**Instructions**: This exam contains 220 points on 11 pages. Please use the space provided, or the back of the previous page if necessary. On questions with choices, all of your attempts will be graded and you will receive the best grade for that question. The last page of the exam provides a codon table.

1. (10 pts) This question involves a 0.1 M buffer solution with a volume of one(1) L whose pH is 7.0.
   i) Which of the following monoprotic weak acids would you use for this buffer: Acetic acid (pKa=4.8), Tris (pKa=8.2), Imidazole (pKa=6.8). Why? (2 pts)
   ii) Do one of the following two choices (8 pts):
   **Choice A:** Describe how you make this buffer using the fully protonated form of the weak acid.
   **Choice B:** The reaction that you are buffering produces protons, dropping the pH to 6.0. How many equivalents of NaOH do you need to add to restore the pH to 7.0?

2. (8 pts) Draw the structure of a tripeptide using three **different** amino acids. You can pick any amino acids that you like, with the exception of Ile, Thr, and Val. You should indicate the sequence of your peptide, the location of peptide bonds, distinguish between mainchain and sidechain atoms, and indicate any chiral centers.

3. (8 pts)
   i) The peptide bond is fixed in one configuration, planer. Why is this so? (4 pts).
   ii) Imagine that the peptide bond is as freely rotatable as the other mainchain bonds in proteins, how would this affect protein stability? Increase or decrease it? You should justify your answer with a **quantitative** estimate of how rotation of the peptide bond would affect stability [Hint: S=RlnW] (4 pts).



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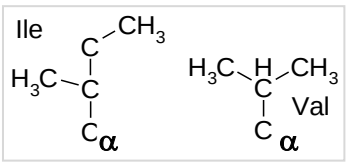
4. (6 pts) An Ile residue in the core of a protein is replaced by a valine residue. You should assume that changing the Ile to Val does not change the overall structure of the protein. Please do **<u>one</u>** of the following choices.




**Choice A:** How will the enthalpy of unfolding be affected by this mutation? Will it increase or decrease? Briefly justify your answer (6 pts).

**Choice B:** How will the entropy of unfolding be affected by this mutation? Will it increase or decrease? Briefly justify your answer (6 pts).

5. (14 pts) Select one of the following enzymes: Trypsin, Chymotrypsin, Elastase, HIV protease, or the Potassium channel (or another one discussed in the course) and answer the following questions. Use the same enzyme for i and ii, you can use a different enzyme for iii if you like.

i) Give the substrate and products of the reaction (1 pt).

ii) Describe the role of functional groups in catalyzing the reaction (4 pts).

iii) Discuss the basis of substrate specificity for the enzyme (4 pts).

iv) Discuss the principal reason why **all** enzymes enhance the rate of reactions (5 pts).



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6. (12 pts) Describe the important features of allosteric systems. Then select one allosteric system and describe why its allosteric features are important for biological function or the expression of proteins in E. coli.

7. (10 pts) What is the hydrophobic effect and describe is its role in **both** protein folding **and** lipid bilayer formation.

8. (5 pts) Glycogen and cellulose are composed of the same monomeric unit. Draw the cyclic structure of that monosaccharide, give its name, and indicate how it is linked together in **either** glycogen or cellulose. Which of these two polymers, glycogen or cellulose, is used for energy storage?



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9. (12 pts)
   i) Outline the major metabolic pathways in yeast cells that are responsible for the complete oxidation of carbohydrates, beginning with monosaccharides and ending with the reduction of water. You answer should focus on the fate of carbon as well how the energy released by these oxidations is captured for ATP formation (8 pts).
   ii) How would your answer change if cells were cultured under low oxygen conditions? (4 pts).

10. (12 pts) Please do **one** of the following two choices:

**Choice A:** You haven't eaten in a while and your liver has been actively metabolizing, consuming ATP. You then have a large influx of glucose due to eating lunch.

i) What will happen to glycogen levels in the liver cell after your lunch? Describe the regulatory events that cause this effect to happen (6 pts).

ii) What will happen to ATP levels in the liver cell, initially, and some time later (i.e. you need to discuss both hormonal and energy regulation of the appropriate pathways (6 pts).

