 Name:_____

This Exam contains 12 pages and consists of 168 Points.

Place your name on the face page and your initials on each page.

You should budget approximately 1 point/minute.

Please use the space provided for your answers. If you place part of your answer for a question elsewhere, please indicate its location.

For questions in which you have a choice, please indicate the question that you are answering.

Points:	
A:	**_____/34**
B:	**_____/12**
C1:	**_____/ 5**
C2:	**_____/ 6**
C3:	**_____/ 8**
C4:	**_____/ 8**
C5:	**_____/ 5**
C6:	**_____/ 3**
C7:	**_____/10**
C8:	**_____/10**
C9:	**_____/10**
C10:	**_____/ 7**
C11:	**_____/ 5**
C12:	**_____/10**
C13:	**_____/ 8**
C14:	**_____/25**
Total:	**_____/168**
%	**_____**

Part A (2 pts each, 34 Pts) ; Multiple Choice. Please circle the best answer.

1. A buffer solution at pH 6.0 is made from an acid with a pK_a of 5.0. What is the ratio of $[A^-]$ to [HA] in this buffer.

a) 10:1
b) 5:1
c) 1:1
d) 1:10

2. The isoelectric point of any amino acid is defined as

a) the pH where the molecule carries no electric charge.
b) the pH where the carboxyl group is uncharged.
c) the pH where the amino group is uncharged.
d) the pH of maximum electrolytic mobility.

3. The peptide bond in proteins is

a) planar, and usually found in a cis conformation.
b) nonplanar, and rotates to three preferred dihedral angles.
c) nonpolar.
d) planar, and usually found in a trans conformation.

4. The denaturation of DNA or protein causes

a) little increase in the entropy of the DNA or protein.
b) large decrease in the entropy of the DNA or protein.
c) no change in the entropy of the DNA or protein.
d) large increase in the entropy of the DNA or protein.

5. If the Gibb's free energy, ΔG, is greater than zero then:

a) the reaction direction will form products.
b) the reaction direction will form reactants.
c) the concentration of the reactants and products must be equal.
d) you cannot predict anything about a reaction with a positive Gibbs energy.

6. A protein that shows infinite *negative* cooperative for binding of *n* ligands will

a) show a Hill coefficient, n_h, of 0
b) only be found in either the unliganded form or the fully liganded form.
c) show a linear Scatchard plot.
d) show a Hill coefficient, n_h, of n.

7. A competitive inhibitor of an enzyme is usually:
   a) a highly reactive compound.
   b) a metal ion such as Hg^{+2} or Pb^{+2}.
   c) structurally similar to the substrate.
   d) a drug.

8. An allosteric *activator* of an enzyme usually
   a) binds to the active site.
   b) participates in feedback regulation.
   c) causes the enzyme to work faster.
   d) precipitates the product.

9. The major problem in the use of drugs to treat HIV infections is:
   a) Drugs that are good inhibitors cannot by synthesized.
   b) The drugs interfere with normal digestion.
   c) The drugs are rapidly degraded.
   d) Virus particles with altered (mutant) proteases arise.

10. Fatty acids *and* phospholipids in water are organized such that the _____ face the solvent and the _____ are directed toward the ____ interior.
   a) hydrophobic tails; hydrophilic heads, polar.
   b) hydrophilic heads; hydrophobic tails, non-polar.
   c) hydrocarbon chains; carboxylic acid groups, polar.
   d) carboxylic acid groups; hydrocarbon chains, non-polar.

11. Which of the following membrane structures function in active transport?
   a) peripheral proteins.
   b) cholesterol.
   c) integral membrane proteins.
   d) trans-membrane proteins.

12. Which of the following would yield the most energy per gram when oxidized?
   a) starch.
   b) glycogen.
   c) fat.
   d) protein.

13. Amino acids whose side chains can interact with the DNA or RNA nucleotide bases via hydrogen bonding include the following:
   a) Asn and Gln.
   b) Val and Ala.
   c) Lys and Leu.
   d) Ile and Val.

14. DNA Gel Electrophoresis is similar to SDS-PAGE of proteins because
    a) Both techniques rely on a constant charge to mass ratio.
    b) Both techniques utilize the sieving properties of gels.
    c) In both cases molecules migrate to the anode.
    d) All of the above are correct.

15. During replication, overwinding or overtightening of DNA is caused by ____ and removed by ____.
    a) DNA ligase, Gyrase
    b) Dna B, DNA polymerase
    c) DnaB, Gyrase
    d) DnaA, Gyrase

16. The two features of the tRNA molecule involved in converting the triplet codon to an amino acid are
    a) in the anticodon loop and the 3'CCA end.
    b) in the anticodon loop and the D stem.
    c) solely in the anticodon loop.
    d) solely at the 3'CCA end.

