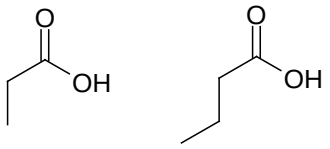
Instructions: This exam contains 250 points in 31 questions on 12 pages. Please use the space provided, or the back of the previous page if necessary. On questions with choices, all of your attempts will be graded and you will receive the best grade for that question.

1. (5 pts) Please do **one** of the following choices:

Choice A: Why are weak acids buffers within one pH unit of their pKa?

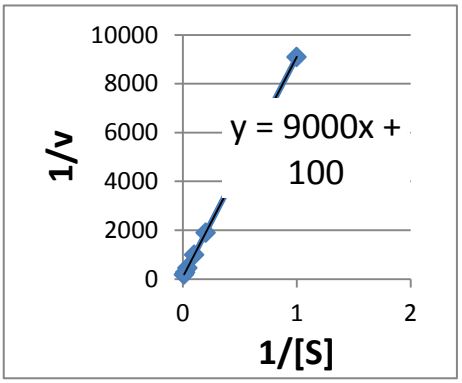
Choice B: Briefly describe how to make a buffer solution, given that the pH of the solution should be equal to the pKa of a monoprotic buffer.

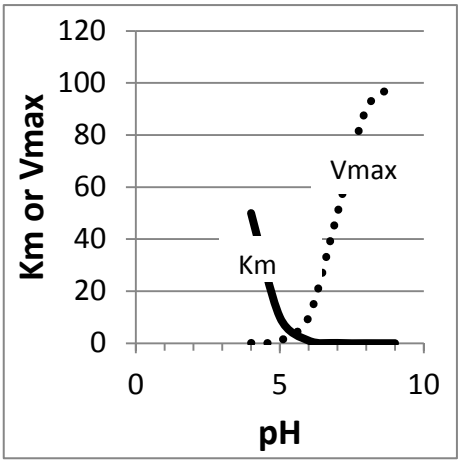
2. (14 pts) The dipeptide, Glu-Asp, is a substrate for a protease. The sidechains of Asp and Glu are shown on the right. *(Part iii of this question is on the next page.)*



i) (6 pts) Draw the substrate (i.e. Glu-Asp) and label the following features on your diagram: a) amino terminus, b) carboxy terminus, c) peptide bond.

ii) (4 pts) The activity of the enzyme against the above substrate was measured at different pH values and the steady-state enzyme kinetic data obtained at pH 3 is are shown on the right. Obtain the Km and Vmax values at pH 3 and plot these values on the graph that shows the effect of pH on Km and Vmax. Please show how you obtained these values.

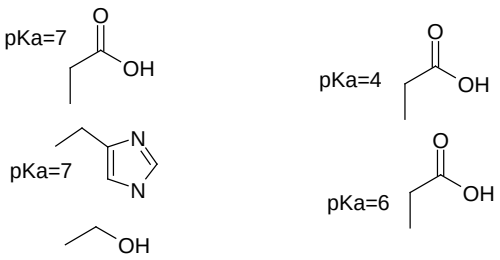




iii) Please do **one** of the following two choices: (4 pts)

Choice A: Based on the dependence of Km/Vmax on pH, what functional groups on the enzyme are most likely to be involved in **binding** of this substrate? Why?

Choice B: The active site residues in serine and aspartate proteases are shown on the right. Is the pH dependence of the Vmax consistent with the mechanism of either a serine protease (e.g. trypsin) or an aspartyl protease (e.g. HIV protease).



