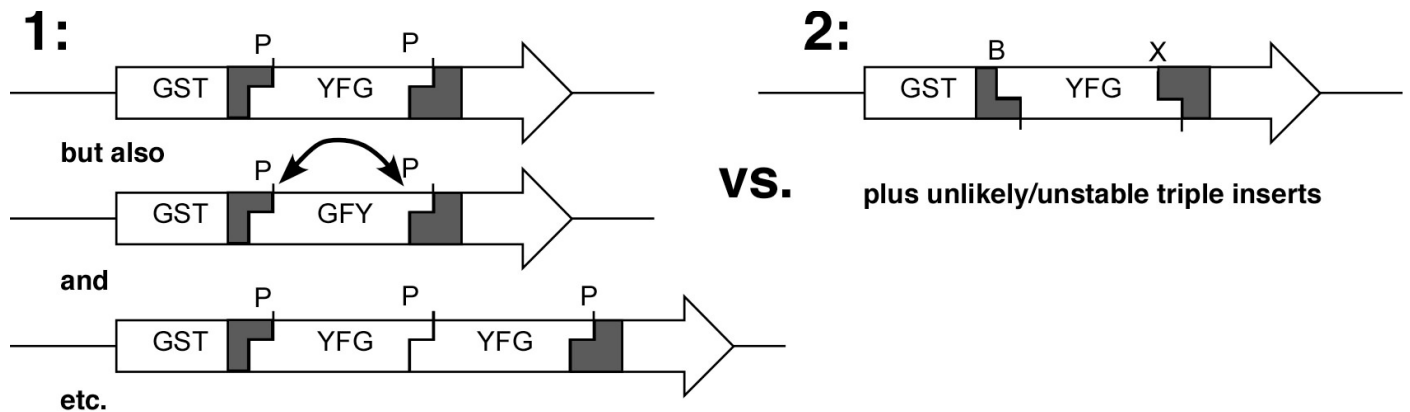
(a; 8 pts) Briefly describe the idea of the DNA microarray. Why is it important to compare two samples when doing these experiments? [Should have been 10 points]

- (+2) The microarray is a slide covered with tiny spots of DNA of known sequence. The spots can be applied with ink-jet-like technology or by stepwise chemical synthesis using photolithography.
- (+2) Samples of RNA (or DNA) are obtained from cells and typically amplified or copied to make fluorescently-labeled copies. This is done in parallel for control and experimental samples.
- (+2) The fluorescent cDNA is hybridized to the chip, and we compare fluorescence intensity between the control fluorophore and the experimental fluorophore. For analysis of gene expression, the results show which genes are activated or repressed under the experimental conditions chosen.
- (+2) The absolute levels of fluorescence are less reliable than the relative levels, so comparative measurements are more reproducible.

(b; 10 pts) You would like to clone a gene into a vector in order to express a fusion protein. The multiple cloning site (MCS) sequence of the vector is sketched, and the YFG insert has the ends shown. Coworker 1 in the lab suggests using Pst I (P) cleavage followed by CIAP and T4 DNA ligase to clone the fragment, Coworker 2 suggests using Bam HI (B) and Xba I (X) instead of Pst I.



Sketch the probable products of trying to clone the gene using each coworker's advice. Which is preferable, and why? Note: this is a simple and straightforward cloning question. Don't worry about reading frames, fusion sequences, compatible digestion buffers, or the NSA.



+2 each for the top-line desired products

+2 for either of the undesired side products for scheme 1

+2 for stating that scheme 2 is preferable

+2 for any explanation to the effect that directional cloning gives fewer possibilities and therefore is more likely to succeed

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