This exam consists of 18 pages. There are a total of 200 points.

Part A: Please circle the best answer (2 pts each. Total =36 pts).

1. Which of the following alcohols would be most soluble in water?
   a) methanol (CH_3OH)
   b) ethanol (CH_3CH_2OH)
   c) butanol ($CH_3CH_2CH_2CH_2OH$)
   d) octanol ($CH_3[CH_2]_6CH_2OH$)

2. In β-pleated sheet structures
   a) neighboring chains cross at right angles.
   b) neighboring residues are hydrogen bonded.
   c) neighboring chains are often connected by disulfide bonds.
   d) neighboring chains are hydrogen bonded.

3. The Standard Gibb's free energy, ΔG^o, is
   a) the residual energy present in the reactants at equilibrium.
   b) the residual energy present in the products at equilibrium.
   c) usually independent of temperature.
   d) The energy required to convert one mole of reactants to one mole of products.

4. If concentration of the reactants is higher than the equilibrium concentration then:
   a) The Gibbs free energy, ΔG, will be positive.
   b) More reactants will form.
   c) The standard free energy, ΔG^o, must be negative.
   d) none of the above.

5. A protein that shows infinite positive cooperative for binding of *n* ligands will:
   a) show a Hill coefficient (n_H) of 1.
   b) only be found in either the unliganded form or the fully liganded form.
   c) show a Hill coefficient (n_H) of *n.*
   d) answers b and c are correct.

6. In an enzyme catalyzed reaction, ____ provides information on ____ and _____ provides information on _____.
   a) K_M, chemical step, V_{MAX}, substrate binding.
   b) K_D, substrate binding, k_{CAT}, chemical step.
   c) K_M, substrate binding, V_{MAX}, chemical step.
   d) k_{CAT}, substrate binding, V_{MAX}, chemical step.

7. Which of the following reactions are *oxidations*?
   a) conversion of an alkane to an alkene.
   b) conversion of an alcohol to an alkene.
   c) conversion of a carboxylic acid to an aldehyde.
   d) all of the above

8. A kinase is an enzyme that:
   a) adds water to a double bond.
   b) uses NADH to change the oxidation state of the substrate.
   c) uses ATP to add a phosphate group to the substrate.
   d) uses ATP to remove phosphate groups from substrates.

9. Which of the following elements of secondary or super-secondary structure are most likely to be found in an integral membrane protein?
   a) single β-strands.
   b) isolated β-hairpin.
   c) α-helices.
   d) β-α-β structure.

A:	/36
B1:	/ 8
B2:	/ 4
B3:	/ 8
B4:	/ 8
B5:	/ 8
B6:	/12
B7:	/ 4
B8:	/ 5
B9:	/10
B10:	/10
B11:	/15
B12:	/10
B13:	/10
B14:	/ 6
B15:	/10
B16:	/ 8
C1:	/14
C2:	/14
TOT:	/200

10. Cholesterol is essential for normal membrane functions because it
    a) spans the thickness of the bilayer.
    b) keeps membranes fluid.
    c) catalyzes lipid flip-flop in the bilayer.
    d) plugs up the cardiac arteries of older men, including Dr. Rule.

11. DNA differs from RNA in the following features
    a) DNA is resistant to base catalyzed hydrolysis; RNA is hydrolyzed by OH^-
    b) DNA residues are linked by 3'→5' phosphodiester bonds; RNA is 2'→5' linked.
    c) DNA has deoxyribose residues; RNA has ribose residues.
    d) All but the second choice are correct differences.

12. The major and minor grooves of B-form DNA correspond to what features of A-form RNA?
    a) minor and major grooves
    b) deep and shallow grooves
    c) deoxyribose backbones
    d) phosphoribose backbones

13. The enzyme that joins DNA fragments cut by restriction enzymes is called:
    a) Primase.
    b) Polymerase.
    c) Ligase
    d) DNA phosphorylase

14. Which of the following statement is *incorrect* about DNA polymerases
    a) require a primer.
    b) synthesize in the 5' to 3' direction.
    c) require a template.
    d) synthesize in the 3' to 5' direction.

