This exam consists of 260 points on 11 pages. Use the space provided or the back of the preceding page.

1. (4 pts) Briefly explain how weak acids can act as pH buffers. Your answer should provide a clear explanation of the buffer region.

2. (12 pts) Please do one of the following two questions. Please indicate your choice.

Choice A: Describe how you would make a one liter of a 0.1 M buffer solution of acetic acid at pH=5.0. You may assume that the pK_a of acetic acid is the same as the sidechain of aspartic acid. Make sure you state the pK_a value for acetic acid that you are using for this problem.

Choice B: A RNA binding protein binds to RNA solely via lysine-phosphate interactions. Sketch the dissociation constant, K_D, as a function of pH. Please state the pK_a value for the lysine sidechain that you are using for this problem.

3. (14 pts)

i) Draw the structure of a tri-peptide whose sequence is X-Gly-Ala, where X represents your choice of a polar but non-ionizing amino acid (please do not use threonine). Assume a pH of 2.0 (6 pts).

ii) Please label all peptide bonds on your diagram and state **two** important features of this bond (2 pts).

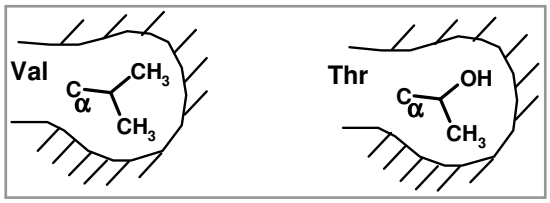
iii) Circle the "mainchain" or "backbone" atoms in your drawing (2 pts).

iv) Will this peptide bind to a cation exchange column at pH 2? Why or why not? (4 pts)

4. (10 pts) Please do **one** of the following choices. Please indicate your choice.

Choice A: Describe how van der Waals forces and hydrogen bonds work together to stabilize protein secondary structures. You should illustrate your answer with a sketch of *one* secondary structure. In this sketch indicate the location of hydrogen bonds and sidechains.

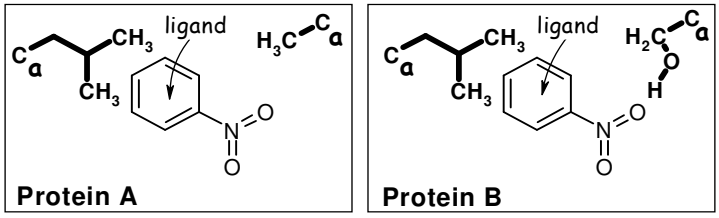
Choice B: A globular soluble protein contains a *buried* valine (Val) reside in its core. If this residue is replaced by threonine (Thr) will the melting temperature increase or decrease? Why? You should consider discussing both enthalpic and entropic terms in your answer.

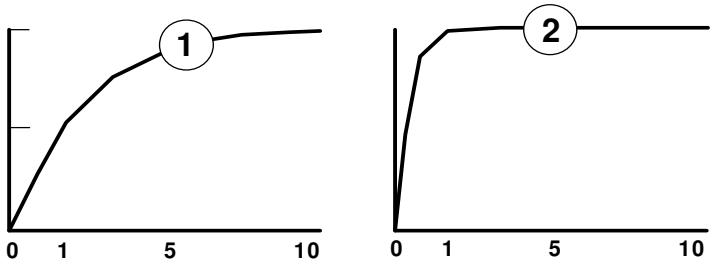


5. (4 pts) Assume that a secondary structure is found on the surface of a soluble globular protein, with one side facing the interior of the protein and the other side solvent exposed. How frequent (e.g. every fifth residue) would you expect to find amino acids with non-polar sidechains? How does this periodicity depend on the secondary structure?

6. (16 pts) What thermodynamic feature of protein unfolding or DNA melting *greatly* destabilizes the folded state? Describe what force or thermodynamic feature is most important for opposing this force and stabilizing the folded protein and double stranded DNA. (Note, you should discuss ***both*** protein and double stranded DNA stability. The features causing destabilization and stabilization may, or may not be, the same for both.)

