1. Removal of the signal peptide from a protein that is translocated across a membrane is accomplished by
   a) Trypsin
   b) Signal Peptidase
   c) HIV protease
   d) the ribosome.

2. 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.

3. Initiation of mRNA synthesis involves
   a) displacement of the lac repressor from the DNA.
   b) binding of RNA polymerase and formation of the open complex.
   c) binding of RNA polymerase followed by binding of helicase.
   d) binding of RNA polymerase followed by binding of single stranded binding protein.

4. 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 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 proofreading exonuclease.

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

6. The sugar that forms a major component of nucleic acids (DNA, RNA) is
   a) Fructose
   b) Galactose
   c) Ribose
   d) Sucrose

7. A non-competitive inhibitor of an enzyme catalyzed reaction
   a) binds to the Michaelis complex (ES).
   b) decreases V_{max}.
   c) can act as an allosteric inhibitor.
   d) 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.
   a) K_I and K_M.
   b) V_{MAX}, K_M.
   c) K_M, V_{MAX}.
   d) V_{MAX}, K_I.

9. In a system that shows negative cooperativity for ligand binding:
   a) the binding of the first ligand has no effect on the binding of subsequent ligands.
   b) the binding of the first ligand raises the K_D for binding of the second.
   c) the binding of the first ligand raises the K_A for binding of the second.
   d) cannot bind more than one ligand.

10. The hormones, glucagon and epinephrine, stimulate glycogen breakdown to G-6-P
   a) directly, by binding to glycogen phosphorylase.
   b) indirectly, by first stimulating adenylate cyclase to make cAMP.
   c) only in the liver.
   e) using ATP as the phosphoryl donor.

11. Energy production involves which of the following
   a) Oxidation of organic molecules.
   b) Transport of electrons on organic molecules.
   c) Generation of proton gradients across membranes.
   d) All of the above.

12. If concentration of the reactants is higher than the equilibrium concentration then:
   a) The Gibbs free energy (ΔG) will be negative.
   b) More reactants will form.
   c) The Gibbs free energy will be positive.
   d) Both b and c are correct.

13. Which of the following would yield the most energy per gram when oxidized?
   a) glucose
   b) glycogen.
   c) fat.
   d) any monosaccharide.

14. The proton gradient and the membrane potential
   a) are sufficient, separately, to make ATP from ADP + Pi.
   b) are both required to make ATP.
   c) usually cancel one another since the system is at equilibrium.
   d) are unrelated to each other.

Part B:

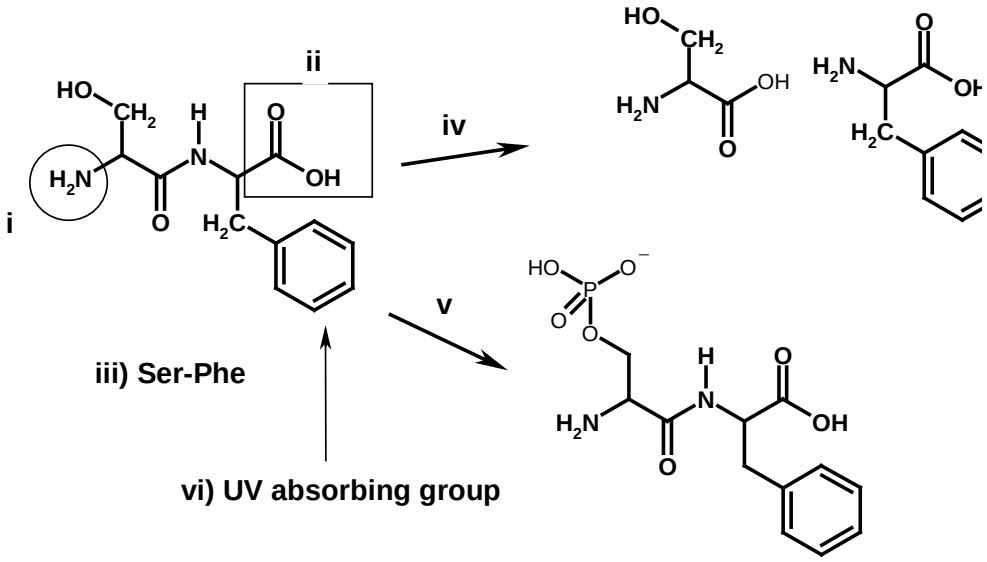
B1. (8 pts) Consider the following dipeptide:

