This exam consists of **17** pages. Be sure that you have all of the pages. There are a total of 152 points on the exam, budget about 1 min/point. There are two bonus questions scattered in the test as well.

A1:	_____/15
B1:	_____/ 5
B2:	_____/ 3
B3:	_____/20
B4:	_____/ 6
B5:	_____/ 3
B6:	_____/10
B7:	_____/20
B8:	_____/ 5
B9:	_____/ 8
B10:	_____/18
B11:	_____/ 5
B12:	_____/ 4
B13:	_____/ 6
B14:	_____/ 6
B15:	_____/ 8
B16:	_____/10
Bonus	_____/ 6
TOTAL	_____/152

Part A: Multiple Choice 1.5 pts each - total of 15

1. DNA absorbs UV light at ____ nm and proteins absorb at ____ nm.
   a) 260, 260
   b) 260, 280
   c) 280, 260 **(+1/2)**
   d) 280, 280

2. Which amino acid has a sidechain that often stabilizes extracellular proteins by the formation of crosslinks in the protein chains?
   a) Alanine
   b) Cysteine
   c) Methionine
   d) Tyrosine

3. A ligand that binds to a protein more strongly than another ligand will
   a) Have a smaller dissociation constant (K_D).
   b) Have a larger dissociation constant (K_D).
   c) Have a smaller association constant (K_A).
   d) Have a slower kinetic on-rate.

4. In both hemoglobin and myoglobin the oxygen is bound to.
   a) the iron atom in the heme group.
   b) the nitrogen atoms on the heme.
   c) a hydrophobic pocket in the protein.
   d) the surface of the protein.

5. During any successful purification scheme, you would expect
   a) the number of different proteins in the sample to decrease. **(+1/2)**
   b) the specific activity to decrease.
   c) the specific activity to increase. **(+1/2)**
   d) both a and c are correct.

6. In gel electrophoresis both proteins and DNA are separated on the basis of their:
   a) charge-to-mass ratio.
   b) molecular weight.
   c) positively charged sidechains.
   d) different isoelectric points.

7. Which of the following fatty acids would have the lowest critical micelle concentration (CMC)? (The CMC is the highest concentration of monomeric fatty acid that can be present in solution before micelles appear.)
   a) C_4-COOH
   b) C_5-COOH
   c) C_6-COOH
   d) C_7-COOH
   e) C_8-COOH

8. If the Gibbs free energy, ΔG, of a system is positive then:

   a) More reactants will spontaneously form.
   b) More products will spontaneously form.
   c) It is not possible to extract any energy from the system.
   d) both a and c are correct.

9. T_M refers to:
   a) the temperature at which 50% of a DNA molecule is denatured
   b) the temperature at which 50% of a protein molecule is denatured
   c) the temperature at which membranes are 50% fluid.
   d) all of the above.

10. During replication, overwinding or overtightening of DNA is caused by _____ and removed by _____:
   a) DNA ligase, Gyrase
   b) Dna B (Helicase), DNA polymerase
   c) DnaB (Helicase), Gyrase
   d) Single Stranded Binding Protein, DnaB (Helicase)

Part B:

1. Protein Structure (5 pts)

   i) Complete the chemical structure of the dipeptide shown to the right by adding the correct atoms to the mainchain. (i.e. don't draw any sidechains). (1 pt).

   ii) Draw the sidechain for a non-polar residue and a positively charged reside on your peptide, indicate which residue is of which type (2 pts).

   iii) Name your dipeptide (1 pt):

   Leu-Lys

   iv) Circle the bond that is planer and trans (1 pt).

   You should have circled the peptide bond.

Non-polar

Peptide bond

Pos. Charge
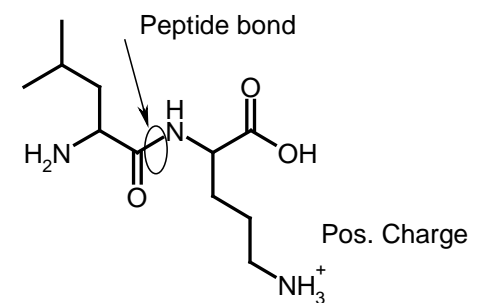
2. Complete the following 'fill in the blanks', the first example has been done for you as an example (3 pts).

   i) Amino acid side chains are to proteins as <u>nucleotide bases</u> are to DNA.

   ii) The mainchain atoms in protein are represented by <u>deoxyribose</u> sugars in DNA.

   iii) The peptide bond in proteins is analogous to the <u>phosphodiester</u> bond in DNA.

   iv) The amino acid phenylalanine has the same number rings as <u>pyrimidine (C,T)</u> bases in DNA.

   v) The bond between the C_α and C_β atoms in a protein serves the same role as the <u>glycosidic</u> bond in DNA.

   vi) The amino terminus of a protein is analogous to the <u>5'</u>- end of a DNA strand.

   vii) A dimeric protein has the same number of chains as <u>double</u> stranded DNA.

3. Please do **four** of the following five choices. *Read each choice carefully, some ask you to discuss two items while others give you a choice between one item or another.* (20 pts) (Two choices are on the next page)

Choice A: Discuss the role of the hydrophobic effect in the formation of the folded state of globular water soluble proteins *and* biological membranes. You answer should provide a clear molecular description of the hydrophobic effect and the relative importance of the hydrophobic effect in each process (5 pts).

