There are a total of 170 pts on this exam in 12 pages. 1 min/pt is a very comfortable pace.

1. (7 pts) Hydrogen bonds play an important role in many biochemical interactions. Please answer the following questions:

   i) Define, or give a general description, of a hydrogen bond (4 pts)

   ii) Give an example that illustrates the biochemical and thermodynamic importance of hydrogen bonding in **either** a) protein structure, or b) DNA structure.(3 pts)

2. (8 pts) In addition to hydrogen bonding, the following thermodynamic factors:
   i) van der Waals, ii) electrostatics, iii) hydrophobic effect, iv) conformational entropy,
   play a role in the stability of proteins, biological membranes, and DNA.

   i) For each of four interactions, state whether the interaction is primarily an enthalpic or entropic effect and briefly describe the molecular nature of the factor (4 pts).

   ii) Select **one** of the following **three** structures and discuss the relative importance of all of the above four factors in stabilizing the native state (4 pts).
   a) the tertiary structure of globular proteins
   b) the double stranded structure of DNA
   c) phospholipids bilayers.

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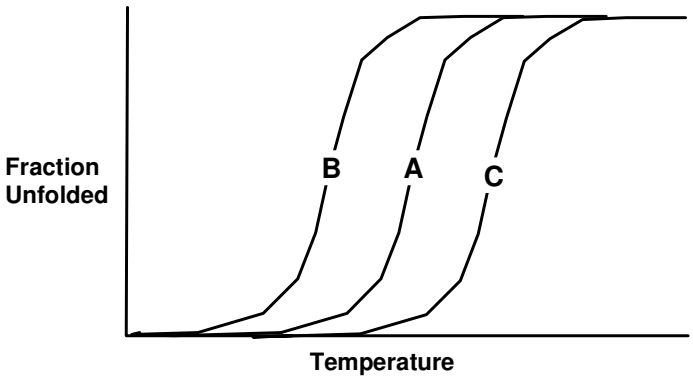
3. (3 pts) Draw any dipeptide and label the peptide bond. Name one feature of the peptide bond.

4. (8 pts) Compare and contrast the **primary** structure of DNA and proteins. Your answer should include a brief description of the monomeric units (including mainchain and backbone), how they are linked together, and the standard convention for naming these polymers.

5. (6 pts) Please do one of the following two choices.

Choice A: A 40 residue amino acid sequence spontaneously forms a helical hairpin (two helices packed against each other, joining by a short turn) in a lipid bilayer. Discuss the general features of the amino acid sequence of this protein.

Choice B: A protein contains an isoleucine residue in its hydrophobic core. The temperature dependence of unfolding of two mutants and the wild type were measured, giving the curves to the right. One mutant contained an isoleucine to valine change, the other an isoleucine to tryptophan change. Which unfolding curves (A, B, C) corresponds to which proteins (Ile, Val, Trp)? Briefly justify your answer.



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6. (1 pt) Give **one** important use of heme-iron ($Fe^{2+/3+}$) in biochemical processes.

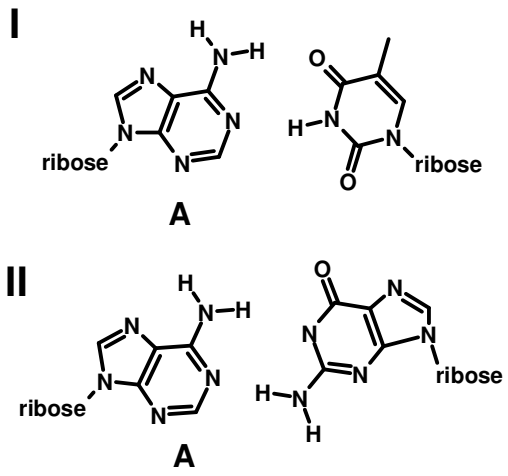
7. (5 pts) Compare and contrast the properties of HIV reverse transcriptase to polymerase III (Pol III is used to replicate the DNA found in E. coli).

