Biochemistry 465 **Your Name:** Key

Biological Information Processing **Prof. Jason Kahn**

Exam I (100 points total) ~~March 5, 2008~~ October 7, 2008

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. N = 74

You need a calculator for this exam, and no other study aids or materials are permitted.

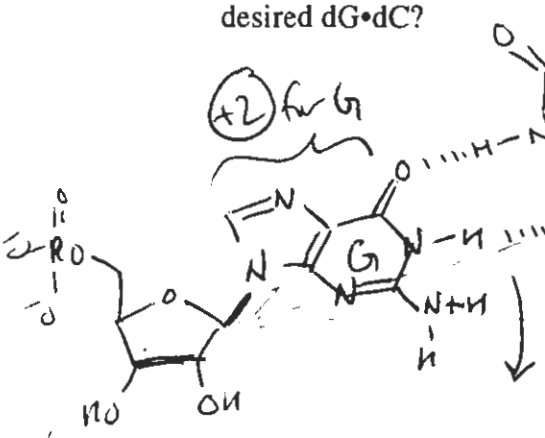
Generous partial credit will be given, *i.e.*, if you don't know, guess.

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. DNA Structure and Base Pairing (30 pts):

(a; 10 pts) Draw a rG•rU wobble pair in RNA, showing the sugar for one of the nucleotides. Given that the related dG•dT base pair is quite stable, how do you think biology distinguishes it from the desired dG•dC?



+2 for G

+2 for U [+1 for T]

+1 for each good H-bond [total +2]

+1 for correct wobble pair

The G will protrude into the minor groove – there fore can be recognized.

or – glycosidic bond angles are not the same as regular dsRNA

+2 for either

+1 for ribose

(b; 10 pts) We discussed DNA symmetries. Each base pair has a pseudodyad axis (rotation by 180° about a dyad axis means rotates an object onto itself). What is "pseudo" about the base pair pseudodyad – what part of the base pair is rotated onto itself? Explain why the helix axis is necessarily perpendicular to the pseudodyad. Circle Yes or No to answer whether the helix axis necessarily perpendicular to the base pair plane.

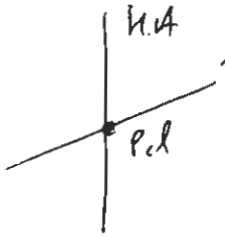
+1

pseudodyad

relates glycosidic bonds +2

not the complete bp or structure +3

+3 If the H.A. were not ⊥ to the p.d., then it would not be rotated into itself.

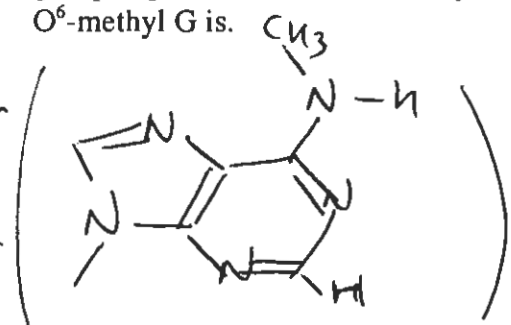


] base plane – need not be ⊥ to the helix axis

(c; 10 pts) Draw the structures of N^6-methyl A (i.e. an A with a methyl group attached to the amino group at position 6) and O^6-methyl G. Explain why N^6-methyl-A is not a miscoding lesion, whereas O^6-methyl G is.

+1 for A

+1 for $-CH_3$ on N^6



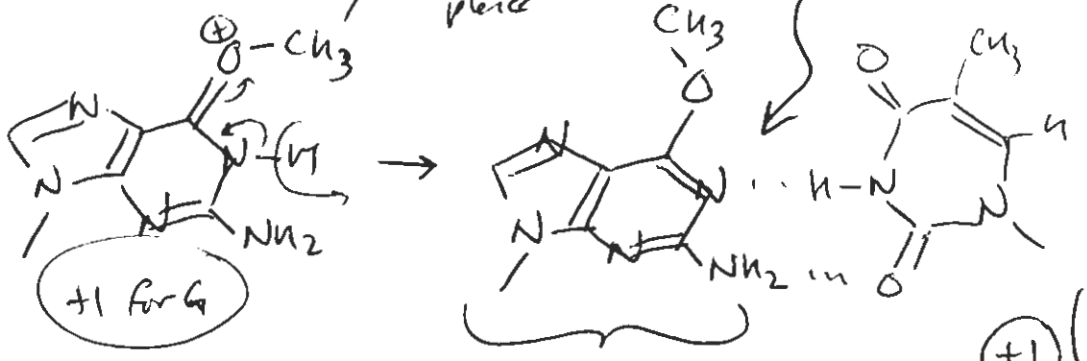
N^6-methyl-A

Same W-C face +1

+2

W-C H-bonding face is different – what was a donor is now an acceptor – can H-bond NOT instead of C +1

