

This exam consists of **12** pages. Be sure that you have all of the pages. There are a total of 253 points on the exam, budget about 1 min/2 points. There are two bonus questions worth 5 points.

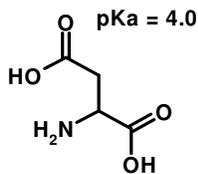
**Part A: Multiple Choice 2 pts each - total of 14**

- DNA absorbs UV light at \_\_\_\_ nm and proteins absorb at \_\_\_\_ nm.
  - 260, 260
  - 260, 280**
  - 280, 260
  - 280, 280
- Which amino acid has a sidechain that often stabilizes extracellular proteins by the formation of crosslinks in the protein chains?
  - Alanine
  - Cysteine**
  - Methionine
  - Tyrosine
- In both hemoglobin and myoglobin the oxygen is bound to.
  - the iron atom in the heme group.**
  - the nitrogen atoms on the heme.
  - a hydrophobic pocket in the protein.
  - the surface of the protein.
- During any successful purification scheme, you would expect
  - the number of different proteins in the sample to decrease.
  - the specific activity to decrease.
  - the specific activity to increase.
  - both a and c are correct.**
- Cellulose consists of \_\_\_\_\_ in \_\_\_\_\_ linkages, while glycogen contains \_\_\_\_\_ in \_\_\_\_\_ linkages.
  - glucose,  $\beta$ (1-4), fructose,  $\alpha$ (1-4).
  - glucose,  $\beta$ (1-4) glucose,  $\alpha$  (1-4).**
  - glucose,  $\alpha$ (1-4), glucose,  $\beta$  (1-4).
  - glucose,  $\alpha$ (1-6), glucose,  $\alpha$  (1-4).
- $T_M$  refers to:
  - the temperature at which 50% of a DNA molecule is denatured
  - the temperature at which 50% of a protein molecule is denatured
  - the temperature at which membranes are 50% fluid.
  - all of the above.**
- RNA is more easily hydrolyzed than DNA in basic solutions because:
  - The uracil in RNA can function as a catalyst.
  - The 2'-OH group in RNA can act as a nucleophile.**
  - The linkages between the bases are intrinsically weaker in RNA.
  - The deoxyribose can deprotonate to neutralize the base.

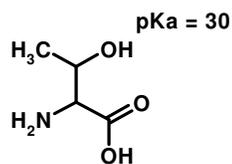
A1 :	_____ / 14
B1 :	_____ / 7
B2 :	_____ / 10
B3 :	_____ / 8
B4 :	_____ / 5
B5 :	_____ / 15
B6 :	_____ / 8
B7 :	_____ / 12
B8 :	_____ / 18
B9 :	_____ / 10
B10 :	_____ / 6
B11 :	_____ / 41
B12 :	_____ / 5
B13 :	_____ / 8
B14 :	_____ / 10
B15 :	_____ / 8
B16 :	_____ / 8
B17 :	_____ / 10
B18 :	_____ / 8
B19 :	_____ / 28
B20 :	_____ / 12
Bonus	_____ / 5
<b>TOTAL</b>	_____ / 251

**Part B:**

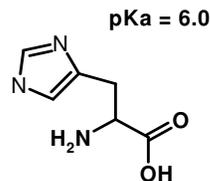
1. (7 pts) Pick *one* of the following four amino acids.



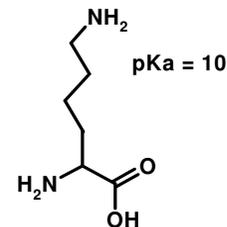
Aspartic Acid



Threonine



Histidine



Lysine.

i) Name the amino acid that you have chosen (1 pt).

ii) Draw any one of the above amino acids in its correct ionic form (*all* ionizable groups), assuming that the pH of the solution is 6 [indicate the protonation state on the above diagram] (4 pts).

- All would have the mainchain amino group protonated.
- All would have the mainchain carboxyl group deprotonated.
  - Aspartic acid - the pH  $\gg$  pKa, so the sidechain is deprotonated.
  - Threonine - the pH  $\lll$  pKa, so the sidechain is protonated.
  - Histidine - The pH = pKa, so the sidechain would be  $\frac{1}{2}$  protonated.
  - Lysine - the pH < pKa so the sidechain amino group is protonated.

iii) Provide a *brief* explanation for the pK<sub>a</sub> value of the side chain for *any* of the above amino acids. Feel free to justify your answer by comparing one amino acid to another. (2 pts).