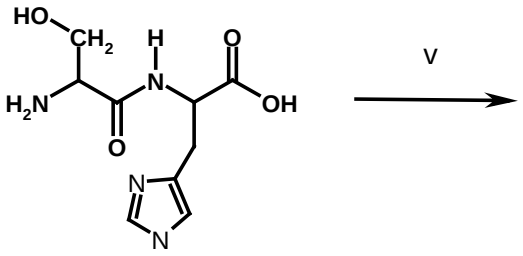
15. The rapid appearance of HIV-1 strains that are resistant to AIDS drugs is due in part to this property of its reverse transcriptase:
    a) the RNase domain of the enzyme causes error prone synthesis.
    b) it lacks a 5' → 3' exonuclease.
    c) it has low affinity for the correct dNTP's.
    d) it lacks a 3' → 5' exonuclease.

16. Okazaki fragments are
    a) short fragments composed entirely of DNA.
    b) an intermediate in the synthesis of the lagging strand.
    c) an intermediate in the synthesis of the leading strand.
    d) short fragments composed entirely of RNA.

17. During replication, overwinding or overtightening of DNA is caused by ____ and removed by ____:
    a) DNA ligase, Gyrase
    b) Helicase, DNA polymerase
    c) Helicase, Gyrase
    d) Single stranded binding protein, Gyrase

18. Removal of the leader (signal) peptide from a protein that is translocated across a membrane is accomplished by
    a) Trypsin
    b) Signal Peptidase
    c) HIV protease
    d) Protein phosphatase

B1. (8 pts) Consider the dipeptide shown below:



Name of peptide:

i) Circle the amino terminus (1 pt)

ii) Put a box around the carboxy-terminus (1 pt)

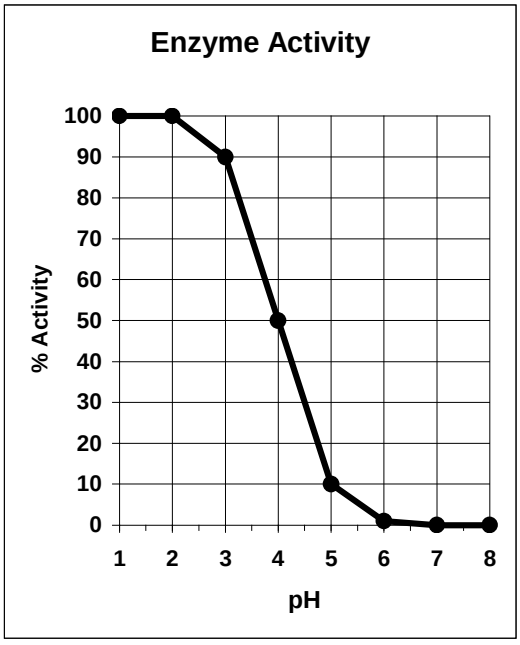
iii) Indicate the correct ionization state (charge) of this peptide at pH=7.0 (1 pt).

iv) Write the correct name for this peptide, using the full name of each amino acid or the three letter code. if you can't remember the name of the amino acids, describe the general method by which peptides are named. (1 pt)

v) Draw to the right of the arrow labeled 'v' the products of the reaction if this peptide was treated with a protease. Indicate all reactants/products (2 pts)

vi) Identify the bond that does *not* freely rotate, what is the name for this bond? Why does this bond not rotate? (2 pts)

B2. (4 pts) The activity of an enzyme is measured as a function of pH, giving the data shown in the graph to the right. Which amino acid is likely to be involved in catalysis? Briefly justify your answer.



B3. (8 pts) A partial list of energetic terms or 'forces' that play a central role in Biochemistry is given below:

1. Hydrogen bond
2. Electrostatics
3. Van der Waals
4. Configurational Entropy

Pick **any two (2) of the above four** and give a brief description of its molecular nature. Your answer should indicate whether the term is largely enthalphic (ΔH) or entropic (ΔS).