+1 for methyl in the right place



O6-methyl G

+2 for recognition of tautomerization

Score for the page_____

2. Secondary and tertiary structure (20 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) - Non-Watson-Crick hydrogen bonds

- Metals chelating phosphates that approach each other
  Divalent ← (+1)
  ~~+2~~ ↑ (+1)
  (+1)

(+3) (- Either: chelate away all divalent mtls M^{++}
  or
  thermal denaturation [may not work in real life]

(b; 12 pts) We gave the analogy of the DNA trajectory as the path of a drunk being forcefully ejected from a bar; for the purposes of the problem, assume that the bar's door faces due west. In this analogy, what corresponds to the behavior of short vs. long lengths of DNA? How would one measure the persistence length for the drunk-ejection process. [Do not try this experiment in college!]

random motion biased to the west by initial shove

(+3) Short DNA → travels along the initial direction due to impetus from bouncer's foot – projection ≈ contour length

(+3) Long DNA – random motion leading to no net average movement in any direction

Measure persistence length –

(+3) for repeated trials + taking average (Eject 100's of drunks with same initial velocity [or come back night after night], measure where they end up after a long time, average displacement to the west = P,

(+3) for displacement in initial direction

Score for the page_____

3. DNA topology (30 pts):

(a; 8 pts) Consider a plasmid of 3000 bp. Assume the helical repeat is 10.5 bp/turn. Calculate Lk_0, the ideal (non-integral) relaxed linking number and Lk_m, the actual (integral) linking number closest to Lk_0. Plasmid DNA as isolated from cells typically has $\sigma \equiv \Delta Lk / Lk_0 = (Lk - Lk_0)/Lk_0 = -0.06$. Calculate ΔLk for this plasmid. (The ΔLk will be approximately equal to the writhe, as DNA is torsionally stiff.)

+1 for setting up calculation

$Lk_0 = \frac{N}{bp} = \frac{3000\ bp}{10.5\ bp/turn} = 285.7$ (+2) for #

$Lk_m = 286$ (+2)

[+1 if sign is wrong]

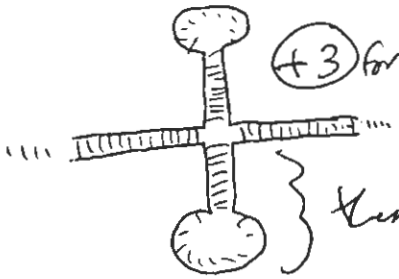
$\sigma = \frac{\Delta Lk}{Lk_0} = -0.06 \quad \Delta Lk = -0.06 \times 285.7 = -17.1$ (+3)

So the most common $\Delta Lk = -17$ – there will be ~~topos~~ topoisomers with $\Delta Lk = -15, -16, -17, -18, -19$

$Wr \approx -17$ (actually more like −14, with the rest being ΔTw)

(b; 9 pts) Sketch a DNA cruciform structure. When a cruciform is extruded from a plasmid, the ~~linking~~ twist number is substantially reduced even though most of the DNA is still helical – why? In the example above, if 5 helical turns are extruded as a cruciform, and assuming that the ~~4~~ superhelical strain in the rest of the dsDNA is manifested exclusively as writhe, what is the value of Wr for the plasmid? (The decrease in writhe and hence bending is the driving force for cruciform extrusion.)

oops!



(+3) for sketch

(+3) These are self-crossings – don't count toward the linking number

Extruding 5 helical turns → $\Delta Tw = -5$, so $\Delta Wr = +12$ (+3)

Score for the page_____

(c; 9 pts) What are the two main differences between 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 I: Cuts + rejoins one strand, so $\Delta Lk = \pm 1$, does not burn ATP

Type II: Cuts + rejoins both strands, so $\Delta Lk = \pm 2$, does burn ATP

(+3) for either/both - they are the same essentially

(+3)

[+4 if these are given as 2 reasons]

(+3) The topoisomerase is still linked to DNA - the protein itself becomes a massive DNA adduct that disrupts replication - it's not simply the loss of a needed activity.

(Solenoidal)

(d; 4 pts) Draw a toroidal superhelix with Wr = -4.

