Biochemistry I - Spring 2000 - Final Exam

This exam has a total of 163 points and is divided into three sections. To do well, you should do all of the questions in sections A and B. Section C provides four pairs of questions, you are to only do one question from each pair.

There are a total of 14 pages in this exam, including this one. Please write your name, or at least your initials, on each page before you begin.

Please be as succinct as possible in your answers!

Grade:
Part A: _____
Part B:
- **B1** _____
- **B2** _____
- **B3** _____
- **B4** _____
- **B5** _____
- **B6** _____
- **B7** _____
- **B8** _____
- **B9** _____

Part C:
- **C1/2** _____
- **C3/4** _____
- **C5/6** _____
- **C7/8** _____

Section A: Mutiple Choice. Please circle the BEST answer (24 questions, 48 points total, 2pts each).

1. A hydrogen bond is:
   a) only found in water.
   b) only found in DNA .
   c) only found in proteins.
   d) found in both DNA and proteins.

2. For all practical purposes, a buffer will control pH over which range:
   a) at pH values = pKa ±1.
   b) at pH values = pKa ±2.
   c) at pH values = pKa ±0.1.
   d) at any pH value.

3. The peptide bond in proteins is
   a) planar, but rotates to three preferred dihedral angles.
   b) cleavable by restriction endonucleases.
   d) planar, and usually found in a trans conformation.
   e) cleavable by lysozyme.

4. Which of the following elements of secondary or super-secondary structure are most likely to be found in an integral membrane protein?
   a) single b-strands.
   b) isolated β-hairpin.
   c) α-helices.
   d) β-α-β structure.

5. An "oil drop with a polar coat" is a metaphor referring to the three dimensional structure of:
   a) an integral membrane protein.
   b) collagen.
   c) a globular protein.
   d) glycogen.

6. Immunoglobulins are proteins that
   a) are involved in the determination of blood groups
   b) only bind small molecules with little specificity
   c) only bind large proteins with little specificity
   d) commonly perform catalytic functions.

7. The active site of an enzyme
   a) contains amino acids that confer substrate specificity.
   b) is flexible.
   c) contains residues important for catalysis.
   d) all of the above are true.

8. The transition state of a reaction is
   a) highly populated.
   b) lowered in energy in enzyme catalyzed reactions.
   c) raised in energy in enzyme catalyzed reactions.
   d) bypassed in enzyme catalyzed reactions.

9. Allosteric enzymes
   a) are specific for the allo stereoisomer of amino acids.
   b) must be tetrameric.
   c) always display positive cooperativity
   d) can be monomeric.

10. The high rate of the formation of HIV viruses that are resistant to drugs is due to:
    a) Induction of mutations in the viral genome (DNA) by the drugs.
    b) Interference of drugs with proofreading ability of PolI.
    c) Interference of drugs with proofreading ability of HIV reverse transcriptase.
    d) Lack of proofreading by HIV reverse transcriptase.

11. Molecular weight determination of either proteins or DNA by gel electrophoresis relies on which of the following:
    a) A constant charge to mass ratio of the particles.
    b) migration of charged molecules in an electric field.
    c) slower migration of larger particles due to the gel.
    d) all of the above.

12. The subunit molecular weight as well as the number of subunits in the quaternary structure can be determined by:
    a) SDS-PAGE electrophoresis.
    b) Affinity chromatography.
    c) Gel filtration chromatography.
    d) combining information from a) and c).

13. Cholesterol is essential for normal membrane functions because it
    a) spans the thickness of the bilayer.
    b) keeps membranes fluid.
    c) catalyzes lipid flip-flop in the bilayer.
    d) plugs up the cardiac arteries of older men, including Dr. Rule.

14. Which of the following membrane structures function in active transport?
    a) peripheral proteins.
    b) cytochrome C.
    c) integral proteins.
    d) Coenzyme Q.

15. The glycosidic bond
    a) is found in oligosaccharides.
    b) is found in DNA and RNA.
    c) is found in proteins.
    d) both a) and b) are correct.