B4. (8 pts) Compare and contrast the role of any **two of the following three** energetic terms in their contribution to protein **and** DNA Stability. Use the following table to guide your answer. Your answer should contain information on the relative contribution of each term to protein and DNA stability, and a *brief* justification of your answer.

Energetic Term:	**Protein Stability**	**DNA Stability (double stranded)**
Electrostatics		
Van der Waals		
Configurational Entropy		

B5. (8 pts) Briefly describe the hydrophobic effect and briefly describe its role in *either* protein stability or formation of lipid bilayers.

B6: (12 pts)

i) Define/describe allosteric effects. You answer should include a discussion of both homotropic as well as heterotropic allosteric effectors as well as tense (T) and relaxed (R) states. A simple, well labeled, diagram will suffice (6 pts).

ii) Give an example of how an allosteric effect controls a biochemical process. You should identify the allosteric compound (2 pt), the enzyme or protein to which it binds (2 pt), and how this effect is used to control the biochemical process (2 pts).

B7. (4 pts) Briefly state the difference between **one** of the following two choices. You can answer this question by discussing an example. Clearly indicate your choice for grading purposes.

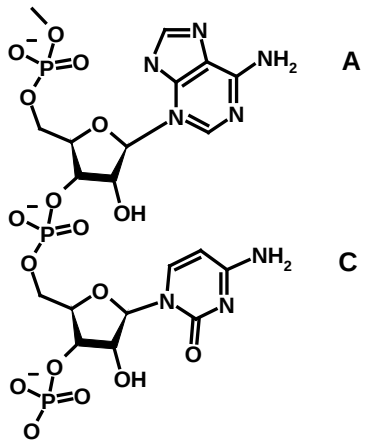
Choice A: Primary structure versus secondary structure of a protein.

Choice B: Tertiary structure versus quaternary structure of a protein.

B8. (5 pts) The following gives the structure of a single stranded section of nucleic acid. Please label, or otherwise indicate on the diagram, the following:

a) a pyrimidine base (1 pt)
b) glycosidic bond (1pt)
c) 5' end (1pt)
d) Phosphodiester bond (1pt)

What is the sequence of this DNA fragment? (1 pt)

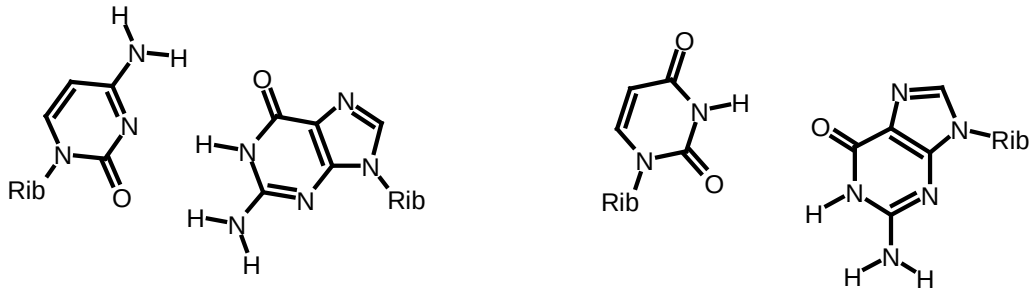


B9. (10 pts) Describe the role of hydrogen bonding in **one** of the following three situations. Be sure to indicate your choice. In the case of the first two choices your answer should include a description of the importance of this interaction in template directed polymer synthesis. In the case of the choice C, you should make a distinction between major and minor groove interactions and provide an example of an interaction between the protein and the nucleic acid. A C-G basepair and a U-G pair have been provided to help illustrate your answer . You need not use both in your answer.

Choice A: Formation of double stranded DNA or RNA.

Choice B: Binding of charged tRNA to mRNA (including wobble basepairing)

Choice C: Recognition of specific DNA sequences by restriction endonucleases.



B10 (10 pts). Discuss **two of the following four** features of enzyme catalyzed reactions. Indicate how these features are important for catalysis or inhibition and provide a specific example of this feature in an existing enzyme. In your examples, you can use any enzyme you like (including ones not discussed in class) and you need not use the same enzyme for all of your answers.

i) Transition state stabilization.

ii) Substrate specificity.

