UC Berkeley, Chem 130/MCB 100A, Fall 2015, Mid-term Exam 1. Your Name_____

UNIVERSITY OF CALIFORNIA, BERKELEY
CHEM C130/MCB C100A
MIDTERM EXAMINATION #1
SEPTEMBER 23, 2015

INSTRUCTORS: John Kuriyan and David Savage

THE TIME LIMIT FOR THIS EXAMINATION: 1 HOUR 50 MINUTES

SIGNATURE:

_____

Please **SIGN** your name on the line above in **INDELIBLE INK**.

YOUR NAME:

_____

PLEASE PRINT your name (**IN INDELIBLE INK**) on the line above (& on the top right hand corner of every page).

PLEASE CIRCLE the name of your GSI:

Eric Greene Helen Hobbs

Madeleine Jensen Robert Louder

Piere Rodriguez

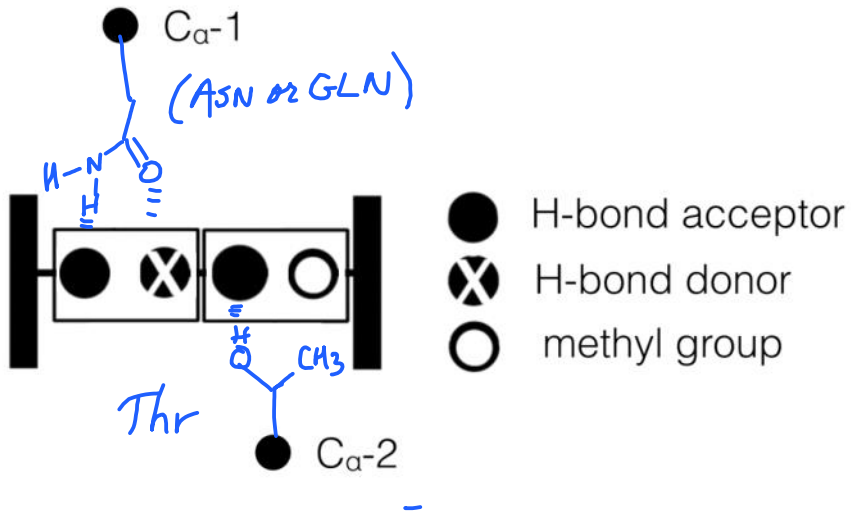
PLEASE WRITE all of your answers **AS LEGIBLY AS POSSIBLE**.
Note that any exam submitted for a regrade should have been written in indelible ink.
SCORING. The exam consists of 5 questions totaling 100 points as broken down in this table:

Question	Part A	Part B	Part C	Part D	Your Total	Max Score
1.						20
2.						20
3.						20
4.						20
5	----------	----------	----------	----------		20
				TOTAL		100

UC Berkeley, Chem 130/MCB 100A, Fall 2015, Mid-term Exam 1. Your Name_____

Q1 (20 points)

Q1A (8 points) The following schematic diagram indicates the **edge** of a Watson-Crick base pair in the major groove (i.e., the DNA axis is vertical, and the two phosphate backbones are indicated by the two vertical black lines, and the base pair is viewed from the side). A protein inserts an α helix into the major groove of DNA and interacts with this base pair using the sidechains of two residues. The Ca atoms of the two amino acid residues are shown as black circles, above and below the bases.



(i) (2 points) Which is the base on the left, and which is the base on the right?

Answer: Base on the left A Base on the right T

(ii) (4 points) There are two sensible choices for sidechain 1 and only one for sidechain 2, to ensure complete and specific interaction with the base pair. Draw the chemical structure of the two sidechains in the diagram above (making any one of the two sensible choices for the first one) and indicate the hydrogen bonds and other contacts, if any, to the basepairs. You do not need to draw out the basepairs in detail.

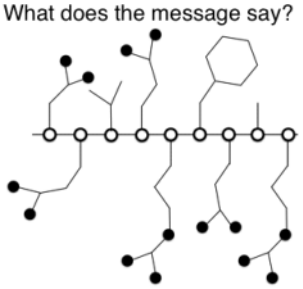
(iii) (2 points) A chemist synthesizes an RNA double helix in which this base pair is present, even though it is not a component of natural RNA. Can the α helix make interactions with the major groove edge of this basepair in the RNA double helix? Explain your answer.