Aspartic acid: It is a strong acid because the negative charge formed on deprotonation can delocalize over the COO group. Alternative answer - it's similar to the mainchain carboxylic acid so it's pKa should be similar.

Threonine: It is a very weak acid because the negative charge cannot delocalize.

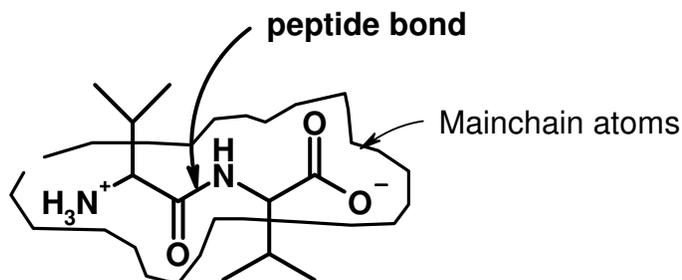
Histidine: It is a stronger acid than Lys because of the aromatic ring.

Lysine: It is similar to the mainchain amino group, whose pKa is close to 10.

2. (10 pts) Draw the structure of a dipeptide that would preferentially insert into the center of a lipid bilayer (5 pts for the drawing).

The two side chains must be non-polar, best if neither are Ala (+ 2)

Structure must be correct, peptide bond in trans (+3 pts)

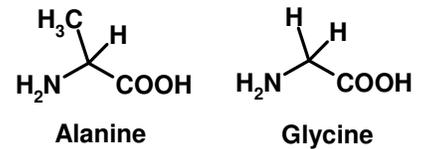


i) Label a peptide bond on your diagram (3 pts).

ii) Indicated the "mainchain" (backbone) atoms (2 pts)



6. (8 pts) A protein has an alanine residue in its core. Briefly describe how the enthalpy *or* entropy associated with unfolding (i.e. the direction of the reaction is assumed to be Native  $\rightarrow$  Unfolded) will change if this Ala is replaced by a Gly. The structures of Ala and Gly are shown on the right.



**Enthalpy:** Since the alanine is in the core, its sidechain is making extensive van der Waals interactions with other residues. Replacement by Gly will cause the loss of these interactions, therefore the enthalpy required for denaturation will **decrease**.

**Entropy:** The sidechain of alanine is more non-polar than glycine. So when the glycine containing protein unfolds there will be less ordered water around its sidechain, thus the entropy change  $N \rightarrow U$  will **increase**. (8 pts) In addition the Gly residue can assume more conformations than the Ala, so the configurational entropy will also increase (not required)

7. (12 pts) Describe allosteric effects and discuss their importance in biochemical processes. Provide one example to illustrate your answer.

Allosteric effects are the change in the activity, or function, of a protein due to the binding of a ligand to another site (6 pts).

They are important because they allow the activity of a protein to be increased or decreased by allosteric effects. In the extreme - an on/off switch (+ 3 pts)

Examples (+ 3 pts)

Hemoglobin - oxygen is a positive activator, enhancing the affinity at high  $O_2$  levels. The shape of the binding curve enhances  $O_2$  delivery to the tissue.

Hemoglobin - bisphosphoglycerate is a negative activator that allows for adaptation to high altitudes.

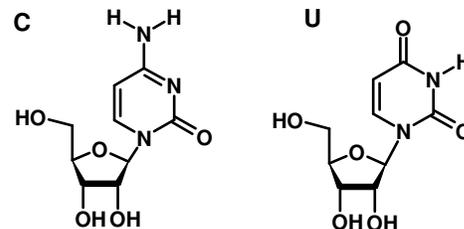
PFK-1 - this is regulated by ATP, AMP, and ADP in response to cellular energy levels and by F26P in response to hormonal signals.

Fructose bisphosphatase -1 this is regulated by ATP and AMP in response to cellular energy levels and by F26P in response to hormonal signals.

Glycogen synthase/phosphorylase: The addition of the phosphate group is a covalent allosteric mediator.

Lac repressor: IPTG binding reduces DNA affinity.