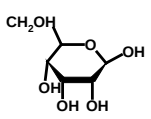
iii) Chemical mechanism.

iv) Competitive & non-competitive inhibitors.

B11 (15 pts). On the right are a series of 20 biochemical structures (A-T), on the left is a list of names or descriptions. Indicate the correct match by writing the letter next to the description or name. Note that a structure *should* only be used once. There may be more than one correct structure. You will get 2 points for each correct grouping of compounds, and then an additional 1 point for having the correct identification of all three compounds within a group. There are five groupings, in order from top to bottom: compounds in anaerobic metabolism, lipids, saccharides, electron carriers, and nucleic acids.

Description	**Match**
1. Product of glycolysis	
2. Product of anaerobic metabolism in humans.	
3. Product of anerobic metabolism in yeast	
4. Unsaturated fatty acid	
5. Triglyceride	
6. Phosopholipid	
7. Six carbon ketose	
8.Saccharide found in bacterial cell walls	
9. Disaccharide from glycogen or starch.	
10. Electron carrier in the TCA cycle and fatty acid oxidation.	
11. Electron carrier in electron transport chain.	
12. Final electron acceptor in electron transport in most species, including humans.	
13. Nucleotide normally found in DNA	
14. Nucleotide normally found in RNA	
15. Nucleotide that is used in DNA sequencing.	

A



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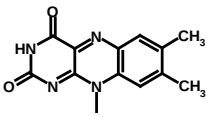

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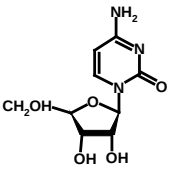

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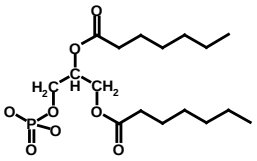

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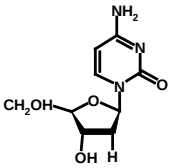

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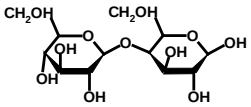

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H O_2

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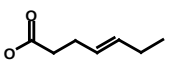

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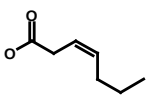

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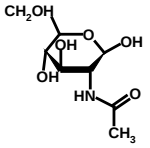

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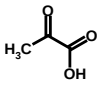

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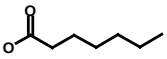

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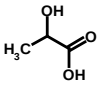

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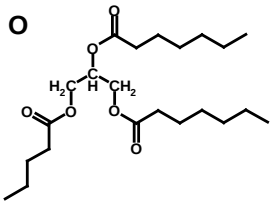

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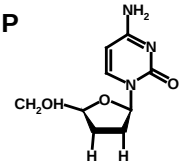

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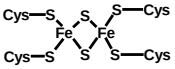

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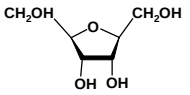

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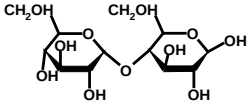

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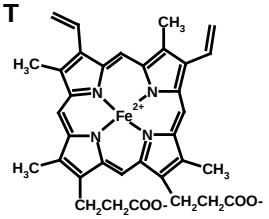


B12. (10 pts) Please do one of the following three choices. Please indicate the choice that you are answering.

Choice A: Individuals with glycogen storage diseases are often missing the enzyme glycogen phosphorylase.

i) How would this deficiency affect the liver's ability to respond to epinephrine? Your answer should include a brief description of hormonal signaling.

ii) What kind of diet should this individual be on? High carbohydrate or high fat? Why?

Choice B: The version of Phosphofructose kinase (PFK) in the muscle is different than that from the liver. Although both catalyze the same reaction, they are *regulated* differently. Based on your knowledge of PFK in the liver, and your knowledge of liver and muscle function, suggest how PFK in the muscle might be regulated by both hormonal as well as energy sensing.

