**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, $\Delta G^o$, 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, $\Delta G$, will be positive.
  b) More reactants will form.
  c) The standard free energy, $\Delta G^o$, 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 **(+1 pt)**
  c) show a Hill coefficient ($n_H$) of *n*. **(+1 pt)**
  **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 (+2 pts)**
  **c) $K_M$, substrate binding, $V_{MAX}$, chemical step (+2 pts)**
  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 **(+1 pt)**
  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 $OH^-$ **(+1 pt)**
    b) DNA residues are linked by 3'→5' phosphodiester bonds; RNA is 2'→5' linked.
    c) DNA has deoxyribose residues; RNA has ribose residues **(+1 pt)**
    **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:



**peptide bond**

V
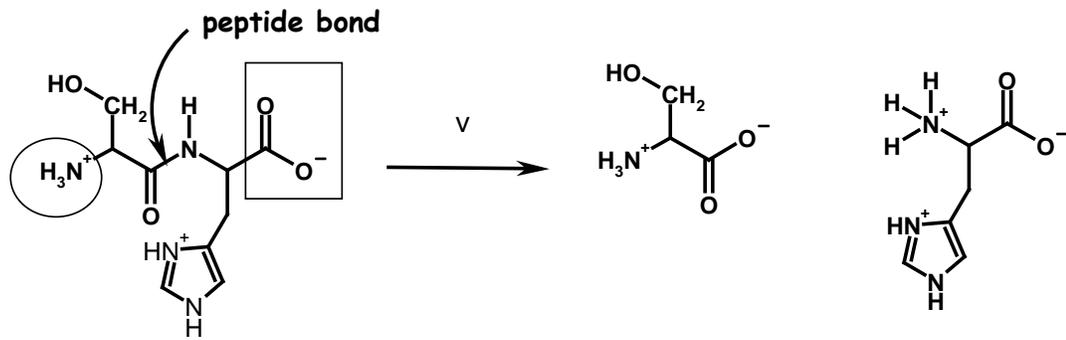
**Name of peptide:** Ser - His

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).

**The amino terminus is protonated since its pKa=10. The carboxy-terminus is deprotonated since its pKa=4.0. Lastly, the His sidechain is 10% protonated since its pKa=6.0.**

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)
**The first residue in the name is the amino terminal one. This peptide is Ser-His**

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) **See drawing, in addition a water molecule is required to hydrolyze the peptide bond.**

vi) Identify the bond that does *not* freely rotate, what is the name for this bond? Why does this bond not rotate? (2 pts) **The peptide bond, it has partial double bond character and is therefore not free to rotate.**

**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.

**At pH=4.0, half of the enzyme is active. Therefore the pKa of this group is 4.0. (+2 pts)**

**Therefore the sidechain is either an Aspartic acid (Asp) or glutamic acid (Glu). (+2 pts)**



**Enzyme Activity**

% Activity

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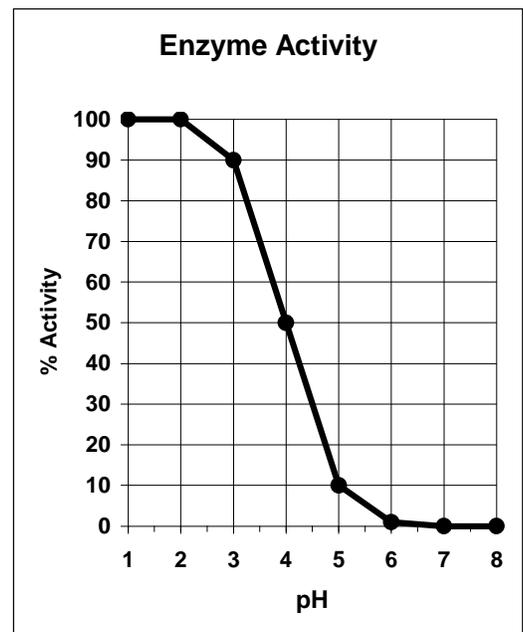
**B3.** (8 pts) A partial list of energetic terms or 'forces' that play a central role in Biochemistry is given below:

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 ($\Delta H$) or entropic ($\Delta S$).