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)

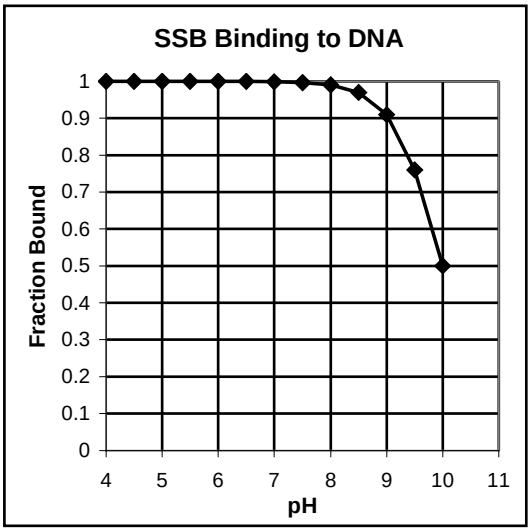
iv) Draw to the right of the arrow labeled 'iv' the products of the reaction if this peptide was treated with a protease. (2 pts)



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 mainchain atoms are shown, simply add the sidechain 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) Yes,

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)

Lysine, because DNA is negatively charged while lysine is positively charged. It can't be His, since there is no change in binding below pH 7.0.

ii) With which part of the DNA (base, sugar, phosphate) is SSB binding to? Justify your answer. (1 pt)

Phosphate

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

Assume fraction bound proportional to charge on Lysine. Therefore need to calculate [HA]=1/(1+R)

pH = pKa + log R

R=10, [HA]= 1/(1+10)= 0.09

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?

Ethanol affects the structure of the water, making non-polar groups more stable when exposed to water (hydrophobic effect). This is a ΔS° term.

Protein: large buried hydrophobic core, therefore ethanol has a large effect.

DNA: Little buried hydrophobic core, therefore small effect.

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).

Electrostatic effect, ΔH° term.

Like charges repel, high energy in DNA phosphate backbone is charged (-) and the two anti-parallel strands repel. Addition of salt shields the phosphate charges, therefore more stable giving a higher Tm.

In proteins, the surface charges do not have a large contribution to the melting temperature.

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.

The primary structure is the order of amino acids in the polypeptide chain, the secondary structure is the conformation of the mainchain atoms, such as an α-helix

Choice B: Tertiary structure versus quaternary structure.

The tertiary structure is the folded form of a single polypeptide chain, for example in myoglobin.

The quaternary structure is the structure of multiple folded chains, such as in hemoglobin, immunoglobulins, EcoR1, HIV protease.

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)

Stabilizing: Formation of H-bonds provide energy since H-bonds between the donor and acceptors are more stable, by about 5 kJ/mol than H-bonds to water. This is an enthalpic term.

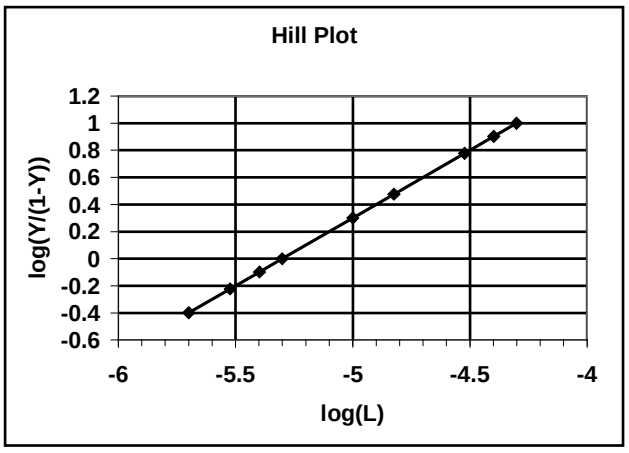
Destabilizing: Configurational entropy. Unfolded chains can assume many configurations and are more stable in the denatured from. ($S=R \ln W$)

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 10μM in the enzyme kinetic experiment.