> The hydrophobic effect is the ordering of water around non-polar groups.
> It decreases the entropy of the system, which is unfavorable. (+3 pts)
>
> In the case of globular proteins, the hydrophobic effect 'drives' the non-polar residues into the core of the protein, releasing water. This is one of the major forces that stabilizes proteins, however, hydrogen bonding and van der waals also play a significant role. (+1 pt)
>
> In the case of membranes, the hydrophobic effect causes the non-polar acyl chains to form the bilayer. This is more important for membranes as the only other force that holds the membrane together is van der Waals, which are weak in fluid membranes. (+1 pt)

Choice B: Describe, or give an example of a hydrogen bond, and discuss the role of hydrogen bonds in the formation of folded proteins *or* double stranded DNA. Your answer should discuss the relative importance of this interaction to the stability of the folded form. (5 pts)

> A hydrogen bond is the bond between a proton attached to an electronegative atom, such as Oxygen or Nitrogen and another electronegative atom. The proton, or hydrogen bond donor, has a partial positive charge and the electronegative atom has a negative charge. As an example, the amide proton in proteins forms a hydrogen bond with the carbonyl oxygen. (+3pts)
>
> Proteins: Important stabilizing term, results in the formation of stable secondary structures, α-helices and β-strands.(+2 pts)
>
> DNA: Watson-Crick hydrogen bonds form between the bases in the inside of the helix. A forms two bonds to T and G forms 3 to C. Moderately important stabilizing term. (+ 2pts)

Choice C: What is 'configurational entropy' and how does it affect the thermodynamics of protein *or* DNA folding. (5 pts).

> The entropy of a molecule is related to the number of conformations that molecule can attain:
>
> $$S=R\ln W. \text{ (+3 pts)}$$
>
> In the case of both proteins and nucleic acids, the folded state has W=1 and the unfolded state has a large number of conformations. Therefore, unfolding is favorable due to the increase in conformational entropy. (+2pts)

Question 3 continued:

Choice D: Explain why the salt concentration can have a large effect of the stability of double stranded DNA, but has little effect on the stability of proteins.(5 pts)

In the case of DNA, electrostatic repulsion between the phosphates on opposite strands is very unfavorable for formation of double stranded DNA. The positively charged ions will screen these charges from each other, making the DNA more stable as the salt concentration is increased. (+3 pts)

In the case of proteins, electrostatic interactions have very little to do with stability, so salt has very little effect on protein stability (+2 pts)

Choice E: A nucleotide base, such as adenosine, will bind *non-covalently* to the blunt end of a double stranded piece of DNA , assuming a position similar to as it would if were covalently linked to the DNA. The reaction can be described as the following equilibrium: (5 pts)

```
A-G-G-C-G-G         +        A      →      A-G-G-C-G-G A
T-C-C-G-C-G                                T-C-C-G-C-C
```
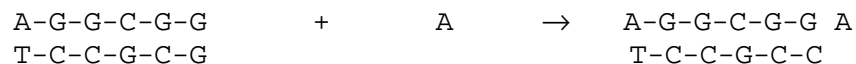
What molecular interaction stabilizes this complex?

pi-pi stacking between the nucleotide base and the end of the DNA. A specific example of a van der Waals interaction.

4. Please do **one** of the following two choices. Please indicate the choice that you are answering. (6 pts)

Choice A: Describe, or draw, the structure of a tRNA. Your answer should also include a discussion of the location of the attached amino acid in a charged tRNAs and the general nature of the interaction of the tRNA with its anticodon.

> Upside down L-shaped structure (+2 pts) with amino acid attached to 3' end (+2 pts) and the three bases of the anticodon interacting with the three bases of the codon (+2 pts).

<div align="center">

OR

</div>

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.

> When base pairing between the codon and anti-codon occurs, the first two bases of the codon generally form normal Watson-Crick hydrogen bonds (+2 pts). In the case of the third codon, alternative hydrogen bonding is often seen, allowing the formation of non-Watson Crick Hydrogen bonds (Wobble base) (+4 pts) . For example, the anticodon on tRNA^{Phe} is 5'GAA'3' which forms normal Watson-Crick base pairs with all three bases of the codon UUC, but a wobble base pair with UUU.

5. Please do one of the following two questions. Please indicate the choice you are answering (3 pts):

Choice A: Briefly explain why RNA is more easily hydrolyzed by base (e.g. NaOH) than DNA. Your answer should include a discussion of the mechanism of hydrolysis.

> RNA contains the sugar ribose. Ribose has a 2'OH that when deprotonated, can attack the phosphodiester bond.

Choice B: How does the chemical structure of RNA differ from DNA?

> Ribose in RNA replaces deoxyribose in DNA (+1 1/2)
>
> U in RNA replaces T in DNA (+1 1/2)

6. Describe the step associated with *either* lagging strand synthesis in DNA replication *or* the process of elongation during synthesis of proteins on the ribosome. Your answer should include a brief description of the molecules involved, both proteins and nucleic acids, as well as a clear indication of the order of events during the process. A well labeled sketch would be an acceptable answer (10 pts).