7. (15 pts) Two different homodimeric proteins bind the same ligand, nitrobenzene. The structure of the protein-ligand complex in one of the subunits on each protein is shown on the right. The two sidechains from the protein that contact the ligand are in bold.





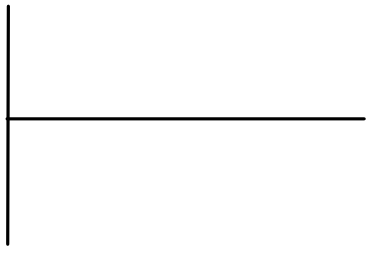
i) Label both the x- and y-axis of one binding curve. Give the correct scale for the y-axis (1 pt)

ii) Match the protein (A or B) to the correct binding curve (1 or 2). Justify your answer in terms of the molecular interactions between the protein and ligand (8 pts).

iii) What does the shape of the binding curve suggest about the nature of the binding, is it cooperative or non-cooperative? Why? (2 pts)

iv) Based on your answer to *part iii*, sketch the Hill plot that you would expect to obtain from the data plotted in curve 1. Use the space to the right. Label the axis of this plot and indicate the important features of the plot (4 pts).

Hill Plot



8. (12 pts) Describe the general features of an allosteric protein. Illustrate your answer using either oxygen transport, regulation of glycolysis or gluconeogenesis, or genetic regulation of protein expression, to demonstrate the importance of allosteric effects in biological systems.

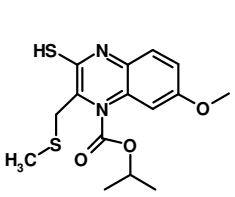
9. (15 pts) What features of the active site of an enzyme leads to an increase in the rate of the chemical reaction ***and*** specificity towards substrates. Illustrate your answer by providing details for one enzyme that was discussed in this course.

10. (14 pts) The following two compounds are currently used to treat AIDS patients. Both of these drugs inhibit the *same* enzyme. This enzyme is used by the virus in one of the first steps in its life-cycle.

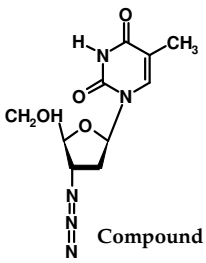
i) Which is the name (or general nature) of the enzyme that is inhibited and which compound is more likely to be a competitive inhibitor and which is more likely to be the mixed-type inhibitor? Briefly justify your answer (6 pts).

ii) Sketch the double reciprocal curves ($1/v_i$ versus 1/[S]) that you would expect to find for data acquired without inhibitor (curve 1) and data acquired in the presence of the mixed-type inhibitor (curve 2). Briefly justify your answer (6 pts).

iii) How would your plot (curve 2) change if you doubled the inhibitor concentration? (2 pts).



Compound I



Compound II

11. (6 pts) Please do one of the following choices. Clearly indicate you choice.

Choice A: Compare and contrast any **one** of the following pairs of methods of protein purification by column chromatography methods. What is similar about the methods? How do they differ? What is the principle of separation? How is the protein eluted from the column?

a) Cation versus anion exchange chromatography.

b) Affinity versus ion exchange chromatography.

c) Gel filtration versus affinity chromatography.

Choice B: Compare and contrast SDS-PAGE (SDS polyacrylamide gel electrophoresis) of proteins versus agarose gel electrophoresis of DNA. How are they similar? In what way do they differ?

Choice C: Define specific activity and briefly discuss its importance in protein purification.

12. (8 pts) Draw the ring form (Haworth representation) of either α-glucose or β-fructose. Be sure to indicate your choice. Which of these two monosaccharides is found in both glycogen and cellulose? How does the linkage between the monosaccharides differ in glycogen versus cellulose?

13. (20 pts)

i) Draw a labeled "cartoon diagram" of a biological membrane, including typical components (4 pts)

ii) Briefly describe (or draw) the chemical features of **one** of the components in your membrane (4 pts).

iii) What thermodynamic force or interaction causes the self-assembly of the membrane? Briefly describe the molecular basis of this force (6 pts).

iv) Which features of the electron transport chain would require that the membrane be fluid and not solid? How might an organism maintain a fluid membrane, even if it grows at low temperatures? (6 pts).