Choice C: Draw a ***simple*** diagram that illustrates the oxidative fate of the principle components of a bagel with cream cheese (i.e. glucose from the bagel, fatty acids and amino acids from the cream cheese). You diagram should resemble a flow chart, showing only the *names* of the major metabolic pathways and how they are connected. The top of your 'flow chart' should begin with the **three** nutrients. The bottom of your 'flow chart' should end with "CO_2".

B13. (10 Pts) Please do **one** of the following two questions. Please indicate your choice.

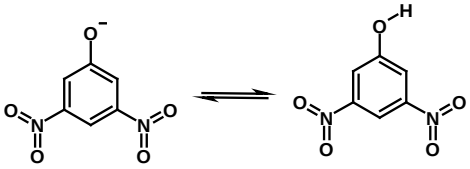
Choice A: Briefly describe how you would determine the quaternary structure of a protein.

Choice B: Describe a purification scheme that would separate the following two proteins. The properties of the proteins are listed in the table below. Briefly describe how the separation technique(s) works.

Protein	Molecular weight	#Asp & Glu	#Lys & Arg
A	50,000	5	5
B	75,000	2	2

B14. (6 pts) Please do **one** of the following two choices. Please indicate your choice.

Choice A: The compound, dinitrophenol is a weak acid. Its protonated and deprotonated form are shown to the right. At one time, this compound was used as a diet pill. This compound is effective at transferring protons across a biological membrane.

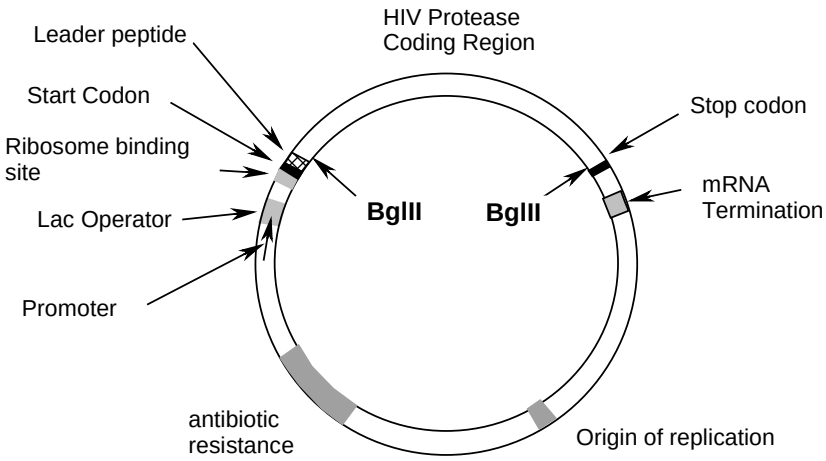


i) Explain why this compound is able to move protons across a biological membrane (2 pts).

ii) Explain why this compound will lead to weight loss (4 pts).

Choice B: If Na^+ were pumped across the inner membrane during electron transport instead of protons, would it still be possible for ATP synthase to couple proton transport to ATP synthesis?

B15. (10 pts). A diagram of the expression vector that produces recombinant HIV protease is shown to the right.
i) In one or two sentences, describe the principal role of any **four** of the following 8 features in the expression of HIV protease (8 pts).



i. Origin of replication:

ii. Antibiotic resistance gene:

iii. Promoter:

iv. Lac operator:

v. Ribosome binding site:

vi. Start codon:

vii. Leader peptide:

viii. Stop codon:

B15 -continued.

Show the results of cutting this plasmid with the enzyme BglII. The recognition sequence for Bgl II is C^GATCG. Your answer should show the detailed structure of the ends of the DNA fragments (2 pts)

B16. (8 pts) Select **either** lagging strand DNA synthesis **or** the elongation of proteins during protein synthesis and discuss the events that lead to template directed polymer synthesis. In the case of DNA, you should describe the process where by ~1000 bases are synthesized at a time, while in the case of protein synthesis you need only discuss the addition of one amino acid. Regardless of your choice, briefly describe the molecular events that occur at each significant step in the process.