> **1. Hydrogen bond.  Bond formed between a proton attached to an electronegative atom and another electronegative atom, i.e.  X-H    Y, were X and Y are electronegative.  This is enthalpic.**

> **2. Charge-charge interactions.  Like charges will repel each other, opposite charges will attract. This is enthalphic.**

> **3. Van der Waals:  Force between any matter - due to dipole-induced dipole and induced dipole-induced dipole interactions. Enthalphic.**

> **4. Configurational entropy: Related to the disorder of the system S=RlnW, where W is the number of conformations the system can assume. This is entropic.**

**Grading: +2 for reasonable description, +2 for correct thermodynamic contribution (enthalpy versus entropy).**

**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 | **Not important, there are few charges on the surface of proteins, and most are balanced.** | **Very destabilizing to the double helix because of electrostatic repulsion between the two phosphate backbones.** |
| Van der Waals | **Moderately important, the tight packing in the core of folded proteins optimizing van der Waals interactions.** | **Very important, the close contact of the $\pi$ orbitals on the bases gives rise to strong van der waals interactions.** |
| Configurational Entropy | **Very destabilizing to the folded state because the unfolded state has a much higher entropy.** | **Very destabilizing to the folded state because the unfolded state has a much higher entropy.** |

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

**The hydrophobic effect is entropic in nature.**

**When non-polar groups, such as non-polar amino acid sidechains or lipid acyl chains, are exposed to water, they order the water around them, reducing the entropy of the water, which is unfavorable (+3 pts)**

**When the non-polar group is buried in the core of the protein or bilayer, the water is released. The released water has high entropy, which is favourable (+3 pts)**

**Therefore the hydrophobic effect stabilizes the folded form of proteins and bilayers (+2 pts)**

**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).

**Relaxed conformation of the protein is the active form.**

**Tense conformation of the protein is the inactive form.**

**Allosteric activators stabilize the active relaxed (R) form.**

**Allosteric inhibitors stabilize the inactive tense (T) form.**

**Homotropic - the ligand affects the binding of other ligands that are the same.**

**Heterotropic - the ligand affects 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 (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).

| 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. |

**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.

> **Primary structure is the sequence of amino acids.**

> **Secondary is the conformation of the backbone in space (e.g. α-helix, β-sheet.**

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

> **Tertiary is the conformation of the backbone and sidechain atoms for a single chain.**
> **Quaternary is the conformation of the backbone and sidechain atoms for multiple chains.**

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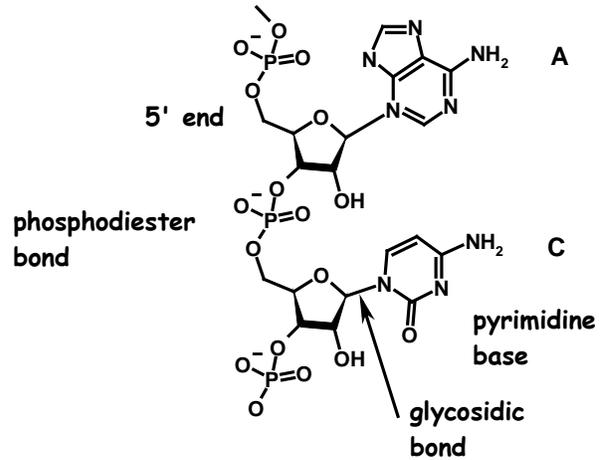
    What is the sequence of this DNA fragment? (1 pt)

    **Beginning at the 5' end: AC**



**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.

**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.**

Major groove
Restriction endonucleases would form hydrogen bonds with the indicated groups