The A-form double helix in RNA has a narrow major groove, so an alpha helix cannot enter it readily. So, the answer is NO.

UC Berkeley, Chem 130/MCB 100A, Fall 2015, Mid-term Exam 1. Your Name_____

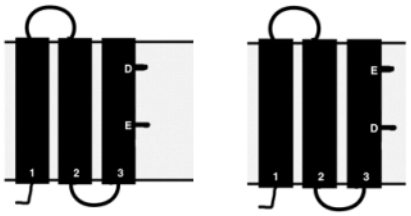
Q1B (4 points) One spy communicates with another spy by sending them DNA that can be decoded to give peptide sequences, which correspond to messages. She sends the spy a message, which is decoded as shown schematically below. In this diagram, the backbone is shown as a horizontal line, with only the Ca atoms indicated by white circles. For the sidechains, oxygen and nitrogen atoms are shown as black circles, and no hydrogens are shown.

What does the message say?



Message: NEVER FEAR

Q1C (8 points) Two membrane proteins are so constructed that there is one D and one E, located two turns apart on one of the transmembrane helices, within the lipid bilayer. The E and D sidechains are on the outside of the protein, as shown in the schematic diagram. One protein has the E first, then the D and the order is flipped in the other protein. The positions of the E and D sidechains are shown schematically. No other polar groups are near the E and D sidechains. The protein forms a very strong dimer in the membrane, and helix 3 from each protein is at the dimer interface, with the D from one interacting with the E from the other, and vice versa.



(i) (2 points) Both proteins are stable in the membrane when expressed together, but if expressed separately they are unstable. Provide a reasonable explanation for why the original proteins are stable and form a strong dimer, whereas they are unstable separately.

Helix 3 exposes polar/charged sidechains to the membrane environment. These sidechains lose interactions with water, and cannot regain good hydrogen bonding interactions with each other, since they are located 2 turns apart. So, on their own, the proteins are unstable in the membrane.
Presumably, the proteins are able to dimerize so that the D and E sidechains form hydrogen bonds with each other. This compensates for the loss of interactions with water.

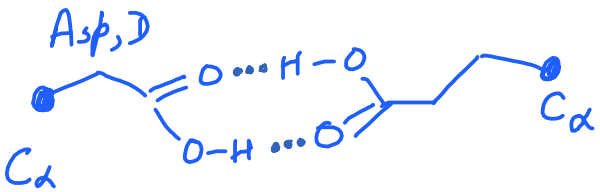
Wednesday, September 23, 2015 9:19 AM

UC Berkeley, Chem 130/MCB 100A, Fall 2015, Mid-term Exam 1. Your Name_____

(ii) (2 points) Do you expect the pKa values for the E and D sidechains to be lower or higher than their normal values? Explain your answer.

The E and D sidechains have to be neutral in order to hydrogen bond. If they were charged, they would have an even stronger penalty for insertion into the membrane. So, they are harder to *deprotonate*, and so the pKa values become *higher*.

(iii) (2 points) Draw the most likely configuration of an E interacting with a D at the dimer interface. The diagram should include the chemical structure of one E and one D sidechain. Show all hydrogen bonds clearly, indicating them with dashed lines.



(iv) (2 points) The particular protein shown in the diagram cannot serve as a proton transporter but a scientist thinks that she can design a proton transporter by simply adding more acidic residues to helix 3. How many more acidic residues would she need to add, given the typical length of a transmembrane helix? Explain your reasoning.

If we assume that the helix is straight, there are 35/1.5 = 23.3 residues in the membrane. This corresponds to 23.3/3.6 = 6.5 turns (roughly 6 or 7 turns). If there is an acidic residue at each turn, then they could form a relay for protons. So, she would have to add 4 to 5 additional residues.

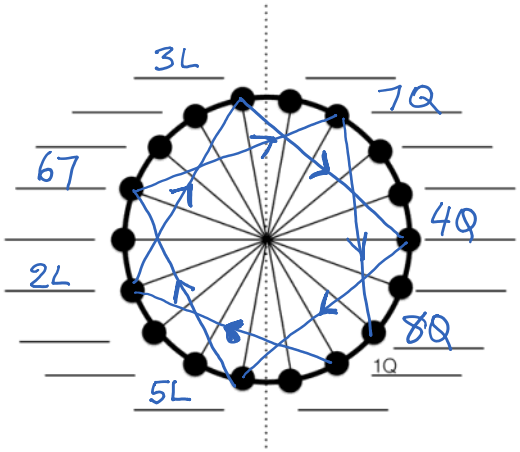
Page 4 of 15

UC Berkeley, Chem 130/MCB 100A, Fall 2015, Mid-term Exam 1. Your Name_____

Q2. Q2A (8 points)

