This exam consists of **12** pages. Be sure that you have all of the pages. There are a total of 253 points on the exam, budget about 1 min/2 points. There are two bonus questions worth 5 points.

A1:	_____	/16
B1:	_____	/ 7
B2:	_____	/10
B3:	_____	/ 8
B4:	_____	/ 5
B5:	_____	/15
B6:	_____	/ 8
B7:	_____	/12
B8:	_____	/18
B9:	_____	/10
B10:	_____	/ 6
B11:	_____	/41
B12:	_____	/ 5
B13:	_____	/ 8
B14:	_____	/10
B15:	_____	/ 8
B16:	_____	/ 8
B17:	_____	/10
B18:	_____	/ 8
B19:	_____	/28
B20:	_____	/12
Bonus	_____	/ 5
TOTAL	_____	/253

Part A: Multiple Choice 2 pts each - total of 16

1. DNA absorbs UV light at ____ nm and proteins absorb at ____ nm.
   a) 260, 260
   b) 260, 280
   c) 280, 260
   d) 280, 280

2. Which amino acid has a sidechain that often stabilizes extracellular proteins by the formation of crosslinks in the protein chains?
   a) Alanine
   b) Cysteine
   c) Methionine
   d) Tyrosine

3. In both hemoglobin and myoglobin the oxygen is bound to.
   a) the iron atom in the heme group.
   b) the nitrogen atoms on the heme.
   c) a hydrophobic pocket in the protein.
   d) the surface of the protein.

4. During any successful purification scheme, you would expect
   a) the number of different proteins in the sample to decrease.
   b) the specific activity to decrease.
   c) the specific activity to increase.
   d) both a and c are correct.

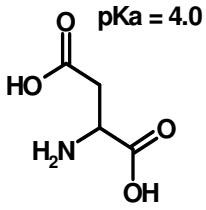
5. Cellulose consists of _____ in _____ linkages, while glycogen contains _____ in _____ linkages.
   a) glucose, β(1-4), fructose, α(1-4).
   b) glucose, β(1-4) glucose, α (1-4).
   c) glucose, α(1-4), glucose, β (1-4).
   d) glucose, α(1-6), glucose, α (1-4).

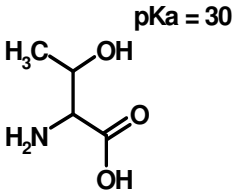
6. T_M refers to:
   a) the temperature at which 50% of a DNA molecule is denatured
   b) the temperature at which 50% of a protein molecule is denatured
   c) the temperature at which membranes are 50% fluid.
   d) all of the above.

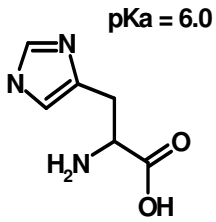
7. RNA is more easily hydrolyzed than DNA in basic solutions because:
   a) The uracil in RNA can function as a catalyst.
   b) The 2'-OH group in RNA can act as a nucleophile.
   c) The linkages between the bases are intrinsically weaker in RNA.
   d) The deoxyribose can deprotonate to neutralize the base.

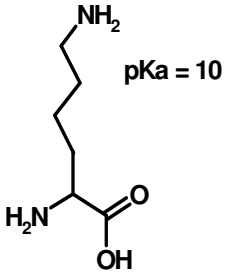
Part B:

1. (7 pts) Pick ***one*** of the following four amino acids.









i) Name the amino acid that you have chosen (1 pt).

ii) Draw any one of the above amino acids in its correct ionic form (*all* ionizable groups), assuming that the pH of the solution is 6 [indicate the protonation state on the above diagram] (4 pts).

iii) Provide a *brief* explanation for the pK_a value of the side chain for ***any*** of the above amino acids. Feel free to justify your answer by comparing one amino acid to another. (2 pts).