8. (8 pts) When a polymerase encounters an "A" in the template, two possible basepairs that could form are shown on the right.

   i) In the top pair (I) which base is a purine? Which is a pyrimidine?

   ii) Identify "Watson-Crick" hydrogen bond donors and acceptors on the A base, and indicate which hydrogen bonds could form during basepairing in the active site of the polymerase for both basepairs I and II.

   iii) Which of the two basepairs indicates the correct base to pair with the "A", the top one (I) or the bottom one (II)? Briefly justify your answer.



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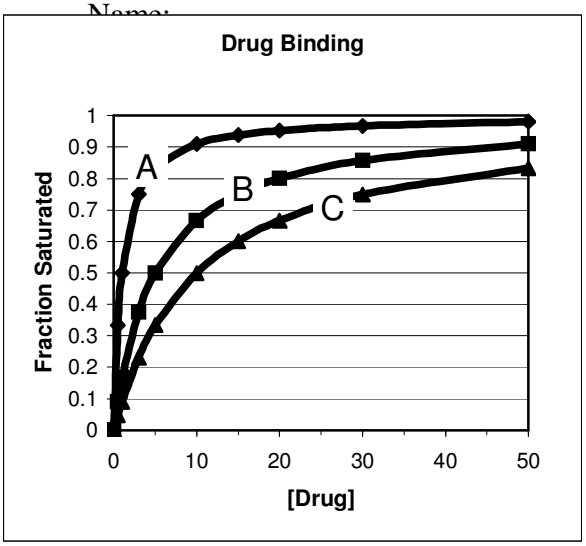
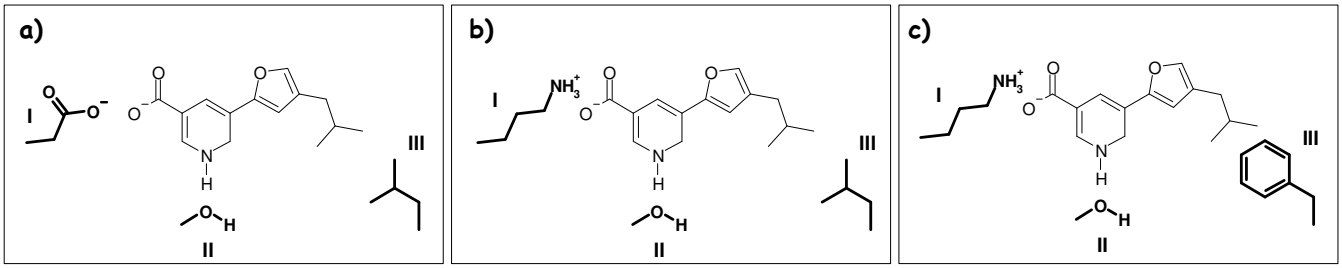
9. (12 pts) The binding of a drug to two mutant and wild-type reverse transcriptases (RT) is being studied. The fractional saturation was measured for all three drug-protein complexes. This drug binds to the wild-type RT with high affinity. Which binding curve (A,B,C) corresponds to which drug-protein pair (a, b, c) in the figure below (I, II, and III are sidechains from RT.) Which pair do you think is the wild-type protein (6 pts).





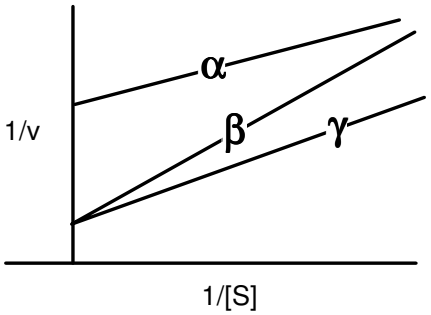
ii) Based on the binding curve, is the binding cooperative or not? What type of data analysis/plot would you do to verify your conclusion? How would you interpret this plot? (2 pts)

iii) If steady-state enzyme kinetics were measured in the presence of the drug and the wild-type enzyme, which of the lines (α, β, γ) in the double reciprocal plot shown to the right would correspond to:

a) the data acquired with the drug present?

b) the data acquired with no drug?

Briefly justify your answer (4 pts).



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10. (8 pts) Provide a brief description of allosteric effects. Your answer should discuss the fundamental basis of allosteric effects and the general nature of homotropic and heterotropic allosteric effectors. Illustrate your answer with any example you like.