Lagging Stand Synthesis:

1. DNA on the lagging strand becomes single stranded due to movement of replication fork (+1 pt)
2. RNA primer synthesized by primase. (+2 pts)
3. Pol III begins synthesis from RNA primer. (+1 pt)
4. Pol III stops when previous RNA primer is reached. (+1 pt)
5. Pol I removes RNA primer using 5'-3' exonuclease activity (+2 pts)
6. Pol I completes synthesis of DNA (+1 pt)
7. DNA ligase seals phosphodiester bond.(+2 pt)

Elongation during Protein Synthesis:

1. The growing peptide, attached to the tRNA is in the P-site on the ribosome (+2 pts).
2. The next amino acid to be incorporated binds to the A-site, due to codon anti-codon interactions.(+2 pts)
3. Peptide bond formation occurs, the peptide now one residue longer, is now on the A-site, attached to a tRNA.(+2 pts)
4. Translocation of the ribosome occurs, moving the uncharged tRNA that was in the P site to the E site. The peptide-tRNA that was in A site is now moved to the P site. (+2 pts)
5. The uncharged tRNA exits from the E site.(+2 pts)

7. (20 pts) The kinetics of EcoR1 cleavage of DNA was measured at three different salt concentrations (0.1 M, 1M and 2M) and the velocity (products formed/unit time) are shown in the following table. The substrate was GAATTC (double stranded, of course). In this enzyme, the rate of hydrolysis of the DNA (k_2 or k_{CAT}) is very slow, such that $K_M \approx K_D$. $V_{MAX} = 10$.

[s] nM	v [NaCl]=0.1M	v [NaCl]=1M	v [NaCl]=2M
1	**5.00**	0.91	0.83
2	6.67	1.67	1.54
5	8.33	3.33	3.12
10	9.09	**5.00**	**4.76**
20	9.52	6.67	**6.45**
70	9.86	8.75	8.64

Salt Dependence of EcoR1
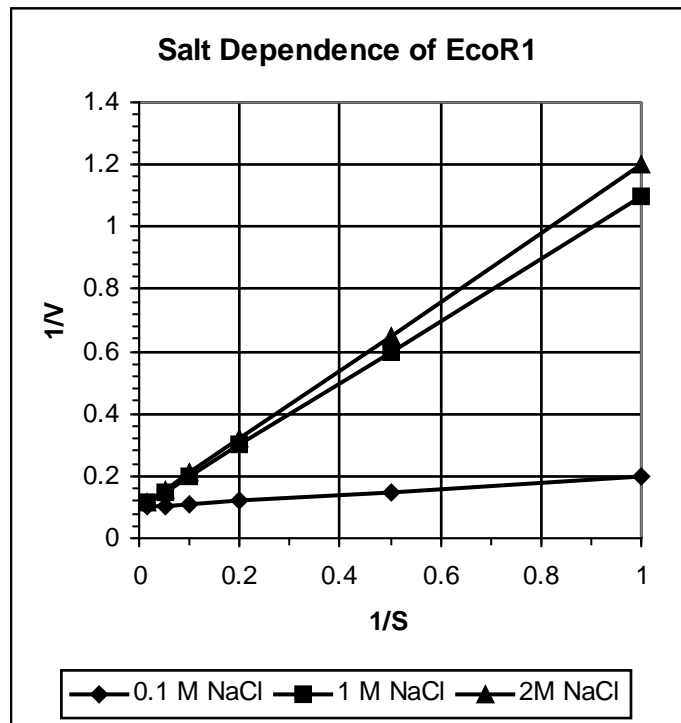
i) Determine the K_M at *each* of these salt concentrations by whichever means you like. Sketch a graph of K_M versus [NaCl] on graph below. (5 pts)

The K_M is the substrate concentration that give 1/2 maximal velocity. Since V_{MAX} was given as 10, the following are the K_M values, obtained from the kinetic data @ an reaction velocity of 5.

[NaCl]	K_M
0.1 M	1 nM
1.0 M	10 nM
2.0 M	>10 nM.

Effect of Salt on Km
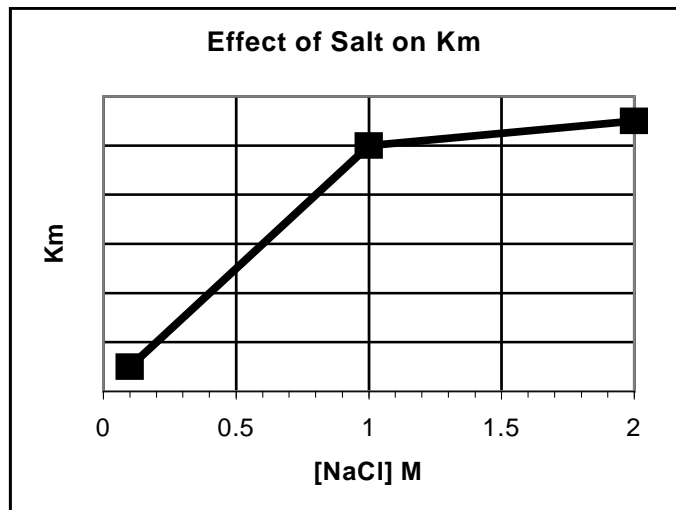
In the case of 2M NaCl, you need to interpolate between 10 and 20 nM of substrate. A guess would be 11 or 12. The best way to get the K_M is from the double reciprocal plot.   The slope is K_M/V_{MAX}. The slope is (1.2-.1)/(1)=1.1. Since V_{MAX} is 10, K_M =11. (3 pts for getting first two K_M values, +2 for third K_M, either by reasonable interpolation or by double reciprocal plot.

ii) Explain the dependence of the K_M on the salt concentration. Your answer should include a discussion of the general nature of the interaction of the restriction enzymes with the DNA and why this interaction may be affected by salt. (3 pts).

The K_M is related to substrate binding. A larger K_M indicates that the substrate binds less tightly as the salt concentration increases. This indicates interference with an electrostatic interaction between the enzyme and the DNA (+2 pts). The most likely interaction is between the negatively charged phosphates on the DNA and Lys and Arg residue on the protein. (+1 pt).