14. (10 pts) Compare and contrasts the standard energy change (ΔG^o) to the Gibbs free energy change (ΔG) (5 pts) and then answer **one** of the following choices (5 pts).

Choice A: The addition of a phosphate to a sugar (e.g. conversion of glucose to glucose-6-P) is unfavorable, with a standard energy change, ΔG^o of +15 kJ/mol, yet reactions of this type in glycolysis occur spontaneously. How is this accomplished?

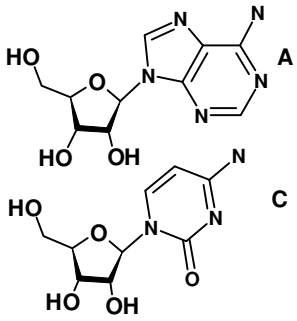
Choice B: The aldolase reaction in glycolysis converts fructose 1-6 bisphosphate to two three-carbon sugars. This reaction is unfavorable, with a ΔG^o of +30 kJ/mol, yet this reaction occurs spontaneously in glycolysis. How is this accomplished?

15. (12 pts) Please do one of the following choices.

Choice A: Assume that you just ate a bagel, which is rich in carbohydrates. Trace the flow of carbon from glucose to CO_2 ***and*** the flow of energy, from glucose to ATP, in the metabolism of the bagel in muscle cells. You should list and briefly describe the pathways involved. How would your answer change if your muscles were operating under anaerobic (no oxygen) conditions?

Choice B: I've just eaten a high carbohydrate meal and shortly afterward I get involved in an intense game of touch football. Describe changes in hormones, glucose-glycogen metabolism in the liver, and the regulation of glycolysis and gluconeogenesis in the liver that would occur, beginning *immediately* after the meal to the end of the football game, when I'm *completely* exhausted.

16. (12 pts) The diagram to the right shows two nucleotides that represent a short dinucleotide.



i) Connect the two nucleotides correctly, as you would find in DNA or RNA.

ii) Indicate the end that would accept additional bases during a DNA polymerase reaction.

iii) Is this dinucleotide DNA or RNA? Why?

iv) Indicate an N-glycosidic bond.

v) Indicate the purine base.

vi) What is the correct sequence of this dinucleotide, AC or CA? Justify your answer.

17. (20 pts) The restriction endonuclease CmuI recognizes and cleaves a six base sequence after the first base. The *incorrect* recognition sequence for the enzyme is shown on the right.

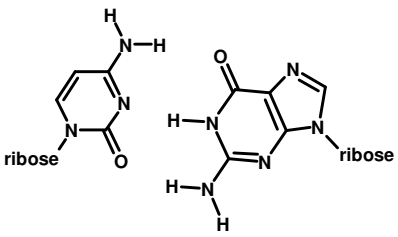
-AGGCCA-
-TCCGGT-

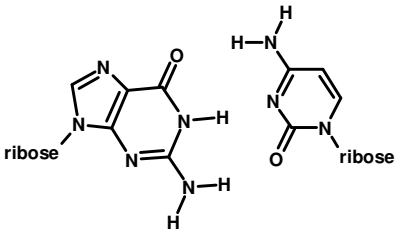
i) Correct the error in the sequence and briefly justify your answer (Note: There may be more than one way to correct the error.) (4 pts).

ii) Show the products that would occur when this DNA is treated with CmuI (2 pts).

iii) What enzyme could be used to rejoin the fragments? Which molecular interaction would serve to hold the two fragments together during the joining process? (4 pts)

iv) The rate of cleavage of DNA by CmuI is insensitive to salt. Based on this observation, the enzyme interacts with which component of the DNA, the bases, ribose, or phosphate? Justify your answer (4 pts).



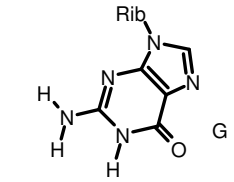


v) CmuI can distinguish between a GC and a CG basepair at the second position. To do so does it bind in the major groove or the minor groove? Briefly justify your answer with reference to the C-G and a G-C basepairs shown to the right (6 pts).

