Instructions: This exam contains 260 points in 29 questions on 12 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.

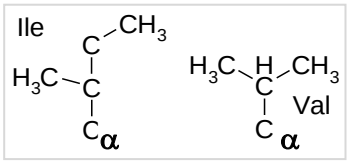
1. (10 pts) This question involves a 0.1 M buffer solution with a volume of one(1) L whose pH is 7.0.
   i) Which of the following monoprotic weak acids would you use for this buffer: Acetic acid (pKa=4.8), Tris (pKa=8.2), Imidazole (pKa=6.8). Why? (2 pts)
   ii) Do one of the following two choices (8 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?

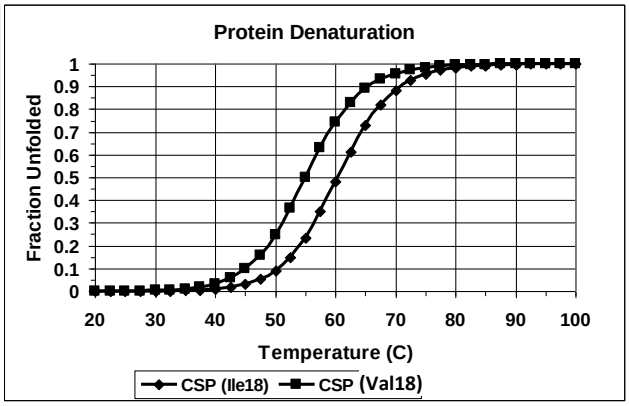
2. (8 pts) Draw the structure of a tripeptide using three different amino acids. You can pick any amino acids that you like, with the exception of Ile, Thr, and Val. You should indicate the sequence of your peptide, the location of peptide bonds, and distinguish between mainchain and sidechain atoms.

3. (10 pts) The amino acid sequence for secondary structures that are found at the exterior of water soluble proteins have a well defined distribution, or order, of polar and non-polar residues.
   i) What is that distribution, for **both** α-helices and β-sheets. Briefly justify your answer with reference to the structure of helices and sheets (6 pts).
   ii) Which groups face the inside of the protein? Polar or non-polar? (2 pts).
   iii) How will the arrangement of polar and non-polar residues change if you were discussing an integral membrane protein? (2 pts).

4. (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).

5. (10 pts) The denaturation curves for wild type cold shock protein (CSP, Ile at position 18) and an Ile18 to Val substitution (mutation) are shown to the right. The thermodynamic parameters for unfolding of the wild-type protein are: ΔH^o = +200 kJ/mol, ΔS^o= +600 J/mol-deg. You should assume that the Ile is buried in the core of the protein.





   i) How will the enthalpy of unfolding be affected by this mutation? Will it increase or decrease? Briefly justify your answer (5 pts).
   ii) How will the entropy of unfolding be affected by this mutation? Will it increase or decrease? Briefly justify your answer (5 pts).

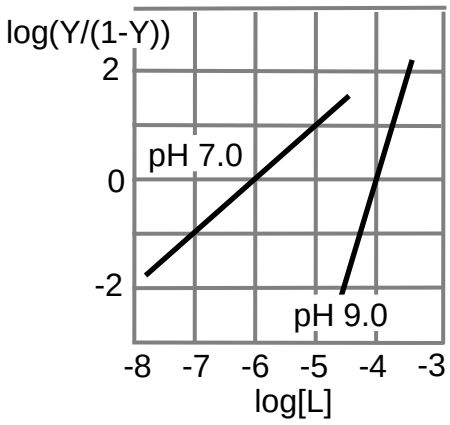
6. (14 pts) Select one of the following enzymes: Trypsin, Chymotrypsin, Elastase, HIV protease, or the Potassium channel and answer the following questions. Use the same enzyme for I and ii, you can use a different enzyme for iii if you like.
   i) Give the substrate and products of the reaction (1 pt).
   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).

7. (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 useful in the regulation of protein expression from expression vectors.

8. (12 pts) What is the hydrophobic effect and what is its role in protein folding, lipid bilayer formation, and the stability of double stranded DNA?