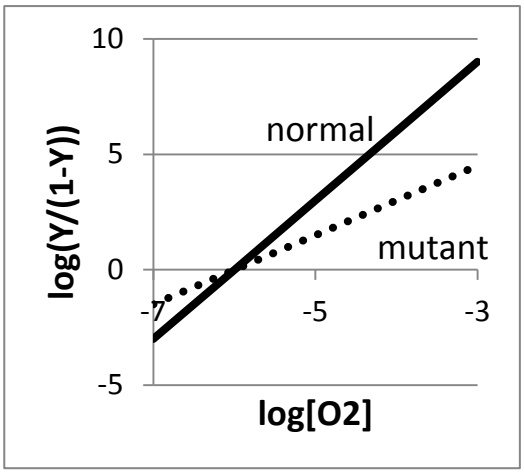
3. (5 pts) The peptide bond is restricted to two conformations, cis and trans, with the trans the preferred lower energy state. Why does it have these properties?

4. (12 pts) The balance between the folded and unfolded state of a protein depends on a number of competing factors, i.e. those that stabilize the folded state and those that destabilize it.
   i) What are these competing factors and are they related to enthalpic (ΔH^o) or entropic terms (ΔS^o) (6 pts).
   ii) How do these factors "shape" the folded form of a protein, i.e. what are the common characteristics of all folded proteins and what thermodynamic forces are responsible for those characteristics? (6 pts)?

5. (6 pts) Compare and contrast the thermodynamic forces that affect the relative stability of folded proteins versus double stranded DNA, **highlight** those forces that play a dominate role in DNA structure. In particular discuss how the concentration of NaCl affects the stability of DNA.

6. (10 pts) What are the characteristics of allosteric systems and how are these characteristics used to control biochemical behavior? Give **one** example from the course where a biochemical process is **controlled** by allosteric behavior.

7. (7 pts) The Hill plot on the right shows the oxygen binding properties of normal hemoglobin and a mutant hemoglobin.
   i) The individual with the mutant hemoglobin has difficulty providing oxygen to their tissues during exercise, why? (4 pts)
   ii) In addition to low oxygen levels, individuals with the mutant hemoglobin also show high levels of lactic acid in their blood during exercise. Why? What is the source of the lactate (3 pts).



8. (10 pts) How do **all** enzymes enhance the rate of chemical reactions?

9. (6 pts) Please do **one** of the following choices:

Choice A: Describe how the tertiary structure of a protein is determined by X-ray diffraction.

Choice B: Describe how the quaternary structure of a protein is determined by gel filtration and gel electrophoresis techniques.

Choice C: What is specific activity and why is it a useful parameter to monitor during protein purification?

10. (12 pts) Briefly describe the structural features of either and alpha helix or a β-barrel. Why are these two structures found in integral membrane proteins? For the secondary structure you selected, how often would non-polar amino acids be found in the sequence, assuming one face of your secondary structure faced the lipid bilayer.

11. (6 pts) Sucrose is β-fructofuranosy-(2→1)-α-glucopyranoside. Draw this structure. What is the name of the bond that joins the two monosaccharides? Can't draw sucrose? Then draw any disaccharide that contains only glucose, and give its name. [Hint: glucose is dud]

12. (6 pts) Briefly discuss how sucrose would be metabolized after hydrolysis into the individual monosaccharides, i.e. through which pathway would the carbon atoms from these sugars flow?

13. (10 pts) A liver cell has high levels of AMP and ADP, but the blood glucose levels in that individual are low.
    i) From the perspective of energy sensing, which pathway should be active, glycolysis or gluconeogenesis, and why. What regulatory features of PFK and bisphosphatase ensure that this would occur (5 pts)?
    ii) From the perspective of the liver's role as a glucose bank, which of those two pathways should be active? How is their activity controlled by hormones (5 pts) ?

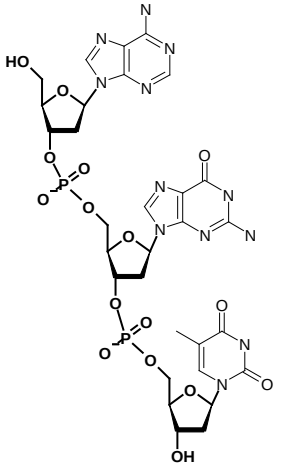
14. (6 pts) How do the structure of glycogen and cellulose differ? In what way are they the same? Which of these is used for energy storage in animals?