L μM	Y
0	0
2	0.29
3	0.38
4	0.44
5	0.50
10	0.67
15	0.75
30	0.86
40	0.89
50	0.91

[S] (mM)	V (I=0)	V (I=10μM)	1/S	1/V (I=0)	1/V (I=10μM)
0	0.00	0			
5	16.67	6.25	0.200	0.060	0.160
10	25.00	10.00	0.100	0.040	0.100
15	30.00	12.50	0.067	0.033	0.080
30	37.50	16.67	0.033	0.027	0.060
50	41.67	19.23	0.020	0.024	0.052
100	45.45	21.74	0.010	0.022	0.046
200	47.62	23.25	.0050	0.021	0.043



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

When Y=0.5, [L]=K_D, therefore K_D=5 uM.

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

No, the slope of the Hill plot is 1. Also, when [L]=10 x K_D, Y=0.91, which is indicative of non-cooperative binding.

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).

Obtain K_I' from α':

α' si the ration of the Y-intercepts on the double reciprocal plot:

Y-int ([I]=0) =0.02

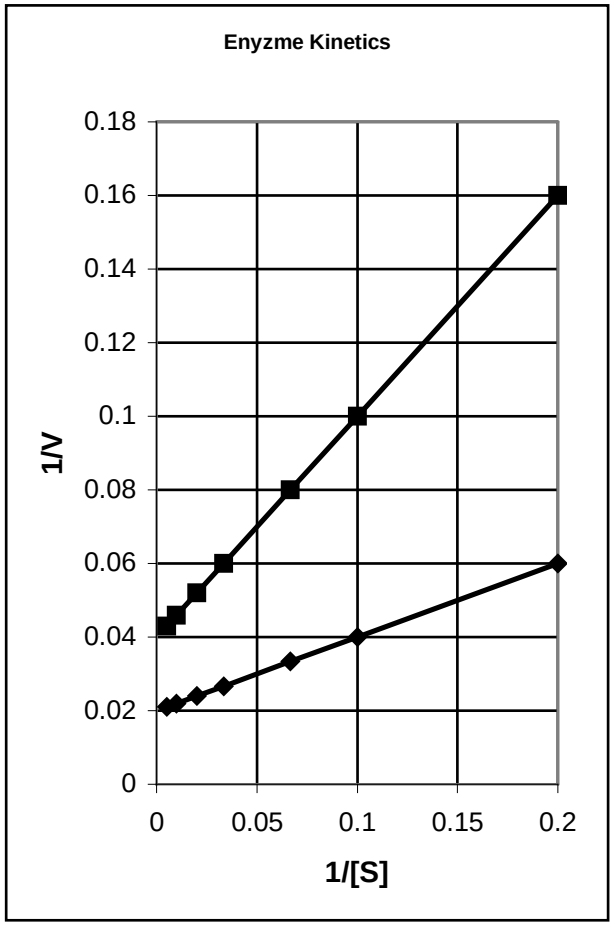
Y-int([I]>0)= 0.04

α'=.04/.02 = 3

$\alpha'=1+([I]/K_I')$

K_I'=10 μM

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)



K_I' refers to the binding to the ES complex. This is different than binding to the free enzyme (K_D). Since the substrate is present which may change the conformation of the F26P binding site.

B7: (10 pts)

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

Allosteric: binding of one ligand affects the affinity of another by changing the relative populatoins of the Tense (inactive) and R (active) states of the protein or enzyme.

Homotropic: same ligand n both cases.

