

This exam consists of 17 pages, including this one. There is a separate formula sheet, make sure you have one. On many questions you have choices. Please indicate which answer is associated with which choice. As always, if you attempt more than one choice all will be graded and the best answer will be used in scoring. **You should allow ~1 min/point.**

Part A: (2 pts each, total 28 pts). Please circle the best answer.

- Removal of the signal peptide from a protein that is translocated across a membrane is accomplished by
 - Trypsin
 - Signal Peptidase
 - HIV protease
 - the ribosome.
- The two features of the tRNA molecule involved in converting the triplet codon to an amino acid are:
 - in the anticodon loop and the 3' CCA end.
 - in the anticodon loop and the D stem.
 - solely in the anticodon loop.
 - solely at the 3' CCA end.
- Initiation of mRNA synthesis involves
 - displacement of the lac repressor from the DNA.
 - binding of RNA polymerase and formation of the open complex.
 - binding of RNA polymerase followed by binding of helicase.
 - binding of RNA polymerase followed by binding of single stranded binding protein.
- The rapid appearance of HIV-1 strains that are resistant to AIDS drugs is due in part to this property of its reverse transcriptase:
 - the RNase domain of causes error prone synthesis.
 - it lacks a 5' --> 3' exonuclease.
 - it has low affinity for the correct dNTP's.
 - it lacks a proofreading exonuclease.
- The enzyme that joins DNA fragments cut by restriction enzymes is called:
 - Primase.
 - Polymerase.
 - Ligase
 - DNA phosphorylase
- The sugar that forms a major component of nucleic acids (DNA, RNA) is
 - Fructose
 - Galactose
 - Ribose
 - Sucrose

A : _____ / 28

B1 : _____ / 8

B2 : _____ / 6

B3 : _____ / 5

B4 : _____ / 4

B5 : _____ / 6

B6 : _____ / 10

B7 : _____ / 10

B8 : _____ / 7

B9 : _____ / 15

B10 : _____ / 5

B11 : _____ / 6

B12 : _____ / 6

B13 : _____ / 4

B14 : _____ / 6

B15 : _____ / 13

B16 : _____ / 10

B17 : _____ / 6

Bonus : _____ / 8

Total : _____ / 155

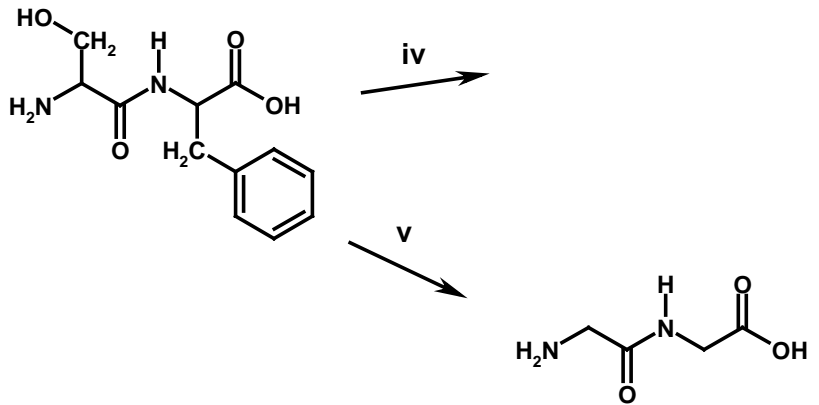
% _____ / 100

7. A non-competitive inhibitor of an enzyme catalyzed reaction
- binds to the Michaelis complex (ES).
 - decreases V_{\max} .
 - can act as an allosteric inhibitor.
 - all of the above.
8. The _____ provides information on the rate of the chemical step of an enzyme and _____ provides information on the affinity of a substrate for that enzyme.
- K_I and K_M .
 - V_{\max} , K_M .
 - K_M , V_{\max} .
 - V_{\max} , K_I .
9. In a system that shows negative cooperativity for ligand binding:
- the binding of the first ligand has no effect on the binding of subsequent ligands.
 - the binding of the first ligand raises the K_D for binding of the second.
 - the binding of the first ligand raises the K_A for binding of the second.
 - cannot bind more than one ligand.
10. The hormones, glucagon and epinephrine, stimulate glycogen breakdown to G-6-P
- directly, by binding to glycogen phosphorylase.
 - indirectly, by first stimulating adenylate cyclase to make cAMP.
 - only in the liver.
 - using ATP as the phosphoryl donor.
11. Energy production involves which of the following
- Oxidation of organic molecules.
 - Transport of electrons on organic molecules.
 - Generation of proton gradients across membranes.
 - All of the above.
12. If concentration of the reactants is higher than the equilibrium concentration then:
- The Gibbs free energy (ΔG) will be negative.
 - More reactants will form.
 - The Gibbs free energy will be positive.
 - Both b and c are correct.
13. Which of the following would yield the most energy per gram when oxidized?
- glucose
 - glycogen.
 - fat.
 - any monosaccharide.
14. The proton gradient and the membrane potential
- are sufficient, separately, to make ATP from ADP + Pi.
 - are both required to make ATP.
 - usually cancel one another since the system is at equilibrium.
 - are unrelated to each other.