C-G

Minor groove

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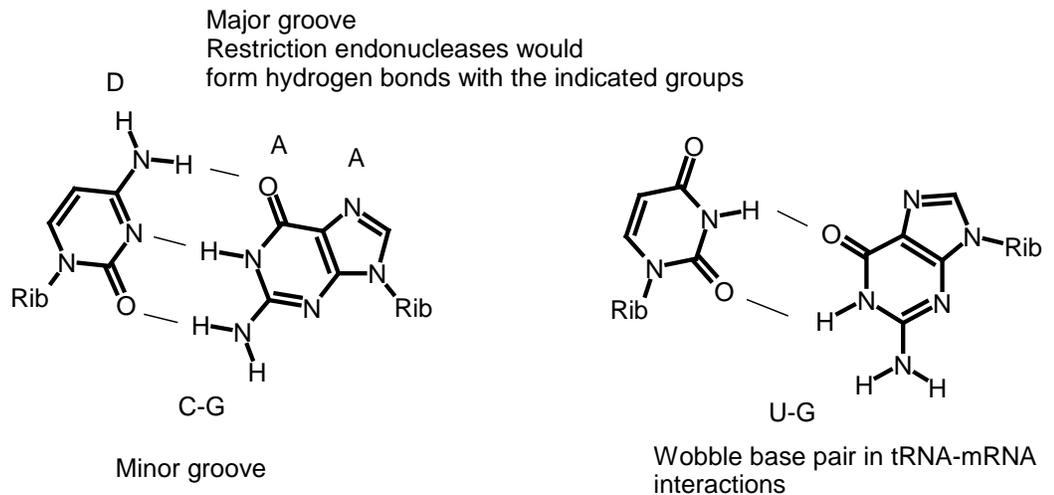
**B10** (10 pts). Discuss **two of the following four** features of enzyme catalyzed reactions. Indicate how these features are important for catalysis or inhibition and provide a specific example of this feature in an existing enzyme. In your examples, you can use any enzyme you like (including ones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

i) Transition state stabilization.

**Enzymes increase the rate of the reaction by stabilizing the high energy transition state. This is done in two ways, by forming enthalpic interactions that are specific for the structure of the transition state. An example of this is the oxy-anion hole in serine proteases.**

**In addition, bring the correct chemical groups together to do catalysis reduces the entropy required to reach the transition state. This method is used by virtually all enzymes.**

ii) Substrate specificity.

**Enzymes have amino acid sidechains in their active sites that are specific for particular substrates. Examples include:**

**Trypsin: Asp residue to interact with the positively charged sidechain on the substrate.**
**Chymotrypsin: non-polar binding pocket.**
**HIV protease: Val82 interacts with non-polar substrates.**
**Lysozyme: specific interaction with N-acetyl groups on polysaccharides in bacteria cell walls.**

iii) Chemical mechanism.

**Specific amino acid sidechains play a role in actually performing the chemistry.**

**Serine proteases - catalytic triad**
**HIV protease - pair of Asp residues**
**Glyceraldehyde dehydrogenase - active site cysteine residue.**

iv) Competitive & non-competitive inhibitors.

**Competitive inhibitors bind in the active site and are chemically similar to the true substrate. They inhibit by simply block the entry of the substrate into the active site.**

**Non-competitive inhibitors bind elsewhere and do not resemble the substrates. They change the conformation of the active site, reducing activity.**

