This exam has **12 pages** and is out of **230 points**. On questions with choices, all of your attempts will be graded and you will receive the best grade. Use the space provided, or the back of the preceding page.

1. (6 pts) Entropy plays an important role in biochemistry. Discuss the role of entropy in **two** of the following:
   a) the solubility of hydro**philic** compounds, such as ions and other polar molecules.
   b) lipid bilayer formation
   c) protein-DNA interactions (non-sequence specific)

2. (12 pts) Please do **one** of the following two choices:
   Choice A: Describe how you would make a buffer solution with the following characteristics. pH=5.0, buffer concentration 0.1 M, volume = 0.5 L. You have the following monoprotic weak acids available, only in their fully protonated form: a) acetic acid (pKa=4.8), b) pyruvate (pKa=3), c) imidazole (pKa=7).
   Choice B: Determine the isoelectric pI of the following peptide: Alanine-Glycine, within 0.1 pH unit.

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3. (8 pts) Briefly explain why solutions of weak acids resist pH changes and are therefore buffers.

4. (8 pts) An enzyme has a lysine residue in its active site that is involved in substrate binding. The enzyme also contains an aspartic acid residue in its active site that must be deprotonated for activity. Please do **<u>one</u>** of the following choices:
   Choice A: The normal pKa for Aspartic acid is 4.0. Do you expect the pKa of the Asp residue in the active site to be higher or lower? Briefly justify your answer.
   Choice B: Sketch the pH dependence for the K_M. Briefly justify your answer.
   Choice C: Sketch the pH dependence for k_{CAT}. Briefly justify your answer.

5. (10 pts) Draw the chemical structure of a tetra (4 residue)-peptide that has the following sequence properties:
   a) the sequence would be found in a **solvent exposed β-strand** of a soluble globular protein.
   b) one of the residues can be phosphorylated.
   c) one of the residues absorbs UV light.

Please answer the following questions:
   i) Give the name (sequence) of your tetra-peptide: (1 pt)
   ii) What type of enzyme would add a phosphate to the residue you drew to satisfy b)? What is the source of that phosphate? (2 pts)

   iii) Which residue would be released in the first cycle of Edman degradation (used for protein sequencing)? (1 pt)

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6. (10 pts) DNA is stabilized in its double stranded form by molecular interactions that also stabilize the tertiary structure of proteins.
   i) Compare the relative importance of enthalpic factors (van der Waals, H-bonds, electrostatics) on the stability of proteins and double stranded DNA. Which factors stabilize folded proteins and double stranded DNA? Which destabilize them?

   ii) Compare the relative importance of entropic factors (chain entropy, hydrophobic effect) on the stability of proteins and double stranded DNA. Which factors stabilize folded proteins and double stranded DNA? Which destabilize them?

7. (6 pts) The peptide bond is has unique properties. What are those properties and how do they affect protein folding?

8. (4 pts) You have two unlabeled samples, one is a globular protein and the second is a segment of double stranded DNA. You need to determine which sample is the protein and which is the DNA. How would you distinguish between the two samples?

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9. (10 pts) Allosteric effects play a critical role in functioning of biological systems. Please answer **both** of the following questions (parts i and ii, you have a choice for part ii).
   i) Describe the general framework for allosteric effects. Your answer should include a description of the properties of T and R states and how these properties lead to allosteric effects (6 pts)

   ii) Please do **one** of the following two choices (4 pts)
   Choice A: Describe how allosteric effects are important in oxygen delivery (or the adaptation to high altitude)
   Choice B: Describe how allosteric effects are important in metabolic regulation (regulation of one pathway is sufficient).

10. (8 pts) Explain how **all** enzymes increase the rate of reaction. Support your answer with a brief description of the active site of any enzyme.

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11. (21 pts) You are measuring the binding of a homodimeric protein to the following DNA segment. The protein also binds the amino acid tryptophan (Trp). You measure the binding of DNA to the protein in the absence and presence of tryptophan and RNA polymerase (RNAP) and obtain the binding data shown in the table on the right and plotted in a Hill plot (log (Y/(1-Y)) versus log[L]). Both the Trp binding protein and RNAP are from bacteria.