**Choice B:** You had an enormous breakfast and your liver hasn't been too busy, so it has adequate ATP levels. After some time your blood glucose levels begin to drop.

i) How will your liver respond to the drop in blood glucose? What will happen to glycogen levels in the liver cell? Describe the regulatory events that cause this effect to happen (6 pts).

ii) What will happen to ATP levels in the liver cell, initially, and some time later (i.e. you need to discuss both hormonal and energy regulation of the appropriate pathways (6 pts).



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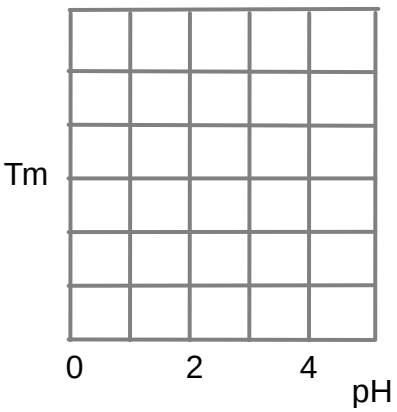
11. (8 pts) Please do both parts of this question.
   i) Draw the chemical structure of any triglyceride or any phospholipid, be sure to indicate your choice.
   ii) A closed phospholipid vesicle is placed in a solution of glucose. What happens to the size of the vesicle, and why?

12. (6 pts) How would you determine whether a protein had a quaternary structure (a definition of a quaternary structure would be a good way to start your answer).

13. (8 pts) The phosphate group on DNA has a $pK_a$ of 1.0.
   i) What is the charge on DNA at pH 7.0? (2 pt)
   ii) Sketch a graph of the $T_M$ for double stranded DNA as a function of pH in the space to the right. Justify your answer with a discussion which molecular force/interaction would be most affected by changing the pH. Hint: you may find it useful to also plot the fraction protonated versus pH (6 pts)



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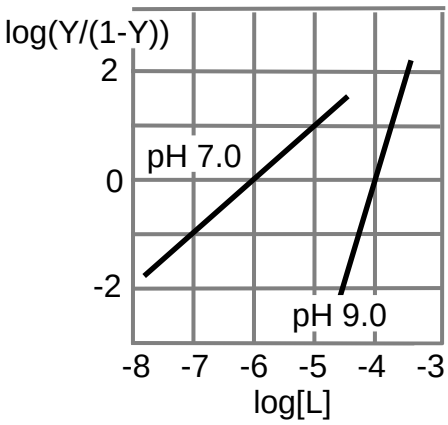
14. (12 pts) Single stranded binding protein binds to single stranded DNA. In this question, you should consider the protein to be the ligand (L) and the DNA to be the macromolecule (M). Many proteins can bind to one DNA molecule, i.e. possible liganded species are ML, $ML_2$, $ML_3$, …, and it is possible to have cooperative protein-protein interactions between adjacent bound proteins. The Hill plot for this system is shown on the right, obtained under two different pH values, pH 7.0 and pH 9.0.

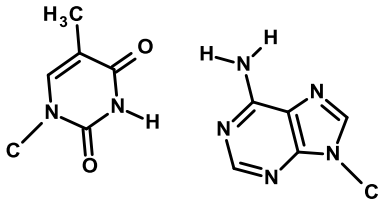



i) What is the $K_D$ for binding at pH 7.0? Justify your approach (2 pts).

ii) Explain the effect of pH on the $K_D$ for the protein-DNA interaction, i.e. what interaction between the protein and the DNA is most likely being affected by pH? State the most likely functional groups (5 pts).

iii) How does pH affect the cooperativity? Provide a possible explanation for the effect (5 pts)

15. (8 pts) The nucleotide base portion of a basepair is shown on the right.




i) Draw the Watson-Crick hydrogen bonds between the bases (1 pt)

ii) Circle the purine base (1 pt)

iii) Draw the complete structure of one of the bases, i.e. draw the ribose (or deoxyribose) and show the correct linkage to the nucleotide that would be above and below the base. The C1' carbon on the sugar is drawn for you (5 pts) (Q18 may be helpful).

iv) Which edge, top or bottom, is the major groove (1 pt)?