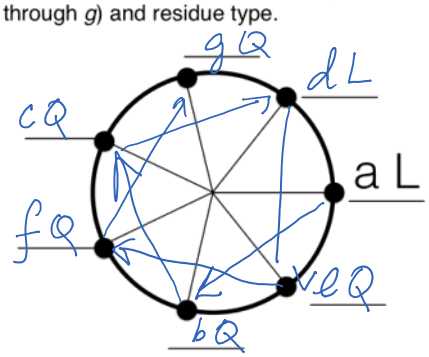
(i) (3 points) You are asked to design a straight (i.e., not coiled-coil) α-helix that is perfectly amphipathic. The helix contains 4 leucine and 4 glutamines, and no other residues. In the helical wheel shown below, the face of the helix to the right of the dotted line is polar, and that to the left is non-polar. The Cα atoms of residues on the helical wheel are shown as black circles. Notice that the helical wheel is divided into 18 equal sectors.

Starting from the Cα atom labeled "1Q", complete the helical wheel by identifying the positions of the other 7 residues. For each residue, put down the sequence number and the identity of the residue (L or Q) on the appropriate horizontal line. Connect sequential Cα atoms by arrows, in the direction of the chain. For your diagram, the helix should point *into the page (i.e., the N-terminus should be above the page and C-terminus below the page*). Helical wheels for you to practice on are given on the next page.



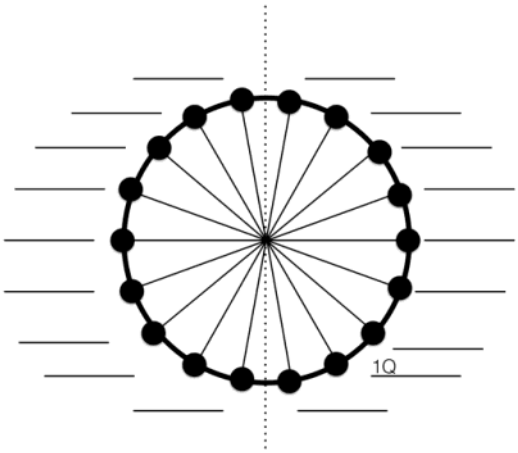
Note
Each Cα is separated by a ~100° rotation

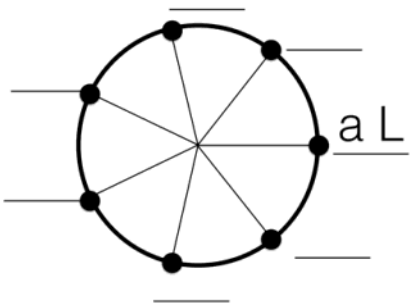
(ii) (3 points) Next, you are asked to design a helix that can dimerize through coiled-coil formation, again using only leucine and glutamine. Use the helical wheel shown below to draw the sequence of seven residues in the coiled coil (denoted *a* through *g*), such that no leucine residues are solvent exposed when the dimer is formed. The residue at the *a* position is indicated. Draw arrows connecting subsequent residues, and label each one with the position (*a* through *g*) and residue type.



UC Berkeley, Chem 130/MCB 100A, Fall 2015, Mid-term Exam 1. Your Name_____

Practice helical wheels. Provide your final answer to Q2, parts i and ii on the previous page. Any drawings or writings on this page will be ignored during grading.