16. Which of the following are forms of energy storage in biochemical systems?
    a) NADH & $FADH_2$
    b) Concentration gradients across membranes.
    c) ATP.
    d) all of the above.

17. The hormones, glucagon and epinephrine, stimulate glycogen breakdown to G-1-P
    a) directly, by binding to glycogen phosphorylase.
    b) indirectly, by first stimulating adenylate cyclase to make cAMP.
    c) only in the liver.
    d) only in muscle cells.

18. The sequence of DNA and RNA molecules is always written:
    a) beginning at the 5' end and going to the 3' end.
    b) beginning at the 3' end and going to the 5' end.
    c) both of the above are acceptable.
    d) neither of the above.

19. The major reason for A pairing with U is:
    a) complementary hydrogen bonds.
    b) a purine-pyrimidine pair fits well in the double helix.
    c) efficient stacking of this arrangement of bases in the helix.
    d) recognition of non-'Watson-Crick' hydrogen bonds by DNA polymerases

20. An expression vector or expression plasmid
    a) always contains an origin of replication.
    b) usually contains a gene that confers antibiotic resistance to the bacterial host.
    c) always contains DNA segments for the regulation of mRNA production.
    d) all of the above.

21. Restriction fragments cut with Sau3A (X/GATCX; X is any base) and BamHI (G/GATCC)
    a) can be efficiently joined because they both cut the DNA at the same location.
    b) can be efficiently joined because they both give the same cohesive ends.
    c) can not be joined because they both give different cohesive ends.
    d) can be efficiently joined because they both begin with G and end with C.

22. Replication in E. coli is initiated by the generation of short RNA primers because
    a) RNA polymerase is a more efficient enzyme at starting replication.
    b) DNA polymerases require a priming strand with a 3'-OH group.
    c) The initial error rate is lower with RNA than with DNA.
    d) DNA polymerases don't become activated until phosphorylated by DnaA.

23. During replication, overwinding or overtightening of DNA is removed by:
    a) Ribosomes.
    b) DNA polymerase.
    c) DnaB.
    d) Gyrase.

24. A promoter is a
    a) a specific sequence of DNA to which core RNA polymerase binds.
    b) a specific sequence of DNA to which holo RNA polymerase binds.
    c) a specific sequence of DNA to which a repressor binds.
    d) a specific DNA sequence to which DnaA binds.

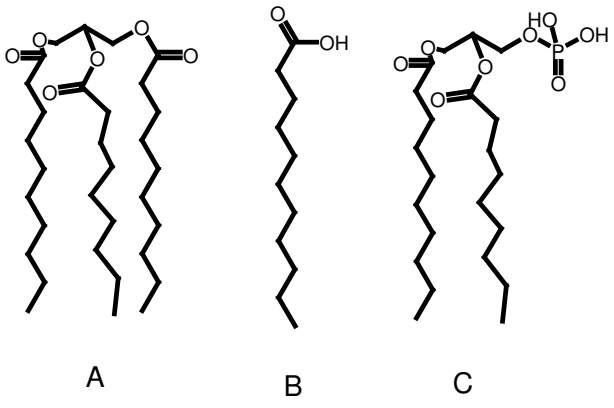
Part B: Please do All of the following Problems.

B1: (5 pts) Three molecules are shown to the right, labeled A, B, and C.

i) Below are the names of two lipid molecules. Write the letter that corresponds to the structure of the molecule.