11. (8 pts) Enzymes serve to catalyze the chemical transition of specific substrates to products. Please answer **one** of the following two choices:

Choice A: Using the framework of transition state theory, discuss the method by which enzymes increase the rate of chemical reactions. Provide one example of a reaction mechanism to illustrate your answer.

Choice B: Enzymes are specific for their substrates. How is substrate specificity achieved by enzymes? Illustrate your answer by discussing **two** enzymes with **different** substrate specificities.

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12. (1 pt) Most enzymatic data is analyzed using steady-state kinetics. During the measurement of product formation, which of the following are assumed to be constant? [Circle correct choice]
   a) the substrate, [S]
   b) the enzyme-substrate complex [ES]
   c) the product, [P]
   d) the enzyme-product complex [EP]
   e) all of the above.

13. (2 pts) Please do **one** of the following two choices:

Choice A: You wish to separate hemoglobin from ribosomes. What separation technique would you use?

Choice B: In a protein purification scheme the specific activity increases. Define the specific activity and discuss why it increases during purification.

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

Choice A: The diagram on the right shows a collection of monosaccharides. Indicate which monosaccharide (a – f) best characterizes the following descriptions:

i) Is found in DNA, but not RNA.

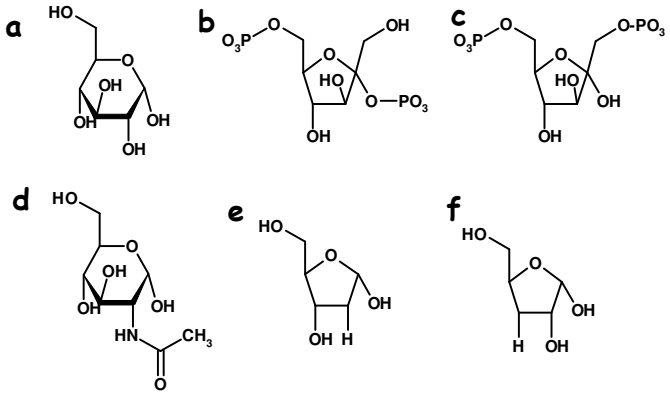
ii) Is found in cellulose

iii) Is found in glycogen

iv) Is found in bacterial cell walls

v) Is the substrate of a key regulatory step in glycolysis.

vi) Is a compound that activates a key regulatory step in glycolysis



Choice B: How does a triglyceride differ from a phospholipids (a labeled drawing is sufficient). What is the normal biological function of each of these compounds?

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15. (3 pts) Briefly discuss how the permeability properties of biological membranes are important for **one** of the two choices:

Choice A: oxygen transport. **Choice B:** production of ATP in the mitochondria.

16. (10 pts) Briefly discuss the steps (pathways & major intermediates) that occur in the complete oxidation of glucose to produce ATP when oxygen is present. Your answer should also discuss how the energy that is released by oxidations during this process is captured for subsequent conversion to ATP.

17. (8 pts) Protein kinases and phosphatases play an important role in the regulation of carbohydrate metabolism. Please answer both i) and ii).

i) Compare and contrast the reactions catalyzed by these two enzymes (6 pts).

ii) Selecting conditions of **either** low or high blood glucose levels, discuss how kinase/phosphatase activity controls **either** glycogen synthesis/degradation **or** glucose synthesis/degradation.

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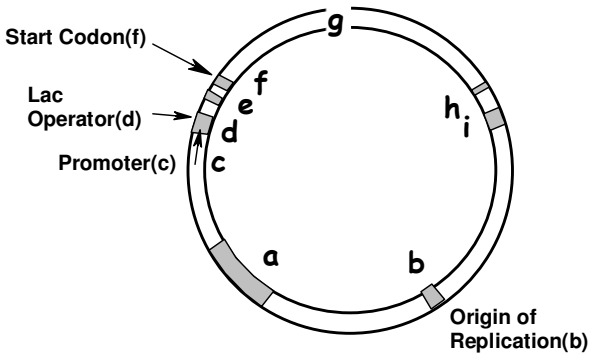
18. (10 pts) The following is diagram of an expression vector. Several of the labels are missing from this diagram, however all of the elements are in the correct position.



i) Provide the missing labels for a, e, g, h, and i.

a)

e)

g)

h)

i)

ii) Discuss the role of any **4 of the 9** labels in the expression of recombinant protein. Be sure to indicate your choices.

iii) What would you add to the DNA sequence of the vector to cause the protein to be exported out of the cell?