8. (18 pts) The binding of cytosine, deoxycytosine, or uracil nucleotides to a protein has been measured by equilibrium dialysis and the binding curves for all three ligands are shown below. The structures of two of the three ligands are shown to the right. This protein has one binding site.



i) Determine the  $K_D$  for all three ligands from the binding curves. Briefly justify your approach (6 pts).

The  $K_D$  is the ligand concentration that gives  $\frac{1}{2}$  saturation (+ 3pts).

Cytosine nucleotide: 5  $\mu$ M

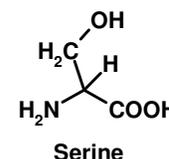
Cytosine deoxynucleotide: 50  $\mu$ M

Uracil nucleotide: 100  $\mu$ M

ii) Based on your  $K_D$  values – which ligand binds more tightly? Which is the weakest binder? Briefly justify your answer (4 pts).

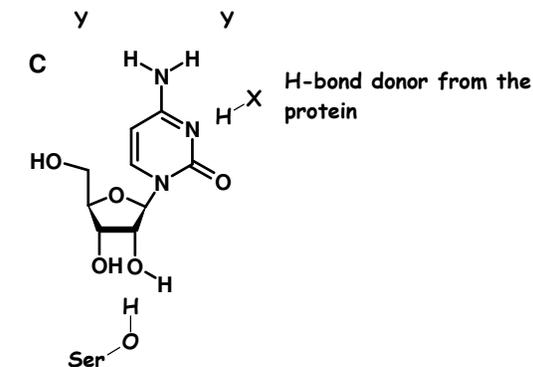
Cytosine binds most tightly since its  $K_D$  is the lowest (5  $\mu$ M). Uracil is the weakest binder, since its  $K_D$  is the highest.

iii) Replacement of a serine residue in the protein with an alanine residue causes the binding curve for cytosine nucleotide to become indistinguishable from that of cytosine deoxynucleotide. Based on this data, plus the  $K_D$  values from above, suggest how this protein is interacting with the cytosine nucleotide (labeled “C” in the above figure). Feel free to use the above figure in your answer (6 pts).



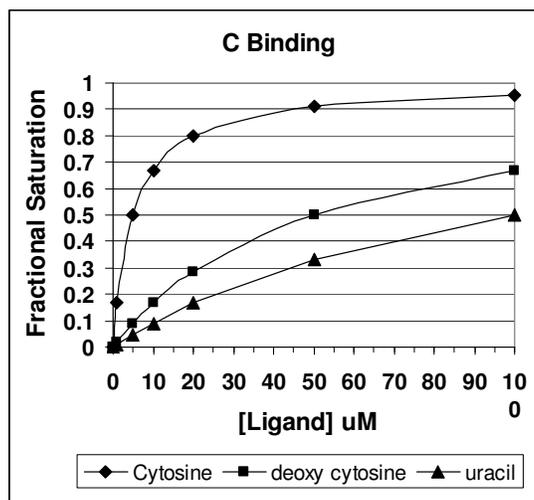
- The reduction in affinity to that of the deoxynucleotide by replacement of Serine with Alanine suggests that the serine is forming a hydrogen bond with the cytosine nucleotide. Since the  $K_D$  drops to that of the deoxynucleotide, this serine is probably forming a hydrogen bond with the 2'OH.
- The very weak binding to uracil nucleotide also suggests that the base is being recognized, again by specific hydrogen bond donors and acceptors from the protein. A plausible model is shown on the right.

H-bond acceptors from the protein.



iv) The data for the cytosine nucleotide is plotted on a Hill plot. What is the slope of the line when the curve crosses the x-axis? Briefly justify your answer (2 pts).

Since there is only one site, the binding must be non-cooperative, therefore the Hill plot will have a slope of 1.



9. (10 pts) A statement often heard at parties is that enzymes increase the rate of reactions by lowering the energy of the transition state. Please do **one** of the following two choices.

**Choice A:** Briefly describe why lowering of the transition state increases the rate of the reaction.

**Choice B:** Briefly describe how an enzyme lowers the energy of the transition state. Illustrate your answer with an example.

**A**

- The rate of the reaction is proportional to the concentration of the transition state:  $v = k [X]$  (6 pts).
- The transition state is in equilibrium with the reactants. This equilibrium constant will be larger if the energy of the transition state is lower, giving a larger concentration of  $[X]$ . The actual fraction of  $[X]$  is  $K_{eq}/(1+K_{eq})$  (4 pts).

