This exam has 14 pages and contains 220 points. Allot 1 min/2 pts.

Part A: Multiple Choice. Please circle the best answer. 1.5 pts/question 18 pts total.

1. 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.

2. Which of the following statements is incorrect about *most* DNA polymerases
   a) require a primer.
   b) synthesize in the 5' to 3' direction.
   c) require a template.
   d) synthesize in the 3' to 5' direction.

3. DNA Gel Electrophoresis is similar to SDS-PAGE of proteins because
   a) DNA and proteins are separated according to their molecular weight.
   b) Both techniques rely on a constant charge to mass ratio.
   c) Both techniques utilize the sieving properties of gels.
   d) All of the above are correct.

4. A PCR reaction contains
   a) mRNA.
   b) tRNA.
   c) DNA polymerase.
   d) dideoxy-NTPs.

5. In the chain-terminator method of DNA sequencing, an "A" will be read on a sequencing gel when
   a) ddATP is included in the DNA synthesis reaction.
   b) A is present on the template stand.
   c) ddTTP is included in the DNA synthesis reaction.
   d) T is present on the primer stand.

6. The RNA polymerases that transcribe bacterial DNA are
   a) multisubunit enzymes.
   b) monomeric and very large.
   c) interchangable with DNA polymerases.
   d) only active inside the cell.

7. Removal of the signal peptide from a protein that is translocated across a membrane is accomplished by
   a) A serine protease, such as Trypsin.
   b) Signal Peptidase.
   c) HIV protease.
   d) fMet aminopeptidase.

8. During replication, overwinding or overtightening of DNA is caused by ____ and removed by ____:
   a) DNA ligase, Gyrase.
   b) Helicase, DNA polymerase.
   c) Helicase, Gyrase.
   d) DNA polymerase, Gyrase.

Part A:	_____/ 18
1:	_____/ 10
2:	_____/ 7
3:	_____/ 8
4:	_____/ 18
5:	_____/ 12
6:	_____/ 16
7:	_____/ 6
8:	_____/ 6
9:	_____/ 4
10:	_____/ 16
11:	_____/ 18
12:	_____/ 8
13:	_____/ 12
14:	_____/ 14
15:	_____/ 4
16:	_____/ 10
17:	_____/ 6
18:	_____/ 8
19:	_____/ 19
Total:	_____/220

9. Which of the following chromatography methods would be appropriate to use if the pH of the solution was lower than the isoelectric pH (pI) of a protein?
   a) anion exchange.
   b) cation exchange.
   c) Gel filtration.
   d) Affinity chromatography..

10. Which of the following 'forces' is the most unfavorable for protein folding and the formation of double stranded DNA?
   a) Configurational Entropy.
   b) Hydrophobic Interactions.
   c) Van der Waals Interactions.
   d) Hydrogen Bonds.

11. Energy released by the *total* oxidation of glucose is ultimately stored as:
   a) a concentration gradient across a membrane.
   b) on NADH and $FADH_2$.
   c) as ATP.
   d) as ADP.

12. Direct coupling of metabolic reactions requires that
   a) the product of one is the substrate of the next step.
   b) ATP be involved in the reaction.
   c) the favorable and unfavorable reactions occur on different enzymes.
   d) the favorable and unfavorable reactions occur on the same enzyme.

Part B:

1. (10 pts)
   a) Draw the structure of a dipeptide in the space to the right. The first amino acid can be any non-polar amino acid and the second can be any polar or charged amino acid (4 pts).
   b) Name the peptide that you have drawn (1 pt).
   c) Indicate the peptide bond with an arrow and list two important characteristics of the peptide bond (3 pts).

   d) Indicate the phi and psi angles associated with one residue (2 pts).

2. (7 pts)
   a) Provide a brief, one or two sentence description or definition of the following (6 pts):

      i) primary structure of a protein:

      ii) tertiary structure of a protein:

      iii) quaternary structure:

   b) Give *one* example of a protein that has a quaternary structure (1 pt).

3. (8 pts) Two important forms of secondary structure are the α-helix and the β-sheet (formed from β-strands). Please answer the following questions.
   a) What interaction(s) stabilizes these secondary structures (2 pts).

   b) Compare and contrast the structure of the mainchain (backbone) and sidechain groups in these two structures (a labeled drawing is fine) (5 pts).

   c) What is a Ramachandran plot? What is its relationship to a α-helix and β-strand (1 pt).

