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?

(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.

(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.

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?

(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!]

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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 $\sigma \equiv \Delta Lk / Lk_0 = \left(Lk - Lk_0 \right) / Lk_0 = -0.06$. Calculate ΔLk for this plasmid. (We assume that ΔLk will be approximately equal to the writhe, as DNA is torsionally stiff; in real life about 80% of ΔLk partitions into writhe.)

(b; 9 pts) Sketch a DNA cruciform structure. When a cruciform is extruded from a plasmid, the twist (Tw) is substantially reduced even though most of the DNA in the cruciform is still helical – why? In the example above, if 5 helical turns of the original plasmid are extruded as a cruciform, and assuming that the 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.)

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(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)?

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

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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.

(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?

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