Part B:

B1. (8 pts) Consider the following dipeptide:

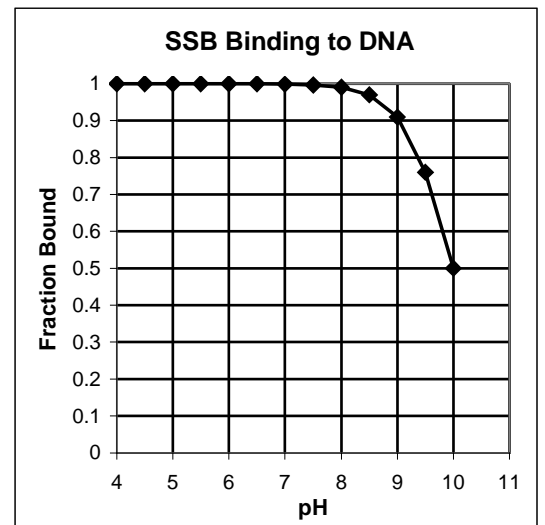
- i) Circle the amino terminus (1 pt)
- ii) Put a box around the carboxy-terminus (1 pt)
- iii) Write the correct name for this peptide, using the full name of each amino acid or the three letter code. If you can't remember the name of the amino acids, describe the general method by which peptides are named. (1 pt)



- iv) Draw to the right of the arrow labeled 'iv' the products of the reaction if this peptide was treated with a protease. (2 pts)
- v) What would be the effect of treating this peptide with a protein kinase? Please complete the drawing that I have started for you to the right of the arrow labeled 'v'. In this drawing the main chain atoms are shown, simply add the side chain and the appropriate modification. What other products/reactants would be required in this reaction? (2 pts).
- vi) Does this dipeptide absorb UV light (Yes/No)? If so, draw an arrow to the group that would absorb UV light. (1 pt)

B2. (6 pts) The graph to the right shows the effect of pH on the binding of single stranded binding protein (SSB) to single stranded DNA. SSB contains Aspartic acid ($pK_a=4$), Histidine ($pK_a=6$), and Lysine ($pK_a=10$) residues, as well as other *uncharged* amino acids.

- i) On the basis of the binding data, which of these three residues is likely to be used by SSB to bind to DNA. Why? (2 pts)
- ii) With which part of the DNA (base, sugar, phosphate) is SSB binding to? Justify your answer. (1 pt)



- iii) Predict how much SSB would be bound to DNA at pH 11. Please show your work. (3 pts)

B3: (5 pts) Do any one of the following two questions. In answering the questions, be sure to fully discuss the thermodynamic principle and whether the effect is primarily an enthalpic or an entropic one.

Choice A: The addition of a small amount of ethanol to a protein solution causes denaturation of the protein. However a solution of double stranded DNA is hardly affected. Why?

Choice B: The addition of NaCl to a protein solution causes little change in the T_M of the protein, but the same concentration of salt can have a large effect on the T_M of double stranded DNA. Why? Your answer should state the direction of the change of T_M for the DNA when salt is added (e.g. higher or lower).

B4: (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.