2. (10 pts) Draw the structure of a dipeptide that would preferentially insert into the center of a lipid bilayer (5 pts for the drawing).

i) Label a peptide bond on your diagram (3 pts).

ii) Indicated the "mainchain" (backbone) atoms (2 pts)

3. (8 pts) Please do ***one*** of the following two choices.

Choice A: Describe the "hydrogen bond" with reference to the chemical properties of the atoms that comprise a hydrogen bond. Which atoms are considered to be the donor atoms? Which are considered to be the acceptor atoms?

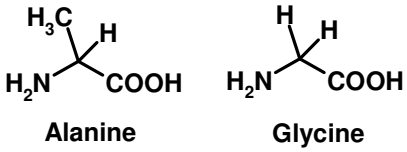
Choice B: Briefly discuss molecular ***and*** thermodynamic aspects of the hydrophobic effect.

4. (5 pts) Briefly describe ***one*** of the four levels of protein structure (primary, secondary, tertiary, quaternary).

5. (15 pts) *Briefly* describe the role of all of the following on the stability of ***both*** globular proteins and double stranded DNA. Indicate the relative contribution of each effect to the stability or instability of the folded form. Use the back of the preceding page if you need additional room.

i) the hydrophobic effect,
ii) van der Waals,
iii) electrostatics,
iv) hydrogen bonding,
v) conformational entropy.

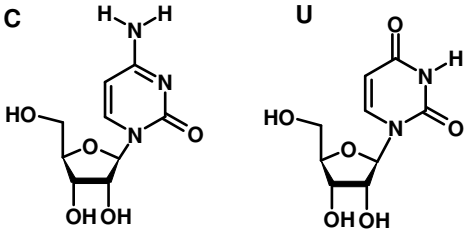
6. (8 pts) A protein has an alanine residue in its core. Briefly describe how the enthalpy ***or*** entropy associated with unfolding (i.e. the direction of the reaction is assumed to be Native → Unfolded) will change if this Ala is replaced by a Gly. The structures of Ala and Gly are shown on the right.



Alanine **Glycine**

7. (12 pts) Describe allosteric effects and discuss their importance in biochemical processes. Provide one example to illustrate your answer.

8. (18 pts) The binding of cytosine, deoxycytosine, or uracil nucleotides to a protein has been measured by equilibrium dialysis and the binding curves for all three ligands are shown below. The structures of two of the three ligands are shown to the right. This protein has one binding site.

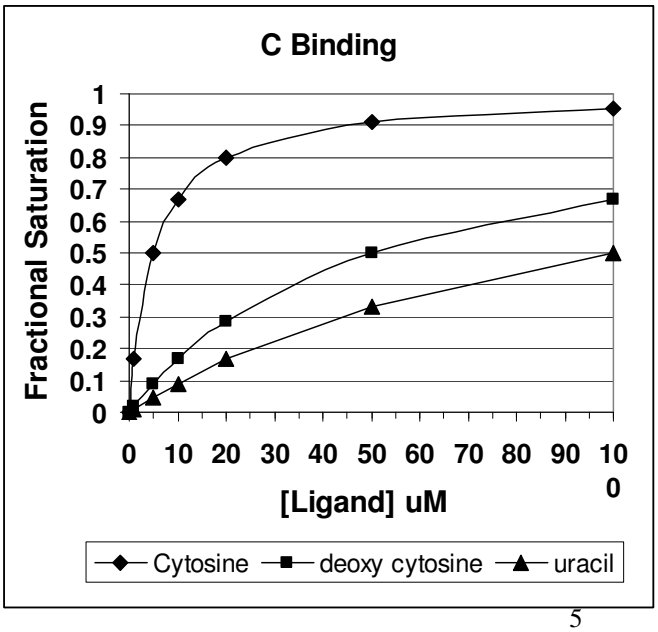


i) Determine the K_D for all three ligands from the binding curves. Briefly justify your approach (6 pts).

ii) Based on your K_D values – which ligand binds more tightly? Which is the weakest binder? Briefly justify your answer (4 pts).

iii) Replacement of a serine residue in the protein with an alanine residue causes the binding curve for cytosine nucleotide to become indistinguishable from that of cytosine deoxynucleotide. Based on this data, plus the K_D values from above, suggest how this protein is interacting with the cytosine nucleotide (labeled "C" in the above figure). Feel free to use the above figure in your answer (6 pts).

iv) The data for the cytosine nucleotide is plotted on a Hill plot. What is the slope of the line when the curve crosses the x-axis? Briefly justify your answer (2 pts).