Triglyceride:_____
Phospholipid:_____

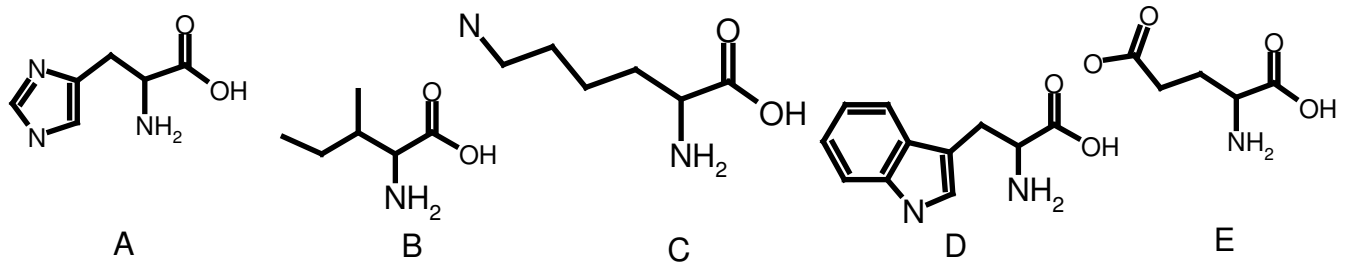
ii)Which compound(s) is(are) charged at neutral pH?



B2: (4 pts) The structural hierarchy of proteins and nucleic acids (i.e. DNA and RNA) ranges from primary to quaternary structure. The following table lists this hierarchy for proteins and Nucleic acids. Two of the entries have been provided, supply **four of the remaining 6**. You can give either a short description or provide an example.

	Primary	**Secondary**	**Tertiary**	**Quaternary**
Protein			*Folded form of proteins, ie. myoglobin*	
Nucleic Acids				*Protein-nucleic acid complexes*

B3: (10 pts) The following are the structure of five amino acids.:



i) Write, underneath **two(2)** of the structures, the name of the amino acid (three letter code is quite acceptable)

ii) For the following statements, write the letter of the amino acid to which the statement best applies. Note, you may want to use the same amino acid more than once. If two (or more) are equally acceptable, write both of them. In some cases none of these may apply. If so, just write 'none'.

a) Sidechain is hydrophobic and found buried in the core of protein: _____

b) Sidechain absorbs UV light strongly: _____

c) Sidechain found at active sites of some enzymes: _____

d) Sidechain can form electrostatic interactions with DNA: _____

e) Sidechain can form hydrogen bonds with nucleotide bases: _____

f) Sidechain that has a pK_a of around 4: _____

g) Sidechain that has a pK_a of around 7: _____

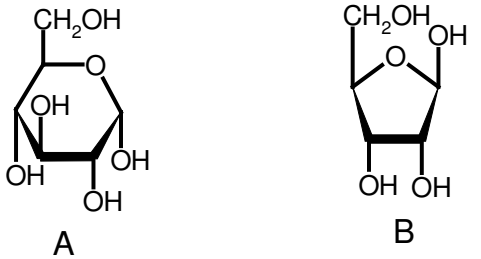
h) Can measure its sidechain pKa with NMR _____

B4. (5 pts)

i) Which of the following two sugars is ribose (A or B)?

ii) Draw a box around the 2-OH of ribose.

iii) Circle the carbons on ribose that participate in the formation of phosphate esters in the backbone of DNA and RNA.



B5. (14 pts) A list of energetic terms or 'forces' that play a central role in Biochemistry is given below:

1. Hydrogen bond
2. _____
3. Electrostatics
4. Van der Waals
5. Configurational Entropy

i) Line 2 is blank, which *very important* energetic term has been omitted?(2pts)

ii) Pick **any two of the above five** and give a brief description of its molecular nature in relationship to biochemical structures. (4 pts)
Choice #1

Choice #2

iii) Compare and contrast the role of **two(2) of the above five** energetic terms in their contribution to protein and DNA Stability. Use the following table to guide your answer. Indicate in the left column the energetic terms you have selected to discuss (Each box is worth two points, *be brief*).

