This exam consists of 236 points in 24 questions. Allot 1 min/2 pts. There are a total of 16 pages, with four bonus questions.

1. (8 pts) Briefly discuss the general nature of hydrogen bonds. What are their properties? Why do they form? Give an example of a hydrogen bond in biochemistry and briefly discuss why it is important.

2. (11 pts)
   i) (8 pts) Briefly compare and contrast van der Waals forces and the hydrophobic effect. Your answer should include thermodynamic attributes (e.g. enthalpic or entropic).

   ii) (3 pts) Describe how the hydrophobic effect stabilizes either soluble proteins ***or*** biological membranes.

3. (4 pts) What feature or property of the ***unfolded*** state of proteins (or DNA) is responsible for stabilizing the unfolded state? Is this an enthalpic or entropic term?

4. (8 pts) Peptide Structure.
Draw a dipeptide using any two amino acids (4 pts). One residue should be polar, the other ionizable. Label your diagram with (1 pt each):
a) the location of the peptide bond
b) ***all*** pK_A values
b) circle the mainchain atoms
c) the name of your dipeptide

5. (8 pts) The peptide bond is planer and assumes two conformations, *cis* or *trans*.
i) Why is the peptide bond planer? (4 pts)

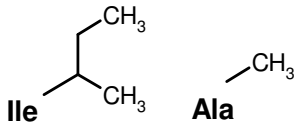
ii) Which of the two conformations is more common or stable? Why? Which conformation did you draw for problem 4? (4 pts)

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

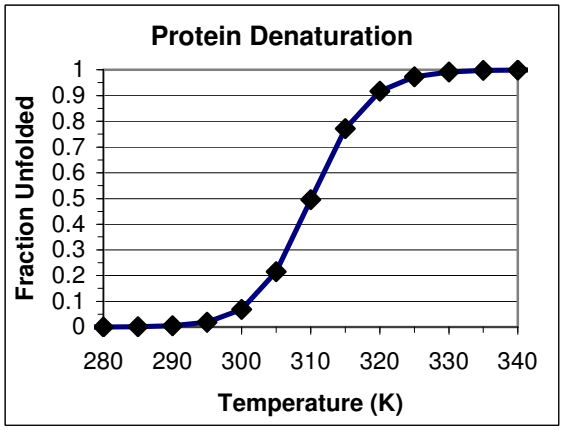
Choice A: What is the principal difference between secondary and tertiary structure?

Choice B: Briefly compare and contrast an α-helix to a β-sheet.

7. (8 pts) The thermal stabilities of a wild-type and mutant small globular protein were measured. The original, or wild-type protein contains an isoleucine residue while the mutant contains an alanine residue. The thermal denaturation curve for the wild-type protein is shown below.



Assuming that the Ile group is buried in the core of the protein, sketch the denaturation curve you would expect to obtain for the ***alanine*** containing protein. Be sure to consider **all** thermodynamic factors. Briefly justify your answer.



8. (6 pts) Briefly describe the oxygen binding site on hemoglobin and myoglobin (4 pts). Besides oxygen, what else is transported by other proteins using the same chemical group that binds oxygen? Where does this transport process occur (2 pts)?

9. (16 pts) What features does the active site of an enzyme possess? Explain how these features confer ***both*** substrate specificity and catalytic ability (12 pts). Illustrate your answer with one example of an enzyme (4 pts).

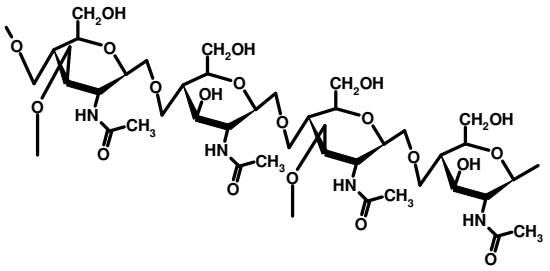
10. (15 pts) Provide a *brief* description of allosteric effects (8 pts) and then provide ***one*** example of an allosteric effect in any of the systems we have discussed in this course (2 pts). Discuss the biochemical importance of allosteric effects for your choice (5 pts).

11. (8 pts) Please do ***one*** of the following three choices. Please indicate your choice.