9. (10 pts) A statement often heard at parties is that enzymes increase the rate of reactions by lowering the energy of the transition state. Please do ***one*** of the following two choices.

Choice A: Briefly describe why lowering of the transition state increases the rate of the reaction.

Choice B: Briefly describe how an enzyme lowers the energy of the transition state. Illustrate your answer with an example.

10. (6 pts) Describe the reaction that is catalyzed by ***two*** of the following four enzymes. List any cofactors/cosubstrates.

i) kinase:

ii) phosphatase:

iii) dehydrogenase:

iv) DNA ligase:

11. (41 pts) The restriction endonuclease HaeIII recognizes and cleaves the seq. shown on the right:

-GG^CC-
-CC^GG-

i) If you try to use AGGCCT as a substrate for kinetic measurements you find that the V_{MAX} for this substrate is high at low temperatures, but decreases to zero at higher temperature. Assuming that the enzyme is *not* being denatured at the higher temperature, explain this result (Hint: Read *part ii)* (4 pts).

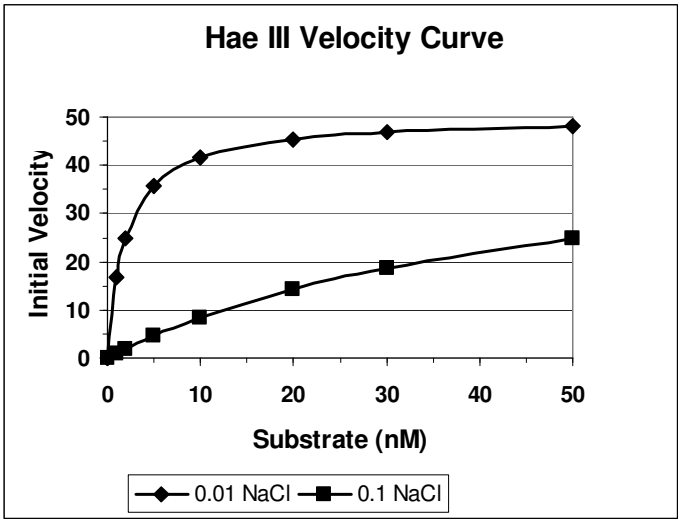
ii) The substrate AGGGGCCCCT produces high V_{MAX} values over all temperature ranges. Draw the *products* after the treatment of this substrate with HaeIII (4 pts).

iii) How could you use gel electrophoresis to monitor product formation in this reaction (4 pts)?

(this question continues on the next page)

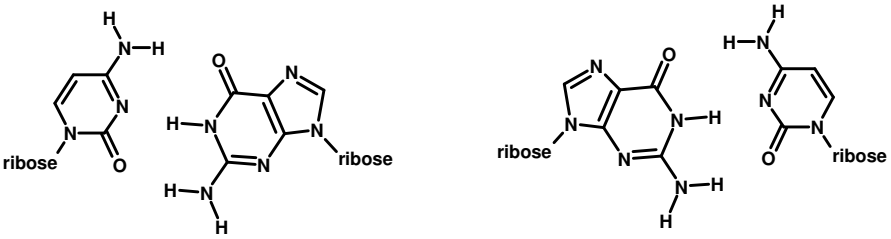
Question 11 – continued.

iv) Steady state enzyme kinetic measurements are obtained at 0.01 M and 0.1 M NaCl concentrations and these data are shown to the right, in the form of velocity curves. What are the K_M values at these two salt concentrations? Justify your approach (Assume that V_{MAX} = 50 at both salt concentrations.) (6 pts).