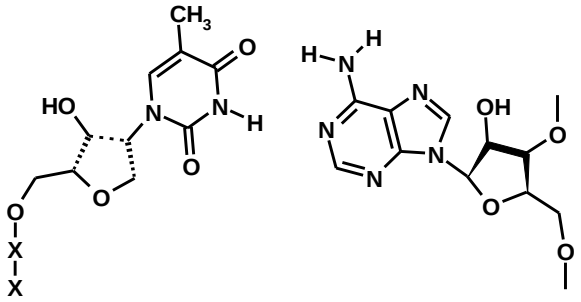
9. (12 pts) The phosphate group on DNA has a pK_a of 1.0.
   i) What is the charge on DNA at pH 7.0? (1 pt)
   ii) Sketch the titration curve for a segment of double stranded DNA that is 10 nucleotides long. Be sure to indicate the number of equivalents on the x-axis. (5 pts)
   iii) Sketch a graph of the T_M for double stranded DNA as a function of pH. Justify your answer with a discussion which molecular force/interaction would be most affected by changing the pH. (6 pts)

10. (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? (3 pts)
   iv) Explain the effect of pH on cooperativity by postulating the nature of the interaction between the individual proteins while bound to the DNA (2 pts).

11. (9 pts) A TA basepair in DNA shown on the right. The "T" is the first base in a longer sequence, i.e. TXXXX.
    i) Although the relative position of the TA bases is correct, the diagram contains at least three errors. Identify and correct three of these errors (3 pts).
    ii) Sketch the phosphate linkage that you would observe linking the T to the next base (2 pts)
    ii) Indicate the "Watson-Crick' hydrogen bonds involved in AT basepairing (1 pt).
    iii) Indicate the major and minor groove (1 pt).
    iv) Label all hydrogen bond donor and acceptors in both grooves (2 pts)



12. (10 pts) A protein binds to the following DNA sequence with a K_D of 1 nM

    TTTTTT
    AAAAAA

    and it binds with a lower affinity to

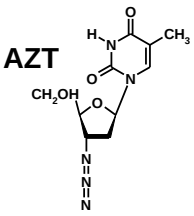
    AAATAA
    TTTATT

    i) Is this protein likely a homodimer or a heterodimer? Why? (2 pts)
    ii) Is this protein binding via the major or the minor grove? Justify your answer (5 pts).
    iii) Estimate the affinity for binding to TTTATT. Be sure to state any assumptions that you make (e.g. the strength of any bonds) in your answer. Assume T=300K. (3 pts)

13. (8 pts) Does the T_M increase or decrease with respect to GC content? Why?

14. (8 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).
    iii) What do most DNA polymerases do if they do make an error and incorporate the wrong base? (2 pts)

15. (5 pts) The drug ATZ is shown on the right. This drug is a competitive 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?



Note: The next questions (16-19) are related.

16. (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
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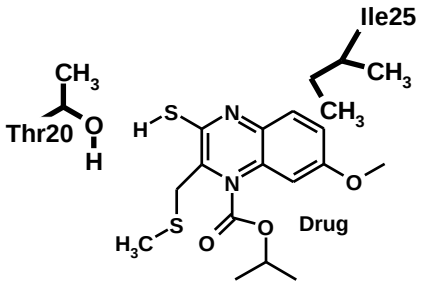
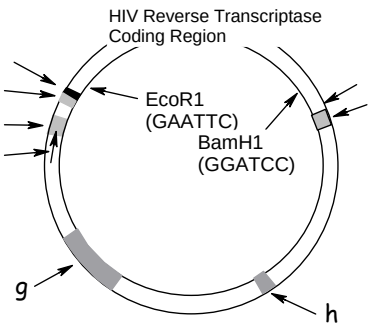
i) What thermodynamic forces or interactions are responsible for the stabilizing the bound form of this drug. Justify your answer with reference to functional groups on both the drug and the enzyme (6 pts).



17. (23 pts) A mutation has arisen in the gene for reverse transcriptase that has reduced the drug binding, producing a drug resistant strain.

i) 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 a bacterial lysate (6 pts).

ii) 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

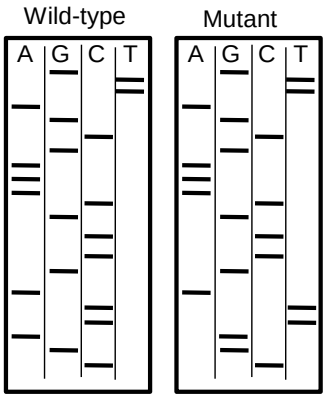
(Note: this question continues on the next page).

iii) Select any 4 of elements a-g from part ii and provide a brief description of their function (4 pts).