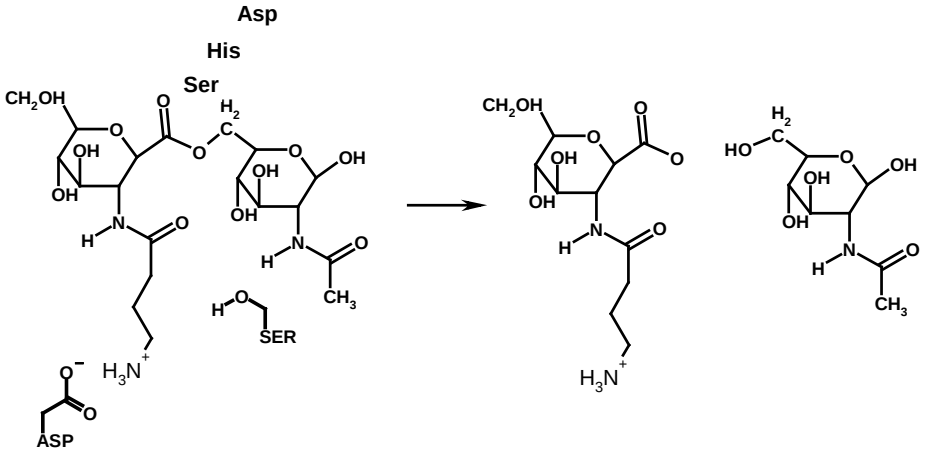
Heterotropic: one ligand controls the binding of a different ligand.

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).

Compound	**O_2**	**BPG (Bisphosphoglycerate)**	**F-2,6-bis phosphate (F26P)**	
Protein	**Hemoglobin**	**Hemoglobin**	**PFK**	**F16 bis phosphatase**
Control	positive cooperativity gives S-shaped binding curve that enhances delivery of oxygen to tissues	Negative cooperativity reduces oxygen affinity, but gives higher levels of delivery at higher altitudes	Activates glycolysis when glucose is plentiful	Inhibits gluconeogenesis when glucose is plentiful.

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)

Enzymes have amino acid side chains that interact specifically with functional groups on the substrate. These interactions include H-bonds, electrostatics, hydrophobic effect and van der waals forces. In the above example, an Asp residue forms an electrostatic interaction and a Serine residue forms a H-bond to the substrate.

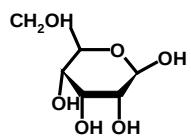
Choice B: Briefly discuss the *general* methods of rate enhancement by *any* enzyme (5 pts) For this particular enzyme, speculate, based on reaction mechanisms discussed in this course, as to the nature of the amino acids in the vicinity of the cleaved bond. You should sketch some amino acid side chains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

Enzymes lower the energy of the transition state by either direct interactions (ΔH effect) as well as placing functional groups close to in space to the substrate (ΔS effect). The above reaction is the hydrolysis of an ester, likely performed by a serine protease/esterase. Thus the enzyme provides a Ser, a His and an Asp residue (the catalytic triad) to enhance cleavage.

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 or name. 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	**I**
2. Product of anaerobic metabolism in humans.	**K**
3. Product of anerobic metabolism in yeast	**G**
4. Fatty acid	**J**
5. Triglyceride	**L**
6. Phosopholipid	**D**
7. Six carbon aldose.	**A**
8.Saccharide found in bacterial cell walls	**H**
9. Disaccharide	**F**
10. Electron carrier in the TCA cycle and fatty acid oxidation.	**B**
11. Electron carrier in electron transport chain.	**N**
12. Final electron acceptor in electron transport in most species, including humans.	**O**
13. Nucleotide normally found in DNA	**E**
14. Nucleotide normally found in RNA	**C**
15. Nucleotide that is used in DNA sequencing.	**M**

A



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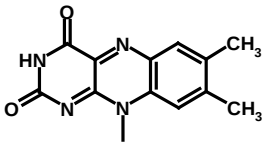