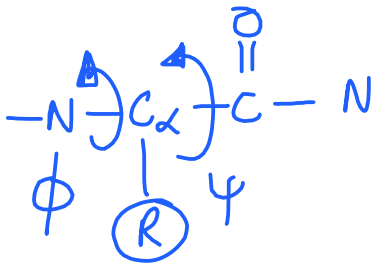


UC Berkeley, Chem 130/MCB 100A, Fall 2015, Mid-term Exam 1. Your Name_____

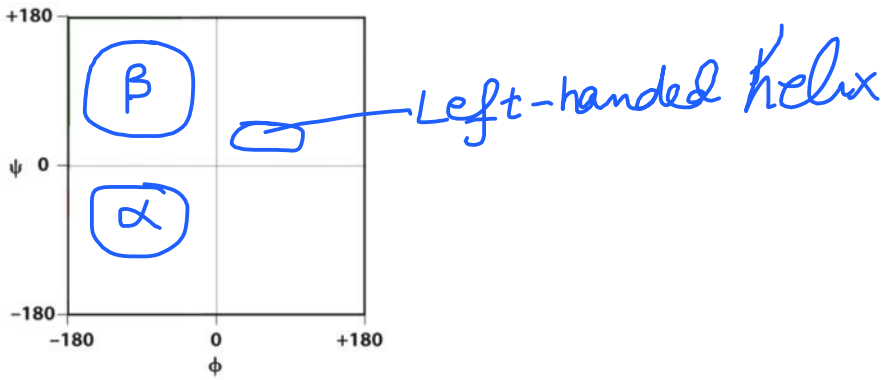
Q2A, continued.
(iii) (2 points) You wish to ensure that the coiled-coil is necessarily parallel rather than antiparallel, by making just two sequence changes. Which positions would you mutate, and what change would you make?

In a parallel coiled-coil, the *e* position of one helix can form ionic interactions with the residue at the *g* position of the other helix. So one way to do this is to put a lysine or arginine at *g* and an aspartate or glutamate at *e*.
Or the other way around. See Figure 4.42 in TMOL.

Q2B (8 points)
(i) (3 points) Draw the structure of the peptide backbone connecting two residues, and indicate the ϕ, ψ backbone torsion angles. Indicate the angles clearly by drawing curved arrows around the bonds. The direction of rotation is not important.



(ii) (3 points) Shown below is a graph for the ϕ and ψ backbone angles. Sketch, roughly, and label the allowed regions corresponding to the α, β and left-handed helical conformations for an alanine-alanine dipeptide.



(iii) (2 points) Structural biologists who experimentally determine protein structures generate a Ramachandran diagram for their new protein structure to ensure that it is correctly determined. A scientist uses NMR to determine the structure of a protein from an organism that grows at high temperatures, and finds that his structure has many residues in disallowed regions of the Ramachandran diagram. He reasons that this is due to the special properties of the high-temperature protein, rather than carelessness on his part. Do you think his reasoning is correct? Justify your answer.

His reasoning is incorrect. The van der Waals repulsions, which defines the allowed regions of the Ramachandran diagram, are so strong that they cannot be overcome even at the higher temperatures that this organism might be growing in. So, the disallowed regions would be roughly the same. A correctly determined protein structure will not violate the Ramachandran diagram.

Q3C (4 points)
Consider two proteins, A and B. The molecular weight of B is twice that of A.

(i) (2 points) When separating these proteins on an ion exchange column containing a positively charged resin, A elutes first, followed by B, at pH 7.0. At pH 5.0, B elutes first, followed by A. Which protein contains more histidine residues? Explain your answer.

At pH 5.0, B elutes first, so it is more positively charged. But at pH 7, B is less positively charged. So B must have more groups that titrate and become protonated at pH 5.0. This is likely to be due to having more histidines (which has a pKa in this region).

(ii) (2 points) A scientist decides to separate these proteins on a gel filtration (size-exclusion) column. Which protein would elute first?

In a gel filtration column, the larger protein would elute first. So, B would elute first.

UC Berkeley, Chem 130/MCB 100A, Fall 2015, Mid-term Exam 1. Your Name_____

Q3A (10 points). In the schematic below, a nucleotide, denoted A, is interacting with a edge of a Watson-Crick base pair formed by bases B and C. The bases of B and C occupy the plane denoted by the box.

(i) (1 points) What is the name of the nucleotide labeled A?