15. (5 pts) In catabolic biochemical pathways, such as glycolysis, energy is released by the _____ (type of reaction) of organic compounds. This energy is stored directly on _____ or _____. The stored energy is then used to generate a _____ during _____(a pathway), which is ultimately used to generate ATP.

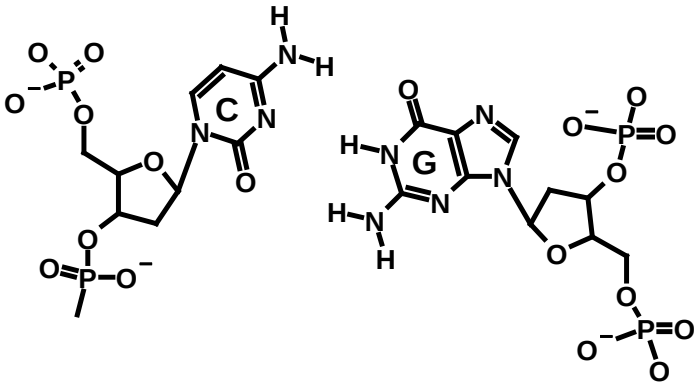
16. (4 pts) Compare and contrast the structural properties of any **two** of the following lipids (you may sketch a chemical structure if you like): a) waxes b) triglycerides, c) phospholipid, d) cholesterol.

17. (10 pts) Describe, or sketch, the structure of a biological membrane. What are the properties of the membrane in terms of permeability and fluidity? What are the general functions of the proteins found in the membrane?

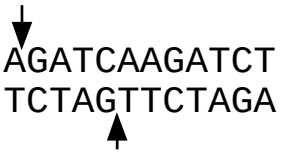
18. (10 pts) The following is a short segment of single stranded DNA, that contains A, G, and T.
    i) Identify the 5' end.
    ii) Circle the purine base(s)
    iii) What changes to this diagram would you make to convert this DNA to RNA?
    iv) If this were double stranded DNA, what is the orientation of the complementary strand, parallel or anti-parallel?



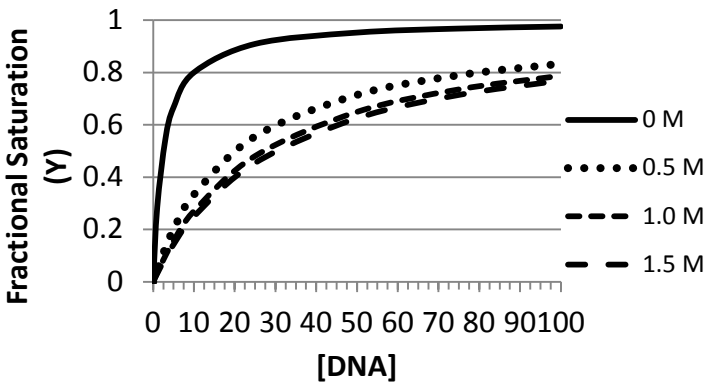
19. (9 pts) A CG basepair in DNA shown on the right.
    i) Indicate the "Watson-Crick' hydrogen bonds involved in this basepair (1 pt).
    ii) Indicate the major and minor groove (1 pt).
    iii) Label all hydrogen bond donor and acceptors in both grooves (2 pts)
    iv) Is it possible for a protein to distinguish a CG basepair from a GC basepair (i.e. interchanging the bases) if it binds in the minor groove? Justify your answer (5 pts).



20. (6 pts) A new restriction endonuclease, CmuII recognizes the sequence shown on the right, and cuts at the arrows.
    i) Is this enzyme a homo- or heterodimer? Why? (2 pts)
    ii) Could you ligate DNA fragments generated by CmuII to DNA fragments generated by BamHI (G^GATCC) [Hint: Draw the products after digestion with each enzyme.] (4 pts). Why?