17. A change in the *middle* base of the anticodon triplet would most likely
    a) prevent the tRNA from becoming charged with an amino acid.
    b) cause the tRNA to become charged with the incorrect amino acid.
    c) cause the incorporation of the wrong amino acid into the protein sequence.
    d) have no effect on the final protein sequence since this is the wobble position.

B (3 pts each/9 pts). Listed below are generalizations that apply to biochemical structures or functions. **Pick any three of the five**. In many, but not all, there are clear exceptions to the rule as it is stated. In those cases, provide a single good example of an exception in the space provided. If the rule actually applies without exception, state " No exceptions" in the space provided.

1. Oxidative phosphorylation in *all* organisms requires the presence of oxygen as an electron acceptor.

2. *All* heme containing proteins transport oxygen.

3. *All* polymerases have a 5'-3' polymerase activity.

4. *All* membrane proteins are α-helical.

5. *All* carbohydrates can be found in 5 or 6-membered ring forms.

C1 (5 pts): Do part a or part b, but not both.

Choice a: Sketch the overall structure of **either** an α-helix **or** a β-sheet. Please indicate your choice. You do not need to show a detailed drawing of the chemical structure, but do indicate the direction of hydrogen bonds relative to the mainchain direction in your drawing.

OR

Choice b: Compare and contrast tertiary and quaternary structures of proteins. Give an example of each type.

C2 (6 pts): Do **one** of the following **two** questions:

Choice a: Select **one** of the following two intermolecular forces and compare and contrast their importance to the stability of folded proteins and double stranded DNA..

- Van der Waals interactions
- Electrostatic interactions

OR

Choice b: Provide a brief description of the hydrophobic effect. Indicate whether this effect is favorable or unfavorable for protein folding and phospholipid bilayer formation.

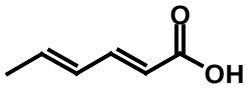
C3 (8 pts): Provide a *general* conceptual framework that explains the underlying molecular mechanism of allosteric affects. Briefly describe how allosteric effects are used to regulate **either** phosphofructose kinase activity **or** hemoglobin **or** glycogen synthesis. Give an example of an allosteric regulator for your chosen example and briefly indicate its regulatory role.

C4 (8 pts): Do only **one** of the following two questions:

Choice a: Most bacteria can produce lactate when fed glucose. Lactate is a valuable commercial product. What growth conditions would you employ to maximize the production of lactate from bacterial cultures? Support your answer by a brief description of the metabolic steps involved in lactate production.

OR

Choice b: How many $FADH_2$, NADH, and acetyl-CoA molecules would be produced by β-oxidation of the fatty acid shown to the right. Please show your work.



C5 (5 pts): After eating several candy bars the glucose level in the blood can rinse to 1 mM. In a normal individual, these levels drop to about 0.5 mM within 30-45 min and then a constant glucose level is maintained. *Briefly* discuss how the regulation of blood glucose is accomplished by **hormonal control**. (You need **not** give extensive details about phosphorylation pathways, etc.)

C6 (3 pts): Do only **one** of the following two questions:

Choice a: Distinguish between ΔG^o and $\Delta G^{o'}$ (the latter is ΔG^o *prime).*

OR

Choice b: Distinguish between ΔG and ΔG^o.

C7 (10 pts): Do **either** part a **or** part b, but not both!

Choice a: In question 6, the concentration of glucose will initially be different across the cell membrane, with a concentration of 1 mM on the outside and 0.5 mM on the inside. The membrane potential across the cell membrane is -0.01 volts, with the inside more negative.

i) Calculate the Gibbs free energy, ΔG, associated with this concentration gradient (5 pts).

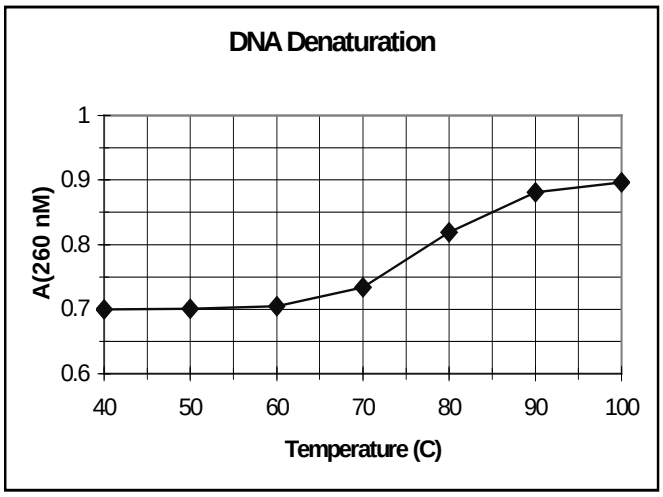
ii) Based on the sign of ΔG, is the movement of glucose into the cell spontaneous or not (2 pts)?

iii) How many glucose molecules would have to be transported to provide sufficient energy to synthesize an ATP (2 pts)?

iv) If the transport of glucose does indeed lead to ATP synthesis, what *must* exist in the membrane for this to occur (1 pts)?