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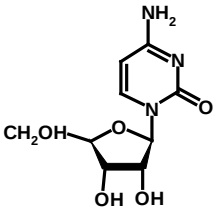

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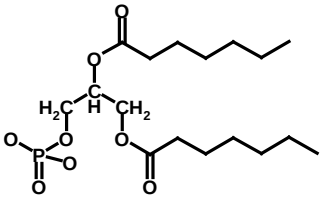

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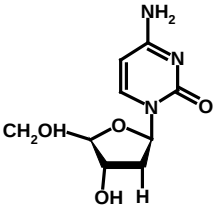

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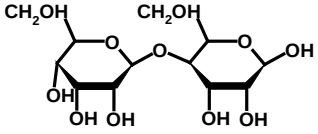

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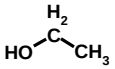

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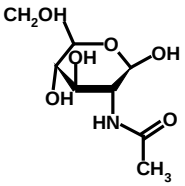

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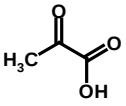

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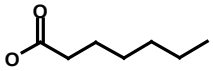

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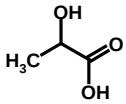

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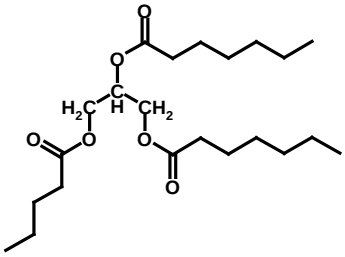

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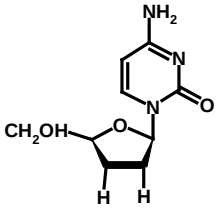

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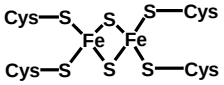


O

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^o? Illustrate your answer with an example of the use of either of these two thermodynamic parameters.

ΔG is the Gibbs free energy. It gives the amount of energy that can be extracted from a system when not at equilibrium. When $\Delta G<0$ the reaction is spontaneous in the direction written. For example, the proton gradient across the mitochondrial membrane can be used to generate energy.

ΔG^o is the energy required to convert one moel of reactants to one mole of product. It defines the equilibrium position of a reaction: $\Delta G^o = -RT \ln K_{EQ}$.

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 enthalphy associated with that transition.

$\ln K_{EQ} = (-\Delta H^o/R)(1/T) + (\Delta S^o/R)$. Plotting $\ln K_{EQ}$ versus (1/T) gives a straight line with a slope of $-\Delta H^o/R$.

Sharp transitions imply that the unfolding of protein, lipid, or DNA occurs over a narrow temperature range. Since the amount of unfolded is related to the equilibrium constant: $f_u = K_{EQ}/(1+K_{EQ})$. This implies that K_{EQ} changes rapidly over a narrow temperature range. Since the temperature dependence of K_{EQ} is proportional to ΔH^o, a large ΔH^o will cause K_{EQ} to change rapidly over a narrow temperature range.

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? You 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).

Since the insulin receptor is not-functional protein dephosphorylation won't occur and proteins are likely to remain phosphorylated all of the time. Since glycogen syntahse is inactive when phosphorylated, glycogen levels will be low.

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).

Insulin will lead to protein dephosphorylation, this will raise F26P levels since PFK-2 is active when dephosphorylated. F26P stimulates glycolysis which will enhance the conversion of glucose to acetyl CoA. Acetyl CoA can be used to synthesize fatty acids. Which are then stored in triglycerides (fat).

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).

Start with acomplex mixture, separate according to size, charge, salt solubility, affinity. For example, gel filtratoin columns have beads with pores in them, small proteins enter beads and elute later.

Specific activity = units of desired Enzyme/total protein. This should increase during a purification scheme.

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

SDS-gel denatures proteins into single chanis and the molecular weight of each chain can be obtained from the distance migrated. The quaternary structure is obtained using the fact that the native molecular weight from gel filtration is the sum of all subunits. For example, if a single band with a molecular weight of 10 kDa is obtained from SDS page, and a native molecular weight of 30 kDa is obtained from gel-filtration, the protein is a trimer.