**B**

- The general method is pre-ordering of reactive groups. Therefore, when the transition state "assembles" on the enzyme, there is no entropy cost for ordering the catalytic groups. This cost was paid when the protein folded. For example the two Asp residues in HIV protease are held in the correct position for catalysis (5 pts)
- The second method, which is specific to certain enzymes, involves direct interaction with just the transition state. This occurs in serine proteases where the oxyanion hole interacts with the negatively charged tetrahedral intermediate (5 pts)

10. (6 pts) Describe the reaction that is catalyzed by **two** of the following four enzymes. List any cofactors/cosubstrates.

i) kinase: Adds a phosphate group to its substrate. ATP required, ADP produced.

ii) phosphatase: Removes a phosphate group by hydrolysis.

iii) dehydrogenase: Catalyzes a redox reaction, an electron acceptor such as FAD or NAD<sup>+</sup> is required.

iv) DNA ligase: Joins DNA that was cut by restriction endonucleases. ATP required.

11. (41 pts) The restriction endonuclease HaeIII recognizes and cleaves the seq. shown on the right: -GG<sup>^</sup>CC-  
-CC<sup>^</sup>GG-

i) If you try to use AGGCCT as a substrate for kinetic measurements you find that the  $V_{MAX}$  for this substrate is high at low temperatures, but decreases to zero at higher temperature. Assuming that the enzyme is *not* being denatured at the higher temperature, explain this result (Hint: Read *part ii*) (4 pts).

The 4 basepair substrate melts, or denatures at the higher temperature.

ii) The substrate AGGGGCCCT produces high  $V_{MAX}$  values over all temperature ranges. Draw the *products* after the treatment of this substrate with HaeIII (4 pts).

The two products are:

AGGGG	CCCCCT
TCCCC	GGGGA

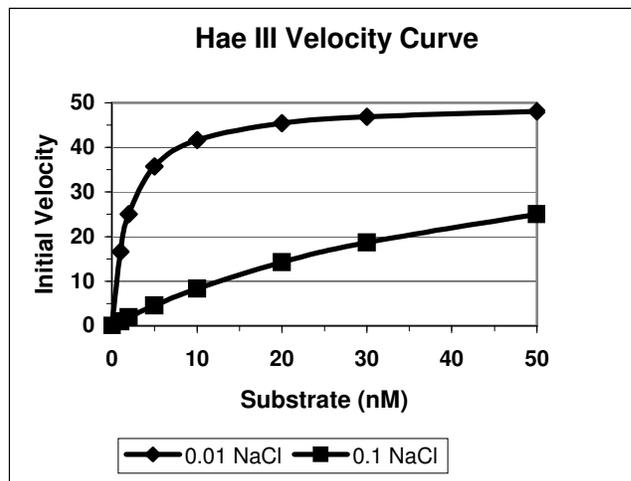
iii) How could you use gel electrophoresis to monitor product formation in this reaction (4 pts)?

Gel electrophoresis separates DNA molecules by size. The substrate is 10 long and the two products are 5 long, thus the products will migrate faster on the gel and it will be possible to distinguish between the two.

(this question continues on the next page)

**Question 11 – continued.**

iv) Steady state enzyme kinetic measurements are obtained at 0.01 M and 0.1 M NaCl concentrations and these data are shown to the right, in the form of velocity curves. What are the  $K_M$  values at these two salt concentrations? Justify your approach (Assume that  $V_{MAX} = 50$  at both salt concentrations.) (6 pts).



The  $K_M$  is the substrate concentration that gives  $\frac{1}{2}$  maximal velocity (3 pts).

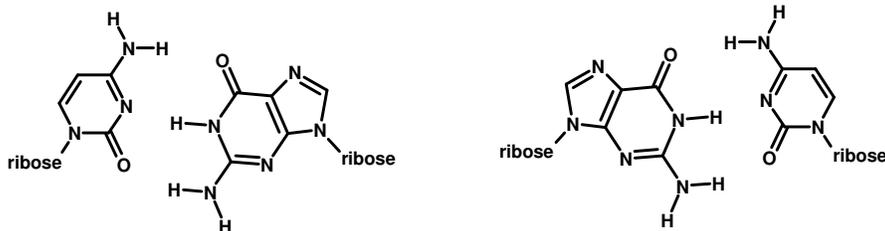
At 0.01 NaCl  $K_M = 2$  nM

At 0.10 NaCl  $K_M = 50$  nM.

iv) What protein-DNA interaction is being affected by the change in salt concentration? i.e. What type of amino acids on the protein are interacting with which groups on the DNA? Support your answer by reference to the  $K_M$  values obtained in part iv (8 pts).