Effect of CCCGGG

7, continued.....

The first experiment (lowest salt concentration) was repeated in the presence of the following DNA molecule: CCCGGG (double stranded), at a concentration of 9 nM. The resultant enzyme kinetic data is plotted on a double reciprocal plot shown to the right.

iii) Is CCCGGG a competitive or non-competitive inhibitor of the enzyme? Justify your answer *either* by reference to the structure of CCCGGG *or* by analysis of the enzyme kinetic data. (4 pts)

It is a competitive inhibitior (2 pts)

i) It is very similar to the substrate (i.e. double stranded DNA) (2 pts)

ii) The y-intercept of the double reciprocal plot is the same in both cases, therefore V_{MAX} is not changed. (2pts).

iv) Determine the dissociation constant (K_D) of CCCGGG to Eco R1 from the enzyme inhibition data. (4 pts)
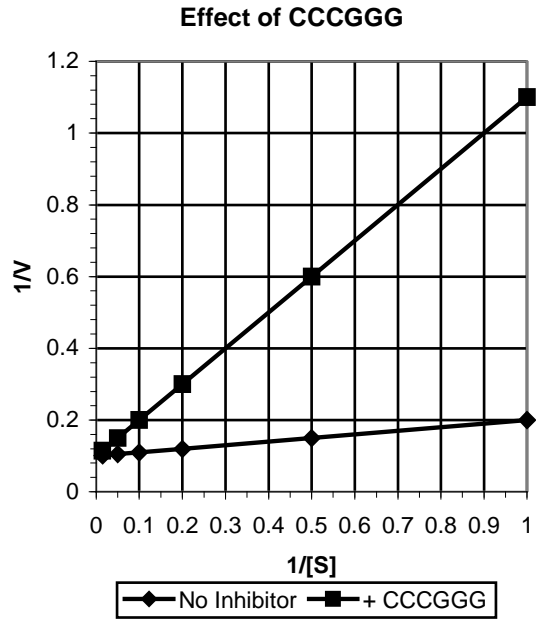
In the case of an inhibitor, $K_I = K_D$. K_I is obtained from $\alpha=1+([I]/K_I)$, and α is just the ratio of the slopes:

Slope ([I]=9 nM): (1.1-.1)/1 = 1.0
Slope ([I]=0 nM): (.2-.1)/1 = 0.1
α=1/0.1 = 10   (10-1) = [I]/K_I K_I = 1 nM.

v) Using your value from part iv, sketch as accurately as possible, *either* the binding curve *or* the Hill plot for the binding of CCCGGG to EcoR1. Be sure to label the axis, including units, and include a numerical scale as well. You should comment on whether you think the binding of CCCGGG to EcoR1 is cooperative or not *and* how the cooperativity (or lack of) is reflected in your plot. If you were unable to obtain a binding constant from *part iv*, state an assumed value and proceed with the problem. (4 pts).

The binding should be non-cooperative, since Eco R1 has only one binding site, therefore the binding curve will be hyperbolic and the Hill plot will have a slope of 1.0 as the plot crosses the x-axis. (+1 pt)

Binding Curve: This will be hyperbolic, with Y=0.5 when [DNA]=1 nM.(1 pt, +2 pts for axis)

Hill Plot: Slope of 1, crosses x-axis at log(10^{-9})= -9 (+1 pt, +2 pts for axis)
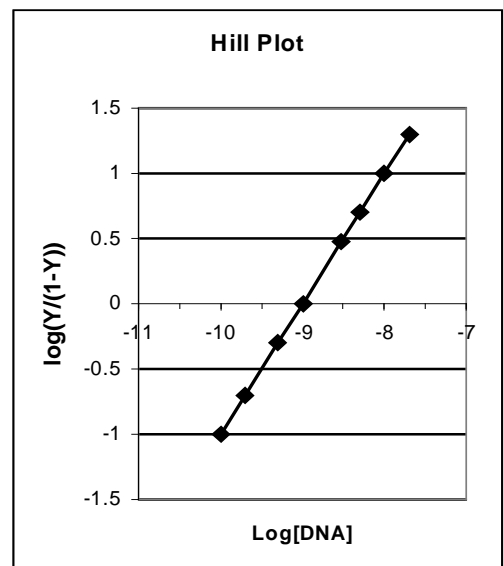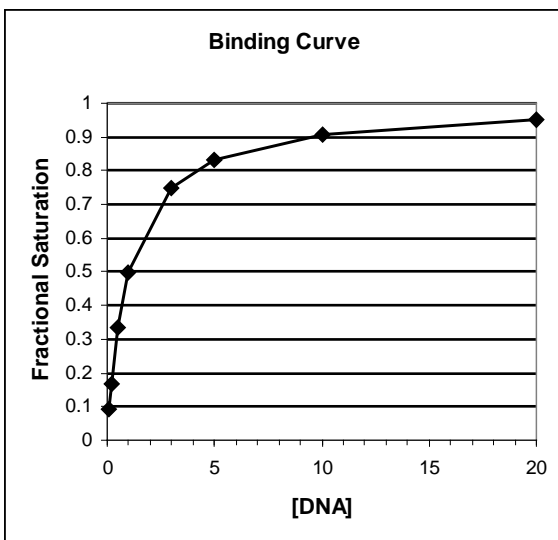
8. Please do **one** of the following two questions. Please indicate your choice. (5 pts).
   Choice A: Suggest a method to purify EcoR1 from a complex mixture of proteins by affinity chromatography. Discuss how would you elute the enzyme off of the column.