21. (8 pts) The binding of the lac repressor protein to DNA was measured as a function of salt concentration and the binding curves are shown on the right. Based on these data, what type(s) of interactions are used by the lac repressor to bind to DNA [Hint: It may be useful to sketch a plot of K_D versus [NaCl]]



22. (12 pts) DNA and RNA Polymerases:

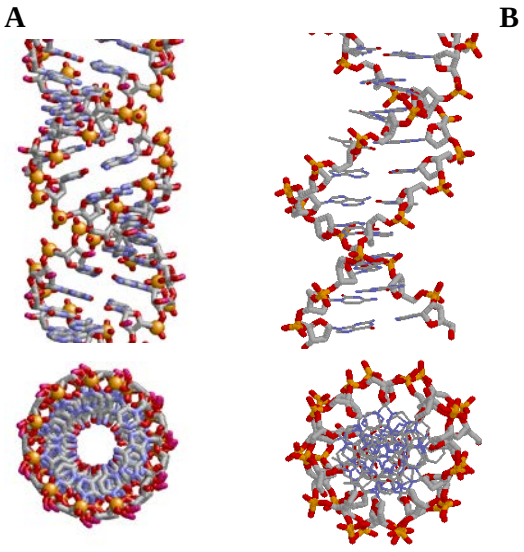
i) What are the factor(s) the affect the selection of the new base that is added to the growing chain?

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

iii) The standard energy change between the reaction and product (addition of a base) is approximately zero, yet polymerization is spontaneous. What feature of the reaction ensures that it is spontaneous?

iv) What is the most significant difference between DNA polymerases and RNA polymerases, besides the fact that one generates DNA and the other RNA.

23. (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.



24. (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.

25. Please do **one** of the following choices (8 pts):

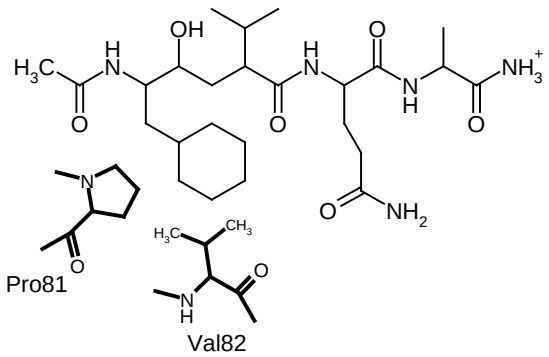
Choice A: Describe the steps associated with the elongation step of protein synthesis.

Choice B: What events occur during termination of protein synthesis?

Choice C: 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?

Note: The remaining questions on this exam are related.

26. (6 pts) The structure of an inhibitor of HIV protease is shown on the right. Residues from the enzyme are shown in bold. 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.

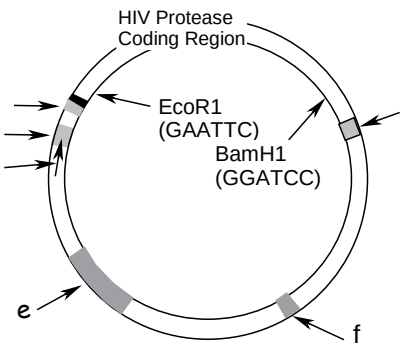


27. (16 pts) A mutation has arisen in the gene for HIV protease that has reduced the drug binding, producing a drug resistant strain.

i) Why are mutations in the viral genetic information quite prevalent (3 pts)?

ii) Describe in general (e.g. with a flowchart), 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).

iii) An image of the expression vector is shown on the right. The arrows on this diagram indicate the location of the control elements that are present on this plasmid. These elements are listed on the right, labeled a-f. Place the label in the correct position. The last two (e, f) have been done for you (3 pts).