**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 | L |
| 2. Product of anaerobic metabolism in humans. | N |
| 3. Product of anerobic metabolism in yeast | G |
| 4. Unsaturated fatty acid | I or J |
| 5. Triglyceride | O |
| 6. Phosopholipid | D |
| 7. Six carbon ketose | R |
| 8.Saccharide found in bacterial cell walls | K |
| 9. Disaccharide from glycogen or starch. | S |
| 10. Electron carrier in the TCA cycle and fatty acid oxidation. | B |
| 11. Electron carrier in electron transport chain. | Q or T |
| 12. Final electron acceptor in electron transport in most species, including humans. | H |
| 13. Nucleotide normally found in DNA | E |
| 14. Nucleotide normally found in RNA | C |
| 15. Nucleotide that is used in DNA sequencing. | P |
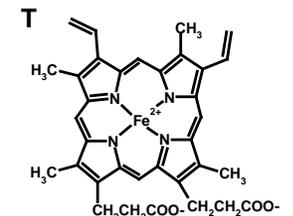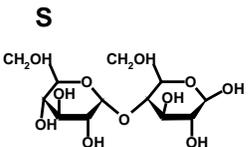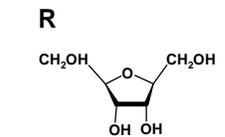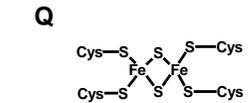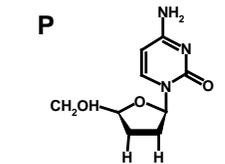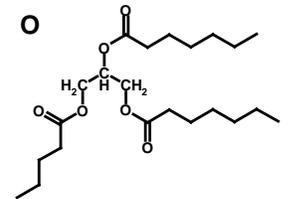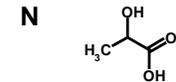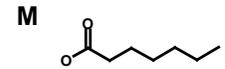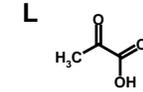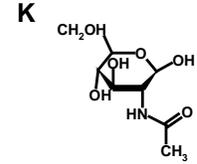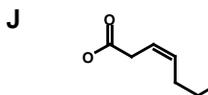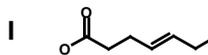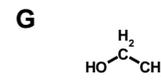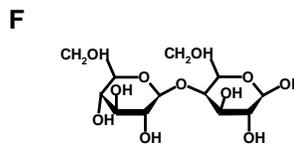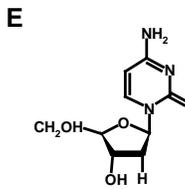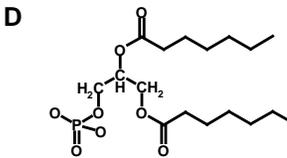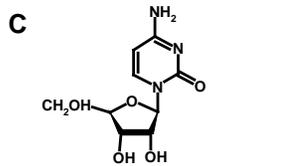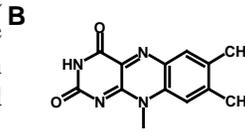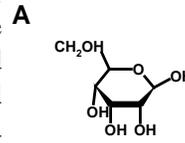
**B12.** (10 pts) Please do one of the following three choices. Please indicate the choice that you are answering.

**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.

  Epinephrine binds to its receptor, a G-protein coupled receptor. This leads to activation of adenyl cyclase, elevation cAMP levels. This leads to phosphorylation of enzymes. Normally glycogen phosphorylase would be phosphorylated and become active. But since the enzyme is missing, it is not possible for the liver to release glucose.

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

  **This person should be a low carbohydrate, high fat diet. Otherwise, any excess carbohydrates will be converted to glycogen and will not be released. The glycogen will accumulate, produces dysfunction of the liver.**

**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.

  **The simplest example is the response to epinephrine. In this case the liver will make glucose by gluconeogenesis to send to the muscle for energy production. The muscle must do the opposite, since it will need ATP to generate movement. The two enzymes that are controlled in glycolysis and gluconeogenesis, PFK and F1,6 bis phosphatase, must be regulated by F2,6P in an opposite fashion.**

  **In the liver: PFK is activated by F2,6 P levels**
  **In the muscle: PFK is inhibited by F2,6 P levels**
  **(in actual fact, muscle PFK is activated directly by cAMP)**

  **You would expect regulation by energy levels to be the same in both tissues, as neither tissue will undergo glycolysis if there is plenty of ATP.**

**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 "$CO_2$".
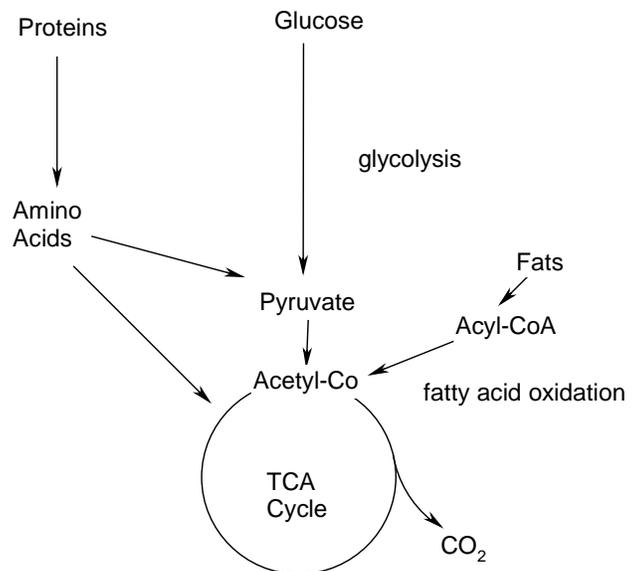
**B13.** (10 Pts) Please do **one** of the following two questions. Please indicate your choice.