   Attach *CCCGGG* (double stranded form) to a bead (resin). Eco R1 will bind. (+3 pts). You could elute with high salt concentration. (+2 pts)

   Choice B: Explain how you would prove that restriction enzymes are homodimeric using SDS-PAGE gel electrophoresis and gel filtration chromatography.

   SDS-page will give the denatured molecular weight of each chain (+2). Since this is a homodimer, only one band, corresponding to a single molecular weight, will be observed (+1 pt)

   Gel filtration will give the native molecular weight (+1 pt), in this case <u>twice</u> that of each subunit.(+1 pt)

9. Allosteric effects play an important role in the regulation of biochemical processes.   Briefly describe the nature of allosteric effects and then select *one* example from the following list and describe how allosteric effects control its function. Your answer should include a description or structure of the allosteric activator or inhibitor. (8 pts)
   1. Hemoglobin
   2. PFK
   3. lac repressor

   An allosteric protein exists in two forms, a tense (T) or inactive form, and a relaxed (R) or active form. (3 pts) When an allosteric ligand binds to an allosteric protein:
       i)   allosteric activators will stabilize the R form, increasing the activity (2 pts)
       ii)   allosteric inhibitors will stabilize the T form, reducing activity. (2 pts)

   Examples (+2 pts for any of the following +1 for example +1 for correct description)

| Hemoglobin	PFK	Lac Repressor
• Oxygen is an allosteric activator, increasing its own affinity. • BPG (bis phosphoglycerate) and H^+ are allosteric inhibitors, decreasing oxygen affinity	• F2,6P is an allosteric activator. • ADP, and AMP are also allosteric activators. • ATP is an allosteric inhibitor	• If you consider the form bound to DNA as the active form then IPTG is an allosteric inhibitor, because it stabilizes the non-DNA binding state.

10. (18 pts) The following is a short segment of human DNA that contains the DNA sequence that encodes a human growth hormone. This hormone, if produce in large quantities in bacteria, can be used to treat a growth deficiency in people. The first three codons of the gene have been translated into the protein sequence for you. The *entire* gene for the growth hormone is in italics and consists of 11 codons, beginning with ATG and ending with TGA.

```
AGGCGTAGTGCTTTGC ATG TTT TGT CAT CAC CGT AGT GCT GAT GGG TGA TGTAGTCTG
                 Met Phe Cys
```

The above gene needs to be inserted into an expression vector/plasmid such that human growth hormone can be produced effectively in bacteria. This vector has the structure shown to the right.

The actual sequence of this vector between the promoter (-35 & -10) and the HindIII restriction site (boxed) is:

```
 -35                    -10              SD
TTGACATTTATGCTTCCGGCTCGTATAATGTGTGGAACAGGAAAG AAGCTT
```
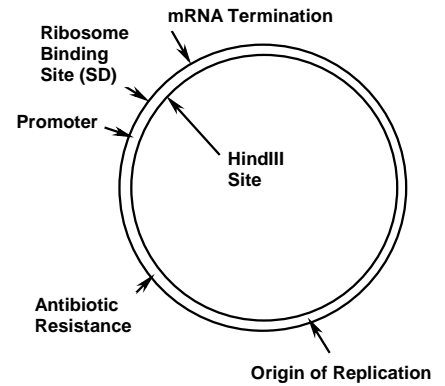
i) The sequence of the growth hormone shows two codons that are underlined, ATG and TGA. Explain the role of *either* of these two triplets of bases in protein synthesis. (2 pts)

ATG: start codon - it is the first codon used in the synthesis of the protein. Codes for fMet

TGA: Stop codon - signals release of the completed protein from the ribosome.

ii) Explain the role of *either* the antibiotic resistance gene *or* the origin of replication in the *maintenance* of this plasmid in the bacteria cell (2 pts).

Antibiotic resistance gene: Only bacteria that have the plasmid can grow in the presence of antibiotic. Bacteria that do not have the plasmid will die.

Origin of replication: insures that the plasmid DNA will be replicated along with the bacterial chromosomal DNA such that well a cell divides, the plasmid DNA will be found in both daughter cells.

iii) Explain the role of *either* the -35 and -10 regions *or* the role of the SD sequence in the production of the human growth hormone. (2 pts)

The -35 and -10 are part of the promoter, where RNA polymerase binds to initiate mRNA synthesis.

The SD sequence (ribosome binding site) binds the mRNA to the rRNA (+1 pt) on the 30 s subunit (+1 pt).

iv) The recognition sequence for HindIII is A^AGCTT. Write the double stranded form of the six base sequence and show this DNA after treatment with HindIII. (1 pts)

```
        AAGCTT          →          A              AGCTT
        TTCGAA          →          TTCGA              A
```

Question 10, continued:

v) The following product was obtained after polymerase chain reaction using the growth hormone DNA as template.