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16. (4 pts) Please do **one** of the following choices.

**Choice A:** A protein binds to the following DNA sequence: TTTAAA
AAATTT

Is this protein likely a heterodimer or a homodimer? Why?

**Choice B:** A protein that binds in the minor groove can bind to double stranded C or G with equal affinity, Why?

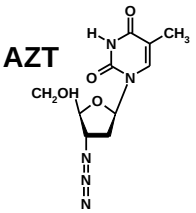
**Choice C:** DNA protein interactions are often affected by salt, why?

17. (6 pts) DNA polymerases usually insert the correct residue in the newly synthesized strand.

i) What are the factor(s) the affect the selection of the new base? (3 pts)

ii) How is the new base added? Give a brief description of the reaction (3 pts).

18. (6 pts) The drug ATZ is shown on the right. This drug is an inhibitor of HIV reverse transcriptase, the enzyme involved in copying the viral RNA to DNA. AZT is very effective at preventing HIV replication, even at relatively low concentrations. Why is this drug very effective at interfering with the life cycle of the HIV virus (Hint: a similar concept is exploited in dideoxy sequencing of DNA).

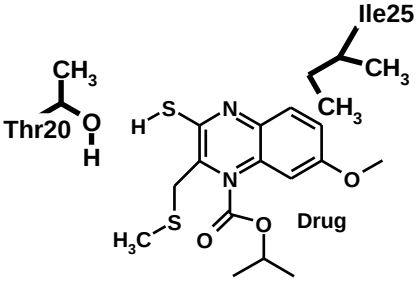



***Note: The next questions (19-23) are related.***

19. (6 pts) A mixed-type inhibitor of reverse transcriptase is shown on the right. Residues from the reverse transcriptase (Thr20, Ile25) are shown in bold. The beginning, middle, and ending sequence of the 600 basepair reverse transcripase gene is shown below:

```
     1  2  3  4     17 18 19 20 21 22 23 24 25 26            200
     MetTyrValHis---AlaGlyProThrSerArgLysAlaIleGlu---SerSerTyrPhe
CGCG ATG TATGTTCAT---GCGGGCCCGACCAGCCGCAAAGCGATTGAA---AGTAGTTACTTT TAA
GCGCTACATACAAGTA---CGCCCGGGCTGGTCGGCGTTTCGCTAACTT---TCATCAATGAAAATT
```

What thermodynamic forces or interactions are responsible for the stabilizing the bound form of this drug. Justify your answer with reference to functional groups on both the drug and the enzyme.



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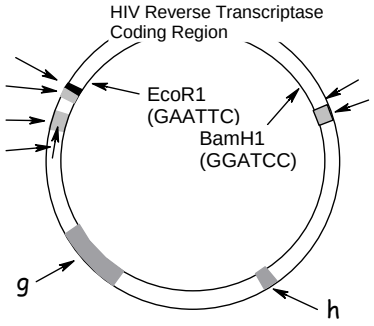
20. (22 pts) A mutation has arisen in the gene for reverse transcriptase that has reduced the drug binding, producing a drug resistant strain of HIV virus.
   i) Why are high levels of mutations found in the HIV virus (1 pt)?
   ii) Describe in general, all of the steps that you would need to take to produce this mutant protein in E. coli, beginning with the viral RNA and ending with purification of the protein from bacteria (6 pts).

iii) An image of the expression vector is shown on the right. The arrows on this diagram indicate the location of all of the control elements that will be required to produce the reverse transcriptase intracellularly. These elements are listed on the right, labeled a-h. Place the label in the correct position. The last two (g, h) have been done for you (3 pts).