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?

- Both give molecular weights
- Both rely on constant charge to mass ratio
- Both use sieving properties of gels
- In the case of SDS, the negative charges on the proteins have to be generated using SDS.

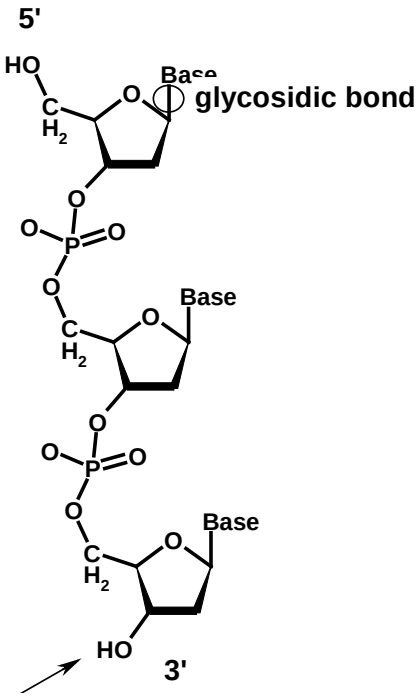
B13: (4 pts) A small segment of a *primer* is shown to the right. This primer would be annealed to a larger template strand.

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.

Watson-Crick hydrogen bonds are important for replication fidelity. When DNA is synthesized the correct base is added to the growing strand based on these hydrogen bonds.

A-T two hydrogen bonds

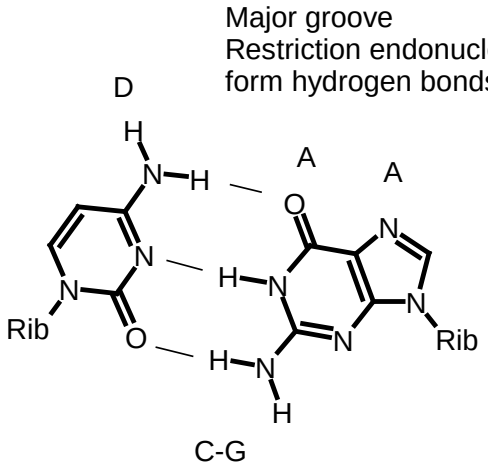
G-C three hydrogen bonds.

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

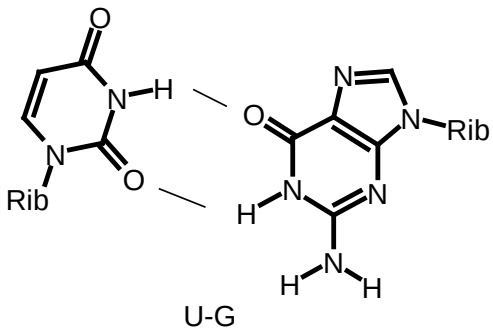
The anticodon on tRNA forms H-bonds with the triplet bases in the codon. Translating the mRNA information to an amino acid. In some codons tRNA interacts with all three base using normal Watson-Crick hydrogen bonds. However, in some codons the third base of the codon does not make normal Watson -Crick hydrogen bonds. Hydrogen bonds are formed but not in a different way (see diagram).

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

Restriction enzymes recognize specific sequences by forming H-bonds with DNA in the major grrove. Donor/acceptors on the protein interact with acceptors/donors on the edge of the bases, as indicated on the diagram.



Minor groove



Wobble base pair in tRNA-mRNA interactions

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.

1. Use Reverse transcriptase to convert single stranded viral RNA to DNA/RNA double stranded
2. Treat with RNase to digest RNA, giving single stranded DNA
3. Use DNA polymerase to generate double stranded DNA

Choice B:

Sequence of Vector:

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

Sequence of PCR product

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

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

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. 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.