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19. (5 pts) Restriction endonucleases always recognize sequences that are the same on the top and the bottom strand and cut in the same location on each strand. With reference to the structure of the protein, briefly describe why this type of sequence specificity and cutting site occurs.

20. (8 pts) Please do **one** of the following two choices:

Choice A: Briefly explain how dideoxy nucleotide triphosphates are used to determine the sequence of DNA. Your answer should include a description of the reagents/compounds that are needed for this reaction.

Choice B: Briefly explain how PCR (polymerase chain reaction) leads to the amplification of DNA segments. Your answer should include a description of the reagents/compounds that are needed for this reaction.

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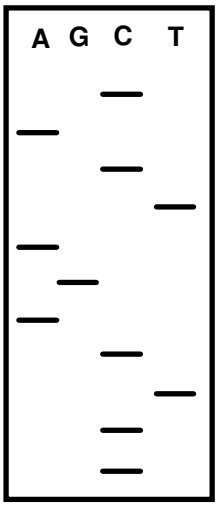
21. (6 pts) Please do one of the following two choices.

Choice A. The following two DNA molecules were mixed together and used for a DNA sequencing reaction. Draw the resultant sequencing gel.

5'-G-T-C-T-G-3'

3'-A-T-G-C-A-G-A-C-G-T-G-5'

Choice B: Read the sequence from the sequence gel shown on the right. Be sure to indicate the 5' and 3' ends of your answer.



22. (7 pts) The HIV protease gene from a mutant (inactive) HIV virus was sequenced and part of the sequence that was obtained from the sequencing gel is shown below. The complete nucleotide and protein sequence of HIV protease is also given. Identify the nucleotide and amino acid change (6 pts) and briefly explain why this virus is inactive (1 pt).

DNA sequence from gel: 5'-AAAGGAAGCTCTATTAACAACAGGA

HIV protease.

5'-CCTCAGATCACTCTTTGGCAA
ProGlnIleThrLeuTrpGln$_7$

CGACCCCTCGTCACAATAAGGATAGGGGGGCAACTAAAGGAAGCTCTATTAGATACAGGA
ArgProLeuValThrIleArgIleGlyGlyGlnLeuLysGluAlaLeuLeuAspThrGly$_{27}$

GCAGATGATACAGTATTAGAAGAAA**T**GAATTTGCCAGGAAAATGGAAACCAAAAATGATA
AlaAspAspThrValLeuGluGluMetAsnLeuProGlyLysTrpLysProLysMetIle$_{47}$

GGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTACGATCAGATACCTGTAGAAATCTGT
GlyGlyIleGlyGlyPheIleLysValArgGlnTyrAspGlnIleProValGluIleCys$_{67}$

GGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCT**GTC**AACATAATTGGAAGA
GlyHisLysAlaIleGlyThrValLeuValGlyProThrProValAsnIleIleGlyArg$_{87}$

AATCTGTTGACTCAGATTGGTTGTACTTTAAATTTCcccattagtcctattgaaact-3'
AsnLeuLeuThrGlnIleGlyCysThrLeuAsnPhe

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23. (12 pts) The following shows the complete nucleotide sequence of one of the surface proteins from swine flu virus (the HA protein). The entire length of this gene is 1700 bases, beginning with ATG and ending with TAA. Note that there is a BamHI restriction site (GGATCC) at position 230. You wish to produce this protein in bacteria for the purposes of making a vaccine to save the world from a potential pandemic.