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

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

28. 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. You need not worry about the annealing temperature; make your primers 15 bases in length. Pay close attention to control elements that may, or may not, be present on the vector and whether they should be included in the PCR product (6 pts). The sequence of the HIV gene is shown below:

```
  1  2  3  4  5  6         78 79 80 81 82 83 84 85         94 95 96 97 98 99
ProGlnIleThrLeuTrp--------ProThrProValAsnIleIleGly-------GlyCysThrLeuAsnPhe
CCTCAGATCACTCTTTGG--------CCTACACCTGTCAACATAATTGGA-------GGTTGCACTTTAAATTTT
GGAGTCTAGTGAGAAACC--------GGATGTGGACAGTTGTATTAACCT-------CCAACGTGAAATTTAAAA
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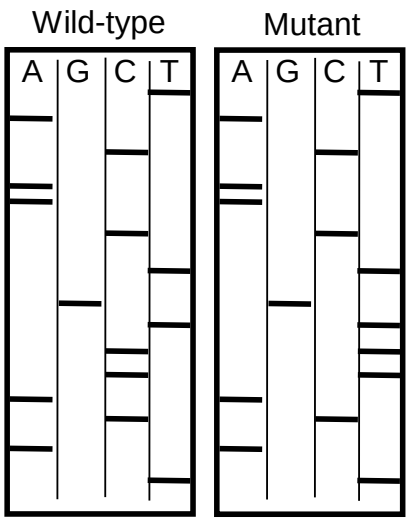
29. (4 pts) After construction of the completed expression vector, you tested four different plasmids to determine which one was correct. The plasmid DNA was digested with EcoR1 and BamH1 and electrophoresis was performed. The gel contained the original PCR product, and the digestion product of the four plasmids. Which plasmid is correct, and why.

30. (16 pts) After determining which plasmid is correct, you sequence the DNA to determine the location of the mutation. A section of the sequencing gel of the wild-type and mutant DNA is shown on the right.



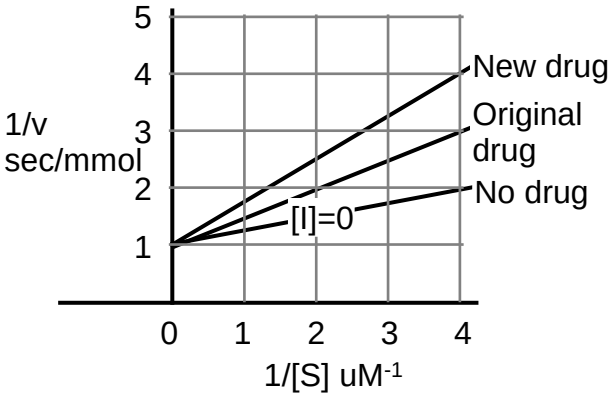
i) What would the "A" lane of the gel look like if the ddATP was accidentally omitted from the reaction? Briefly justify your answer (2 pts)

ii) 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 a codon table is given with the formula sheet (4 pts).

```
  78 79 80 81 82 83 84 85         94 95 96 97 98 99
--ProThrProValAsnIleIleGly-------GlyCysThrLeuAsnPhe
--CCTACACCTGTCAACATAATTGGA-------GGTTGCACTTTAAATTTT
--GGATGTGGACAGTTGTATTAACCT-------CCAACGTGAAATTTAAAA
```

iii) Draw the structure of the altered amino acid. How would you alter the structure of the **drug** to increase its affinity to the mutant enzyme? Be sure to discuss any interactions that might be important for binding your modified drug to the mutant enzyme (4 pts)

iv) Steady state enzyme kinetics was performed using the original drug and your modified drug, in the presence of 1 nM of the inhibitor. How much stronger does the new drug bind to the enzyme? [Hint: Determine K_i] (4 pts)



v) Is this a competitive or mixed type inhibitor? [Hint: how does the inhibitor affect the double reciprocal plot?](2 pt)

31. (5 pts) Please do **one** of the following choices.

Choice A: How might you modify the expression vector to export the HIV protease out of the cell?

Choice B: If the following codons were appended to the end of the coding sequence, how could this facilitate the purification of the HIV protease? CACCACCACCACCACCAC

Bonus (3 pts each)(Use the back of the page to answer).

B1. If you ran in the Pittsburgh marathon and hope to run another 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. In what way is the ribosome like an apple?

B3. In what way are peppermint patties like IPTG?