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 and BamH1 sites. You need not worry about the annealing temperature; make your primers 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).

v) How could you use gel electrophoresis & restriction digests to confirm that your final expression vector was correct? (4 pts)

18. (14 pts) A section of the sequencing gel of the 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 (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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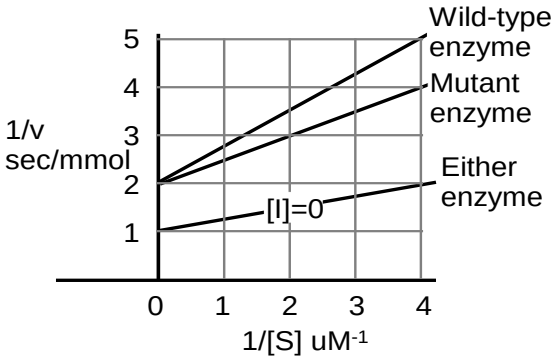
ii) How would you alter the drug to increase its affinity to the mutant enzyme? (4 pts)

(Note: this question continues on the next page).

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)



19. (5 pts) Please do **one** of the following three choices.

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

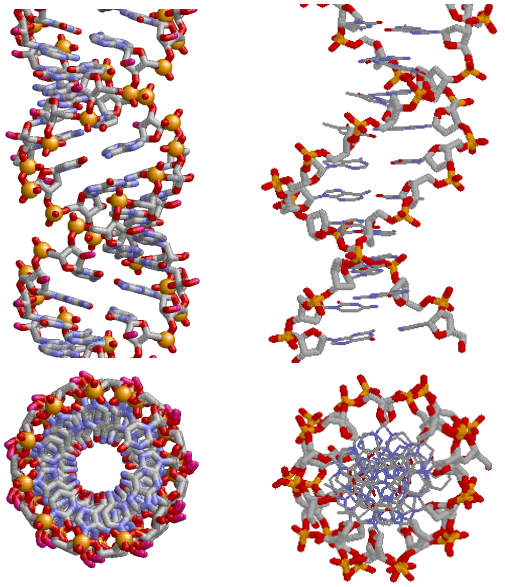
Choice B: how might you modify the expression vector to export the HIV reverse transcriptase out of the cell?

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

20. (3 pts) Although expression of proteins in bacteria has many advantages, it is not the method of choice for proteins that are to be used as drugs, such as insulin or human growth hormone. Why?

21. (5 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.

A **B**



22. (6 pts) The codon table shows that many amino acids are encoded by more than one codon. However, there is usually only one tRNA per amino acid. How can a single tRNA interact with multiple codons?

23. (5 pts) Please do one of the following choices:

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

Choice B: Explain why the first amino acid is N-formyl methionine instead of methionine.

Choice C: Explain how indirect coupling is used to make nucleic acid polymerization and tRNA charging spontaneous.

24. (6 pts) Glycogen and cellulose are composed of the same monomeric unit. Draw the structure of that monosaccharide, give its name, and indicate how it is linked together in **either** glycogen or cellulose.

25. (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).

26. (12 pts) Please do **one** of the following two choices:

Choice A: You haven't eaten in a while but 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? 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).

25. (6 pts) The concentration of potassium ions outside a membrane is 0.1 M and the concentration inside is 0.05 M. The membrane potential is +100 mV, with the inside positive. What direction will the potassium ions flow? Briefly justify your answer (assume T=300K)

28. (2 pts) Define specific activity as it relates to protein purification.

29. (7 pts) You are measuring the binding of a DNA fragment to a protein. The off-rate of the protein from the DNA is very, very, slow such that it is possible to separate the free protein and DNA from the protein-DNA complex using standard purification schemes.
   i) Describe how you might separate the three different components, i.e. the free DNA, free protein, and the protein DNA-complex. (5 pts)
   ii) Explain how you could measure the fractional saturation using UV absorbance of your three separated samples. You should give a clear explanation of how you would obtain the relevant extinction coefficients (2 pts).

Bonus (4 pts) 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?