DNA concentration (Ligand) (nM)	Fraction of DNA bound	Fraction of DNA bound (+ Trp)	Fraction of DNA bound (+ Trp +RNAP)
0.1	0.01	0.10	0.50
1.0	0.10	0.50	0.90
10.0	0.50	0.90	0.99
100.0	0.90	0.99	1.00
K_D			

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GAGCGAAATTTCGCACTTGACATTTATGCTTCCGGCTCGTATAATGTGTGGAATTGTGAGAGGAGGAACAGCTATGA
CTCGCTTTAAAGCGTGAACTGTAAATACGAAGGCCGAGCATATTACACACCTTAACACTCTCCTCCTTGTCGATACT
                          -35                  -10
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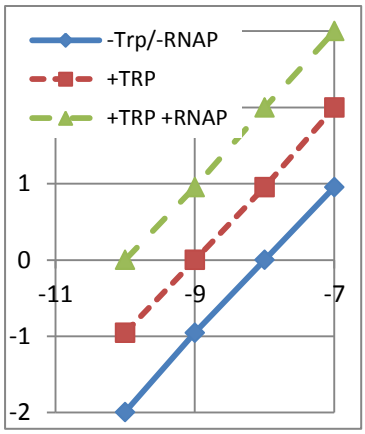
i) What do the -35 and -10 signify on the DNA strand (1 pt)?



ii) What is the K_D for binding of the protein to the DNA (protein alone, protein + Trp, protein + Trp & RNAP). Write these values in the table above and briefly indicate how you arrived at these values (5 pts).

iii) Is the binding of the DNA to the protein cooperative? Justify your answer with reference to the Hill plot (3 pts).

iv) The protein, when bound to the DNA, greatly *increases* the amount of mRNA production. Based on this information, and the fact that the protein is a homo-dimer, identify the DNA sequence that is the binding site for the protein. *Justify your answer*. [Hint: restriction enzymes are also homodimers, what is the key characteristic of the sequence they recognize?] (4 pts)

v) How would you prove your answer to part iv? Discuss how you would modify the DNA and the type of experiment you would design (4 pts)

Part vi) of this question is on the following page:

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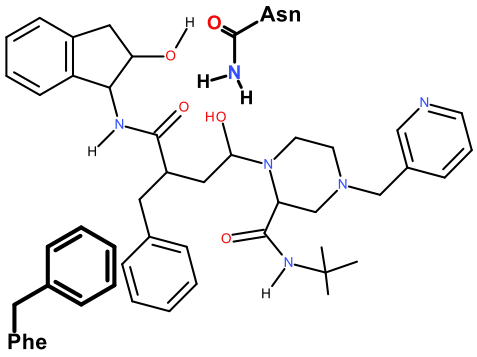
Continuation of Question 11:

vi) Could you use this protein to regulate the expression of proteins from a plasmid? What sequences would you need on the plasmid and how would you turn on production of the protein (4 pts)?

12. (20 pts) You are screening a large number of compounds to find new inhibitors of HIV protease. A candidate is shown on the right, interacting with two residues (Asn & Phe) on the protease (in bold).

i) How would you determine whether the inhibitor was competitive or mixed type? What data would you collect and how would you analyze the data (6 pts)?



ii) After confirming the type of inhibition, you obtain the structure of the protein-drug complex. What technique would you have used to obtain the structure? (1 pt)

iii) An Asn and a Phe residue in the protease makes several energetically favorable contacts with the bound drug.

a) What thermodynamic interactions between the Asn and the drug are responsible for enhancing the drug binding? (2 pts)

b) What thermodynamic interactions between the Phe and the drug are responsible for enhancing the drug binding? (2 pts)

Parts iv) and v) of this question are on the following page.

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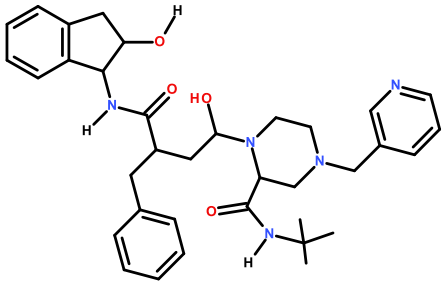
iv) You are using the drug to treat patients infected with HIV. During treatment, a patient develops viruses that are resistant to the drug. You wish to identify the changes in the protease that affect drug binding. You sequence the wild-type and mutant HIV protease genes and obtain the following sequences. What amino acid change has occurred that prevents drug binding? (The entire sequence of the HIV protease gene is on the formula sheet. The primer that was used to generate this sequence data is also indicated on the formula sheet.) Draw the structure of the altered amino acid in the mutant protease (6 pts).

Wild-type sequence: ACCTACACCTGTCAACATAATT
Mutant sequence: ACCTACACCTGTCGAAATAATT

v) How would you modify the drug so that it would effectively bind to the mutant HIV protease? State whether this modification should increase or decrease K_D. Clearly indicate which group you would change on the **drug**, and how you would change it (3 pts).



13. (4 pts) What is specific activity and why is it useful in protein purification?

14. (6 pts) The following is a list of names. On the right are a number of structures, indicate the correct structure next to the name.