- The  $K_M$  increases at higher salt, therefore the binding of the substrate (DNA) is becoming weaker as the salt increases (4 pts)
- Changing the salt concentration is most likely to affect electrostatic interactions, likely between Lys or Arg groups on the protein and the negatively charged phosphate group on the DNA. As the salt concentration increases, this interaction weakens because the sodium and chloride ions reduce the electrostatic interaction (4 pts)

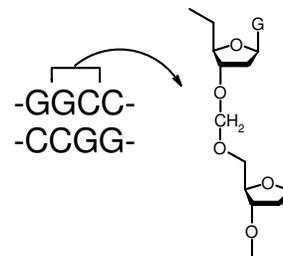
v) HaeIII can clearly distinguish between a GC and a CG basepair. To do so does it bind in the major groove or the minor groove? Briefly justify your answer with reference to the C-G and a G-C basepairs shown to the right (6 pts).



It must be using the major groove. The pattern of hydrogen bond donors and acceptors in the major groove are D, A, A for the CG basepair and A, A, D for the GC basepair. These are different.

In the minor groove the pattern is A, D, A for both CG and GC basepairs.

vi) The diagram to the right shows an inhibitor of HaeIII. The chemical structure of the region that is different from normal DNA is shown on far right. Is this a competitive or mixed type inhibitor? Why (5 pts)?



This is a competitive inhibitor because:

- A) it is similar to the substrate (3 pts).
- B) it lacks a phosphodiester bond between the nucleotides, so it can't be cleaved (2 pts)

vii) Will this inhibitor affect  $K_M$ ,  $k_{CAT}$ , or both? Briefly justify your answer (4 pts).

Just  $K_M$ . Since it binds at the same site as the substrate, at high substrate concentrations, all of the inhibitor will be displaced and therefore it cannot affect the rate of substrate conversion to product ( $k_{cat}$ )

12. (5 pts) Briefly describe how X-ray diffraction *or* NMR spectroscopy can be used to determine the structure of proteins or nucleic acids.

**X-ray:**

X-rays are scattered by electrons ( 2 pts).

Interference between the scattered x-rays depends on the relative positions of the atoms (2 pts)

The interference is converted to an electron density map into which atoms are placed (1 pt).

**NMR**

Interproton distances are measured ( 3 pts)

Interproton distances can be converted to 3D coordinates (2 pts)

13. (8 pts) Select the purification scheme that will separate protein "C" from a mixture of the following three proteins. Justify your answer by showing that the scheme will actually work (8 pts).

Protein	Molecular Weight	Solubility in ammonium sulfate (conc required to ppt 50% of the protein).	#Asp + Glu	#Lys + Arg
A	50,000	4.0	5	10
B	100,000	3.0	10	5
C	50,000	4.0	10	5

Scheme 1: Gel filtration chromatography → precipitation with 4 M ammonium sulfate.

Scheme 2: Gel filtration chromatography → ion exchange chromatography at pH=7.

Gel filtration separates by size, so the first step in either scheme will remove B, leaving A and C (2 pts)

Since both A and C have the same solubility in ammonium sulfate, the second step in scheme 1 will not separate them.

At pH 7.0 the charge on the two proteins is:

$$A: -5 (\text{Asp and Glu}) + 10 (\text{Lys and Arg}) = +5$$

$$C: -10 (\text{Asp and Glu}) + 5 (\text{Lys and Arg}) = -5$$

It was not necessary to do a detailed calculation, an approximate one is fine.

Since the charges are different, it should be possible to separate them by ion exchange ( 6 pts)

14. (10 pts) Please answer **one** of the following three choices:

**Choice A:** How does the presence of *cis* double bonds in unsaturated fatty acids affect the phase transition of the membrane? What intermolecular interaction is affected by the presence of these groups in the bilayer?

**Choice B:** Compare and contrast the structure of a membrane protein (e.g. bacteriorhodopsin) to that of a soluble protein (e.g. myoglobin)?