4. (18 pts)

a) Provide a molecular description of the hydrophobic effect. Is it an enthalphic or entropic term (6 pts).

b) Briefly discuss the role of the hydrophobic effect in *four* of the following. If the hydrophobic effect has no *significant* role, briefly explain why and describe the major energetic term that is involved. (12 pts)

i) Stability of double stranded DNA.

ii) Well-packed core of globular proteins.

iii) Formation of lipid bilayers.

iv) Melting of lipid bilayers.

v) Non-polar interior of globular proteins.

vi) Phe-Ala as a substrate for Chymotrypsin *or* HIV protease.

vi) Lys-Ala as a substrate for Trypsin.

5. (12 pts) Please do **one** of the following two choices.

Choice A: Define or describe allosteric effects and discuss the importance of these effects in biochemical systems. Illustrate your answer with an example.

Choice B: Explain, in general terms, why enzymes enhance the rate of reactions. Illustrate your answer with an example of a specific enzyme.

6. (16 pts) I was working in my garden on Saturday using my arms to lift a very heavy tool.

a) (2 pts) After about 10 min of *very* vigorous lifting my arms became quite weak. What compound(s) did I deplete in my muscles after 10 min?

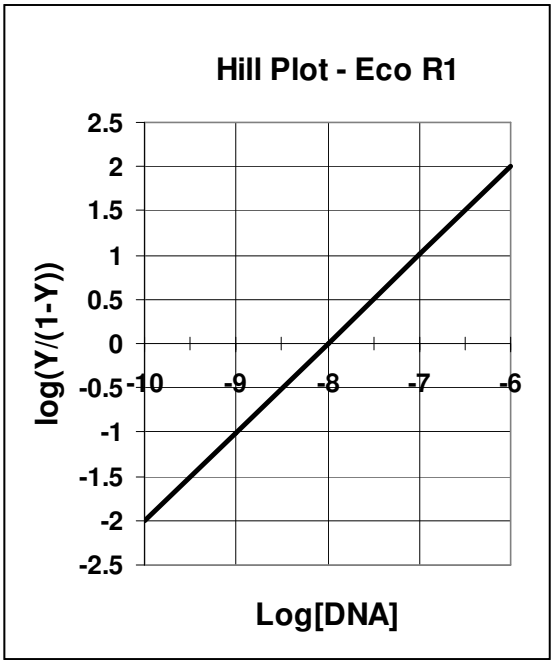
b) (10 pts) After resting for about 2-5 min, my arm strength was pretty much back to normal. Describe the events that transpired to restore energy to my arms. You should *briefly* discuss (i.e. name) *all* of the metabolic pathways that would be involved, how they might be regulated, and the involvement of any hormones.

c) (4 pts) After about 2 hours of this activity I noticed that it became increasingly more difficult to restore the energy levels in my arms and maintain this activity. How did my metabolism change, and why?

7. (6 pts) Compare and contrast feedback versus product inhibition of metabolic pathways. Provide *one* example of a feedback inhibitor.

8. (6 pts) The Hill plot for the binding of the restriction endonuclease EcoR1 to GAATTC (double stranded) is shown to the right. The slope of the line is equal to 1.

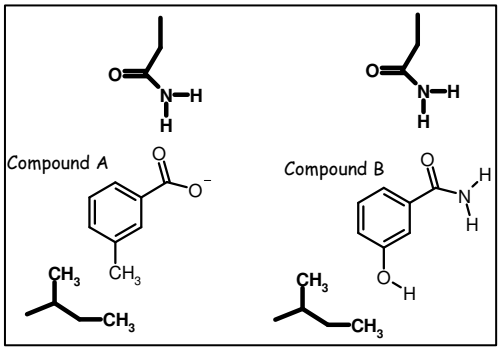
   a) What does this result tell you about the cooperativity of binding? (4 pts)