OR

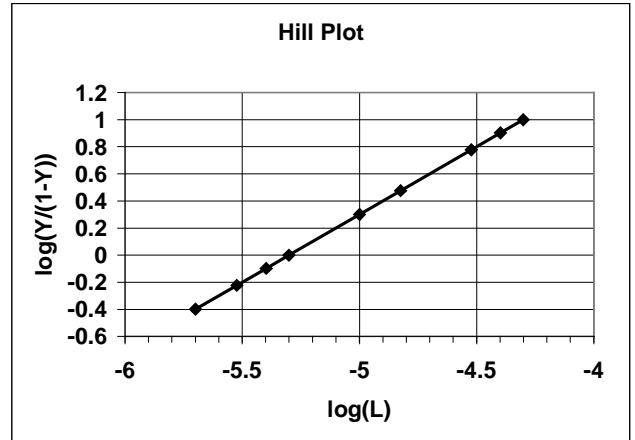
Choice B: Tertiary structure versus quaternary structure.

B5: (6pts) Briefly discuss the structure of an α -helix, **or** a β -sheet, **or** double stranded DNA. You should provide a *simple* sketch of the structure (2 pts). Then discuss the *common* interaction that stabilizes *all* of these structures. Also discuss the *most destabilizing* factor for of all these structures. Be sure to state whether these interactions/factors are ethalpic or entropic in their nature. (4 pts)

B6. (10 pts) The interaction of fructose-2,6-phosphate with fructose-1,6-bisphosphatase was characterized by equilibrium dialysis as well as by measuring the effect of F-2,6-P on the kinetics of the phosphatase. This particular phosphatase is from the Gila monster and happens to be *dimeric*.

Ligand binding data is shown on the left side of the table and enzyme kinetic data is shown in the right side of the table. The ligand binding experiment was performed by equilibrium dialysis with the enzyme and F-2-6-P alone. The enzyme kinetic data was performed with the enzyme, F-2-6-P, and fructose-1,6-P as the substrate. A Hill plot and a double reciprocal plots have been provided for you. The concentration of F-2,6-P was 10 μ M in the enzyme kinetic experiment.

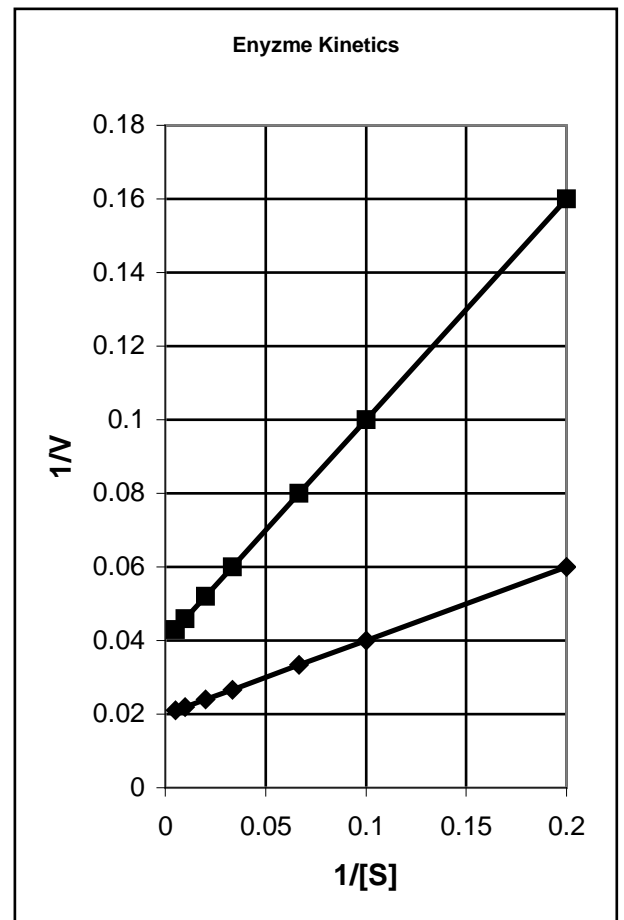
L μ M	Y	[S] (mM)	V (I=0)	V (I=10 μ M)	1/S	1/V (I=0)	1/V (I=10 μ M)
0	0	0	0.00	0			
2	0.29	5	16.67	6.25	0.200	0.060	0.160
3	0.38	10	25.00	10.00	0.100	0.040	0.100
4	0.44	15	30.00	12.50	0.067	0.033	0.080
5	0.50	30	37.50	16.67	0.033	0.027	0.060
10	0.67	50	41.67	19.23	0.020	0.024	0.052
15	0.75	100	45.45	21.74	0.010	0.022	0.046
30	0.86	200	47.62	23.25	.0050	0.021	0.043
40	0.89						
50	0.91						



i) Determine the K_D from the ligand binding data. Briefly explain how you arrived at your answer (2 pts).

ii) Is the binding of F-2-6-P to this enzyme cooperative or not? Justify your answer.(2 pts)

iii) Show, by determining the K_I for the inhibition of F-1-6bis phosphatase by F-2,6-P, that the K_D and K_I values are *different*. [You can assume $K_I = K_D$] (4 pts).