Choice A: Explain ring formation in monosaccharides, including multiple configurations.

Choice B: Briefly describe how *any* dietary disaccharide or polysaccharide enters glycolysis.

Choice C: Cellulose cannot be digested by any human enzyme. However, the human enzyme lysozyme readily cleaves the carbohydrate portion of bacterial cell walls. Briefly explain how this might occur. The carbohydrate portion of a bacterial cell wall is shown on the right. You should compare the structure of cellulose to the carbohydrate portion of the bacterial cell wall in your answer.



12. (6 pts) Please do ***one*** of the following two choices. Please indicate your choice.

Choice A: Briefly describe how the permeability properties of the biological membrane are essential for the synthesis of ATP by ATP synthase in the mitochondria.

Choice B: Briefly describe how the structure of membrane proteins differs from water soluble proteins. Your answer should comment on restrictions on secondary structure.

13. (10 pts) Please do ***one*** of the following five choices. Please indicate your choice.

Choice A: What type of chemical change generates energy in degradative metabolic pathways? Provide one example of this change, including cofactors/cosubstrates, and give the *generic* name of the enzyme that catalyzes reactions of this type.

Choice B: Briefly describe one way by which metabolic pathways can be regulated. Illustrate your answer using either glucose storage/release from glycogen, as regulated by hormones, or glycolysis or the TCA cycle, as regulated by energy sensing.

Choice C: Briefly describe how corn starch or cane sugar can be converted to ethanol using yeast.

Choice D: Individuals on a high protein diet often complain that they tire easily during *vigorous* exercise. Why might this occur?

14. (8 pts) Please do ***one*** of the following two choices. Please indicate your choice.

Choice A: What is the difference between the Gibbs free energy, ΔG, and the standard energy ΔG^o? Which provides a more useful description of energy changes in metabolic pathways? Why?

Choice B: Briefly describe coupling with respect to metabolic pathways. Why is coupling usually required for the operation of a metabolic pathways?

15. (6 pts) Please do ***one*** of the following two choices. Please indicate your choice.

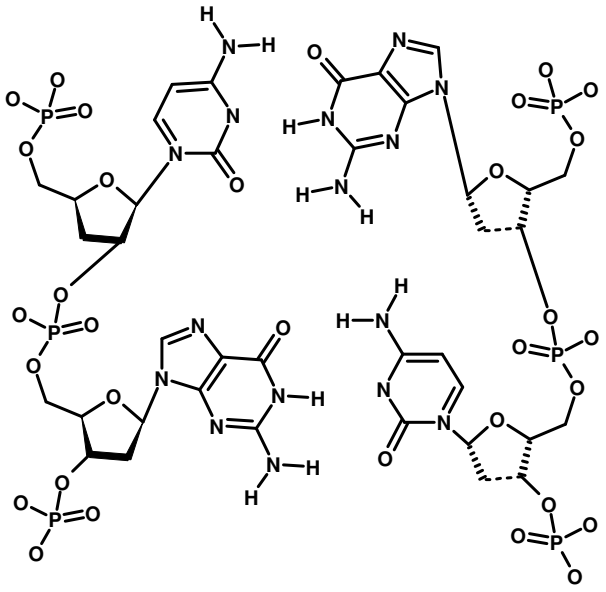
Choice A: Compare and contrast any ***two*** of the following parameters:

a) K_D c) K_I'

b) K_I d) K_M

Choice B: Define, or explain how to obtain, the Hill coefficient, n_h. How is it used to characterize ligand binding?

16. (14 pts) The diagram on the right shows two basepairs in a double stranded nucleic acid.



i) (5 pts) Label, on the diagram to the right, all of the following:

a) ***both*** 5' ends

b) a single glycosidic bond

c) a single ribose (or deoxyribose)

d) a single phosphodiester bond

e) a single purine

ii) (4 pts) Using the ***lower*** basepair:

a) indicate the edge of the basepair that represents the major groove,

b) the edge that represents the minor groove,

c) draw all Watson-Crick H-bonds.

d) Indicate additional donor/acceptors in the minor groove.

iii) (3 pts) Is this DNA or RNA? Justify your answer.

iv) (2 pts) There are two significant errors in this diagram? What is one of them?