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

**Use SDS-gel electrophoresis in the presence of β-mercapto ethanol to get subunit molecular weight.**

**Use gel-filtration to get native molecular weight.**

**Generate a model of quaternary structure that is consistent with both**

**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 |

- **By gel filtration chromatography, the sizes of the proteins are different. The gel filtration resin contains small pores. The smaller protein (A in this case) will enter the pores and will take longer to move down the column.**

<p style="text-align:center">**OR**</p>

- **Ion exchange chromatography.  At pH=7.0 both protein have the same charge (0). But at pH=3, protein A will have a charge of ~+5 and protein B will have a charge of ~+2.  Therefore they will separate on a cation exchange column. The resin in a cation exchange column will have a negatively charged group, causing positively charged protein to bind.**

**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.
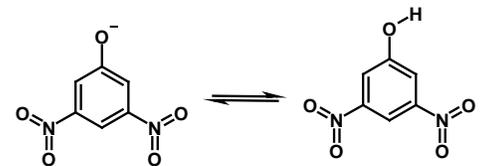


   i) Explain why this compound is able to move protons across a biological membrane (2 pts).

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

   **i) When protonated, it is no longer charged. Although protonated DNP is polar, it can still cross the membrane.**

   **ii) The energy that is used to pump protons across the inner mitochondrial membrane will be dissipated by the DNP, thus instead of going towards ATP synthesis, it will just generate heat.**

**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?

**Yes. The pumped Na⁺ ions would generate a membrane potential, or voltage difference, across the membrane. The inner membrane space would be positively charged, therefore the membrane potential would be negative. Since $\Delta G = RT \ln [H^+_{IN}]/[H^+_{OUT}] + ZF\Delta\psi$, the Gibbs free energy for proton transport would be negative, i.e. spontaneous flow into the mitochondrial matrix. As the protons flowed throught ATP synthase, it would be possible to generate ATP.**

**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).
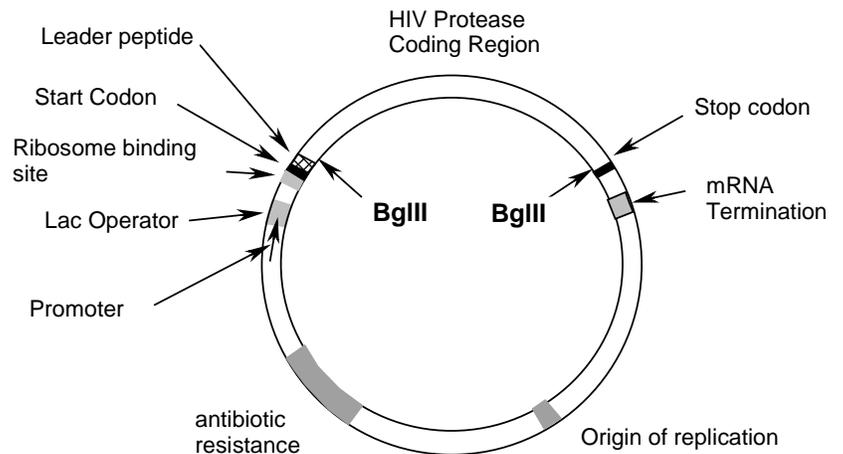


i.   Origin of replication: **This insures that the plasmid will be replicated in the bacteria.**

ii.   Antibiotic resistance gene: **This section of DNA produces an enzyme or protein that makes the bacteria resistant to an antibiotic. This insures that the bacterial will retain the plasmid after it is placed into them.  If they lose the plasmid, they die.**

iii.   Promoter: **RNA polymerase binds here and initiates the production of mRNA**

iv.   Lac operator: **The lac repressor binds at this site , preventing the production of mRNA, until the lac repressor is removed from the DNA by binding to lactose or IPTG.**

v.   Ribosome binding site: **Binds the mRNA to the 30S ribosome subunit during initiation of protein synthesis.  Defines the reading frame by positioning the first codon, AUG, at the right place on the ribosome.**

vi.   Start codon: **Signals the ribose where the protein coding begins**.