_____: Ribose

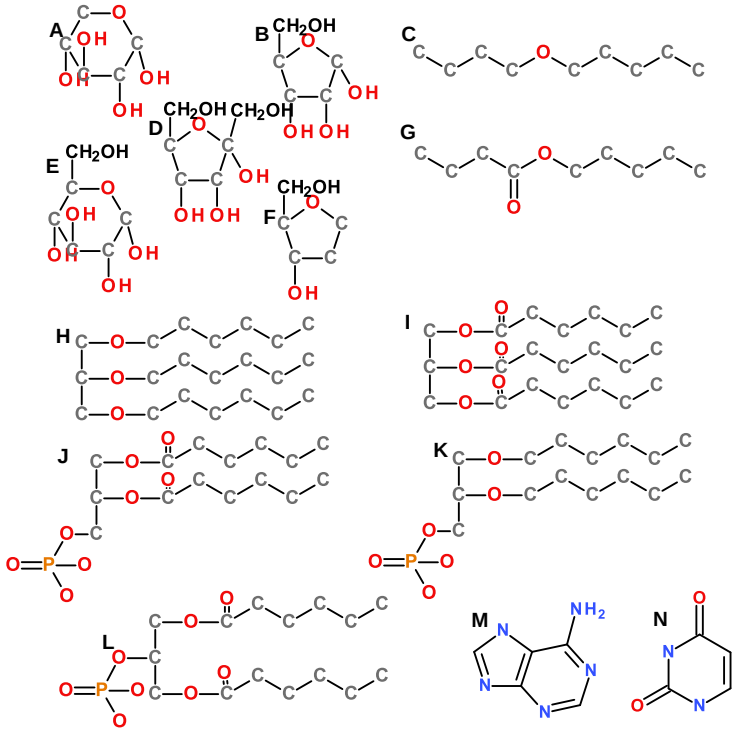
_____: Glucose

_____: Triglyceride

_____: Wax

_____: Phospholipid

_____: Pyrimidine



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15. (10 pts) In two weeks I am going on my annual "rim-to-rim" hike across the Grand Canyon where we cover 22 miles (and 2 miles of elevation loss and gain) in about 12 hours.

i) Briefly describe the metabolic pathways that are responsible for energy production during the hike. Which pathways would be operating at the very start of the hike and which would likely become more active later on in the hike (6 pts)

ii) In order to do this hike I carry an ultra-lite pack. What type of food should I carry in my pack, carbohydrates or fats? Which provided more energy/gram and why? (2 pts)

iii) When climbing up to the rim, I consume all of the oxygen in my leg muscles. What other metabolic pathway is occurring so that I can continue to produce ATP. What is the product of that pathway? (2 pts)

16. (2pts) A base is attached to the sugar of a nucleotide through a _____bond, while

nucleotides are linked together by _____bonds (a type of covalent bond).

17. (4pt) Both RNA and DNA can form a double helix, describe two similarities and two differences in these structures.

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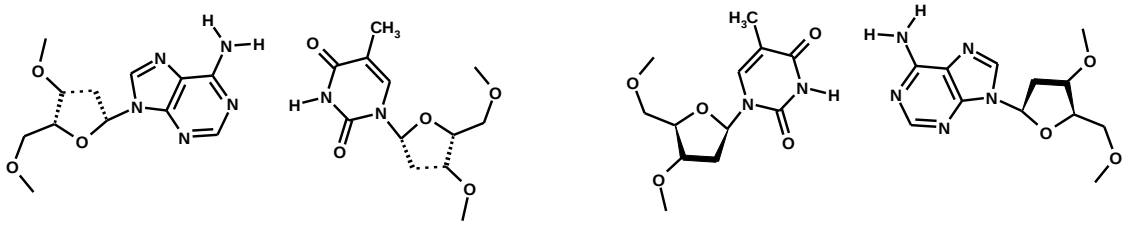
18. (2pts) What structural/chemical feature of RNA makes it less stable than DNA in a basic solution and why?

19. (4pt) Describe two similarities and two differences of DNA polymerase and RNA polymerase in the synthesis of DNA and RNA, respectively.

20. (4 pt) Explain the unique features of restriction site sequences and how a restriction enzyme can cut both strands of the DNA.

21. (8pt)Two different base pairs are shown below.



i) Indicate the hydrogen bonds that can occur between the bases and where the major and minor groove would be located.
ii) Label the hydrogen bond donors and acceptors on the bases in both grooves.
iii) Draw an example of an amino acid side chain that would likely interact with a hydrogen bond acceptor.
iv) Explain whether a protein that interacted with the basepair on the right through the minor groove would be capable of a similar interaction with the basepair on the left and why.