U

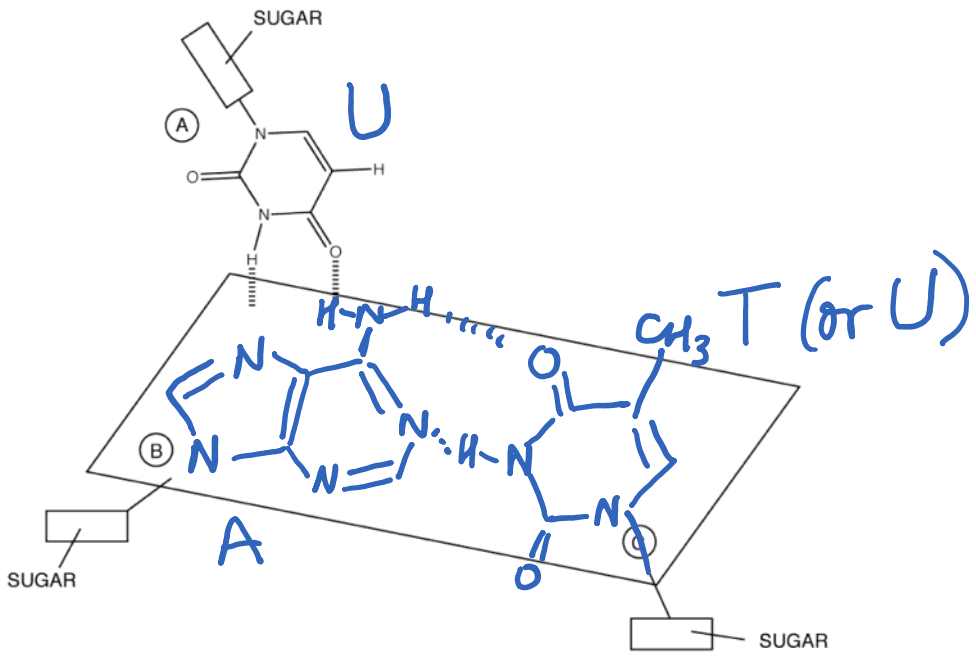
(ii) (2 points) What are the nucleotides labeled B and C?

A and T (or U)

(iii) (1 points) Which groove of the double helix is A interacting with?

major

(iv) (6 points) Draw the chemical structures of B and C and indicate all hydrogen bonds. Note the hydrogen bonds between A and B are indicated by dashed lines.



UC Berkeley, Chem 130/MCB 100A, Fall 2015, Mid-term Exam 1. Your Name_____

Q3B (10 points) A circular double-stranded plasmid DNA is synthesized. The plasmid has 1000 basepairs. To begin with, it is a perfectly relaxed circle.

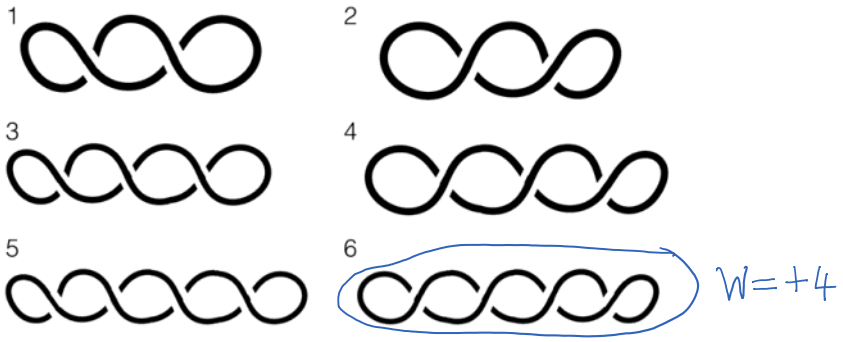
(i) (2 points) Assuming that there are 10 base pairs per turn of DNA, what is the linking number, *L*, of the relaxed plasmid?

L = 100

(ii) (2 points) A topoisomerase enzyme acts on the DNA, and it introduces four extra turns into the DNA (i.e., it *overwinds* the DNA by four turns). What is the linking number of the DNA if it is held perfectly flat, i.e., not allowed to form supercoils?

L=104

(iii) (2 points) The plasmid is then allowed to relax into a supercoiled structure. Circle the diagram below that best describes the resulting structure.



(iv) (2 points) What is the value of the twist, *T*, after the DNA has relaxed into a supercoiled form?

The twist will relax back to the value for the normal DNA, which is 100.

Q3C (2 points) In RNA, single-stranded structures often form helical conformations. Explain why.

The single-stranded regions can still form base-stacks, which stabilizes the helical conformation.

T

UC Berkeley, Chem 130/MCB 100A, Fall 2015, Mid-term Exam 1. Your Name_____

Q4. (20 points)

Q4A. (8 points) A folding/refolding experiment is carried out using a protein that has 16 cysteine residues. The protein contains four "zinc-fingers". In the correctly folded protein, each zinc-finger consists of four specific cysteine residues that coordinate one zinc ion.