HA gene sequence

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   1 atgaaggcaa tactagtagt tctgctatat acatttgcaa ccgcaaatgc agacacatta
  61 tgtataggtt atcatgcgaa caattcaaca gacactgtag acacagtact agaaaagaat
 121 gtaacagtaa cacactctgt taaccttcta gaagacaagc ataacgggaa actatgcaaa
 181 ctaagagggg tagccccatt gcatttgggt aaatgtaaca ttgctggctg gatcctggga
 241 aatccagagt gtgaatcact ctccacagca agctcatggt cctacattgt ggaaacatct
     ...
1441 tgctttgaat tttaccacaa atgcgataac acgtgcatgg aaagtgtcaa aaatgggact
1501 tatgactacc caaaatactc agaggaagca aaattaaaca gagaagaaat agatggggta
1561 aagctggaat caacaaggat ttaccagatt ttggcgatct attcaactgt cgccagttca
1621 ttggtactgg tagtctccct gggggcaatc agtttctgga tgtgctctaa tgggtctcta
1681 cagtgtagaa tatgtattta a
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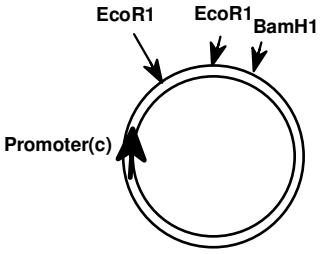
i) (4 pts) Design primers to amplify the coding region for this protein, placing EcoR1 sites (GAATTC) sites at both ends of the PCR product. After PCR, your final product should be (EcoR1 sites underlined):

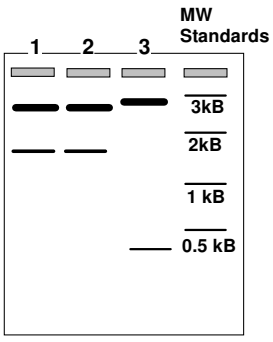
GAATTCATGAAGG…~1700 bases….TATTTAAGAATTC.

Ignore the melting temperature requirement and make your primers a total of 12 bases in length, including the six bases from the EcoR1 sites. Note that only the first and last 300 bases, and only the top strand, is shown above; you may need to generate part of the complementary strand in your primer design.

ii) (4 pts) You have the following expression vector at your disposal. Briefly describe how you would insert the HA gene (PCR product) into the expression vector.



iii) (4 pts) After inserting your PCR product into the expression vector you transform the DNA into bacteria and check for production of the HA protein. You find that some bacterial produce the HA protein while others do not. You isolate the DNA from a number of bacteria, digest it with BamH1, and separate the DNA fragments by gel electrophoresis. Note that the HA gene contains an internal site for BamH1, at position 230. On this gel samples 1 and 2 produce the protein, while sample 3 does not. Explain why a sample 3 does not produce protein. [use the back of the previous page if you need more space.]



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24. (8 pts) Briefly describe the structure of the ribosome (4 pts) and then answer **one** of the following three choices (4 pts):

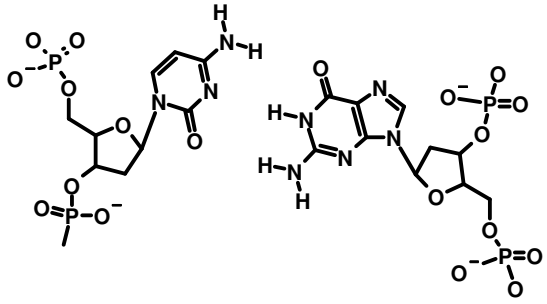
Choice A: Describe the events that occur during elongation of the growing polypeptide chain.

Choice B: Describe the events that occur during termination of protein synthesis.

Choice C: Although both AAA and AAG code for lysine (Lys) there is only one $tRNA^{LYS}$. Briefly explain how a single tRNA can recognize both codons.

25 (10 pts) The following binding data was obtained for a protein that binds to double-stranded nucleic acid. Discuss how this protein recognizes the nucleic acid, including which groove the protein binds to. Support your answer by reference to the experimental data. The following diagram of a basepair may be useful to illustrate your answer.

Nucleic Acid	Conditions	K_D
AACTT	0.1 M NaCl	10^{-9} M
AAGTT	0.1 M NaCl	10^{-9} M
AATTT	0.1 M NaCl	10^{-8} M
AACTT	0.5 M NaCl	10^{-7} M



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