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22. (12pt) The sequence below shows a portion of the DNA and protein sequence for the N-terminus and C-terminus of the HIV protease. Your job is to clone this protein into the expression vector shown on the right. (note this vector is referred to in questions 24 & 25 below)

Answer the following questions:

```
5'CGCCTCAGATCACTCTTTGGCAA.......ACTTTAAATTTTCCG3'
3'GCGGAGTCTAGTGAGAAACCGTT.......TGAAATTTAAAAGGC5'
   ProGlnIleThrLeuTrpGln-------ThrLeuAsnPhe
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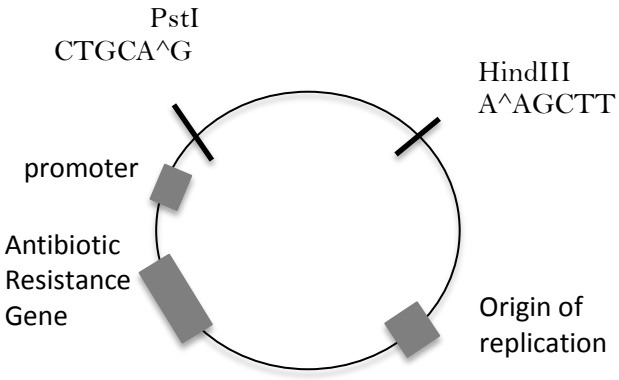

i) If you were going to utilize the start and stop codons present in the vector, write the sequence of both primers (limit 12 base pairs) you would design for PCR (in the 5'->3' direction).

ii) Calculate the Tm for the left primer and state what temperature you would use for the annealing step of PCR.

iii) Describe the steps that you would need to perform to clone the resulting PCR products into the vector that is shown above.

23. (12pt) Describe the three steps of PCR and explain how repeating these steps leads to exponential amplification of a target DNA (be sure to describe relevant features of cycle temperatures, templates, primers). You may draw a figure to support your explanation.

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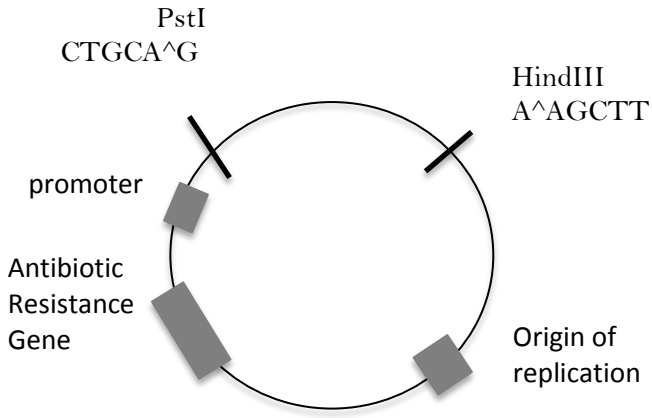
24. (7pts) The expression vector shown in problem 22 (shown again on the right) is missing several of the regulatory transcription and translation sequences that are listed below. Place these in the correct order (5' to 3') by numbering 1 through 7 (fill in the blanks), including the placement of the inserted PCR product.

    i) mRNA terminator _____
    ii) PCR product _____
    iii) Start codon _____
    iv) Lac operator _____
    v) Stop codon _____
    vi) Ribosome binding site _____
    vii) Promoter _____



25. (4 pts) Answer one of the following two choices:

Choice A. In lecture we discussed two additional sequences that can be added to expression vectors to aid in protein purification. Describe one of these two sequences in terms of where they would be placed in the vector shown in question 24 and how the sequence can aid in purification.

Choice B. There are two sequences in the expression vector shown in question 24 that are needed for maintenance in bacteria. Describe one of these two sequences and explain why they are needed for the plasmid to be maintained.

26. (4 pt) DNA sequencing requires the incorporation of a modified nucleotide called a dideoxyNTP. Describe the modification and explain how this is utilized in Sanger sequencing.

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27. (8 pts) Describe the steps involved in "charging" of tRNAs, include all enzymes and molecules needed for these reactions.

28. (10 pts) Answer one of the following three choices (for each feel free to include well-labeled diagrams with an explanation):

Choice A: Describe the steps involved in the initiation, elongation and termination of transcription in bacteria. Be sure to include information regarding the sequences needed and factors involved in these reactions.

Choice B: Describe the lac operon and how it can be used to regulate transcription in bacteria. Be sure to include information regarding the DNA sequences involved and the molecules needed to turn on and off transcription.

Choice C: Describe the steps involved in the initiation, elongation and termination of translation in bacteria. Be sure to include information regarding sequences needed for these steps and factors involved in the reactions.

29.(6 pt) Explain why there are 20 amino acids and 64 codons, but only 25-35 types of tRNAs on average per cell.

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