vii.   Leader peptide: **Will  cause export of the protein out of the cell.**

viii.   Stop codon: **Termination signal for protein synthesis.**

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**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)

**You would get two fragments, one large and one small. The ends the fragments would look like:**

```
---CGATCG-----------CGATCG---          ---C       GATCG-----------C      GATCG---
---GCTAGC-----------GCTAGC---          ---GCTAG        C-----------GCTAG      C---
     (Before cutting with Bgl II)                    (After cutting with Bgl II)
```
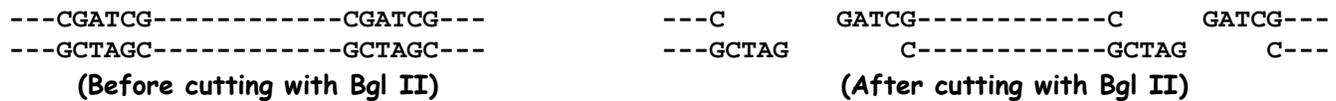
**B16.** (8 pts) Select **either** lagging strand DNA synthesis **or** the elongation of proteins during protein synthesis and discuss the events that lead to template directed polymer synthesis. In the case of DNA, you should describe the process where by ~1000 bases are synthesized at a time, while 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.

**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.

**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)

**The $K_D$ is the ligand concentration to give Y=0.5. This occurs when [SSB]=1 uM.**

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)
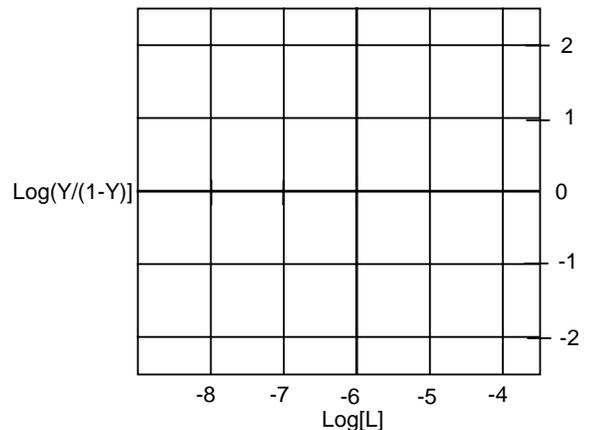


**The Hill coefficient is obtained from the slope when the line crosses the x-axis can be calculated using the above numbers:**
**$\Delta Y/\Delta X$ = (1.70-0.0)(-5 - (-6)) = +1.7**

**Since this is greater than 1, the binding shows positive cooperativity.**

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

**Using the same approach as part i, $K_D$ = 10 μM**

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

**Since the $K_D$ at higher salt is larger, the binding has gotten weaker.**

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)

**The additional salt has weakened an electrostatic interaction, therefore the SSB likely interacts with the phosphate.**

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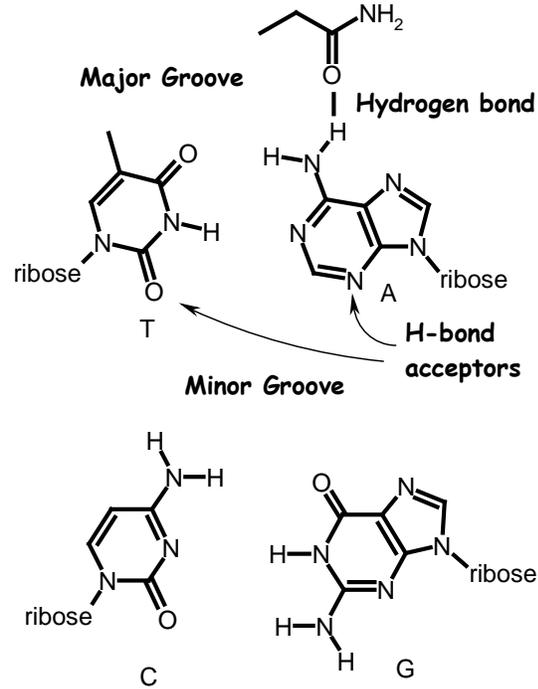
i) Label the major and minor grooves of the TA basepair and draw any hydrogen bonds that might occur between the protein and the DNA (2 pts).