**Choice C:** Explain why it is important for biological membranes to be fluid, and discuss the role of cholesterol in this property of the membrane.

**A:** A *cis* double bond will cause a "kink" in the fatty acid chain, reducing van der Waals interactions. This will lead to a decrease in the melting temperature.

**B:** The solvent exposed surface of myoglobin will contain polar, charged, and some non-polar sidechains. The equivalent surface of a membrane protein will only contain non-polar residues for those regions that contact the non-polar acyl chains of the lipids.

**C:** Small non-polar electron carriers, such as Coenzyme Q must diffuse within the bilayer for electron transport. This can occur if the membrane is fluid. Cholesterol causes the membrane to be fluid over a broader temperature range.

15. (8 pts) Indirect coupling is often used to insure that reactions proceed spontaneously to products. Briefly describe how indirect coupling accomplishes this goal and give an example from **either** nucleic acid biochemistry **or** from metabolic pathways.

- In the pathway  $A \rightarrow B \rightarrow C$ . If the reaction from B to C is very favorable, then the concentration of B will be reduced to below its equilibrium concentration (5 pts).
- Consequently the Gibbs free energy of the A to B reaction will become negative, and it will be spontaneous in the direction written (1 pt)
- Examples include (2 pts)

- Aldolase in glycolysis
- Malate dehydrogenase in the TCA cycle
- Fatty acid activation, formation of acyl CoA.
- DNA polymerase addition of nucleotide triphosphates
- Charging of tRNA.

16. (8 pts) I've referred to the liver as a "glucose bank" because it stores excess glucose and releases glucose into the blood when there is a request for glucose. Please do **one** of the following two questions.

**Choice A:** Name **one** hormone that is responsible for regulating glucose metabolism in the liver. Briefly describe how it regulates glycogen synthesis and degradation, glycolysis, and gluconeogenesis. You need not describe in detail the signaling pathways.

**Choice B:** In the muscle, regulation of glycogen is similar to that in the liver. The regulation of glycolysis is similar to the liver in terms of energy sensing, but opposite in terms of hormonal regulation. Briefly describe why this form of regulation is important for the normal function of muscle tissue.

**A:**

**Glucagon and epinephrine** - release of glucose into the blood.

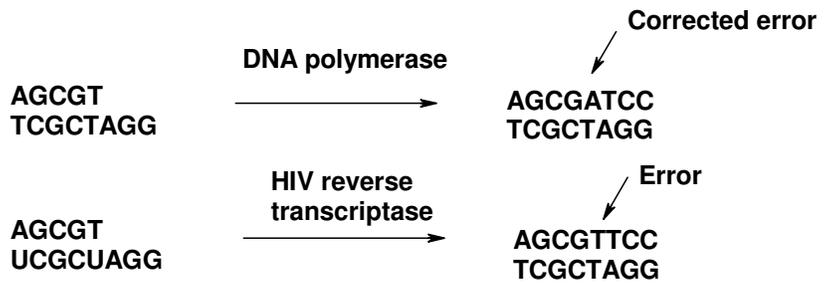
- Both of these activate glycogen breakdown by phosphorylation of glycogen phosphorylase.
- Gluconeogenesis is also activated by the low levels of F26P, producing glucose from pyruvate.

**Insulin** - storage of excess glucose in the blood.

- Activated glycogen synthase by dephosphorylation of the enzyme
- Glycolysis is activated (if ATP is required) due to the high F26P levels.

**B:** In the muscle, you would want a hormone like epinephrine to stimulate glycolysis such that the glucose can be used to produce ATP for the muscle to use for mechanical work. In the liver, you want epinephrine to shut down glycolysis and activate gluconeogenesis since the liver is to release glucose into the blood.

17. (10 pts) The following primer-templates were incubated with either DNA polymerase III or HIV reverse transcriptase plus all four nucleoside triphosphates (dNTPs) and Mg<sup>2+</sup>.



i) Draw the final product in **both** cases. Briefly justify your answer (6 pts).

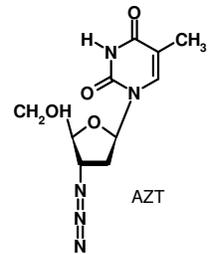
- The 3' base of the primer is not the correct base for a T.
- The DNA polymerase 3'→5' exonuclease activity would remove the incorrect base, and then complete the top strand correctly.
- The HIV reverse transcriptase can't correct errors, so the T is left in place, and the next T is added, etc.

ii) How would your answer to **either** case differ if dTTP was *completely* replaced by dideoxyTTP (ddTTP) in the reaction mixture (4 pts)?