AAGCTTATGTTTTGTCATCACCGTAGTGCTGATGGG<u>TGA</u>**AAGCTT**
TTCGAATACAAAACAGTAGTGGCATCACGACTACCCACT**TTCGAA**

Describe, or draw a suitable sketch, how this fragment is inserted into the expression vector. Include a brief description of how the covalent bonds are reformed after the gene is inserted into the plasmid (2 pts)

Both the PCR product and the plasmid would be treated with Hind III, giving the following products: (+1 pt)

AGCTT<u>ATG</u>TTTTGTCATCACCGTAGTGCTGATGGG<u>TGA</u>**A**   (PCR Product)
        ATACAAAACAGTAGTGGCATCACGACTACCCACT**TTCGA**

-----A   AGCTT-----
-----TTCGA   A-----

The single standed regions can basepair via hydrogen bonds, to give the following: (+1/2 pts)

-----**AAGCTT**<u>ATG</u>TTTTGTCATCACCGTAGTGCTGATGGG<u>TGA</u>**AAGCTT**-----
-----**TTCGAA**TACAAAACAGTAGTGGCATCACGACTACCCACT**TTCGAA**-----

The covalent bonds are reformed using DNA ligase (+1/2 pt)

vi) (3 pts) After inserting the growth hormone gene into the vector you note that the bacterial cells that produce growth hormone die quickly such that very little growth hormone can be produced. The cell death is due to the toxic effects of the production of human growth hormone within the bacteria. Briefly describe how you would modify the expression vector to *either*:
   • regulate (ie. turn on and off) the production of the human growth hormone, *or*
   • export the hormone out of the bacterial cell.

   Place the lac operator sequence between the promoter and the SD sequence, the lac repressor will bind preventing mRNA synthesis (off) until IPTG is added (on) (+3 pts)

   Include the coding seqeuence for the leader peptide at the amino terminus of the gene. This will result in export of the protein out of the cell. (+3 pts)

Bonus! (3 pts). In fact, only 50% of the plasmids produced growth hormone (killing the cells), while the other 50% did not produce any growth hormone at all! Explain this observation.

   The Hind III fragment can be inserted into the expression plasmid in both orientations, with a 50/50 chance. One orientation gives expressed protein. In the other case the gene is 'backwards' and will not produce protein.

Question 10, continued

vii) Please do **one** of the following **two** choices (6 pts).

  Choice A: A DNA sequencing reaction was performed using the primer:
  5'-AGGCGTAGTGCTTTGC-3', with the human growth hormone DNA as the template.
  The sequencing gel is shown to the right.

  a) The DNA sequence for the hormone gene shown on the previous page contains an error.
    Using the sequencing gel, identify the error and indicate its location on the DNA sequence.
    The sequence is repeated here (2 pts):
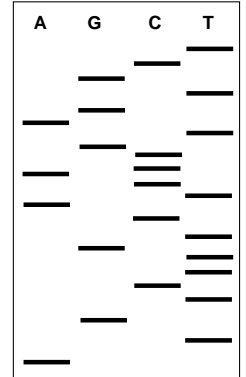
AGGCGTAGTGCTTTGC<u>ATGT</u>**T**TTGTCATCACCGTAGTGCTGATGGG<u>TGA</u>TGTAGTCTG

> The sequence from the sequencing gel is ATG TCT T, i.e. the second
> codon has been changed from TTT to TCT

  b) Does this error change the protein sequence? Briefly, justify your answer. (1 pt)

> Yes, TTT codes for Phe, TCT for Ser.

c) Briefly describe the reaction components that were used to generate the DNA bands in the 'A' lane.(3 pts)

> 1. Primer (AGGCGTAGTGCTTTGC) (+1/2)
> 2. Template (The above DNA, in double stranded form) (+1/2)
> 3. DNA polymerase (+1/2)
> 4. All four 'normal' nucleoside triphosphates (dNTP) (+1/2)
> 5. Dideoxy A, to cause chain termination when it is incorporated. (+1)

Choice B:

a) Show the PCR primers that would be required to generate the PCR product shown at the top of the previous
  page. You should give the sequence of the first 10 bases of each primer. (3 pts)

> **AAGCTT**<u>ATG</u>TTTTGTCATCACCGTAGTGCTGATGGGTGA**AAGCTT**
> **TTCGAA**TACAAAACAGTAGTGGCATCACGACTACCCACT**TTCGAA**

The left primer would be: 5'-**AAGCTT**<u>ATG</u>TT-3'

The right primer would be: 3'-CACT**TTCGAA-5'**

b) Briefly describe the components that would be required for PCR and indicate how the process works. Feel
  free to use a diagram to illustrate your answer.(3 pts)

> 1. Template (human DNA) (+1/2)
> 2. The above two primers (+1/2)
> 3. dNTPs (+1/2)
> 4. DNA polymerase (+1/2)

The region between the two primers is amplified by repeated cycles of (+1 pt)
>   i) Thermal denaturation of DNA
>   ii) Annealing of primers
>   iii) Extension of primers by DNA polymerase.

11. (5 pts) The following shows an AT base pair and a TA base pair.   The first would be found in the sequence:

<div align="center">XAX   while the second would be found in the sequence: XTX
XTX   XAX</div>

Restriction endonucleases can easily distinguish between these two sequences due to the formation of hydrogen bonds in the major groove. If restriction endonucleases bound in the minor groove would they be able to differentiate between an AT and a TA basepair?