This question is continued on the next page.

B6 continued:

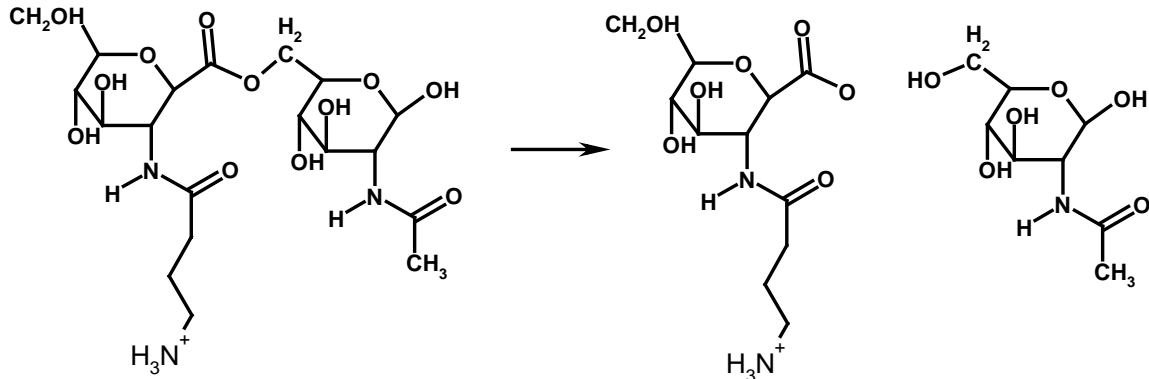
iv) Provide an explanation as to why the K_D and K_I are different, since both represent the binding of F-2,6-P to the enzyme. [Hint: You are comparing K_D to K_I *prime*, not to K_I](2 pts)

B7: (10 pts)

i) Define allosteric effects. Your 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 depicting the change will suffice (6 pts).

ii) Give an example of how an allosteric effect controls a biochemical process. You should identify the allosteric compound (1 pt), the enzyme or protein to which it binds (1 pt), and how this effect is used to control a biochemical process (2 pts).

B8. (7 pts) The following compound is a substrate for an enzyme that was isolated from a Potto [A Potto is a small furry mammal. This fact is totally irrelevant to this problem, but they are cute.]. The product is shown to the right.



Answer **one** of the following two questions:

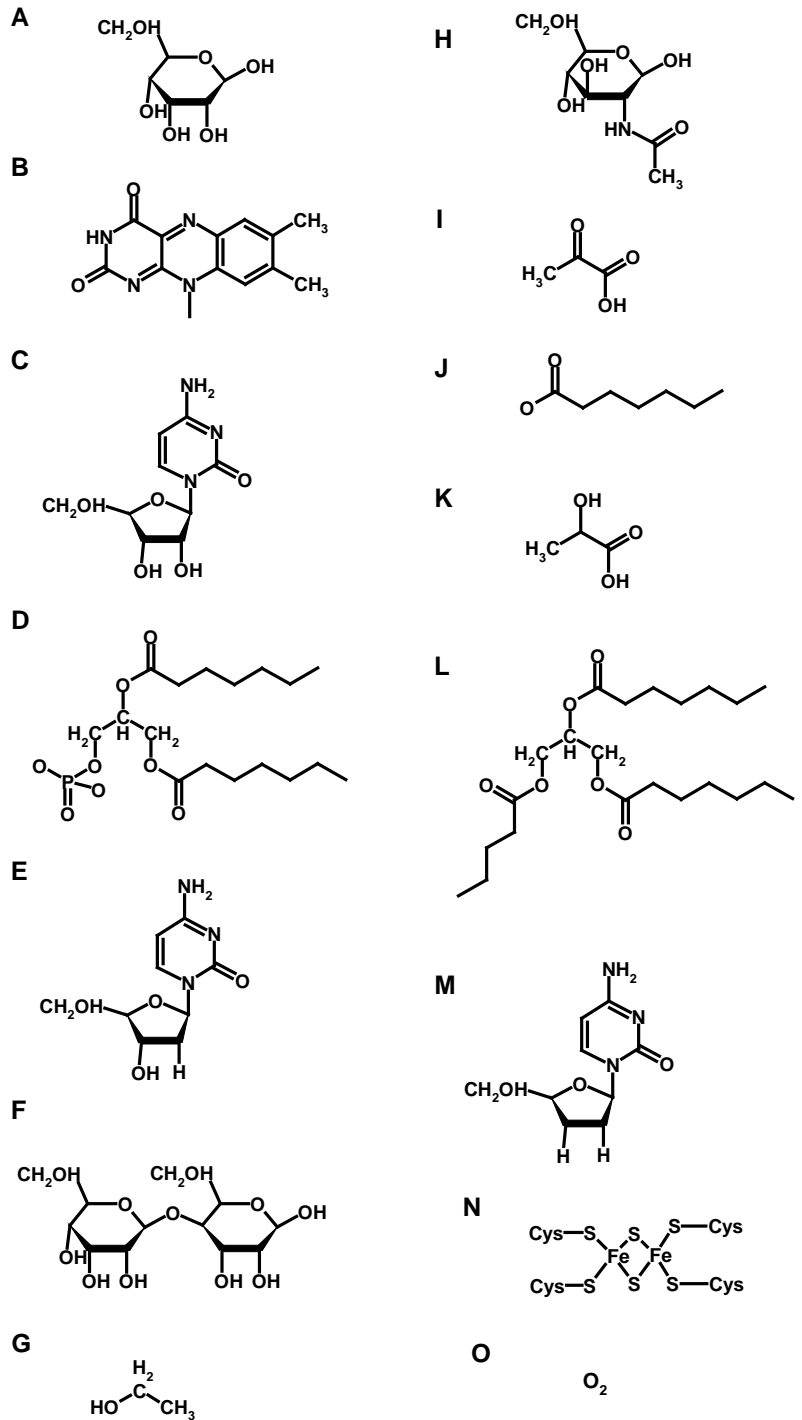
Choice A: Briefly discuss substrate specificity in general (5 pts) Then speculate as to why this enzyme is specific for this particular substrate. You should sketch some amino acid side chains in the active site of the enzyme to show how they interact with this substrate (2 pts)

OR

Choice B: Briefly discuss the *general* methods of rate enhancement by *any* enzyme (5 pts) For this particular enzyme, speculate, based on reaction mechanisms discussed in this course, as to the nature of the amino acids in the vicinity of the cleaved bond. You should sketch some amino acid side chains to illustrate their spatial relationship between the sidechains and the substrate (2 pts)

B9 (15 pts): On the right are a series of 15 biochemical structures (A-O), on the left is a list of names or descriptions. Indicate the correct match by writing the letter next to the description. Note that a structure *should* only be used once. 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 anaerobic metabolism in yeast	
4. Fatty acid	
5. Triglyceride	
6. Phospholipid	
7. Six carbon aldose.	
8. Saccharide found in bacterial cell walls	
9. Disaccharide	
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.	



B10. (5 pts) Please answer **one** of the following two questions. Please indicate the choice that you are answering.

Choice A: What is the difference between ΔG and ΔG° ? Illustrate your answer with an example of the use of either of these two thermodynamic parameters.

OR

Choice B: Briefly explain how the ΔH of a reaction can be obtained from measurements of K_{EQ} . Then explain the relationship between the sharpness of a temperature induced transition and the enthalpy associated with that transition.

B11. (6 pts) Please answer **one** of the following two questions, please indicate the choice that you are answering.

Choice A: One form of diabetes is due to a non-responsive, or non-functional, insulin receptor. Do you expect individuals with this form of diabetes to have high or low glycogen levels in their liver? Your answer should include a *brief* discussion of the signal transduction/protein phosphorylation cascade (3 pts) as well as how glycogen synthesis/degradation is regulated by protein phosphorylation (3pts).

OR

Choice B: A person on a high carbohydrate diet can become fat even though they do not eat fatty foods. Ingested carbohydrates will be broken down to glucose, thus leading to high glucose levels in the blood and the release of insulin. *Briefly* describe the metabolic pathways that have to occur in order for glucose to be converted to fats (3 pts). Your answer should include a discussion of how insulin indirectly controls the flow of sugars through glycolysis.(3 pts).