The addition of the first T would cause chain termination, since there is no 3'OH to add the next base. In the case of DNA polymerase, this would occur after the A that replaced the incorrect T is added.

**Bonus.** (3 pts) AZT, whose structure is shown to the right, is a drug that is used to treat individuals infected with HIV. How does this drug interfere with viral replication?

Like ddTTP, it is lacking a 3'OH for elongation. Therefore, if it is incorporated into the DNA during conversion of the viral RNA to DNA, the conversion will halt and the virus can't replicate.



18. (8 pts) Please do **one** of the following two choices. Please indicate the choice that you are answering.

**Choice A:** The aminoacyl synthetase that attaches Ala to the correct tRNA can also, by mistake, attach the amino acid Gly. If Gly is attached, then it is removed by hydrolysis at a separate editing site on the enzyme. Based on the structure of these amino acids, provide a sketch or description of the site which adds the amino acid to the tRNA **and** the separate site that will remove Gly but not Ala from the incorrectly charged tRNA (The structure of these amino acids is shown in question 6).

**Choice B:** A number of amino acids are associated with more than one codon. For example, the amino acid Phe can be incorporated into a peptide chain whether the codon is UUU or UUC, yet there is only one tRNA molecule that is charged with Phe. Briefly explain how this occurs.

**A:**

- The charging site must be large enough to accommodate the sidechain of Alanine. Since glycine is smaller, it can also fit here and be added to the wrong tRNA.
- The editing site: If it is too small to allow the alanine sidechain to enter, the Alanine will not be cleaved off the correct tRNA. Glycine, being smaller, can enter the editing site and be removed from the tRNA.

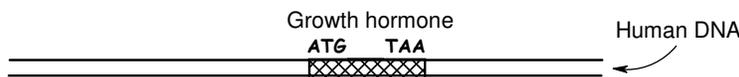
**B:**

The anticodon loop on the tRNA forms standard Watson-Crick H-bonds with the first two bases of the codon. In the case of the third base of the codon, normal Watson-Crick h-bonds form for one codon and a "wobble" (not quite Watson-Crick) basepair forms.

19. (28 pts) The diagram below shows a segment of human DNA with the gene for a small, four amino acid, human growth hormone indicated as a stippled box. The start codon (ATG) and stop codon (TAA) are indicated. A diagram of an expression vector is also provided. The vector has a *single* BamHI site, whose recognition sequence is G<sup>A</sup>GATCC. You desire to produce recombinant human growth hormone that will remain inside the bacteria after induction of transcription. Please answer the following questions.

i) There are five labels on the expression vector. The table below either gives the name of labeled items or their function. Please complete the missing entries for *three* of the five (6 pts).

Label	Name	Function
1	Origin of replication	Allows the plasmid to be replicated in the bacteria
2	Antibiotic resistance gene	Forces the bacteria to keep the plasmid. Loss of the plasmid would cause the bacteria to be killed by the antibiotic that the bacteria are grown in.
3	Promoter	RNA polymerase binds here.
4	Lac operator	This binds the lac repressor, which inhibits mRNA production. mRNA production can be turned on by removing the lac repressor from the DNA.
5	Ribosome binding site	Causes the mRNA to bind to the small (30s) ribosomal subunit.

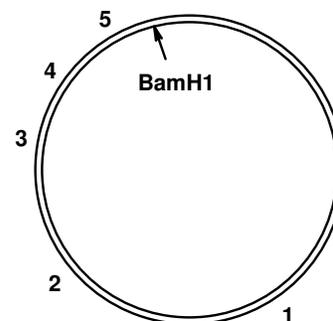


ii) Briefly describe how you would generate PCR primers to amplify the DNA segment that codes for the growth hormone. You should include the start and stop codons in your final product because these are not present in the expression vector. Since you do not know the entire sequence of the growth hormone you should not worry about determining the exact length of the primer (6 pts).