Begin by marking *all* non-watson crick hydrogen bond donors (D) and acceptors (A) in the major *and* minor groove of the following AT and TA base pairs. (3 pts).   And then justify your answer by discussing how an enzyme would interact with each basepair via the minor groove. (2 pts)

<div align="center">Major Groove</div>
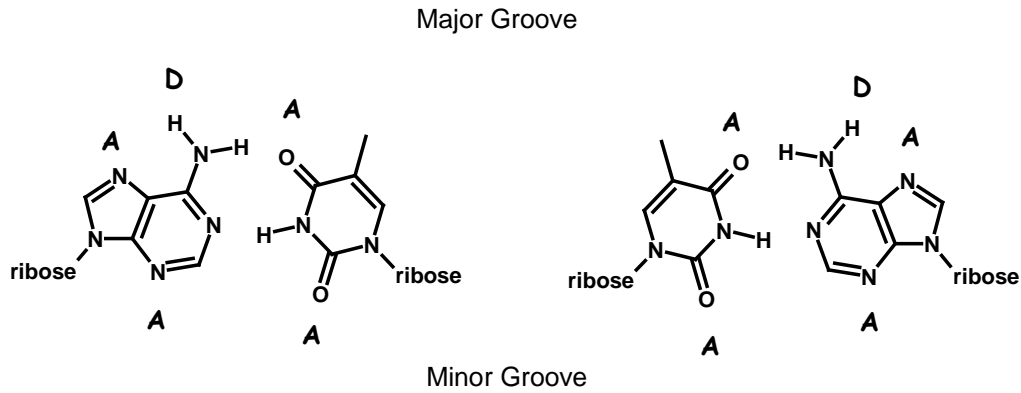
<div align="center">Minor Groove</div>

+1 1/2 pts for identifying all H-bonds in major groove, -1/2 if Nitrogens on 5 membered ring of A were not identified.
+1 1/2 for identifying all H-bonds in minor groove.

The AT base pair has the following pattern of H-bonding: A---D----A---
While a TA base pair has the following: -----A----D--A
where the dashes between the letters indicates the approximate spacing between the donors and acceptors. The donors and acceptors are clearly in different locations in the major groove!

In the minor groove both AT and TA show two acceptors in roughly the same position, thus the enzyme could not tell one from the other (+ 2pts)

12. List the major metabolic pathways (e.g. TCA cycle, fatty acid oxidation (FAO), glycolysis, oxidative phosphorylation (OxPhos), in the correct order, that the following compounds would be processed by while being converted to energy (ATP) .

You need only do *two* of the four. (4 pts).

Sucrose (table sugar): Glycolysis → TCA cycle → OxPhos

Olive oil (triglyceride): FAO → TCA cycle → OxPhos

NADH: → OxPhos

Amino acids: TCA cycle → OxPhos

13. Provide a *general* description of a biological membrane. You should comment on the nature of the lipids found in the membrane and their important physical properties. Indicate the location of the various classes of membrane proteins with respect to the membrane and provide a *brief* discussion relating the location of each class to its function. You can answer this question with a well labeled diagram. (6 pts).

1. Planar bilayer formed of phospholipids (+1 pt), impermeable barrier. (+1 pt)

2. Contains cholesterol (+1/2 pts) which keeps membranes fluid. (+1/2 pts)

3. Integral membrane proteins:

       Partially buried - perform enzymatic functions (+1 1/2 pts)

       Transmembrane (+1 1/2 pts)
              i) transport proteins
   ii) signaling proteins.

Bonus! Dinitrophenyl (DNP) is a weak somewhat *non-polar* acid, as indicated in the diagram to the right. At one time it was widely used as a diet drug, before the FDA pulled it off the drug store shelf. Its mode of action was to reduce the efficiency of ATP synthesis during oxidative phosphorylation. Suggest how this might occur. (3 pts).
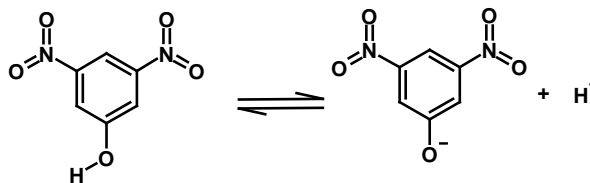
When protonated, DNP can cross the inner mitochondrial membrane, reducing the proton gradient.

14. Please do *one* of the following two questions. (6 pts).

Choice A: In the liver, the hormone epinephrine causes the release of glucose from glycogen as well as the net synthesis of glucose from pyruvate via gluconeogenesis. Explain how both of these processes are coordinately regulated by protein phosphorylation. You answer should include a brief description of the G-protein coupled receptors and the signal transduction pathway.
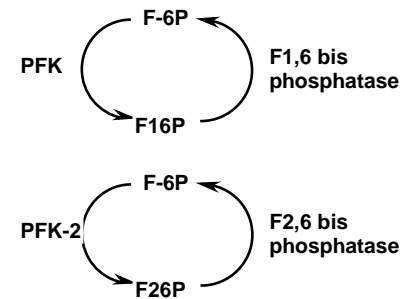
1. Epinephrine cause enzymes to be phosphorylated by the following mechanism (+2 pts)

 i) Epinephrine binds to its receptor on the surface of the cell.
 ii) A conformational change activates a G-protein inside the cell.
 iii) The activate G-protein activates Adenyl cyclase, increasing cAMP levels.
 iv) cAMP activates several protein kinases.
 v) The protein kinases phosphorylate enzymes.

2. Glycogen phosphorylase, which releases glucose from glycogen is active when phosphorylated (+2 pts).

3. Protein Phosphorylation controls F26P levels (+2 pts). F2.6 P levels will be low when glucose levels are low such that glycolysis is inhibited (recall PFK is activated by F26P) and gluconeogenesis is activated (recall F1,6 bis phosphatase is inhibited by F26P) . Therefore, F2-6 bis phosphatase must be active when phosphorylated to convert F26P to F6P.

Choice B: In the muscle tissue, the hormone epinephrine also causes the release of glucose from glycogen. In contrast, glycolysis is activated, instead of gluconeogenesis. Given that the regulation of PFK and 1,6 bisphosphatase are the same in both tissues, explain the most likely difference in effect of protein phosphorylation on enzyme activation in the liver versus the muscle tissue. **(+3 bonus points)**

The muscle enzymes that are responsible for the synthesis and degradation of F26P are regulated in the opposite fashion. i.e. protein phosphorylation activates PFK-2, leading to the production of high levels of F26P when epinephrine is present, which then activate glycolysis.