17. (2 pts) Which interaction is *most* important for stabilizing the double stranded form of DNA?

a) Hydrogen bonds c) van der Waals interactions

b) Electrostatic interactions d) Conformational Entropy.

18. (6 pts) Please do ***one*** of the following two choices. Please indicate your choice.

Choice A: Explain why the melting temperature of DNA increases with GC content.

Choice B: Explain why the melting temperature of DNA increases at higher salt concentration.

19. (14 pts) Please do ***one*** of the following three choices. Please indicate your choice.

Choice A:

a) Discuss the enzymatic features that are associated with DNA polymerases that are used to replicate DNA. Your answer should include information on how the DNA polymerase selects the correct dNTP (10 pts)

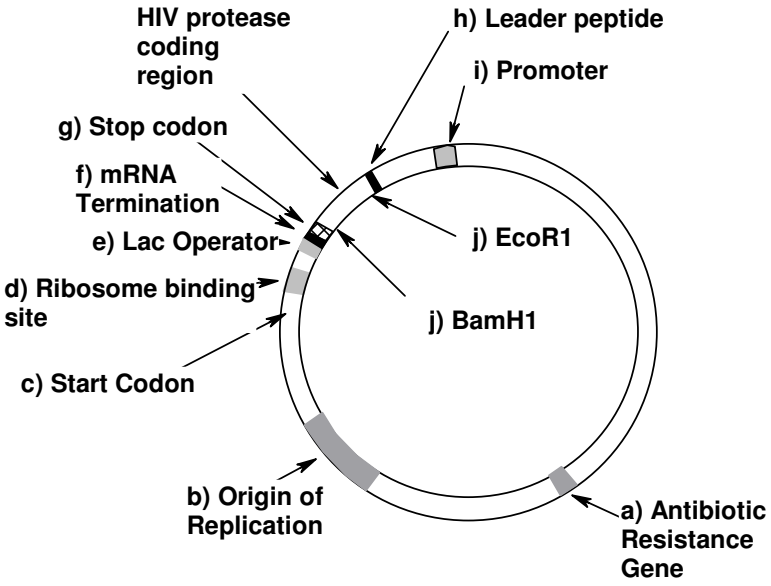
b) How does RNA polymerase differ from a typical DNA polymerase (4 pts)?

Choice B: Briefly discuss how the enzymatic activity of DNA polymerases is used to amplify specific DNA segments (as in PCR).

Choice C: Briefly discuss how polymerases are used to determine the nucleotide sequence of DNA.

20. (14 pts) The diagram to the right shows an expression vector with various DNA segments labeled with letters (a-j).

i) (6 pts) Some, but not all, of these segments must be in a certain order for the successful expression of the HIV protease gene. Give the correct order of these segments with respect to the HIV protease coding region. You may use the diagram below for you answer, just indicate the position of the segment by its associated letter. The start codon (c), stop codon (g), and the mRNA termination(f) have been done for you as an example.



5' C g f

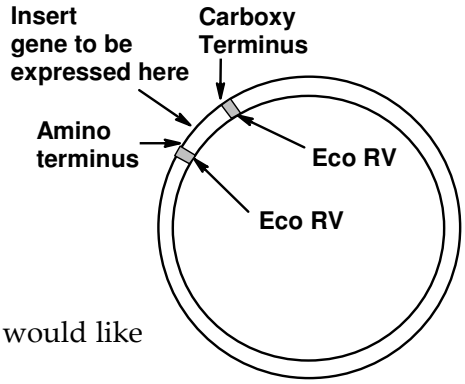
(amino term) HIV protease (carboxy term)

ii) (3 pts) The order for the remaining elements is immaterial, why?

iii) (5 pts) Select any ***one*** of the following five elements and briefly discuss its role in the production of recombinant HIV protease in bacteria. Please indicate your choice.

a) Antibiotic resistance gene
b) Origin of replication
c) Leader peptide
d) Promotor
e) Lac operator

21. (12 pts) Assume that you have the expression vector shown on the right. The restriction sequence for Eco RV is GAT^ATC, i.e. cleavage by this enzyme produces blunt ends. Although not shown, this vector contains all of the required elements for expression of proteins, with the ***exception*** of the start (AUG) and stop (UAA) codons and the lac operator sequence. The sequence of the expression vector in the vicinity of the Eco RV sites is:



CCCC**GATATC**XXXXXXXX**GATATC**AAAA
GGGG**CTATAG**XXXXXXXX**CTATAG**TTTT

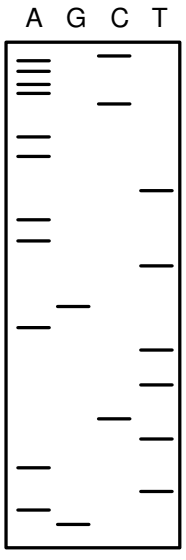
The protein sequence (and the DNA sequence) of the protein you would like to produce using this vector is:

MetPheTrpArgPheCysTyrTyrStop
CGTACATGTTTTGGAGGTTTTGTTATTACTAATCCCCGAC
GCATGTACAAAACCTCCAAAACAATAATGATTAGGGGCTG

i) (4 pts) Give the first 12 bases of ***both*** the left and right PCR primers that would be required to produce a PCR product from the above DNA that could be inserted into this expression vector (list the two primers below, use the back of the preceding page for justification of your answer, don't worry about T_M)

ii) (4 pts) Briefly describe (or diagram) how you would insert the PCR product into the vector.

iii) (4 pts) After constructing the expression vector, you transform the DNA into bacteria such that each bacteria receives one DNA molecule. To your surprise, you find that ***none*** of the bacterial cells produce peptide from the expression vector. Plasmid DNA is isolated from a number of different bacteria and sequenced using a primer (5'-CCCC-3'). Using the partial sequencing gel that is shown on the right, explain why the vector cannot produce a protein.



Bonus question: B1 (4 pts bonus) How might you modify the expression vector such that it would be possible to obtain this protein in bacteria [Hint: Why were only non-functional expression vectors obtained?]. (Use the back of the previous page).

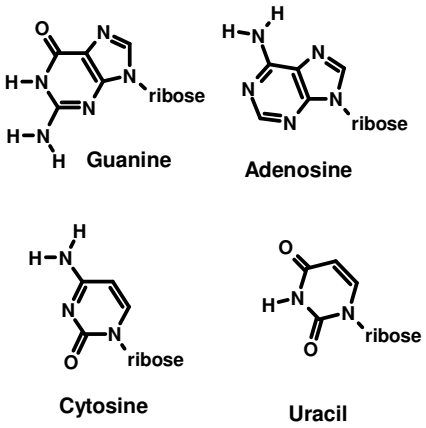
22. (14 pts) Please do ***one*** of the following four choices. Please indicate your choice.

Choice A: Discuss the elongation phase of protein synthesis. Your answer should include a brief description of the structure of the ribosome and the initiation complex.

Choice B: Discuss initiation and elongation of mRNA synthesis.

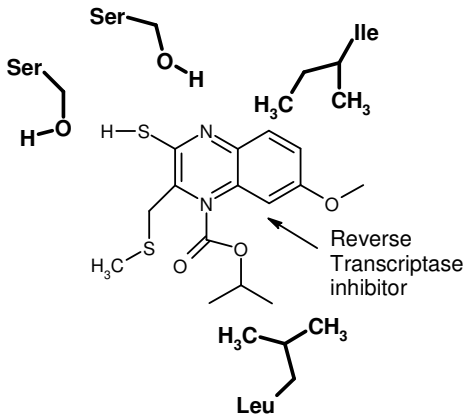
Choice C: The anticodon loop on $tRNA^{TYR}$ contains the sequence 5'-GUA-3'. Explain how this single tRNA can recognize two different Tyr codons (UAU, UAC). The structure of the bases are given to the right.

Choice D: Briefly discuss the role of the ribosome binding (Shine-Delgarno) site on the selection of the correct start codon during protein synthesis. Your answer should include a brief description of the structure of the ribosome and the initiation complex.