18. (8 pts) Please do one of the following choices. Please indicate your choice.

Choice A: In what way does double stranded RNA differ from double stranded DNA? In what way are they similar? Why is RNA more susceptible to cleavage under alkaline conditions?

Choice B: A number of amino acids are associated with more than one codon. For example, the amino acid Phe can be incorporated into a peptide chain whether the codon is UUU or UUC, yet there is only one tRNA molecule that is charged with Phe. Briefly explain how this occurs. The following bases may be helpful.



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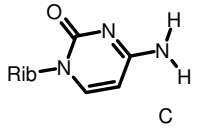

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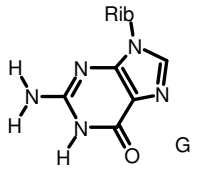

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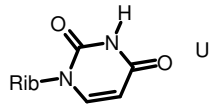

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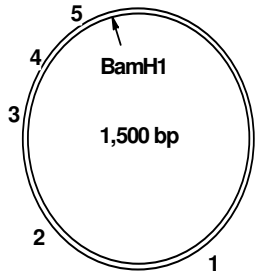
19. (35 pts) The diagram below (next page) shows a segment of human DNA with the gene for a small, four amino acid, human growth hormone indicated as a stippled box. The start codon (ATG) and stop codon (TAA) are indicated. A diagram of an expression vector is also provided. The vector has a *single* BamHI site, whose recognition sequence is G^GATCC. You desire to produce recombinant human growth hormone that will remain inside the bacteria after induction of transcription. Please answer the following questions.

i) There are five numbers on the expression vector. The table below gives the names of three items. Please complete the missing entries (label, or position on the vector, and the function) for ***all*** three (7 pts).

Label	Name	Function
	Lac operator	
	Ribosome binding site	
	Promoter	

ii) Briefly describe how you would generate PCR primers to amplify the DNA segment that codes for the growth hormone. You should include the start and stop codons in your final product because these are not present in the expression vector. Your primers should contain only 6 bases of the growth hormone sequence (8 pts).



iii) How would you verify that you inserted the PCR fragment correctly into the vector, without sequencing the DNA? (4 pts)

iv) Briefly describe how you would induce the production of the growth hormone after placing (transforming) the vector into the bacteria. What compound would you use (4 pts)?

v) How would you modify the expression vector to ***either*** cause export of the protein out of the cell ***or*** to purify the protein using affinity chromatography (2 pts).

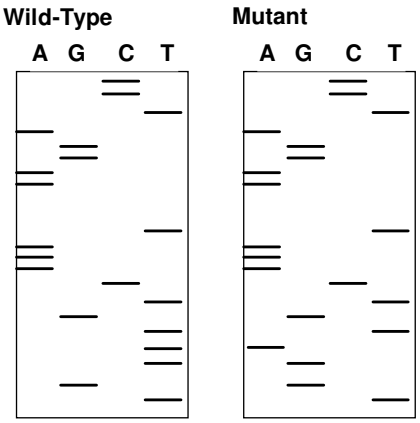
vi) (10 pts) Certain individuals show slow growth due to a mutation in the growth hormone gene. You obtain DNA from one such individual, and amplify the growth hormone gene using your PCR primers. After inserting the mutant growth hormone gene into the vector you obtain the nucleotide sequence of both the normal (wild-type) and mutant gene. The sequencing gels are shown on the right.

a) What DNA sequence would make a suitable primer in this case?

b) What nucleotide change(s) have occurred in the mutant individual?

c) How have these changes affected the amino acid sequence of the growth hormone?

d) Why would this change affect the binding of the hormone to its receptor in the membrane?



20. (12 pts) *Briefly* describe the events that occur in ***either*** DNA transcription (mRNA synthesis) ***or*** protein synthesis. Your answer should include a description of the initiation events, how polymerization occurs, and termination. Feel free to use a well labeled diagram.