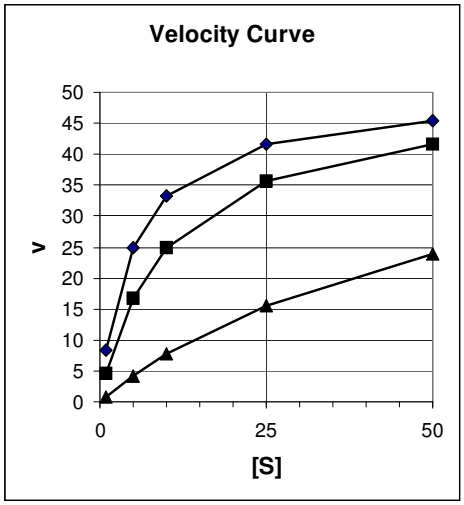


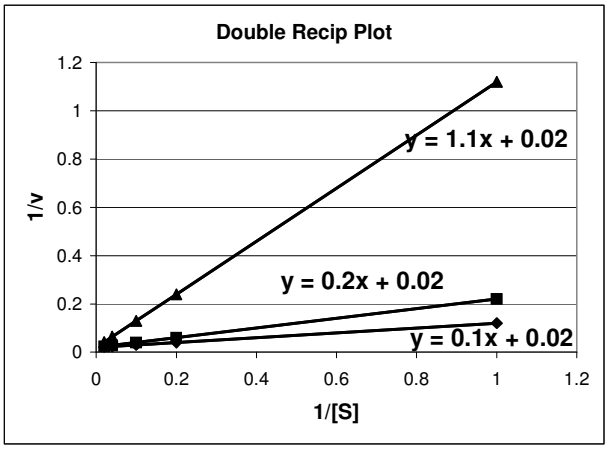
   b) Is this conclusion consistent with the structure of restriction endonucleases and how they interact with DNA? Why? (2 pts)

9. (4 pts) Define specific activity and briefly describe its usefulness in protein purification.

10. (16 pts) Two compounds that bind to the same site on an enzyme are shown to the right, along with some amino acid sidechains from the enzyme (shown in bold). The initial rate of product formation was measured in the absence of either inhibitor, and in the presence of equal amounts (1 μM) of either inhibitor. The data are plotted on both velocity curves and on a double reciprocal plot, however none of the curves are labeled. The equation of each line in the double reciprocal plot is given.







a) On the basis of the kinetic data, are these competitive or non-competitive inhibitors? Why? (4 pts).

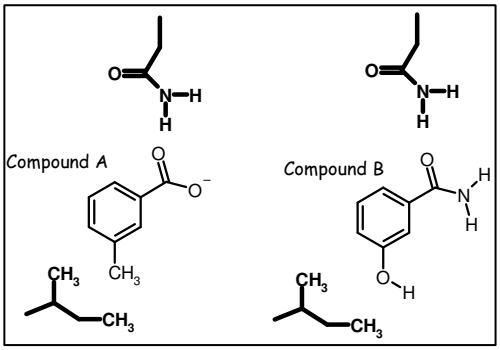
b) Based on your answer to part a), where do you suppose these compounds are binding – to the active site or elsewhere? (2 pts).

c) Determine the K_I for each inhibitor from the double reciprocal plots (4 pts).

Question continues on next page..

Question 10, continues…

d) Which K_I is associated with which molecule? Justify your answer by reference to the interaction between the molecules and the enzyme. The structure of the enzyme inhibitor complexes have been repeated to the right. (4 pts).



e) How would you change the ***enzyme*** to enhance the interaction to the compound that binds less tightly? (2 pts)

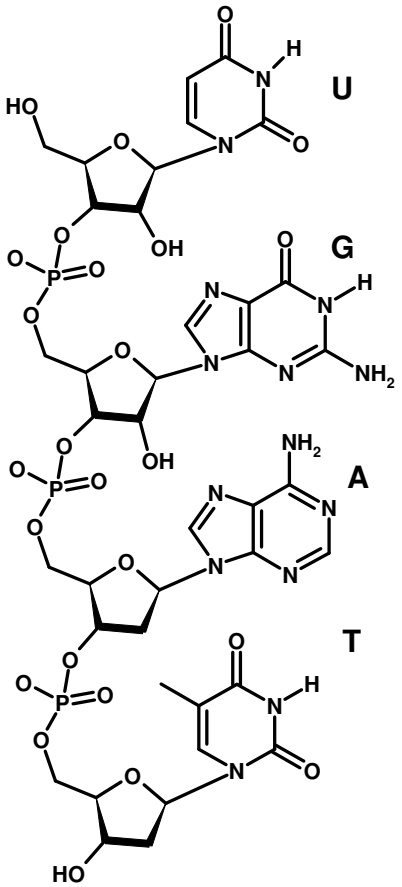
11. (18 pts) The structure of a single stranded nucleic acid is shown on the right.

a) (6 pts) Label the following items on the structure using the Roman numeral and an arrow that clearly points to the item.

i) 5' end. iv) A phosphodiester bond
ii) 3' end. v) A purine.
iii) A ribose. vi) A glycosidic bond.

b) (2 pts) Add *all* missing charges.

c) (2 pts) Write the sequence of this nucleic acid.