Force or Energetic Term:	Protein Stability	DNA Stability
Choice #1:		
Choice #2		

B6. (14 pts) Due to incompatible blood types there is always a shortage of human blood for medical treatment. Your responses to the following questions will lead you through the steps involved in the production of human hemoglobin in bacteria (This has actually been done by Dr. Chien Ho at CMU).

i) Briefly describe the steps involved in obtaining the required DNA fragments for the construction of the library. Discuss how you would obtain the hybridization probe to identify the hemoglobin genes and the requirement for a antibiotic resistance gene on the plasmid (4 pts).

ii) The final expression plasmid has to have several elements, or sequences, that control both the production of mRNA as well as protein. The line drawn below represents the DNA sequence of the expression plasmid that contains the hemoglobin gene as well as the required regulatory features for mRNA and protein synthesis. The start of mRNA synthesis is indicated by the $\rightarrow$. Place the remaining regulatory features on this diagram in the correct order and with approximately the correct spacing. Draw a **neat** square or rectangle around those that are involved in mRNA production (or its regulation) and a circle around those that are involved in protein synthesis (or its regulation) (8 pts).

a) Termination codon
b) mRNA termination site (ρ-factor binding)
c) Start codon
d) -10 region of the promoter
e) operator site
f) -35 region of the promoter

iii) High levels of recombinant hemoglobin would be lethal for the cell. Briefly describe how you would control the expression of the hemoglobin in the bacteria.(2 pts)

iv) *Briefly describe how you would purify this recombinant protein from bacteria *in a single step*. You can assume that both the α and β chains are synthesized in the same bacterial cell. In addition, you can assume that the bacteria cannot provide sufficient heme, thus most of the recombinant hemoglobin in the lysate will be without heme.(2pts).

B7. (9 pts)**.**
i) Sketch the tertiary structure of a tRNA molecule, indicating the location of the anti-codon loop and the acceptor stem.(2 pts).

ii) The assembly step in protein synthesis results in the formation of the complex between the 70S ribosome, the mRNA, and fMet-tRNA in the peptidyl(P) site. A representation of this complex is given in the left-most figure at the bottom of this page. The diagram, proceeding from left to right, show the complete sequence of steps in elongation of the peptide by one amino acid. The first and last diagrams have been completed for you. Do the following:

A) Complete the drawing of the two middle steps.(2 pts)

B) In one of the diagrams include the following labels, in their proper place:(3 pts)

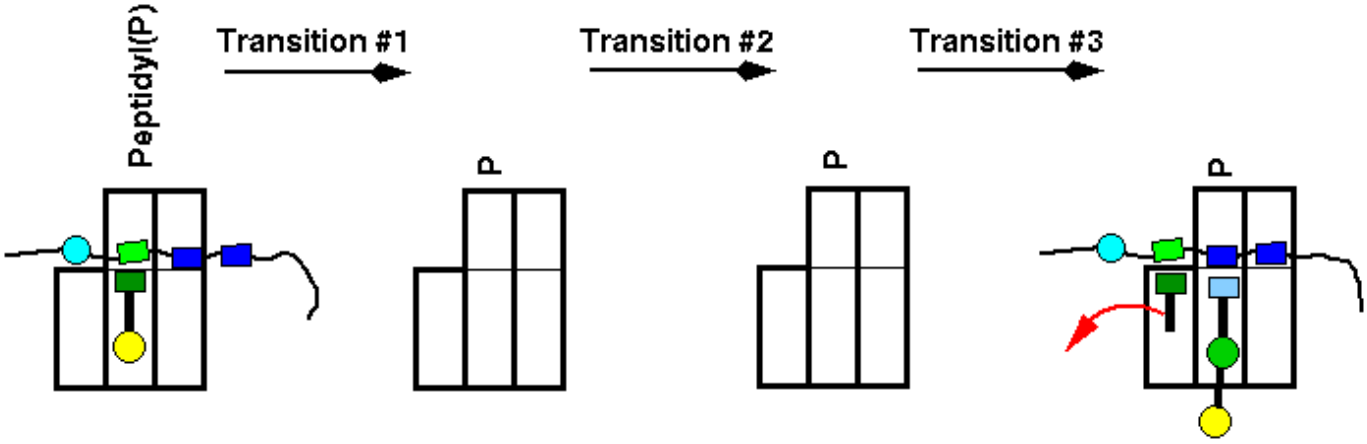
i) Exit Site (E)
ii) Aminoacyl Site(A)
iii) Codon (Start or otherwise)
iv) mRNA
v) 30S subunit of the ribosome
vi) 50S subunit of the ribosome.