- A BamHI site is required on both ends, since there is only a single site in the vector.
- The left primer will be the same as the sequence of the top strand, beginning at the ATG: **GGATCCATG.....**
- The right primer will be the same as the sequence of the bottom strand, beginning at the TTA (complement of TAA): **GGATCCTTA...**

The resultant product will be:

**GGATCCATG.....TAAGGATCC**  
**CCTAGGTAC.....ATTCCTAGG**



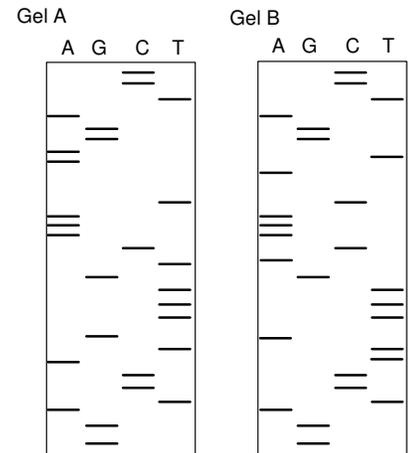
iii) Briefly describe how you would insert the PCR product into the expression vector (4 pts).

1. Cut the PCR product with Bam HI, and the vector with Bam HI.
2. The complementary "sticky ends" will allow the PCR product to form Watson-Crick H-bonds with the vector.
3. Reform the phosphodiester bonds with DNA ligase.

iv) Briefly describe how you would induce the production of the growth hormone after placing (transforming) the vector into the bacteria (4 pts).

1. Grow bacteria containing the plasmid in the absence of IPTG.
2. When sufficient number of bacteria are obtained, add IPTG.
3. IPTG will bind to the lac repressor, causing it to be released from the DNA.
4. mRNA production will begin, leading to production of the growth hormone.

v) (8 pts) After inserting the PCR product into the expression vector you find that some of the vectors produce the recombinant protein as expected but some do not. You sequence the DNA of one that does (gel A) and one that does not (gel B). The DNA sequencing gels for these two vectors are shown to the right. Use the back of the previous page if you need additional space.



- a) Determine the protein sequence of this hormone using gel A (4 pts).
- b) Explain why the vector whose sequence is shown in gel B does not produce any growth hormone (4 pts).

a)

DNA seq. of A is: **GGATCC** ATG TTT GTC AAA TAA **GGATCC**

The prot seq is: Met Phe Val Lys Stop

b)

DNA seq. of B is: **GGATCC** TTA TTT GAC AAA CAT **GGATCC**

There is no start codon, so the mRNA will not initiate the synthesis of the protein (4 pts). This occurred because the PCR fragment was inserted into the vector backwards.

**Bonus (2 pts).** How would you modify the expression vector to cause export of the protein out of the cell?

Add DNA (1 pt) encoding a leader peptide (1 pt) at the amino terminal end of the protein

20. (12 pts) Briefly describe the events that occur in *either* DNA transcription *or* protein synthesis. Your answer should include a description of the initiation events, how polymerization occurs, and termination. Feel free to use a well labeled diagram.

Step	DNA transcription (mRNA syn)	Protein Syn
Initiation	<ul style="list-style-type: none"> <li>RNA polymerize binds to promoter, forming closed complex.</li> <li>Promoter recognized by sigma factor</li> <li>DNA melts (9 bases) forming open complex.</li> </ul>	<ul style="list-style-type: none"> <li>mRNA binds to 30s ribosome using ribosome binding site.</li> <li>tRNA with fMet loaded into the P-site, basepairing to AUG codon.</li> <li>50s subunit added.</li> </ul>
Polymerization	<ul style="list-style-type: none"> <li>Sigma factor leaves.</li> <li>NTPs are added to 3'OH using template to dictate correct base.</li> </ul>	<ul style="list-style-type: none"> <li>Next tRNA+aa loaded into A-site, basepairing to next codon.</li> <li>Amino group of aa in A site attacks ester linkage between 1<sup>st</sup> amino acid (or peptide) and attached tRNA.</li> <li>Extended peptide is now attached to rRNA in A-site.</li> <li>Ribosome translocates, longer peptide is found in the P-site, ready for another cycle.</li> </ul>
Termination	<ul style="list-style-type: none"> <li>When mRNA termination site is reached, RNA polymerase is released from DNA.</li> </ul>	<ul style="list-style-type: none"> <li>Stop codon causes loading of release factor in to A-site.</li> <li>Release factor cause hydrolysis of the ester between the peptide and the tRNA in the P-site.</li> </ul>