The PCR fragment has a BamH1 site on one end and a BglII site on the the other. The vector has the same sites. After digestion of both DNAs with both enzyme you would have the following products

```
--CGATTCCG                GATCGAATTC-----  for the vector
--GCTAAGGCCTAG                CTTAAG-----
```

while the HIV RT gene would look like:

```
GATCC------------ HIV-RT gene -------------C
    G-------------------------------------GCTAG
```

The single stranded regions of both DNAs are complementary, thus they can form hydrogen bonds and can be ligated together to give the following product:

```
--CGATTCCGGATCC------------ HIV-RT gene -------------CGATCGAATTC-----Vector
--GCTAAGGCCTAGG----------------------------------GCTAGCTTAAG-----
```

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.

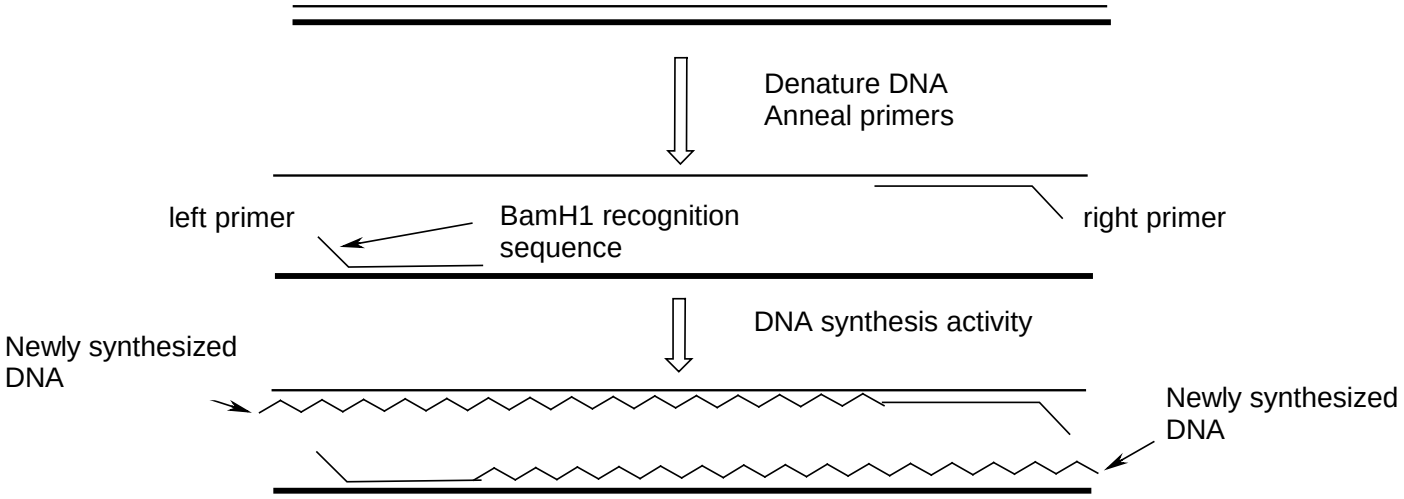
```
aggcttGGCTACGAGTCGGGTACCGTAGTTGAAGCG-------TAGTGCAAAATTTTGGGGCCCGATGTAGccgttaaa
tccgaaCCGATGCTCAGCCCATGGCATCAACTTCGC-------ATCACGTTTTAAAACCCCGGGCTACATGggcaattt
      GlyTyrGluSerGlyThrValValGluAla
```

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)

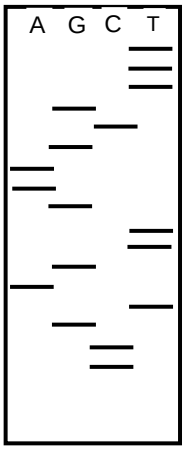
The left primer is: *GGATCC*GGCTACGAGTCGGG

The right primer is: *CGATCG*GTACATCGGGCCCC

The first cycle of the PCR reaction is:



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)