C) There are three transitions between these four complexes. For each of these transitions, state one of the important events that occurs during the transition. The important events associated with the 1st transition are provided as an example, you need to do only transition #2 and transition #3.(2 pts)

Transition #1: *Charged tRNA binds to the aminoacyl-site, basepairing with the 2nd codon.*

Transition #2:

Transition #3:



B8 (8 pts): Enzyme Inhibition.

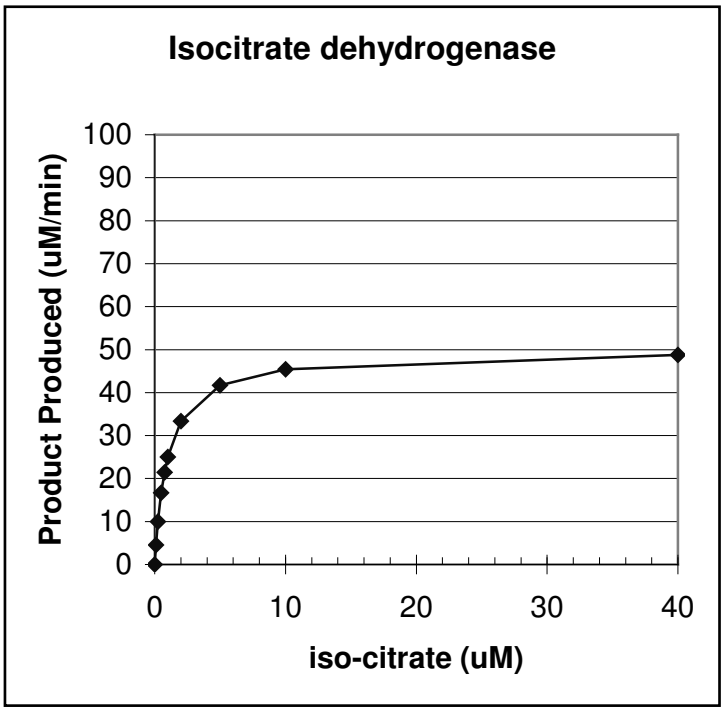
i) How do the enzyme binding sites for a competitive inhibitor and a non-competitive inhibitor differ?

ii) Explain why a competitive inhibitor can only affect K_M and not V_{MAX}. A cartoon diagram of an enzyme might be helpful.

iii) Which type of inhibitor is more likely to serve as a feedback inhibitor in metabolic pathways? Why?

B9:(8 pts) Enzyme Kinetics

The following is a plot of the initial velocity of isocitrate dehydrogenase (a TCA cycle enzyme) as a function of substrate concentration. No ATP or ADP was present in this reaction.



i) Estimate K_M from this graph. Explain your approach.

ii) Sketch, using a **dashed line**, the data you would expect to obtain in the presence of ATP. Briefly justify your answer.

iii) Briefly describe how you would obtain the K_M for this enzyme using a Lineweaver-Burk plot (a simple sketch would be fine)

Part C: Choice Questions:

Do either C1 or C2:(8 pts)

C1. The metabolism of glycogen synthesis and degradation is tightly coupled to glucose synthesis and degradation in both liver and muscle tissues. However, glycogen metabolism is controlled by phosphorylation/dephosphorylation of enzymes while glucose metabolism is controlled by the effects of levels of fructose-2,6-phosphate (F2,6P,FbisP) on the activities of phosphofructokinase (PFK). Briefly describe how these two pathways are coordinately regulated. The following details may be useful in your discussion. F2,6P is synthesized from F-6-P by the enzyme phosphofructose kinase 2 (PFK-2) and degraded to F-6-P by the enzyme fructose-bis-phosphatase-2 (FBPase-2).

C2. Draw a *simple* diagram that illustrates the oxidative fate of the principle components of a bagel with cream cheese (i.e. glucose from the bagel, fatty acids and amino acids from the cream cheese). You diagram should resemble a flow chart, showing only the *names* of the major metabolic pathways and how they are connected. The top of your 'flow chart' should begin with the **three** nutrients. The bottom of your 'flow chart' should end with H_2O.