In the first part of the experiment, the protein is unfolded in urea and zinc is removed. The protein is then allowed to refold in the absence of urea while zinc is added to reform the zinc-fingers. In the second part, the protein is unfolded by urea, then zinc is added in the presence of urea, which allows random configurations of cysteine residues to form zinc-fingers, with four cysteines in each zinc-finger.

(i) (6 points) Assuming that 100% of the activity of the protein is regained in the first part of the experiment, what fraction of the activity would you expect to be regained in the second part? Show all the calculations that you use to work out the answer.

4 Zinc fingers: (16)(15)(14)(13) (12)(11)(10)(9) (8)(7)(6)(5) (4)(3)(2)(1) Ways of Choosing Cys residues

No. of possibilities $= \frac{16!}{(4!)^4 \times (4!)} = \frac{20.9 \times 10^{12}}{8.0 \times 10^6} = 2.6 \times 10^6$

Correct for horizontal rearrangements

correct for vertical reagrrangenets

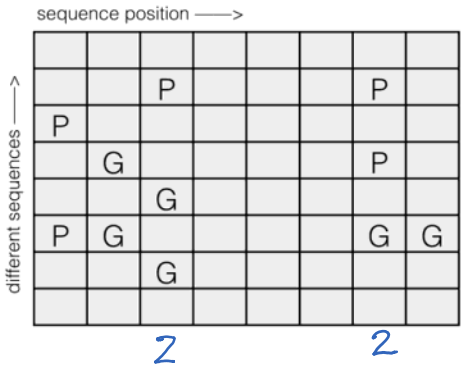
Since only one arrangement is correct, fractional activity would be ≈ 0 [i.e., $\sim 0.5 \times 10^{-6}$]

UC Berkeley, Chem 130/MCB 100A, Fall 2015, Mid-term Exam 1. Your Name_____

Q4A(ii) (2 points) The cysteine residues in the protein used in the experiments described above are quite evenly distributed throughout the protein, with no two cysteines being close to each other. The experiments are repeated with a different protein, in which the cysteines are clustered in groups of four each, with each cluster being well separated from the others in the sequence. Would the outcome of this set of experiments be the same or different than in the first set of experiments? Explain your answer clearly.

The outcome would be different. In the second case, even when the protein is unfolded the correctly matched cysteines are more likely to be close together and therefore form the correct zinc fingers. Hence, the recovered activity would be higher than expected for the experiment.

Q4B (10 points) Shown below is a sequence block of aligned sequences from a set of evolutionarily related proteins. Only proline and glycine residues are shown (the gray squares represent other amino acids).



# of P = 5
# of G = 6
← # of P-G pairings

(i) (2 points) How many P-G pairings are there in this sequence block?

4

(ii) (2 points) How many possible pairings of amino acids are there in this sequence block?

8 rows, 8 columns
In each column, # of pairings = $\frac{8 \times 7}{2}$
Total number = $\frac{8 \times 7 \times 8}{2}$ = 224

UC Berkeley, Chem 130/MCB 100A, Fall 2015, Mid-term Exam 1. Your Name_____

(iii) (2 points) If the sequence block were randomly scrambled, what would be the probability of finding a P-G pairing? Ignore exclusion effects in calculating the probability.

$f_{P,G} = 2 \times \frac{n_P \times n_G}{(64)^2} = \frac{2 \times 5 \times 6}{(64)^2} = 0.0146$

(iv) (2 points) What is the BLOSUM score S_{PG} calculated from this sequence block. Show how you work out the answer.

$f_{PG} = \frac{\text{no. of P,G pairs}}{\text{total \# of pairs}} = \frac{4}{224} = 0.0179$

$L_{P,G} = f_{PG} / f_{PG} = \frac{0.0179}{0.0146} = 1.22$

$S_{PG} = 2 \log_2 1.22$

$= 2 \frac{\log_{10} 1.22}{0.301}$

$= 0.57$

(v) (2 points) Would you expect a BLOSUM matrix calculated from membrane proteins only to be different from a BLOSUM matrix for water-soluble proteins? If so, would the difference be greater for hydrophobic residues or for hydrophilic residues? Explain your answer.

They would be different. Hydrophilic residues would be MORE conserved (have higher diagonal BLOSUM scores) and be less likely to be substituted (lower off-diagonal scores).