**(See diagram)**

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

> **In the minor groove, both T and A present a hydrogen bond acceptor. Since both present the same functional group, an T:A basepair is the identical to a A:T basepair when viewed from the minor groove. In the major groove, the T presents a donor (the keto oxygen) while the A provides both a donor (NH$_2$) group and a acceptor (ring nitrogen).**

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

$\Delta G^o = $ -RT $\ln K_{EQ} = 2.5 \times 23 = $ -57.5 kJ/mol

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).

> **The G base cannot donate a hydrogen bond to the Asn residue of the protein. When the protein is not bound to the DNA, it will form an H-bond to water. When the protein binds, the H-bond to water is broken, but not reformed, costing 20 kJ/mol.**

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).

**The equilibrium constant for binding to GACC is: $K_{EQ} = e^{-\Delta G/RT} = e^{+37.5/2.5} = e^{+15} = 3.3 \times 10^6$, therefore the $K_D$ is 0.3 μM**

**The equilibrium constant for binding to GATC is $10^{10}$, or a $K_D$ of $10^{-4}$ μM.**

**Y=[L]/($K_D$+[L])**
**In the case of GACC, Y = 1μM / (1μM+0.3μM) = 0.76**

**In the case of GATC, Y = 1μM / (1μM + $10^{-4}$ μM) = 1.00**

**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 $\rightarrow$ / $\leftarrow$ T-C-G-A-C-C A-G-C-T-C-C | A-G-C-T-G-G + T-C-G-A-G-G T-C-G-A-C-C A-G-C-T-C-C | $10^o$ C |
| 2 | A-G-C-T-G-G T-C-G-A-G-G $\rightarrow$ / $\leftarrow$ T-C-G-A-C-C-A G-C-T-C-C | A-G-C-T-G-G + T-C-G-A-G-G T-C-G-A-C-C-A G-C-T-C-C | $16^o$ C |
| 3 | A-G-C-T-G G-T-C-G-A-G-G $\rightarrow$ / $\leftarrow$ T-C-G-A-C-C A-G-C-T-C-C | A-G-C-T-G + G-T-C-G-A-G-G T-C-G-A-C-C 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).

- **Molecule one has no single stranded overlap, and therefore the reactant is held together by pi-stacking.**
- **Molecule two has an A-T basepair, adding two hydrogen bonds to the stability of the reactant.**
- **Molecule three has a G-C basepair, which has three hydrogen bonds, plus the pi-stacking.**

- **Therefore molecule 1 should be the least stable, and 3 the most stable.**

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

**Increasing the salt concentration would reduce electrostatic repulsion between the chains, therefore the reactants would be more stable, and the melting temperature would be increased.**

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

**At T=10°C there is an equal population of both species, therefore $\Delta G^o$ = 0 at this temperature. Therefore:**
**$\Delta S^o =\Delta H^o$ /T = 10,000/(283)=35.3 J/mol-deg.**

**At T=20°C, $\Delta G^o$ = 10,000 - 35 × 293 = 10,000 - 10,353 = -353 J/mol**

**$K_{EQ}$ = $e^{-\Delta G/RT}$ = $e^{353/(8.3 \times 293)}$ = 1.156.**

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).

**DNA ligase requires that the two DNA molecules that are to be joined are together. Therefore reaction 1 would be slower since greater than 50% of those molecules will be separated at 20 C ($K_{eq}$ = 1.156). In the case of reaction 3, only 50% are separated at this temperature ($K_{EQ}$ = 1)**

**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).

**Isolate the viral RNA, and using a primer and reverse transcriptase, make a single stranded DNA copy (the reverse transcriptase digests the RNA as it copies it to DNA.**

**Use a primer and a DNA polymerase to convert the ssDNA to dsDNA.**

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).