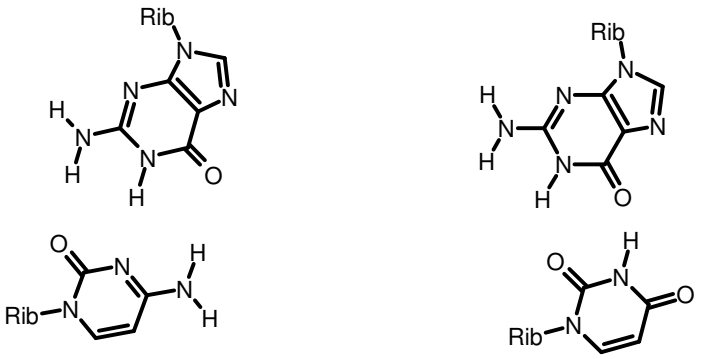
d) (2 pts) Write the sequence of the complementary strand.

e) (2 pts) If this were a primer for DNA synthesis, indicate where the next nucleoside would be added with a **box**.

f) (2 pts) Could this nucleic acid be found in Okazaki fragments during DNA replication? Justify your answer.

g) (2 pts) Indicate, by means of a sketch, where the complementary strand would be located. Be sure to indicate its direction (e.g. 5'-3').

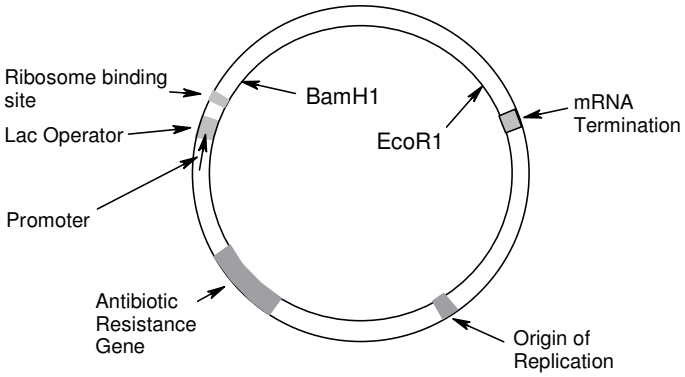
12. (8 pts) Two codons, CAU and CAC, code for the amino acid Histidine. However, in most organisms there is only one tRNA that can be charged with Histidine and recognize these codons. Explain how this occurs. Your answer should include a discussion of tRNA–mRNA interactions and provide specific details about base-base interactions. You may find the diagrams shown to the right useful in illustrating your answer (the left pair is G-C).



13. (12 pts) Select any **one** of the three polymer synthesis reactions that were discussed in the course (DNA replication, RNA synthesis, protein synthesis) and describe the process of synthesis. Your answer should state the template, monomeric units, and the final product. You should also describe the initiation events, polymerization, and termination (briefly).

14. (14 pts) The following show a diagram of an expression vector (plasmid) The nucleotide sequence of a portion of the expression vector, from the promoter to the mRNA termination site is shown below.

The recognition sequence for BamH1 is G^GATCC while that for EcoR1 is G^AATTC.



mRNA Start RBS

TTGACATTTATGCTTCCGGCTCG**TATAAT**GTGTG**G**AAT**TGTGAGCGGATAACAATTTCACACA**GGAC*GGATCC---GAATTC*-----mRNA Term.

Promoter **Lac operator** **BamH1 EcoRI**

a)Explain the role of *four* of the following in the production of recombinant protein in bacteria (12 pts).

i) Origin of replication:

ii) Antibiotic resistance gene:

iii) Promoter:

iv) Lac operator:

v) Ribosome binding site:

b) What two important features that are important for *mRNA translation* are missing from this expression vector? Where should they be located with respect to the other features listed above? (2 pts)

15. (4 pts) If the following DNA was treated with BamH1, what are the products?

GGGGATCCCC
CCCCTAGGGG

16. (10 pts) You want to produce human growth hormone in bacteria. The gene & associated peptide sequence for growth hormone is as listed below.

```
          MetProArgSerArgAspValAlaSerTyrCys
GCCCTTAAATGCCCCGCTCGCGGGACGTCGCCAGTTATTGTTAATTTACTG
CGGGAATTTACGGGGCGAGCGCCCTGCAGCGGTCAATAACAATTAAATGAC
```

a) Give the sequence of the PCR primers that would be required to amplify the gene for human growth hormone such that it would be correctly inserted into the expression vector, as it is shown on the previous page. Don't worry about the T_M, simply make both primers 12 bases long. Be sure to write *both* primers in the 5'-3' direction (5 pts).

b) Briefly describe how you would insert the PCR product into the expression vector (A diagram is not necessary, unless you want to draw it – simply list the steps if you like.) (5 pts).