Q4C. (2 points)

A 20 base pair stretch of sequences likely to form Z DNA is located near a transcription start site. Will the presence of the Z DNA make the transcription region more or less likely to form a bubble (unzippered DNA)? Explain your answer.

Since the presence of Z-form (left-handed) DNA unwinds the DNA, it will tend to make the DNA
double helix (right-handed) less stable near it. So, the answer is: yes, bubbles are more likely.

Q 5. (20 points) Multiple choice and True/False questions. Circle the ***best*** option (or TRUE or FALSE).
+2 points for each correct answer, -1 points for each wrong answer.
To get the maximum score you do not need to answer all the questions, so be careful not to answer questions incorrectly.
Maximum points: 20. Minimum points: 0.

(i) For amino acid residues with neutral R groups, at a pH below the pKa of the isolated amino acid, the net charge on the R group in a folded protein will be:
(a) neutral
(b) positive
(c) negative
(d) determined by its local environment

(ii) RNA does not adopt the B-form double helical structure because:
(a) The extra oxygen atom in RNA collides with a base.
(b) The lack of a methyl group in uracil versus thymidine reduces the hydrophobic stabilization of RNA.
(c) Protein molecules do not have to interact with RNA in the major groove, so the B-form is not needed.
(d) The C2' endo conformation of the sugar is disfavored in RNA.
(e) The C3' exo conformation of the sugar is disfavored in RNA.

(iii) A protein chain forms a random coil structure in water. When the chain is transferred from water to a solvent X, it forms an α helix.
(a) Solvent X is more polar than water.
(b) Solvent X is less polar than water.
(c) Solvent X has a molecular shape that is complementary to that of a helix.
(d) Solvent X mimics the structure of a peptide backbone.

(iv) Active sites in proteins are often located at inter-domain boundaries because:
(a) The structures and sequences of individual domains have to satisfy the constraints of folding, while inter-domain regions can evolve rapidly.
(b) Inter-domain orientations can change easily, allowing evolution to accommodate different ligands at the active site.
(c) The inter-domain regions can provide crevices for the binding of small molecules.
(d) All of the above are true.

(v) Choose the amino acid substitution that results in the greatest change of hydrophobicity:
(a) A—>M
(b) W—>F
(c) N—>Q
(d) F—>R

(vi) RNA structures contain non-Watson-Crick base pairs such as G-U because the constraint of uniform base pair geometry does not apply in RNA as it does in DNA.
TRUE / FALSE

(vii) DNA is the repository of genetic information because the absence of a hydroxyl group at the 2' position makes it less susceptible to cleavage.
TRUE / FALSE

(viii) The structure of the globin fold is relatively unchanged even though the sequences of different globins can be virtually unrelated because:
(a) Heme groups only bind to the globin fold
(b) All the globins have a common ancestor.
(c) The globin fold contains helices, and the stable packing of helices against each other is restricted to specific inter-helical angles.
(d) The globin fold is required to form the hydrophobic core.

(ix) The width of the major groove in DNA is:

(a) ~5 Å
(b) ~15 Å
(c) ~25 Å

(x) Hydrogen bonds are critical for the specificity of DNA base pairing because:

(a) Hydrogen bond formation gives significant stabilization.
(b) Hydrogen bonds are strongly directional, and so they are critical for the imposition of structural constraints.
(c) Hydrogen bonds with water that were lost upon folding are regained by forming the correct base pairs.

(xi) Two non-polar carbon atoms are in favorable van der Waals contact. What is the distance between the carbon atoms?

(a) 2.5 Å (b) 3.5 Å (c) 4.5 Å

(xii) The hydrophobic effect drives protein folding. Choose the *best* explanation:
(a) The numerous van der Waals attraction between non-polar atoms stabilizes the structure.
(b) Exposed non-polar atoms disturb the geometry and energetics of water.
(c) Formation of a hydrophobic core allows polar sidechains to be on the outside, where they can interact with water.
(d) Formation of the hydrophobic core allows secondary structural elements to form.

(xiii) The magnesium ions are usually octahedrally coordinated when they interact with DNA.
TRUE / FALSE

(xiv) In bacteriorhodopsin, when light energy is absorbed, what happens that allows the pump to work? Circle the statement that is **NOT** crucial to the bacteriorhodopsin mechanism.

(a) protonation of the lysine
(b) *cis-trans* isomerization of the retinal
(c) a conformational change in the protein
(d) titration of histidines.