a) lac operator
b) start codon
c) ribosome binding site
d) stop codon
e) mRNA termination
f) promoter
g) antibiotic resistance gene
h) origin of replication

iv) Select two (2) of elements from the above list (a-g) from part ii and provide a brief description of their function (4 pts). **One of your selections must be choice a) (lac operator).**

iv) Provide the sequences of the PCR primers that would be necessary to generate the desired segment of DNA to insert into the vector using EcoR1 (GAATTC) and BamH1 (GGATCC) sites. Make your primers a total of 12 bases in length. Pay close attention to control elements that are already present on the vector and therefore do not need to be part of the PCR product (6 pts).

Left Primer: _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____

Right Primer: _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____

v) Suggest an annealing temperature for PCR, based on the melting temperature of the left primer (2 pts).



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21. (16 pts) A section of the sequencing reaction from wild-type and mutant DNA is shown on the right.

i) Identify changes in the DNA sequence and determine which changes occurred in the protein sequence. A portion of the wild-type sequence is given below and the structure of the enzyme-drug complex is also redrawn below (4 pts).

```
17   18   19   20   21   22   23   24   25   26
Ala  Gly  Pro  Thr  Ser  Arg  Lys  Ala  Ile  Glu
GCG  GGC  CCG  ACC  AGC  CGC  AAA  GCG  ATT  GAA
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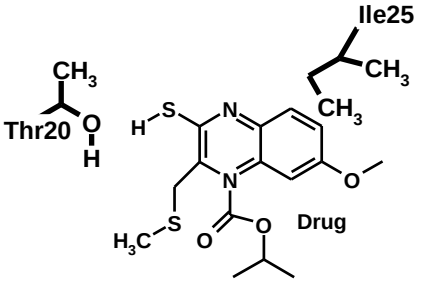
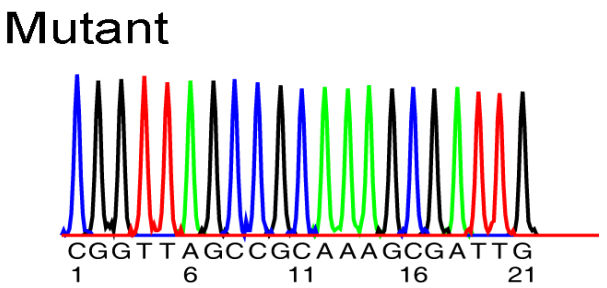
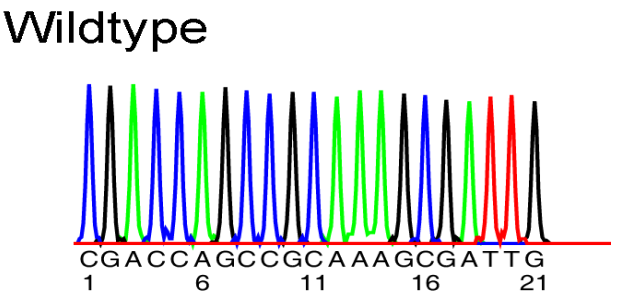
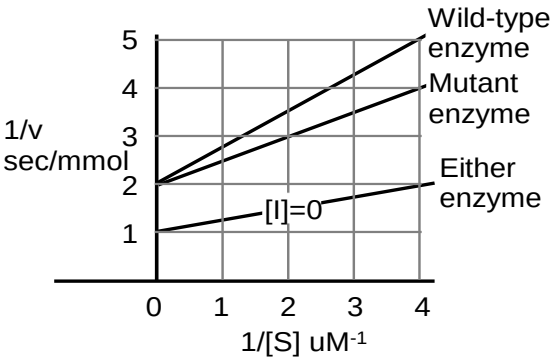



ii) Draw the structure of the changed residue, and indicate how would you **alter the drug** to increase its affinity to the mutant enzyme? (4 pts)




iii) Steady state enzyme kinetics was performed using the wild-type and mutant enzyme in the presence of 1 nM of the inhibitor. Did the mutation increase or decrease the affinity of the drug for the free enzyme ($K_I$)? By how much? (6 pts)




iv) Please do one of the following two choices (2 pts):

**Choice A:** What sequencing primer was used to obtain this sequencing data?

**Choice B:** Briefly explain how the second peak in the data, corresponding to a G, was generated.



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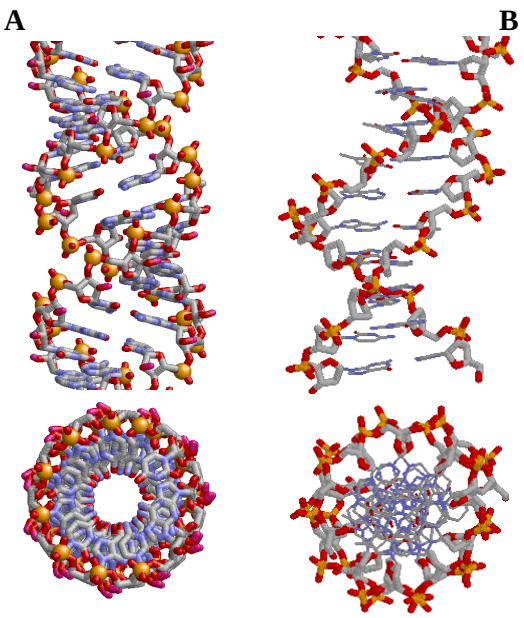