15. (8 pts) Please do *both* sections of this question.
Peptide bond cleavage can be accomplished by any of the following enzymes: trypsin, chymotrypsin or HIV protease. Using one of these enzymes as examples, or any other enzyme that you think appropriate, discuss **both** of the following general attributes of enzyme catalysis.

i) Substrate specificity. Why do enzymes recognize specific substrates. Provide an example. (4 pts).

By forming complementary interaction with their substrates, utilizing favourable hydrogen bonding, van der Waals, hydrophobic effect, and electrostatic interactions (+2 pts).

Examples (+2 for any of these).
- Trypsin cleaves after Lys and Arg residue by virtue of a neg. charge Asp residue in its active site that forms a favourable electrostatic interaction with the substrate.
- Chymotrypin: large non-polar binding pocket that binds Phe, Tyr, Trp substrates by hydrophobic effects and van der Waals interactions.
- HIV protease: non-polar interactions between Val82 and non-polar substrates.
- Lysozyme: formation of hydrogen bonds with N-acetyl group of sugars in bacterial cell wall.
- Eco R1 - recognition of specific base pairs by formation of H-bonds in major groove.

ii) Why do enzymes enhance the rate of chemical reactions? Your answer should include a discussion of the transition state and the role that amino acids play in the catalytic process. Provide an example.

1. The transition state is the high energy intermediate in the chemical reaction (+1 pt)

2. Enzymes lower the energy of the transition state, thereby increasing the rate of the reaction since more molecules can access the transition state. (+1 pt)

3. This is accomplished by (2 pts for either)

    i)   Direct, enthalpic interactions with the transition state, e.g. the oxy-anion hole of Ser proteases.

    ii)   By providing suitable catalytic groups in the correct location. This reduces the entropy by pre-ordering the active site residues, e.g. the catalytic triad of serine proteases, the dual Asp residues of HIV protease.

16. (10 pts) A virus utilizes an enzyme to cleave polysaccharides off the surface of cells as a necessary step prior to infection of cells.   This enzyme catalyzes the reaction shown to the right.
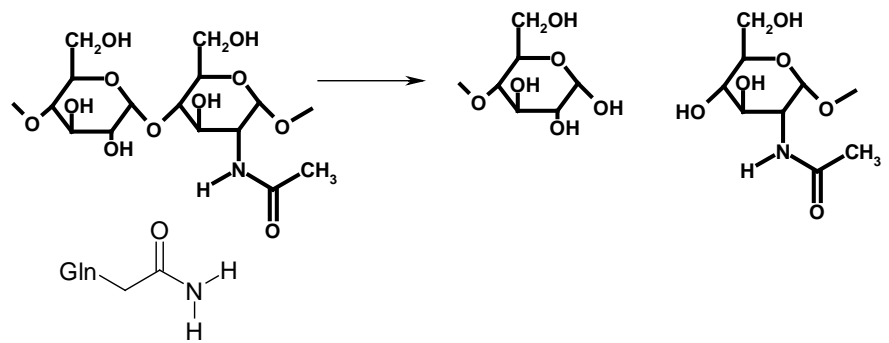
The diagram shows part of the polysaccharide substrate (bold) as well as one of the amino acid sidechains, from a Gln residue, within the active site of the enzyme.   This residue is distant from the cleaved bond and therefore *not* involved in catalysis.

Please answer the following questions:

i) What is the general name for this type of reaction? Briefly justify your answer (2 pts).

> Hydrolysis, since water is added to the bond.

ii) Describe the type of linkage between the saccharides (e.g. β(2-6)). (1 pt)

> α(1-4)

The disaccharide of glucose and N-acetylglucose (shown to the right) can be an effective inhibitor against infection by the virus. As with many other viruses, there is a high rate of mutation in the viral proteins and enzymes. One such mutant enzyme was isolated and the Gln was found to be replaced by a Glutamic acid residue.
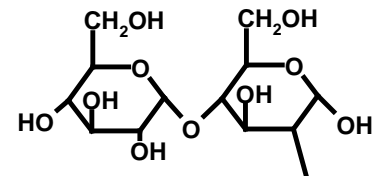
iii) Indicate, on the diagram to the right, ***how you would modify the disaccharide drug*** such that it would bind effectively to the mutant enzyme. The mutant sidechain (Glu) is shown. Briefly explain why your modification of the disaccharide will lead to an drug that is effective at binding to and inhibiting the mutant enzyme (4 pts)
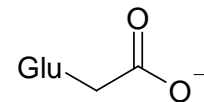
> The Gln residue formed nice hydrogen bonds with the original polysaccharide. One of these will be lost due to changing the amide group to a carboxylic acid. Two general answers are possible:
>
> i)   Protonation of the Glu sidechain will restore activity by allowing the h-bond to form (+2 partial credit)
>
> ii)   Add a positively charged residues (amino) to the disaccharide, giving a favourable electrostatic interaction (+4 pts)

iv) Assuming that this is an RNA virus similar to HIV, explain why high levels of mutations are found in the viral genetic material? Your answer should compare the properties of various DNA polymerases (3 pts).

> Viral polymerases which use RNA as the template, often lack the error checking 3'-5' exonuclease that is found in other polymerases. Without error checking the polymerase makes lots of mistakes in copying its genetic material, leading to mutations.

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