Answer C1 or C2 in the space below:

Do either question C3 or C4 (8 pts)

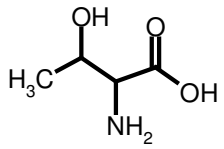
C3.

i) How frequently does the sequence encoding the start codon (AUG) occur in DNA? How does E. coli select the correct starting position to begin translation of mRNA into protein?

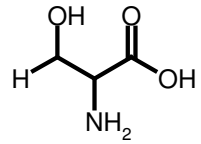
ii) The charging of tRNAs with amino acids is a two step process. Draw, sketch, discuss, etc. these steps using the charging of $tRNA^{Thr}$ with Thr as an example.

iii) How does the cell avoid charging $tRNA^{Thr}$ with Ser? Give a *plausible* description of the active site of the enzyme that charges $tRNA^{Thr}$ (i.e. indicate the amino acids that might be present in the active site of the enzyme). Your description should discuss both activities present in the active site (hint: hydrolytic editing). The structures of Thr and Ser are given to the right.

Thr



Ser



C4. DNA synthesis on the leading strand is continuous, while DNA synthesis on the lagging strand is not. Discuss how E. coli accomplishes synthesis of the lagging strand. Briefly discuss the enzymes involved and highlight the important differences and similarities between the two DNA polymerases.

Answer C3(i-iii) OR C4 In the Space Below

Do either question C5 or C6:

C5. (6 pts) Using either hemoglobin or phosphofructokinase (PFK) as an example, discuss the following aspects of allosteric effects in biological systems (Note, you need not use the same example when answering each part)

i) Necessary structural requirements of the protein for it to exhibit allosteric behavior (i.e. nature of binding sites, etc.).

ii) How the activity of a protein is affected by allosteric effects?

iii) Give an example of an allosteric effector and a brief description of its role in the regulation of the biological process performed by hemoglobin or PFK.

C6 (6 pts): Discuss **two of the following three** features of enzyme catalyzed reactions. Indicate how these features are important for catalysis and provide a specific example of this feature in an existing enzyme. In your examples, you can use any enzyme you like (including ones not discussed in class) and you need not use the same enzyme for both of your answers.

i) Transition state stabilization.

ii) Substrate Specificity

iii) Chemical mechanism.

Do either question C7 or C8: (16 pts)

C7. The binding constant of single-stranded binding protein (SSB) to single-stranded DNA was measured in the following fashion. A number of different concentrations of DNA were added to a 1 uM solution of SSB and the absorbance of the solution was measured at each concentration of the DNA. The original absorbance of the protein solution was 1 absorbance unit. The **change** in absorbance of the protein, due to the addition of the DNA, is given in the 2nd column. A value of 100% would indicate protein that is fully complexed with DNA. The fractional saturation (Y) is also given in the 2nd column.

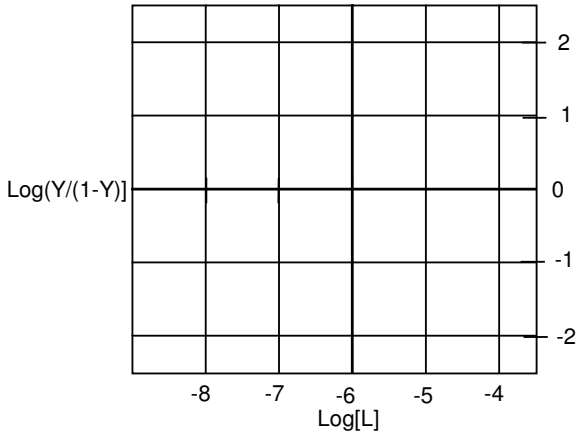
DNA Concentration (uM)	Change in Absorbance (%)	Log(Y/(1-Y))	log [L]
0	0 (Y=0.00)	-	-
0.1	2 (Y=0.02)	-1.70	-7
1.0	50 (Y=0.50)	0.00	-6
10.0	98 (Y=0.98)	1.70	-5
100.0	99 (Y=0.99)	2.95	-4