Left handed toroid -

+2 for a toroid

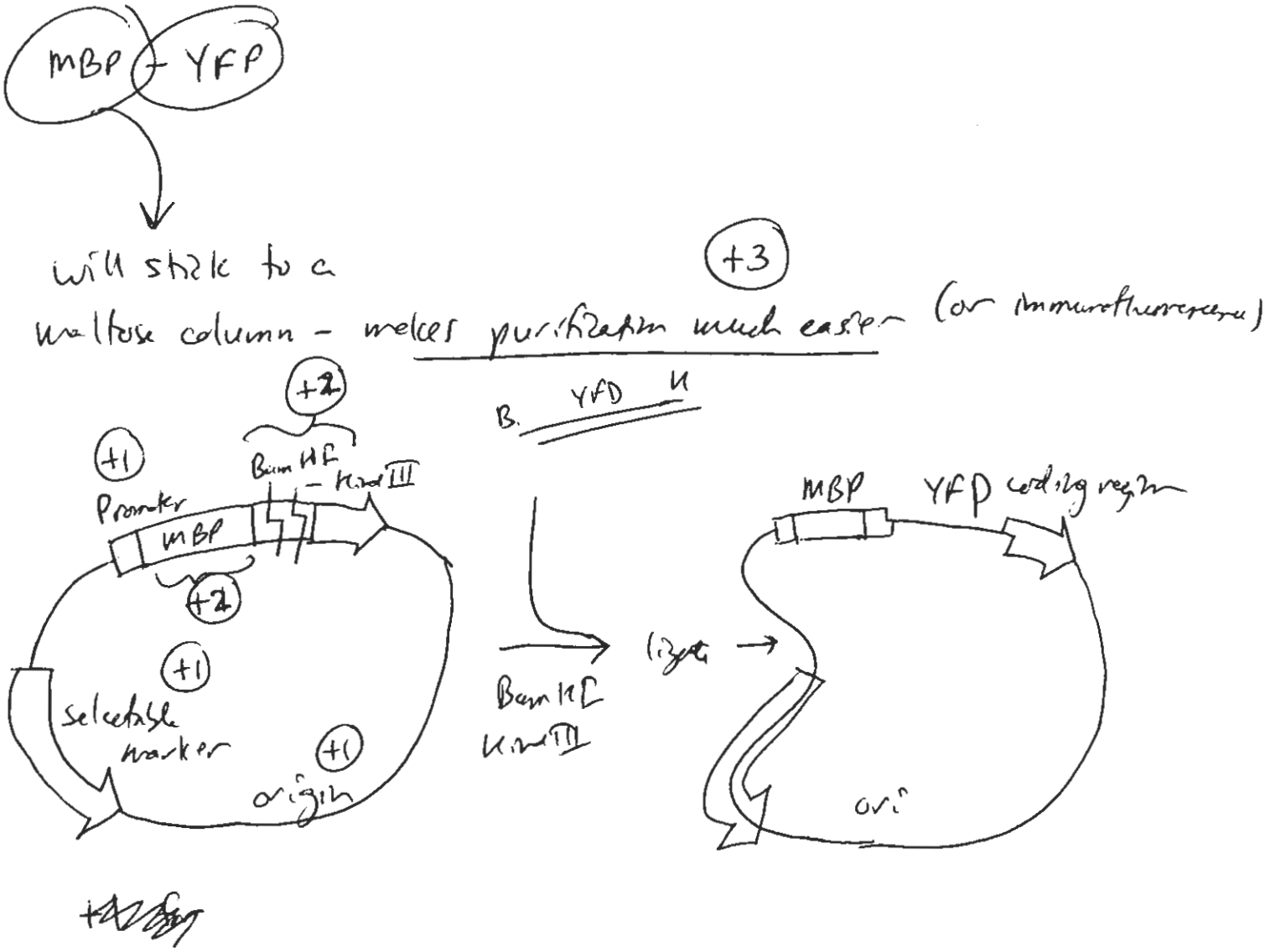
+1 for node signs

+1 for -4

Score for the page_____

4. Methods (20 pts):

(a; 10 pts) Briefly describe the utility of making a fusion protein between MBP=maltose binding protein and YFP=your favorite protein. Assuming that you have a BamH I-Hind III fragment bearing the coding region of YFP, sketch a corresponding vector into which you would clone the fragment to make a fusion protein.

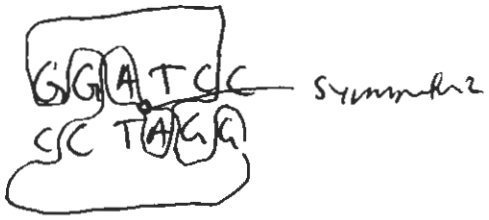


Score for the page_____

(b; 10 pts) What is the natural function of restriction enzymes (REs)? Why do dimeric restriction enzymes typically have palindromic recognition sequences? Do REs require ATP hydrolysis to act? Why or why not?

(+2) -- They destroy foreign DNA that lacks the protective cognate methylation pattern

(+3) – Each subunit makes identical interactions with the target sequence.



(+2) – REs do not require ATP hydrolysis

(+3) – They are catalyzing a downhill reaction – hydrolysis of a phosphodiester to a phosphomonoester

Page	Score
1	
2	
3	
4	
5	
6	
7	
Total	

Score for the page_____