B12: (6 pts) Please answer **one** of the following three questions. Be sure to indicate your choice.

Choice A: Describe the general features of a protein purification scheme. You should describe the starting materials and give an example of any fractionation step and briefly describe how that step works (4 pts). Your answer should also describe how specific activity is used to monitor purity (2 pts).

OR

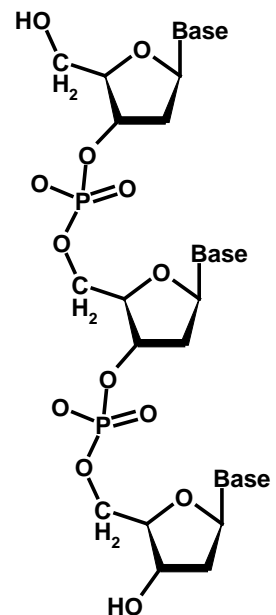
Choice B: Describe how SDS-gel electrophoresis and gel-filtration chromatography can be used to determine the quaternary structure of a protein. Your answer should include a brief description of how these two techniques work.

OR

Choice C: Compare and contrast SDS-gel electrophoresis of proteins and electrophoresis of nucleic acids. What features are similar and which are different? What is the purpose of both of these techniques?

B13: (4 pts) A small segment of a *primer* is shown to the right. This primer would be annealed to a larger template strand.

- i) Label the 3' end of the primer.
- ii) label the 5' end of the primer.
- iii) Circle a glycosidic bond.
- iv) Draw an arrow to the -OH group that would be used by the DNA polymerase to attach the next base to.



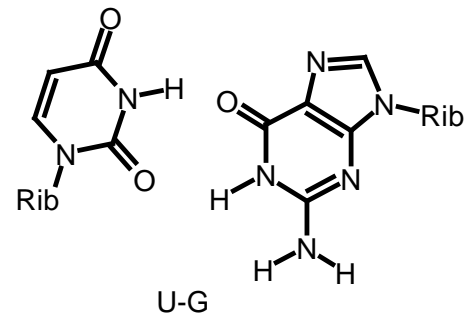
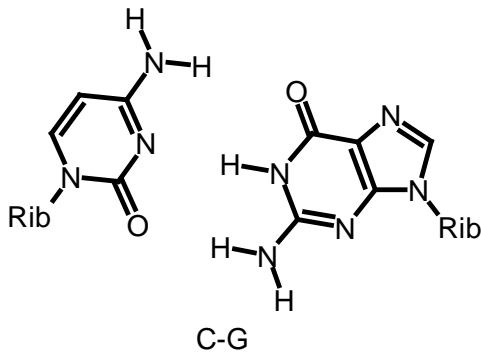
B14: (6 pts) Describe the role of hydrogen bonding in **one** of the following three situations. In the case of the first two choices your answer should include a description of the importance of this interaction in template directed polymer synthesis. In the case of the choice C, you should make a distinction between major and minor groove interactions and provide an example of an interaction between the protein and the nucleic acid. Be sure to indicate your choice.

A C-G basepair and a U-G pair have been provided to help illustrate your answer (i.e. draw some hydrogen bonds!) You need not use both in your answer.

Choice A: Formation of double stranded DNA.

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

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



B15 (13 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.

In this problem you **must** do **both** Parts i and ii. You have a choice within both parts.

Part i (5 pts): Please indicate the choice you are attempting.

Choice A: 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.

OR

Choice B:

Sequence of Vector:

```
--CGATTCGGATCCAA---- HIV-RT  gene to go here! -----GGCCCGATCGAATTC-----
--GCTAAGGCCTAGGTT-----CCGGGCTAGCTTAAG-----
```

Sequence of PCR product

```
GGATCC----- HIV-RT  gene -----CGATCG
CCTAGG-----GCTAGC
```

The upper sequence shows a region of a expression vector into which genes can be inserted for the purpose of obtaining recombinant protein. 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.

BamH1	G/GATCC
BglII	C/GATCG
EcoRI	G/AATTC
HaeIII	GG/CC

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.

Part ii (8 pts):

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.