OR

Choice b: The thermal denaturation curve for the melting of a double stranded DNA molecule is shown in the figure to the right. The absorbance of the double stranded DNA is 0.7 and the absorbance of the denatured DNA is 0.9.



i) Draw a *simple* cartoon that illustrates the change in the structure of the DNA as a result of this experiment (1 pt).

ii) What is the T_M for this DNA? Justify your answer. (2 pt)

iii) What is the GC content of this DNA? Show your work. (2 pts)

iv) What is the ΔG^o for denaturation at 90^{o}C? (5 pts)

C8 (10 Pts): Do **either** part a **or** part b, but not both.

Choice a: Discuss the role of transition state stabilization in enzyme catalysis. Provide one example of transition state stabilization from any of the enzymes that were discussed in this course.

OR

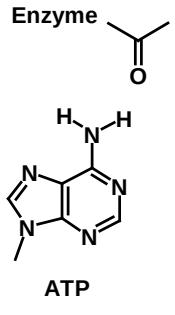
Choice b: Discuss general methods by which enzymes bind specific substrates. Illustrate your answer by providing one example of a specific interaction between an enzyme and its substrate.

C9 (10 pts): Do **either** part a **or** part b, but not both!

Choice a: Describe, *in general*, how you would obtain the inhibition constant, K_I, for any competitive inhibitor. Your answer should include a description of the actual experiments that would be necessary to acquire the raw data. You should also show how a double reciprocal plot is used to obtain K_I by graphical analysis of the data.

OR

Choice b: The enzyme adenyl cyclase converts ATP to cAMP. The interaction of the adenine ring with the enzyme is shown to the right. This diagram also shows the structure of 2AP, an inhibitor of the reaction. The K_M for ATP is 1×10^{-6} M.



i) Is 2AP likely to be a competitive or non-competitive inhibitor? Why? (2 pts)

ii) Using the K_M for ATP, estimate the ΔG^o for the binding of ATP to adenyl cylase. State any assumptions that you made (4 pts)

iii) Based on the difference in the molecular interaction between the enzyme and ATP versus 2AP and your answer to part *ii*, estimate the K_I for 2AP. State any assumptions you have made in solving this problem.(4 pts).

C10: (7 pts) You should do **one** of the following three questions:

Enzyme Name	Recognition Sequence
*Natrat*I	$^{5'}$AA$^{\downarrow}$CGTT
*Tartan*II	$^{5'}$AAA$^{\downarrow}$TTT
*Cut*I	$^{5'}$GGC$^{\downarrow}$GCC
*Cohen*II	$^{5'}$CG$^{\downarrow}$CGCG
*BstE*II	$^{5'}$G$^{\downarrow}$GCGCG

Choice a: The list on the right shows a number of restriction endonucleases and their associated recognition sequences and cleavage sites. Only one strand is shown and the cleavage position is indicated by a downward arrow: $^{\downarrow}$.

i) For *Natrat*I, write the complete sequence (i.e. both strands) and draw the individual products (both strands) after treatment of its restriction sequence with the enzyme (3 pts).

ii) Which of the other four restriction sites would produce fragments that could be readily joined to the fragments produced by cleavage with *Natrat*I? Briefly justify your answer with a diagram of the resultant joined fragment.(4 pts.)

OR

Choice b: Describe the steps involved in converting an mRNA to a cDNA molecule. You should clearly indicate the enzymes involved and any DNA primers that would be required.

OR

Choice c: You have just made a cDNA library from mRNA isolated from human cells. The size of the entire human DNA is approximately 10^9 basepairs. You are interested in isolated the gene for hemoglobin to produce human hemoglobin from bacteria. You probe your cDNA library with a radioactive probe that is 12 nucleotides in length and identical in sequence to a portion of the DNA sequence of the hemoglobin gene. You identify approximately 50 different plasmids that contain this 12 base sequence. What is the likelihood that almost all 50 of these plasmids contain the gene for human hemoglobin? Justify your answer.

C11 (5 pts):

a) Indicate the following on the nucleic acid molecule shown on the right:

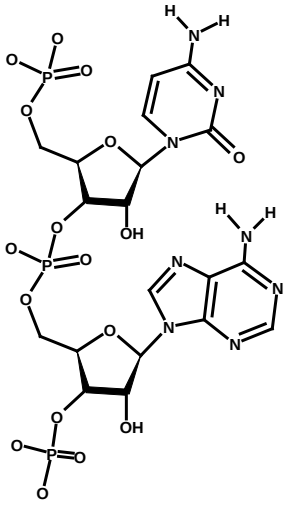
i) The location of the 5' *and* 3' ends.

ii) Any charges that are omitted.

iii) Draw an 'X' through a ribose or deoxyribose.

iv) Draw a circle around the purine base

b) Is this a DNA or RNA molecule? Why?