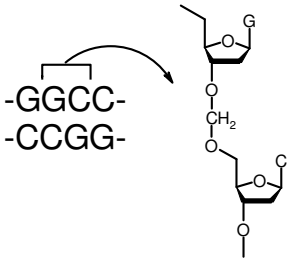


v) What protein-DNA interaction is being affect by the change in salt concentration? i.e. What type of amino acids on the protein are interacting with which groups on the DNA? Support your answer by reference to the K_M values obtained in *part iv* (8 pts).

vi) HaeIII can clearly distinguish between a GC and a CG basepair. 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).



vii) The diagram to the right shows an inhibitor of HaeIII. The chemical structure of the region that is different from normal DNA is shown on far right. Is this a competitive or mixed type inhibitor? Why (5 pts)?



viii) Will this inhibitor affect K_M, k_{CAT}, or both? Briefly justify your answer (4 pts).

12. (5 pts) *Briefly* describe how X-ray diffraction ***or*** NMR spectroscopy can be used to determine the structure of proteins or nucleic acids.

13. (8 pts) Select the purification scheme that will separate protein "C" from a mixture of the following three proteins. Justify your answer by showing that the scheme will actually work (8 pts).

Protein	**Molecular Weight**	**Solubility in ammonium sulfate (conc required to ppt 50% of the protein).**	**#Asp + Glu**	**#Lys + Arg**
A	50,000	4.0	5	10
B	100,000	3.0	10	5
C	50,000	4.0	10	5

Scheme 1: Gel filtration chromatography → precipitation with 4 M ammonium sulfate.

Scheme 2: Gel filtration chromatography → ion exchange chromatography at pH=7.

14. (10 pts) Please answer ***one*** of the following three choices:

Choice A: How does the presence of *cis* double bonds in unsaturated fatty acids affect the phase transition of the membrane? What intermolecular interaction is affected by the presence of these groups in the bilayer?

Choice B: Compare and contrast the structure of a membrane protein (e.g. bacteriorhodopsin) to that of a soluble protein (e.g. myoglobin)?

Choice C: Explain why it is important for biological membranes to be fluid, and discuss the role of cholesterol in this property of the membrane.

15. (8 pts) Indirect coupling is often used to insure that reactions proceed spontaneously to products. Briefly describe how indirect coupling accomplishes this goal and give an example from ***either*** nucleic acid biochemistry ***or*** from metabolic pathways.

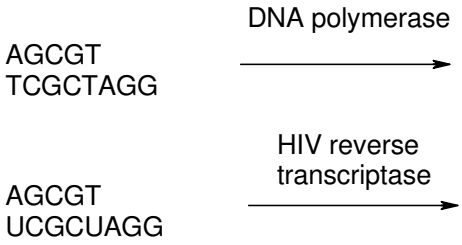
16. (8 pts) I've referred to the liver as a "glucose bank" because it stores excess glucose and releases glucose into the blood when there is a request for glucose. Please do ***one*** of the following two questions.

Choice A: Name ***one*** hormone that is responsible for regulating glucose metabolism in the liver. Briefly describe how it regulates glycogen synthesis and degradation, glycolysis, and gluconeogenesis. You need not describe in detail the signaling pathways.

Choice B: In the muscle, regulation of glycogen is similar to that in the liver. The regulation of glycolysis is similar to the liver in terms of energy sensing, but opposite in terms of hormonal regulation. Briefly describe why this form of regulation is important for the normal function of muscle tissue.

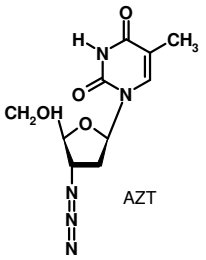
17. (10 pts) The following primer-templates were incubated with either DNA polymerase III or HIV reverse transcriptase plus all four nucleoside triphosphates (dNTPs) and Mg^{2+}.

i) Draw the final product in ***both*** cases. Briefly justify your answer (6 pts).



ii) How would your answer to ***either*** case differ if dTTP was *completely* replaced by dideoxyTTP (ddTTP) in the reaction mixture (4 pts)?