23. (12 pts) Reverse transcriptase (RT) performs an essential step in the life cycle of the HIV virus.

i) The compound on the right is an inhibitor of HIV reverse transcriptase. Amino acid residues from reverse transcriptase that interact with this inhibitor are shown in bold. Is this a competitive or mixed type inhibitor? Why? (4 pts).



ii) Resistance to this drug is caused by a mutation where the leucine (Leu) is changed to glutamic acid (Glu = -CH_2-CH_2-COOH)

a) Why do mutations arise frequently in the HIV virus? (1 pts).

b) Why does the change from Leu to Glu reduce the binding of affinity of the inhibitor (2 pts).

c) How would you alter the drug to be an effective inhibitor of the *mutant* reverse transcriptase (5 pts).

Bonus Question: B2 (4 pts) Describe how you could use the above drug to purify the wild-type (non-mutant) reverse transcriptase.

24. (20 pts) Please do ***one*** of the following three questions.

Choice A: Ligand binding data is obtained for a nucleic acid binding protein using equilibrium dialysis. The concentration of the protein inside the dialysis bag is 1 μM ($[M_T]$). The concentration of nucleic acid bound to the protein ([ML]), in μM, was measured and the data are shown in the table below for various types of nucleic acid under different conditions. **Note**: pK_a = 2.0 for the phosphate group on nucleic acid, 9.0 for Lys/Arg, 6.0 for His, 4.0 for Glu/Asp.

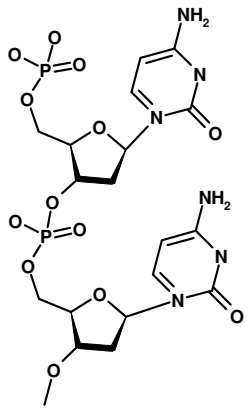
Concentration of Nucleic Acid	rC_6 pH=7 [NaCl]=1 mM	rC_6 pH=10 [NaCl]=1 mM	rC_6 pH=7 [NaCl]=1 M	rC_6 pH=10 [NaCl]=1 M	dC_6 pH=7 [NaCl]=1M
10^{-9} M	0.5	0.1	0.1	0.0	0.0
10^{-8} M	0.9	0.5	0.5	0.1	0.1
10^{-7} M	1.0	0.9	0.9	0.5	0.5
10^{-6} M	1.0	1.0	1.0	0.9	0.9
K_D					

(rC_6 = RNA, all cytosine, 6 residues long; dC_6 = DNA)

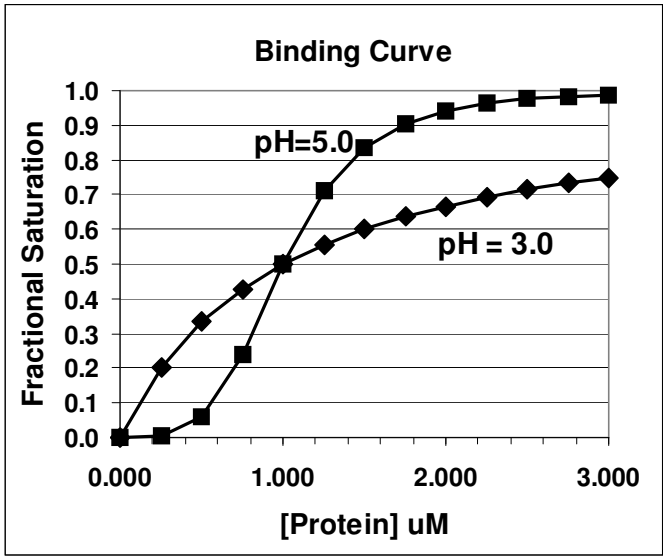
i) Write the K_D for all 5 conditions in the bottom line of the table. Briefly explain how you arrived at your answer (6 pts).

ii) Based on the K_D values, which of the above 5 conditions represents the highest protein-nucleic acid affinity? Briefly justify your answer (4 pts).

iii) Based on the above data, explain the major features by which this protein is interacting with nucleic acid. Your answer should discuss the role of the (deoxy)ribose, phosphate, and base in binding and provide a model of the protein-nucleic acid interaction that accounts for ***all*** of the data. For your convenience, a *partially* complete structure of C_2 is provided on the right (10 pts).