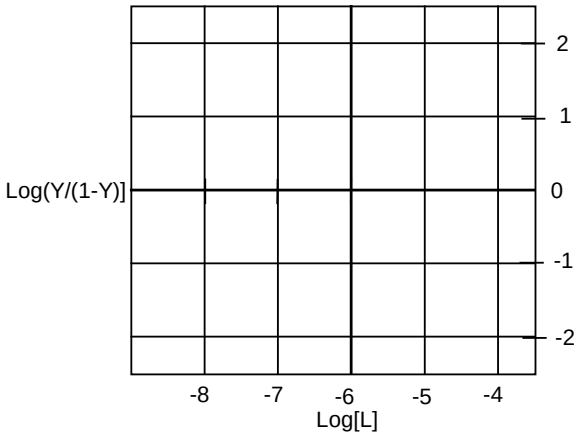
C. (14 pts) Please do any **two** of the following five choices.

Choice A: The binding constant of single-stranded binding protein (SSB) to single-stranded DNA was measured in a solution with a NaCl concentration of 0.1M and 0.5 M, giving the following data. In this problem you should consider the protein to be the ligand (L) and the DNA to be the macromolecule (M)

Protein Concentration (uM)	**Fractional Saturation NaCl=0.1M**	**Log(Y/(1-Y)) NaCl=0.1M**	**log [L]**	**Fractional Saturation NaCl=0.5M**
0	0.00	-	-	0.00
0.1	0.02	-1.70	-7	0.01
1.0	0.50	0.00	-6	0.02
10.0	0.98	1.70	-5	0.50
100.0	0.99	2.95	-4	0.98

i) *Estimate* the dissociation constant (K_D) in 0.1M NaCl from these data. (Hint: You do not need to draw a graph of any sort here). Explain your approach. (4 pts)

ii) Does the binding of DNA to this protein display either positive or negative cooperative behavior? Justify your answer. You should **not** have to do a Hill plot to determine this. However, if you must, the appropriate data (log[Y/(1-Y)], log[L]) are given in the last two columns of the above table. Use the graph to the right. (4 pts)

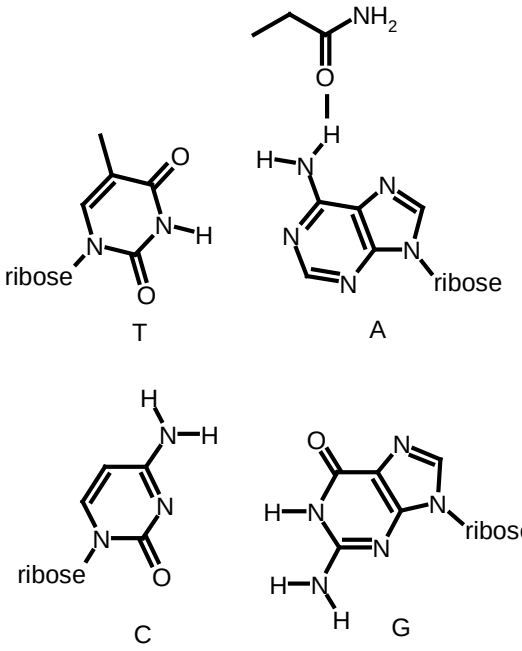


iii) What is the K_D in the presence of 0.5M NaCl? (2 pts)

iv) Does SSB bind more tightly or less tightly to DNA as the salt concentration is raised? (2 pts)

v) Based on the effect of salt of the affinity of SSB to DNA, does SSB interact with the phosphate, ribose, or bases? Briefly justify your answer.(2 pts)

Choice B The restriction enzyme NatRat binds to and cleaves the following DNA sequence: GATC. Below is a diagram of the interaction between an Asn residue of this restriction endonuclease and a TA base pair in its recognition sequence. The position of this base pair in the recognition sequence is indicated in bold and underlined (GA**T**C). Drawn below the TA base pair is a CG base pair to help you visualize what happens when the TA base pair is replaced by a CG basepair.



i) Label the major and minor grooves of the TA base pair and draw any hydrogen bonds that might occur between the protein and the DNA (2 pts).