i) What wavelength of ultra-violet (UV) light was used in this experiment, 260nm or 280nm? (2pt)

ii) What is the extinction coefficient for this protein? (2 pts)

iii) *Estimate* the binding constant from these data, assuming that the binding is **not** cooperative. (Hint: You do not need to draw a graph of any sort here). Explain your approach. (4 pts)

iv) Does the binding of DNA to this protein display either positive or negative cooperative behavior? Justify your answer. You should **not** have to do a Hill plot to determine this. However, if you must, the appropriate data (log[y/(1-y)], log[L]) are given in the last two columns of the above table. Use the graph to the right. (4 pts)

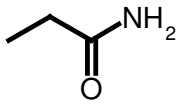


v) Sketch, in the box to the right, the Hill plot you would expect to obtain from these data. You **do not** have to plot the data, just indicate the approximate value of the slope at log(theta)=0. If you did this plot in part iv, go on .(2 pts)

vi) Given the role of SSB in DNA replication, does the particular DNA sequence used for this experiment matter? Why? (1 pt)

viii)**Provide a *plausible* model for the cooperative behavior observed here.(1 pt)

C8 (16 pts): The restriction enzyme ZuluI binds to and cleaves the following DNA sequence: GATC. Below is a diagram of the interaction between an Asn residue of this restriction endonuclease and a TA base pair in its recognition sequence. The position of this base pair in the recognition sequence is indicated in bold and underlined (GA**<u>T</u>**C). Drawn below the TA base pair is a CG base pair to help you visualize what happens when the TA base pair is replaced by a CG basepair.

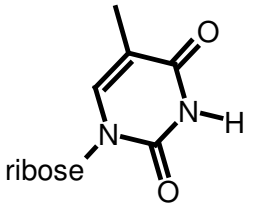


i) Label the major groove of the TA base pair.(1 pt)

ii) Draw in the 'Watson-Crick' hydrogen bonds with **dotted** lines (1pt).

iii) Draw in any hydrogen bonds that might occur between the protein and the DNA with **solid** lines.(1 pt)

iv) The binding constant, K_{EQ}, of ZuluI to the sequence GATC is 10^{10} M^{-1}. Calculate ΔG^o for this binding reaction (assume RT=2.5 kJ/mol; $\ln 10^{10} = 23$)(2 pts).



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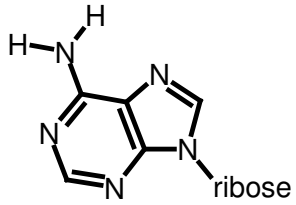

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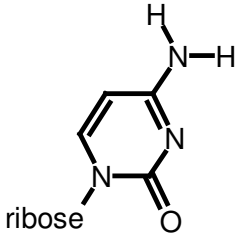

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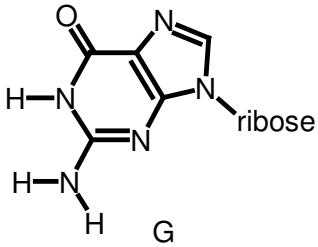

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v)*Replacement of the TA base-pair with a CG base pair will cause the loss of a hydrogen bond between ZuluI and the DNA. What is the energetic cost of this hydrogen bond in kJ/mol? (Be careful when you answer this - don't forget to consider the water molecules)(3 pts)

vi) Replacement of the TA base pair in the sequence GATC with a CG base pair will give the sequence GACC. Calculate the ΔG^o **and** K_{EQ} of binding of ZuluI to GACC. Assume that the only difference in the interaction of ZuluI with the GACC versus its interaction with GATC is the loss of the hydrogen bond to the adenosine residue that was discussed in part v. (5 pts)

vii) Using the above affinity constants, calculate the fraction of ZuluI bound to its normal sequence (GATC) and compare this to the fraction of ZuluI that would be bound to the sequence (GACC) (3 pts).