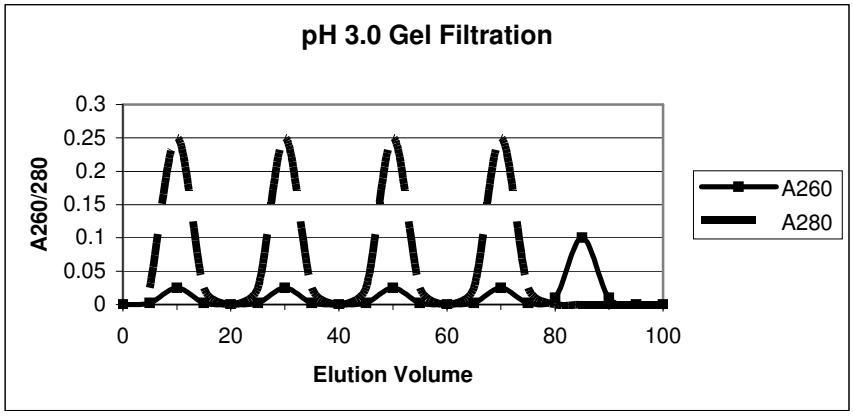


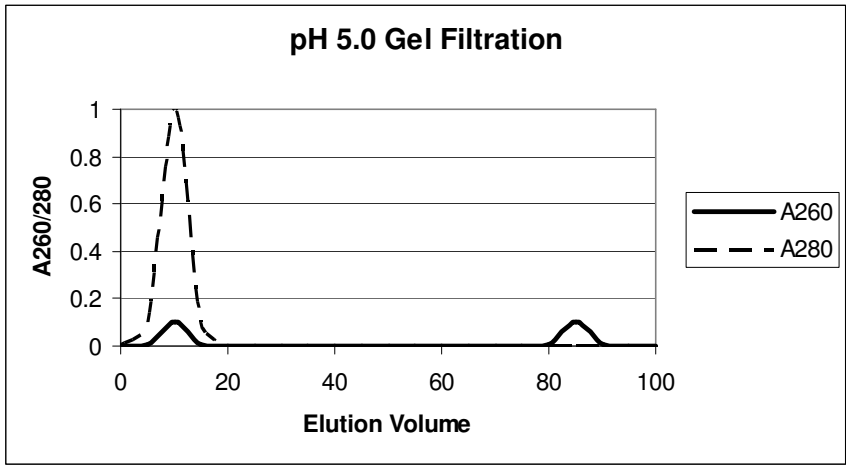
Choice B: The binding properties of a protein to a 100 basepair strand of DNA is measured. In this case the *protein* is considered the ligand (L) and the DNA is considered the macromolecule (M); more than one protein can bind to a single DNA molecule. The fractional saturation as a function of the protein concentration is shown below, measured at two pH values.



The protein-DNA mixture, at a protein concentration of 1 uM, is chromatographed over a gel filtration column and the absorbance at 260 nm and 280 nm is measured as a function of elution volume and these data are shown on the right.

i) Does the change in pH affect the affinity of the protein to the DNA? Justify your answer (6 pts).





Question continues on next page:

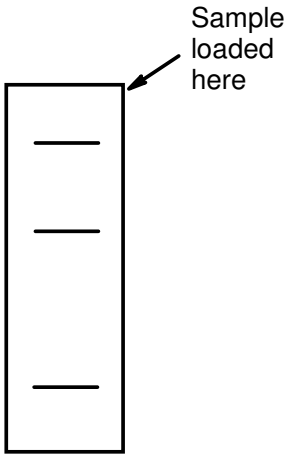
ii) How does the change in pH affect the cooperativity of binding? Justify your answer (6 pts).

iii) Provide a simple model of the protein-DNA complex that would account for the binding ***and*** the gel filtration data at both pH values (8 pts).