Bonus. (3 pts) AZT, whose structure is shown to the right, is a drug that is used to treat individuals infected with HIV. How does this drug interfere with viral replication?



18. (8 pts) Please do **one** of the following two choices. Please indicate the choice that you are answering.

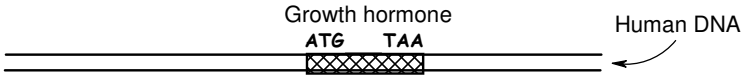
Choice A: The aminoacyl synthetase that attaches Ala to the correct tRNA can also, by mistake, attach the amino acid Gly. If Gly is attached, then it is removed by hydrolysis at a separate editing site on the enzyme. Based on the structure of these amino acids, provide a sketch or description of the site which adds the amino acid to the tRNA ***and*** the separate site that will remove Gly but not Ala from the incorrectly charged tRNA (The structure of these amino acids is shown in question 6).

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.

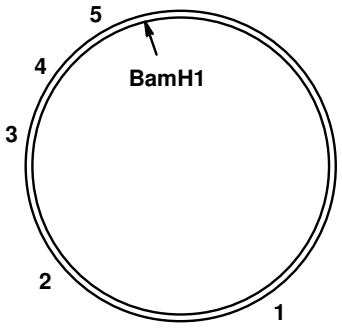
19. (28 pts) The diagram below shows a segment of human DNA with the gene for a small, four amino acid, human growth hormone indicated as a stippled box. The start codon (ATG) and stop codon (TAA) are indicated. A diagram of an expression vector is also provided. The vector has a *single* BamHI site, 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 labels on the expression vector. The table below either gives the name of labeled items or their function. Please complete the missing entries for ***three*** of the five (6 pts).

Label	Name	Function
1	Origin of replication	
2	Antibiotic resistance gene	
3		RNA polymerase binds here.
4	Lac operator	
5	Ribosome binding site	



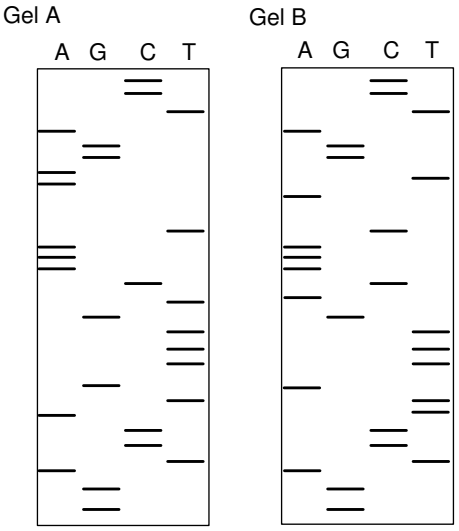
ii) Briefly describe how you would generate PCR primers to amplify the DNA segment that codes for the growth hormone. You should include the start and stop codons in your final product because these are not present in the expression vector. Since you do not know the entire sequence of the growth hormone you should not worry about determining the exact length of the primer (6 pts).



iii) Briefly describe how you would insert the PCR product into the expression vector (4 pts).

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

v) (8 pts) After inserting the PCR product into the expression vector you find that some of the vectors produce the recombinant protein as expected but some do not. You sequence the DNA of one that does (gel A) and one that does not (gel B). The DNA sequencing gels for these two vectors are shown to the right. Use the back of the previous page if you need additional space.
   a) Determine the protein sequence of this hormone using gel A (4 pts).
   b) Explain why the vector whose sequence is shown in gel B does not produce any growth hormone (4 pts).



Bonus (2 pts). How would you modify the expression vector to cause export of the protein out of the cell?

20. (12 pts) *Briefly* describe the events that occur in ***either*** DNA transcription ***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.