iii) Explain why major groove binding allows NatRat to distinguish between a TA basepair and an AT basepair (4 pts).

iv) The binding constant, K_{EQ}, of NatRat to the sequence GATC is $10^{10}\ M^{-1}$. Calculate ΔG^o for this binding reaction (assume T=300K, RT=2.5 kJ/mol; $\ln 10^{10} = 23$)(2 pts).

v) The free energy of binding of NatRat to GACC is -37.5 kJ/mol. Explain this reduction in binding energy with reference to the interaction of the Asn residue in NatRat with the CG basepair. (4 pts).

vii) Assuming that DNA is the ligand, and that the DNA concentration is 1 μM, calculate the fraction of NatRat bound to GATC and GACC at 300 K(2 pts).

Choice C. The dissociation of the following three DNA molecules was studied as a function of temperature. In all cases, the temperature was *below* the melting temperature of the double stranded DNA. Thus the increase in temperature only caused the two double stranded pieces to *reversibly* separate. Equal concentrations of the joined and separate pieces were found at the indicated temperature (T_{MID})

DNA Sequence and Equilibrium						
	Reactant		Products			T_{MID}
1	A-G-C-T-G-G T-C-G-A-G-G T-C-G-A-C-C A-G-C-T-C-C	→ ←	A-G-C-T-G-G T-C-G-A-C-C	+	T-C-G-A-G-G A-G-C-T-C-C	10^o C
2	A-G-C-T-G-G T-C-G-A-G-G T-C-G-A-C-C-A G-C-T-C-C	→ ←	A-G-C-T-G-G T-C-G-A-C-C-A	+	T-C-G-A-G-G G-C-T-C-C	16^o C
3	A-G-C-T-G G-T-C-G-A-G-G T-C-G-A-C-C A-G-C-T-C-C	→ ←	A-G-C-T-G T-C-G-A-C-C	+	G-T-C-G-A-G-G A-G-C-T-C-C	20^o C

i) Briefly explain the relationship between the structure of each DNA molecule and its melting temperature (3 pts).

ii) Predict how T_{MID} would change if the salt concentration of the solution was increased. Justify your answer (3 pts).

iii) Given that ΔH^o is 10 kJ/mol for the first reaction, calculate the equilibrium constant at 20^o C for this reaction (4 pts).

iv) If DNA ligase and ATP were added to reaction 1 and 3, which would give a faster rate of DNA joining? Justify your answer using your answer to part iii (4 pts).

Choice D. (14 pts) The HIV reverse transcriptase (HIV-RT) is also a drug target for AIDS drugs. As with the HIV protease, mutations arise in this enzyme, generating HIV viruses that are resistant to existing drugs. Pharmaceutical companies would like to characterize these altered reverse transcriptases to understand the reduced binding of the drug as well as to perhaps design new drugs to target the mutant viruses. These mutant enzymes would be produced in E. Coli.

i) Briefly describe the three steps in converting the HIV genetic material (RNA) to double stranded DNA. A well labeled diagram would be a fine way to answer this question. You should indicate which enzymes are used in each step (4 pts).

ii) The DNA sequence of the HIV-RT gene, as well as a partial amino acid sequence of the protein, are listed below. The DNA that codes for HIV-RT is shown in upper case letters, the lower case letters are not part of the HIV-RT coding region.

```
aggcttGGCTACGAGTCGGGTACCGTAGTTGAAGCG-------TAGTGCAAAATTTTGGGGCCCGATGTAGccgttaaa
tccgaaCCGATGCTCAGCCCATGGCATCAACTTCGC-------ATCACGTTTTAAAACCCCGGGCTACATGggcaattt
      GlyTyrGluSerGlyThrValValGluAla
```

Describe the PCR primers that you would have to use to give the PCR product shown in part iii of this problem. You need not worry about determining the actual length of the primers, but you should give enough sequence information to indicate the two most important features of each primer (6 pts).