Choice C: Various concentrations of the following DNA molecule:

```
AACGTATGAGAAACAACGTTGACATGGAAGCTTGCGGCC
TTGCATACTCTTTGAAGCTTCTGTACCTTCGAACGCCGG
```

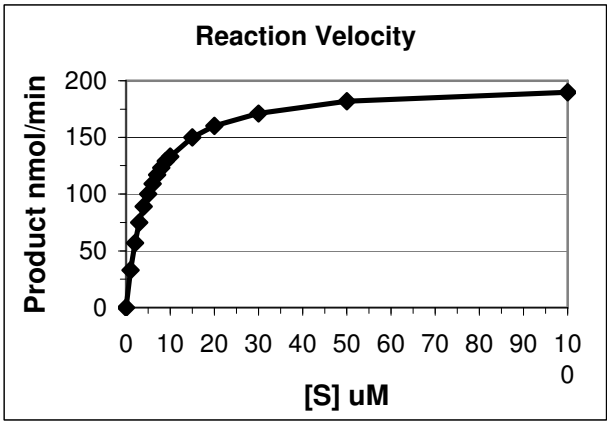
were treated with the restriction endonuclease Ezas3.14 for 1 min. The amount of product produced for each concentration of DNA is given in the graph to the right. The reaction mixture for one of these samples was run on a gel and this gel is shown on the right.



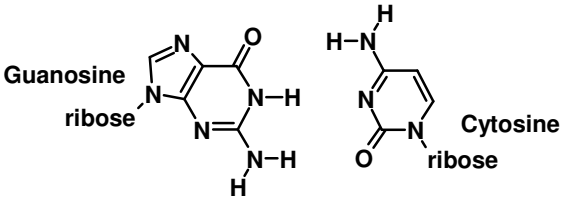
i) Mark on the gel the bands that represent the substrate and the products of this reaction (2 pts). *Briefly justify your answer*.

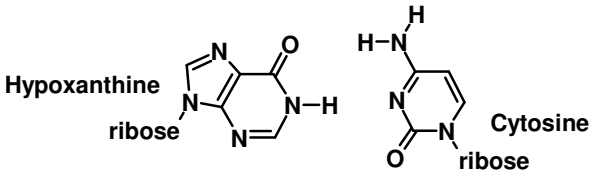
ii) What is the recognition sequence for Ezas3.14? *Briefly* justify your answer. (2 pts)

iii) Determine K_M and V_{MAX} for this substrate. Explain your approach. (6 pts)



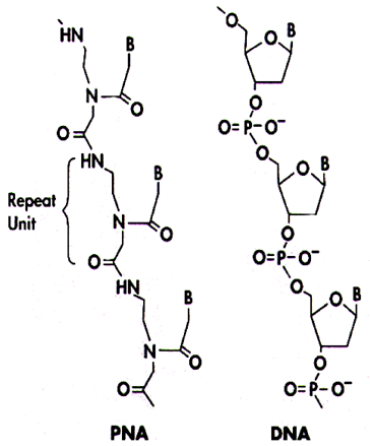
iv) The substrate DNA was modified by replacement of all of the guanosines with hypoxanthine. Would you expect to see a change in K_M or V_{MAX} for this modified substrate? The structure of a GC and G-hypoxanthine basepair are shown below. In this question you should assume that the DNA is double stranded, even if it contains hypoxanthine. Briefly justify your answer (5 pts).





v) (5 pts) The substrate DNA can be modified by replacement of the phosphate-ribose groups in the DNA with a modified polypeptide chain to give a peptide-nucleic acid (PNA). The nucleotide bases (B) remain the same. The PNA could not be cleaved by the restriction endonuclease and is thus an inhibitor.

a) What type of inhibitor is PNA? Why? (2 pts)

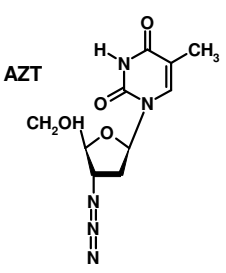


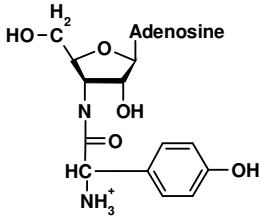
b) Would the presence of the PNA along with the normal substrate affect the measured K_M, the measured V_{MAX}, or both? Why? (3 pts)

Additional Bonus Questions, use the back of the previous page to answer (4 pts each).

B3) The AIDS drug AZT inhibits HIV Reverse transcriptase activity. How does this occur?

B4) The antibiotic puromycin binds to ribosomes in one of the tRNA binding sites and ultimately inhibits protein synthesis. How is protein synthesis inhibited? [Hint: a similar mechanism occurs with AZT].





Puromycin