17. (6 pts) You produce recombinant growth hormone in bacteria, but find that it is inactive in humans. The DNA sequencing gel of the growth hormone gene after it has been inserted into the expression vector is shown to the right. Does this gel explain why the protein is non-functional? Justify your answer. The expected protein and DNA sequence is shown below, with spaces added for clarity.

```
        Met Pro Arg Ser Arg Asp Val Ala Ser Tyr Cys
GCCCTTAAATG CCC CGC TCG CGG GAC GTC GCC AGT TAT TGT TAATTTACTG
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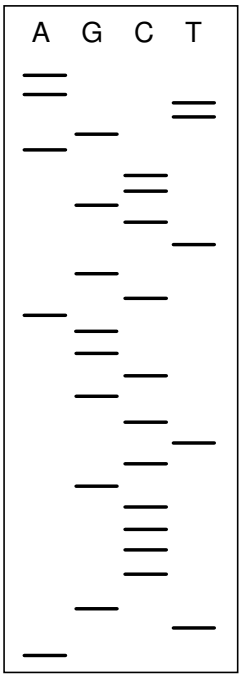
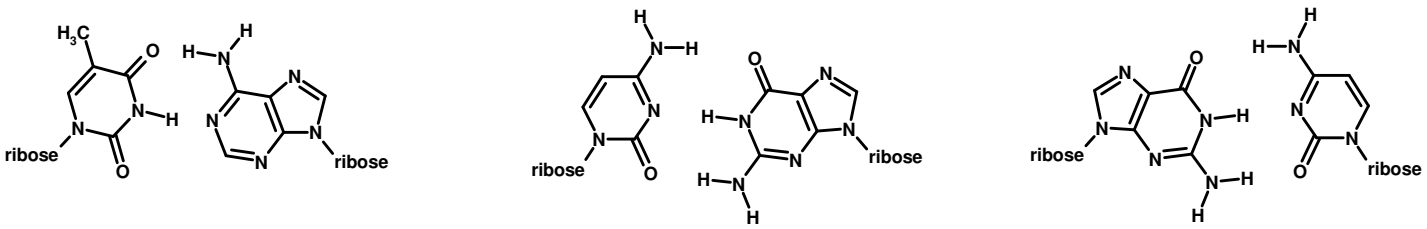


18. (8 pts) After correcting the mistake in the DNA for the growth hormone (see previous problem), you find that it is impossible to purify the human grown hormone from the complex mixture of proteins in the bacterial cells. Briefly describe how you would fix this problem by **one** of the following two approaches. Please indicate your choice.

Choice A: Describe what changes you would make to the expression vector such that the growth factor was exported out of the cell.

Choice B: It is possible to purify proteins using affinity chromatography by including a segment of 6 His residues at either the amino-terminus or the carboxy terminus of the protein. The codon for His is CAC. Describe how you would modify the PCR primers to add these His residues.

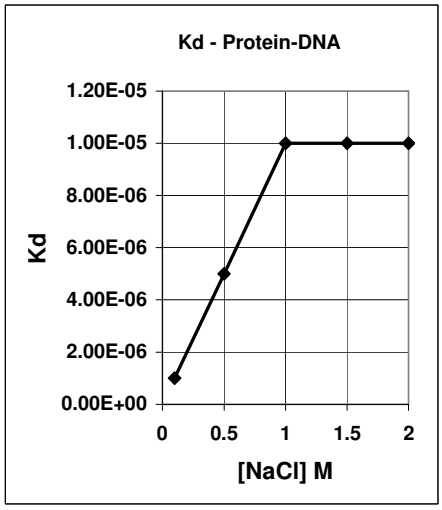
19. (19 pts) A T-A, C-G, and a G-C basepair are shown below.



a) (2 pts) Identify the minor and major groove in all basepairs.

b) (5 pts) A hypothetical protein will bind to *either* GC *or* CG basepairs, but only very weakly to AT or TA basepairs. The protein is using hydrogen bonds to recognize the basepairs. Is it binding in the major or minor groove? Justify your answer.

c) (10 pts) The K_D for the binding of the protein to double stranded DNA composed entirely of GC bases was measured as a function of salt concentration and the data is plotted in the graph to the right.



i) Does the affinity, or binding strength, to the DNA increase or decrease as the [NaCl] increases? Justify your answer. (2 pts).

ii) Based on your answer to *part i*, what additional interactions occur between the protein and the DNA besides hydrogen bonding? Justify your answer. (4 pts)

iii) How much do *each* of these interactions (Hydrogen bonds and the interaction stated in *part ii*) contribute to the standard energy of binding, ΔG^o (Assume RT=2.5 kJ/M). Forgot your calculator? $ln X = 2.3 \log_{10} X$ (4 pts).