Choice D. , continued.

iii)

Sequence of Vector (see question B15)

```
--CGATTCCCGATCGAA---- HIV-RT  gene to go here! -----GGCCCGATCGAATTC-----
--GCTAAGGGCTAGCTT-----------------------------------CCGGGCTAGCTTAAG-----
```

Sequence of PCR product

```
  CGATCG------------ HIV-RT gene -------------CGATCG
  GCTAGC-------------------------------------GCTAGC
```

BamH1	G^GATCC
BglII	C^GATCG
EcoR1	G^AATTC
HaeIII	GG^CC

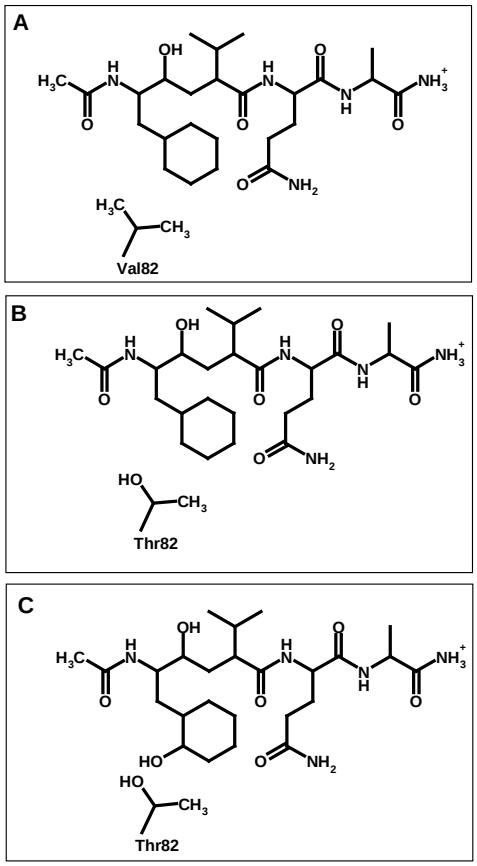
The upper sequence shows a region of the expression vector into which genes can be inserted for the purpose of obtaining recombinant protein. The entire expression vector is shown in question B15. The lower sequence is a double stranded DNA molecule that was made using PCR. This DNA sequence will result in the production of HIV-RT if correctly placed in an expression vector. The table to the right gives the restriction sites for a number of restriction endonucleases. Describe how you would insert the gene for HIV-RT into the expression vector using these restriction enzymes. A simple flow diagram will be sufficient. You should indicate which enzymes are used and clearly show how fragments digested with the restriction enzymes can be rejoined. Beware of the fact that the PCR fragment has the same restriction site at both ends. (4 pts)

Choice E. The diagrams to the right show:

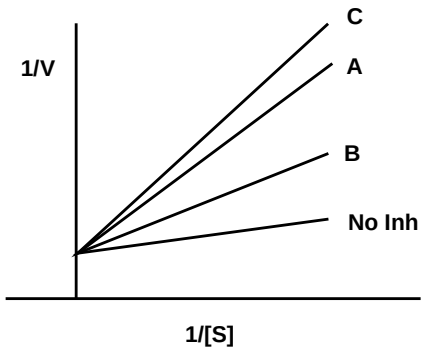
A. Val82 in wild type HIV protease complexed with a cyclohexane containing drug.

B. A mutant HIV protease where Val82 has been replaced by Thr, complexed to the same drug as in "A".

C. The Thr mutant complexed with a modified drug.



i) Double reciprocal plots are shown for each of these combinations. You should assume that the concentration of the inhibitor was the same in all three cases. Determine the *relative* order of K_I values for each of these protein-inhibitor combinations (e.g. The K_I for interaction "A" is lower that "B" which is lower than "C", therefore the order of K_I values is $K_I^A < K_I^B < K_I^C$). *Briefly* justify your answer with reference to the data shown above. (4 pts)



ii) Explain the molecular basis for the relative order of the K_I values with clear reference to the interaction between the enzyme and the inhibitor (6 pts).

iii) The left panel in the following figure shows a DNA sequence gel for the wild type protein for residues 81-83. Sketch the DNA sequence gel for the Thr82 mutant. (4 pts)