**The left primer would be: CGATCGGGCTACGAGTCGGG**

**The right primer would be: CGATCGGTACATCGGGCCC**

**The bold typeface indicates the new DNA that was added from the primers - this is the BglII site required for inserting the PCR product into the expression vector. The non-bold typeface defines the region to be amplified - the starting and ending of the RT-gene.**

**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)

The plasmid and the PCR product would be digested with Bgl II, the DNA mixed and cooled to allow the "sticky ends" to hydrogen bond. The DNA would then be treated with DNA ligase to form the phosphodiester linkage between the DNA fragments.

```
---C        GATCG---RT-Gene--C       GATCG---
---GCTAG         C------------GCTAG      C---
     (After cutting with Bgl II)


---CGATCG---RT-Gene--CGATCG---
---GCTAGC-----------GCTAGC---
     (After cooling & DNA ligase)
```

> **The only complication is that the PCR product can be inserted into the vector in two ways. One way will place the DNA that encodes the amino terminal sequence of the RT protein next to the promoter. The resultant mRNA transcript will give the correct protein when translated on the ribosome.**
>
> **The fragment can also be inserted such that the DNA that encodes the carboxy terminus of the protein is next to the promoter. This will produce a mRNA that is "backwards" and will produce a nonsense amino acid sequence.**
>
> **The easiest way to tell the two apart is the orientation that give the correct protein is the correct one! Alternatively, the positions of other restriction sites in the RT-gene, relative to restriction sties in the plasmid, may allow one to distinguish the two orientation.**
>
> **(Recognition of this problem  +1 pt)**

**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)
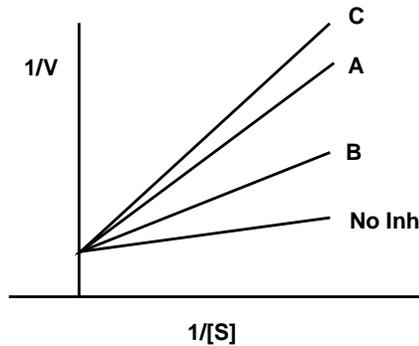
> **The ratio of the slopes ([I]>0 to [I]=0) gives $\alpha$.  Since $\alpha=1+([I]/K_I)$, the larger the slope, the smaller $K_I$. Therefore C has the smallest $K_I$, followed by A, and then by B.**

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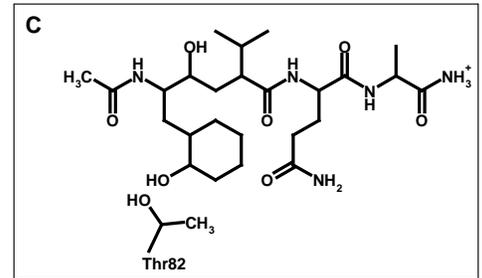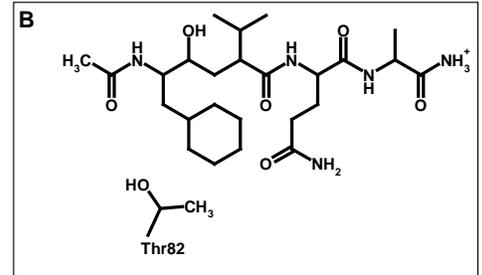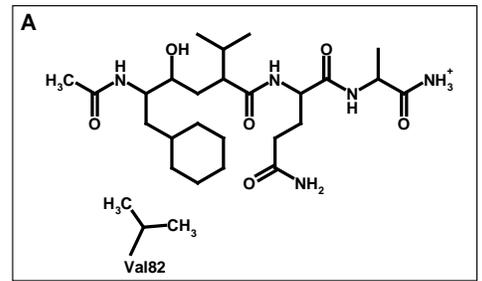
ii) Explain the molecular basis for the relative order of the $K_I$ values with clear reference to the interaction between the enzyme and the inhibitor (6 pts).

> **A small $K_I$ means tighter binding, or a stronger interaction. In C, the -OH of Thr probably makes a hydrogen bond to the OH group on the drug. This interaction is apparently stronger than the non-polar interaction between one of the methyl groups on Val and the cyclohexane ring.  Inhibitor B binds the poorest, because of the polar group on the Thr has no counterpart on the drug.**

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)

> **The possible codons for Thr are: ACX, where X is any base.  The codon for Val (GTC) would be replaced by ACX. The example to the right shows ACC.**