22. (5 pts) Please do **one** of the following three choices.

**Choice A:** why is it advantageous to use different restriction endonucleases (e.g. EcoR1 & Bam H1) for insertion of the PCR product into the vector, rather than a single restriction endonuclease?

**Choice B:** how might you modify the expression vector to export the HIV reverse transcriptase out of the cell?

**Choice C:** how might you modify the expression vector to facilitate purification of the HIV reverse transcriptase by affinity chromatography of beads containing nickel ions?

23. (4 pts) The side view and top view of an RNA molecule and a DNA molecule are shown on the right. Which is which? Briefly justify your answer.




24. (6 pts) Please do one of the following choices:

**Choice A**: Explain the role of the sigma factor in RNA polymerase activity.

**Choice B**: Explain how indirect coupling is used to make nucleic acid polymerization (or tRNA charging) spontaneous.



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25. (6 pts) Please do **one** of the following choices:

**Choice A**: Briefly describe the role of the three tRNA binding sites on the ribosome in protein synthesis?

**Choice B**: How is the reading frame determined by the ribosome during protein synthesis?

**Bonus** (2 pts each)

**B1.** If you ran in the Pittsburgh marathon and hope to run in another one within a week or two, would it be better to eat a high fat/protein diet, or a high carbohydrate diet, in preparation for your next race. Why?

**B2.** If the ribosome is an apple, then the stem is the_____.

| 5' Base | Middle Base | | | | 3' |
|---|---|---|---|---|---|
| | **T** | **C** | **A** | **G** | |
| **T** | Phe | Ser | Tyr | Cys | **T** |
| | Phe | Ser | Tyr | Cys | **C** |
| | Leu | Ser | **Term** | **Term** | **A** |
| | Leu | Ser | **Term** | Trp | **G** |
| **C** | Leu | Pro | His | Arg | **T** |
| | Leu | Pro | His | Arg | **C** |
| | Leu | Pro | Gln | Arg | **A** |
| | Leu | Pro | Gln | Arg | **G** |
| **A** | Ile | Thr | Asn | Ser | **T** |
| | Ile | Thr | Asn | Ser | **C** |
| | Ile | Thr | Lys | Arg | **A** |
| | **Met** | Thr | Lys | Arg | **G** |
| **G** | Val | Ala | Asp | Gly | **T** |
| | Val | Ala | Asp | Gly | **C** |
| | Val | Ala | Glu | Gly | **A** |
| | Val | Ala | Glu | Gly | **G** |



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