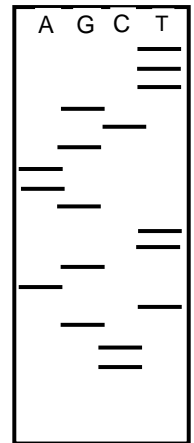
aggcttGGCTACGAGTCGGGTACCGTAGTTGAAGCG-----TAGTGCAAAATTTTGGGGCCCGATGTAGccggttaa
 tccgaaCCGATGCTCAGCCCATGGCATCAACTTCGC-----ATCACGTTTTAAAACCCCGGGCTACATGggcaattt
 GlyTyrGluSerGlyThrValValGluAla

Choice A: Describe the steps that would be required to generate the PCR product in choice B of part i 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. (4 pts for primer design, 4 points for showing PCR cycles)

OR

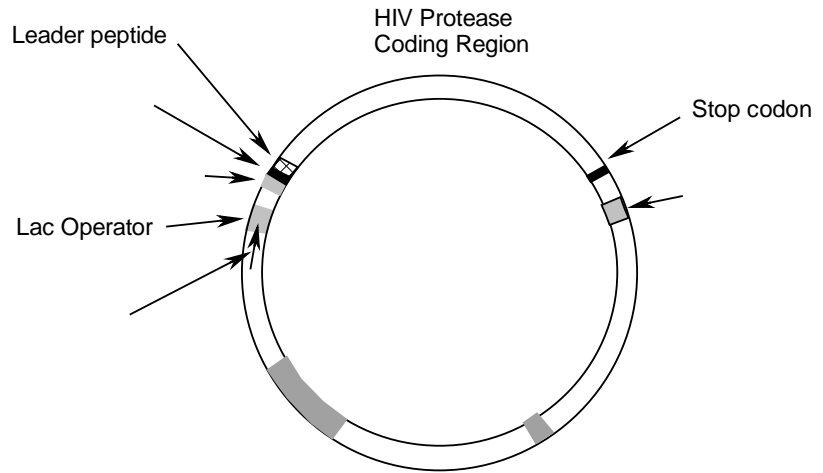
Choice B:

- i) The sequencing gel on the left was obtained from sequencing the HIV-RT DNA shown above. On the basis of this gel, what is the next amino acid following the Alanine residue? Briefly justify your answer. [A codon table can be found on the back of your formula sheet] (3 pts)
- ii) What was the sequence of the *primer* used in this sequencing reaction? (1 pts)
- iii) Briefly describe the reaction components that were used to generate the DNA fragments in the "T" lane. Which of these reagents or components are unique to this lane and which are common to all lanes? (4 pts)



B16: (10 pts) A diagram of the expression vector that produces recombinant HIV protease (or HIV-RT) is shown below:

- A mRNA Termination
- B Start Codon
- C Ribosome binding site
- D Promoter



i) The labels for four items (A-D) have been removed from the diagram. Place **two** of the four of these labels in their *correct* position on the diagram. With clear reference to the function of the two elements, justify your placement of both of them in the space below (1 pt for correct placement, 2 points for justification, 6 pts total)

A:

B:

C:

D:

ii) Select any **one** of the correctly labeled features of the expression vector (e.g. lac operator, leader peptide, stop codon) and describe its role in production of recombinant protein (4 pts)

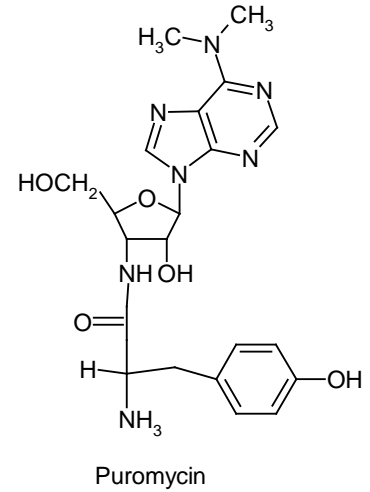
B17: (6 pts) Do **one** of the following two questions.

Choice A: Select **either** DNA synthesis **or** protein synthesis and discuss the molecular events that occur during the *initiation* process.

Choice B: Select **either** lagging strand DNA synthesis **or** 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.

Bonus Questions (4 pts each):

1. The antibiotic puromycin inhibits protein synthesis by termination of the growing peptide chain, explain how this occurs.



2. AZT is a drug that is used to treat HIV. What step of the HIV replication cycle do you think it inhibits? Why?