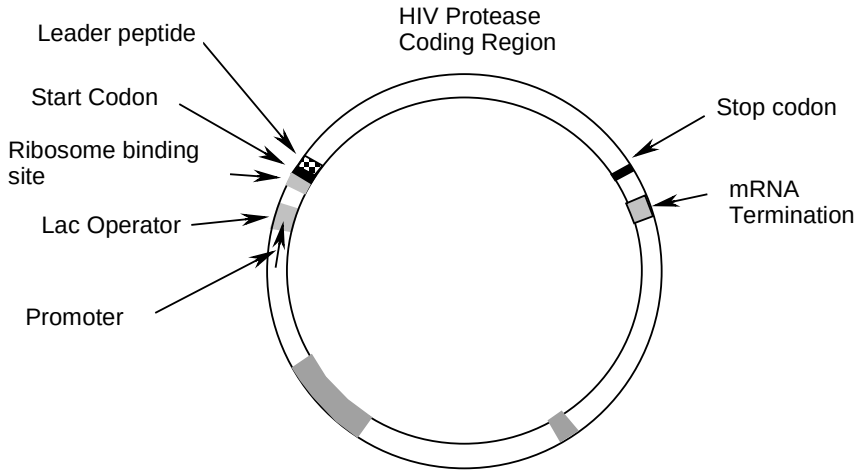
iii) Briefly describe the reaction components that were used to generate the DNA fragments in the "T" lane. Which of these reagents or components are unique to this lane and which are common to all lanes? (4 pts)

i. The Ala codon is followed by TTT, which codes for Phe.

ii. Since the sequencing gel begins with the sequence CCGT, the primer must have the sequence immediately to the left of the this sequence: GGCTACGAGTCGGGTA

iii. The common reagents are dNTPs, primer, template, and DNA polymerase. The unique component is dideoxyT (ddT)

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



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: mRNA Termination: The signals for mRNA termination have to follow the gene, otherwise mRNA that encodes the gene would not be synthesized.

B: Start codon: This has to be the very first amino acid in the protein, and therefore must occur before the leader peptide.

C: Ribosome Binding site: This need to be before the start codon so that it will direct the mRNA to bind on the correct location of the ribosome.

D: Promoter: This sequence element has to be first, since mRNA has to include the ribosome binding site.

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)

lac operator: ON/OFF switch: lac repressor binds to lac operator, the addition of IPTG induces the repressor to come off of the DNA, allowing production of mRNA for the recombinant protein.

leader peptide: targets the expressed protein for secretion outside of the cell.

Stop codon: Ends protein synthesis on the ribosome.

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.

DNA Replication:

i. Specific proteins bind to a specific DNA sequence, the origin of replication.
ii. The DNA is opened up and Single stranded binding protein (SSB) coats the DNA to prevent it from reannealing.
iii. Helicase and Gyrase are loaded on the DNA,.
iv. Helicase activity moves the replication fork, opening up more single stranded DNA for template
v. Gyrase removes the overtightening of the DNA.

Protein Synthesis:

i. mRNA binds to 30 S subunit by virtue of base pairing between the SD sequence and the ribosomal RNA
ii. tRNA-fMet and 50S then bind,
iii. In the inatact initiation complex, the tRNA-fMet is in the P site.

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.

Lagging Strand:

i. Pol III synthesizes DNA from RNA primer until it reaches next RNA primer
ii. RNA primer is removed by Pol I 5'-->3' exonuclease activity
iii. The resultant single strand 'gap' is filled in by PolI 5'-->3' synthesis activity
iv. The remaining single stranded break is fixed by DNA ligase

Protein Elongation:

i. Charged tRNA binds to A site due to basepairing with mRNA codon
ii. Amino acid in A site attacks amino acyl -bond on peptide tRNA in the P site, moving the peptide to the A site.
iii. The ribosome translocates. The uncharged tRNA in the P site moves to the E-site and the peptide-tRNA moves from the A site to the P site
iv. The uncharged tRNA exits from the E site.

Bonus Questions:

You are welcome to email me your answer and I will tell you if it is correct.