C12 (10 pts): The following diagram shows a segment of DNA with the necessary control elements for the production of a recombinant protein (e.g. human insulin) in bacteria.

i) Briefly discuss the role of **three** of the following five features in the production of recombinant protein:

-35 & -10 region:

lac operator:

SD:

AUG & UAA:

ρ:

ii) List in the space to the right the feature(s) that act as signals in protein synthesis?(2 pts)

iii) Indicate, by drawing a *neat* box on the above diagram, the region of the DNA that encodes the information required for the amino acid sequence of the protein (2 pts)

C13 (8 pts): Do part a **or** part b. You can answer this question using simple diagrams or a sequential list of events.
Choice a: Describe the steps involved in the replication of the lagging strand in DNA replication.
Choice b: Describe the steps involved in the addition of one amino acid to a polypeptide chain by the ribosome.

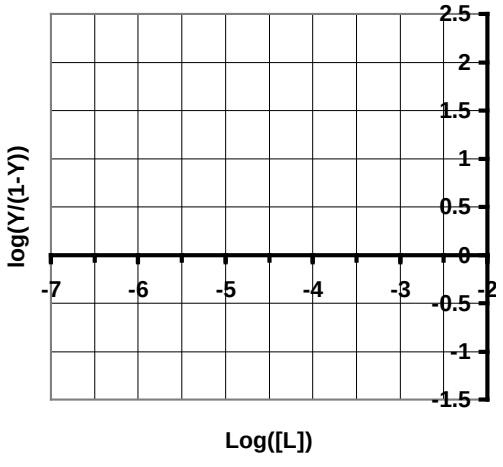
C14 (25 pts): The binding of Single Stranded Binding protein (SSB) to dA_{10} (10 adenosine residues in a single chain) was measured at five different pH values. The binding data for one pH value (10.0) is shown below (left table), as are the K_{EQ} ($=K_A$) values for four of the five pH values (right table).

Experiment #	[DNA]	Y (pH = 8.0)
1	1×10^{-6}	0.10
2	1×10^{-5}	0.52
3	1×10^{-4}	0.92
4	1×10^{-3}	0.99

pH	K_{EQ}	ΔG^o
11.0	$3.0 \times 10^3\ M^{-1}$	
10.0	$4.4 \times 10^3\ M^{-1}$	
9.0	$2.2 \times 10^4\ M^{-1}$	
8.0		
7.0	$1.6 \times 10^5\ M^{-1}$	

i) Estimate the binding affinity (K_{EQ}) at pH 8.0 by direct inspection of the data, enter your value into the right-hand table above. Briefly justify your approach (4 pts).

ii) Is the binding of dA_{10} to SSB at pH = 8.0 cooperative or not? Justify your answer by a quantitative analysis of the data. If you feel the need to construct a Hill plot, please use the graph to the right, although such a plot may not be necessary (4 pts).



iii) Does the binding of SSB to dA_{10} become stronger or weaker as the pH is increased? Justify your answer using the above data. (3 pts)

Continued on next page.....

Question C14, continued:

iv) Based on the effect of pH on the binding affinity, speculate on the nature of the interaction between SSB and the DNA. Make specific reference to the type of amino acid involved and the region of the DNA with which it interacts. (Sample answer: The hydrophobic residues, such as Ala and Val, in SSB interact with the methyl group on the T base). Make specific reference as to why the pH would affect the interaction. (5 pts)

Note that the structure of DNA is shown in question C11. [Hint: The effect of pH on ΔG^o is similar to a titration curve, you may find it useful to calculate ΔG^o values at each pH]

v) Based on your answer to part iv, is the interaction between SSB and DNA likely to be specific for certain base sequences? Speculate why the nature of the interaction between SSB and DNA is important for the function of SSB during DNA replication.(2 pts)

vi) Assuming that the binding was measured by equilibrium dialysis and that the concentration of the SSB was $1x10^{-6}$ M, calculate the UV absorbance at 270 nM of the solution **inside** the dialysis bag for experiment #1 in the above table (Y=0.1, [DNA]=$1x10^{-6}$ M). Please show your work. SSB contains 2 Trp residues, recall that this particular DNA (dA_{10}) contains 10 dA bases.(3 pts)

The following extinction coefficients may be useful: $\varepsilon_{270}^{Trp} = 5{,}000$ $\varepsilon_{270}^{dA} = 10{,}000$

vii) On the basis of the above binding data, design an affinity chromatography step to purify SSB. Briefly describe the nature of the column material and how you would elute the bound SSB off of the column(4 pts).