

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.

A hydrogen bond forms between the following atoms: X-H Y, where X and Y are electronegative atoms (3 pts).

The proton becomes electropositive and interacts with the electronegative Y, creating the hydrogen bond (1 pt)

- Protein secondary structure - stabilized by hydrogen bonds.
- DNA structure - hydrogen bonds stabilize the correct base pair.
- Protein - ligand/substrate interactions can be stabilized by hydrogen bonds.

(4 pts for example)

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).

van der Waals forces occur between all types of matter (polar or non-polar) and represent attractive forces between induced and permanent dipoles on atoms. This interaction is primarily enthalpic. (4 pts)

The hydrophobic effect pertains to non-polar groups. When exposed to water, these groups order water molecules, decreasing their entropy. When the non-polar group is buried, the ordered water is released, which increases the entropy of the system - which is favorable.

(4 pts)

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

Proteins: the core of proteins consists of hydrophobic residues. When proteins fold the water released from these groups causes a favorable entropy change of the system.

Membranes: The hydrophobic effect makes it energetically favorable for the non-polar acyl chains to associate with each other due to the increase in the entropy of the solvent. Thus forming a bilayer structure.

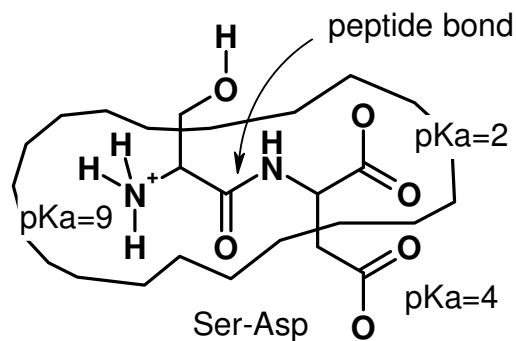
3. (4 pts) What feature or property of the *unfolded* state of proteins (or DNA) is responsible for stabilizing the unfolded state? Is this an enthalpic or entropic term?

Its high conformational entropy.

4. (8 pts) Peptide Structure.

Draw a dipeptide using any two amino acids. One residue should be polar, the other ionizable (4 pts). Label your diagram with:

- the location of the peptide bond (1 pt)
- all** pK_A values (1 pt)
- circle the atoms that make up the mainchain atoms (1 pt)
- the name of your dipeptide (1 pt)



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

The peptide bond has partial double bond character. the overlap between the p orbitals on the sp₂ nitrogen and the p orbitals on the sp₂ carbon cause the four atoms (H-N-C-O) to lie in a plane.

- Which of the two conformations is more common or stable? Why? Which conformation did you draw for problem 3? (4 pts)

Trans (as drawn above), since this reduces steric clash between the atoms.

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

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

The secondary structure refers to the configuration of the mainchain atoms, while the tertiary structure refers to the configuration of both the mainchain and sidechain atoms.

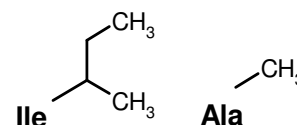
Choice B: Briefly compare and contrast an α -helix to a β -sheet.

In both cases the mainchain hydrogen bonds are fully satisfied.

A helix is helical with H-bonds formed between residues in the same helix

A β -sheet has straight segments that are hydrogen bonded to each other.

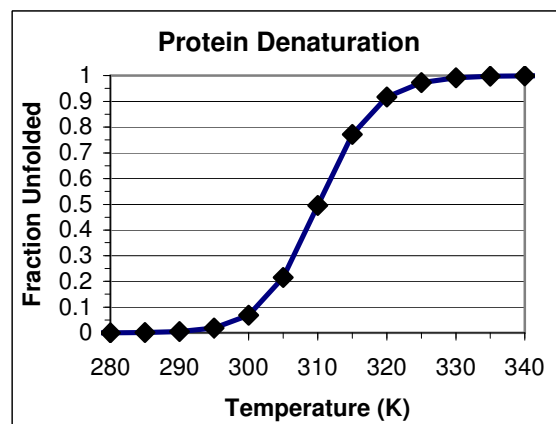
7. (8 pts) The thermal stabilities of a wild-type and mutant small globular protein were measured. The original, or wild-type protein contains an isoleucine residue while the mutant contains an alanine residue. The thermal denaturation curve for the wild-type protein is shown below.



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.

The curve would be displaced to the left, i.e. the Ala containing protein would denature at lower temperatures because (2 pts)

Due to reduced van der Waals interactions, the enthalpy of unfolding for the mutant will be less, making it less stable. (4 pts)



The alanine residue, because of its smaller size, will have a smaller hydrophobic effect. The decrease in solvent entropy due to exposure of the Ala in the unfolded state will be smaller, making the alanine containing protein less stable. (2 pts)

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)?

The oxygen binding site in myoglobin and hemoglobin is an Fe atoms bound to a heme group.

Electrons can also be transported on the Fe atom, as in cytochrome c in electron transport.

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).

The active site of an enzyme contains amino acid side chains that:

- a) interact specifically with the bound substrate, e.g. the negatively charged Asp residue in trypsin, the non-polar residues in chymotrypsin or HIV protease. (6 pts + 2pts for example)
- b) Chemically react with the substrate. They are catalytic because they reduce the energy of the transition state. All enzymes reduce the energy of the transition state by pre-ordering the catalytic groups, e.g. the catalytic triad in serine proteases, the two Asps in HIV protease, the Cys residue in G-3-P dehydrogenase. Some enzymes, such as serine proteases, form enthalpic interactions with the **ONLY** the transition state. (6 pts + 2pts for example, -1 if one method of stabilizing the T state is missing.)

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).

Allosteric effects occur when the binding of one ligand (or phosphorylation) causes a change in the conformation of a protein. This change in shape can affect:

- the binding of other ligands.
- the enzymatic activity.

Examples:

Hemoglobin:

- O_2 affects its own binding, causing positive cooperativity that is essential of optimal oxygen transportation.
- BPG affect the binding of O_2 , allowing altitude adjustment.

Glycogen Metabolism:

- Phosphorylation of proteins causes activation of glycogen phosphorylase and inactivation of glycogen synthase.

Lac Repressor

- Binding of lactose causes it to release from DNA, allowing mRNA to be made. Normally this allows the bacteria to turn on genes required for lactose metabolism.

Glycolysis

- PFK and F16bisphosphatase are allosterically regulated by AMP, ADP and ATP to insure that the correct pathway is operated according to the needs of the cell.

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, if justified.

- **The electropositive aldehyde group is attached by the C5-OH group on C6 aldoses or ketoses, or by the C4-OH group on C5 aldoses, such as ribose. (4 pts)**
- **The newly formed chiral center can have two configurations, α or β . (4 pts)**

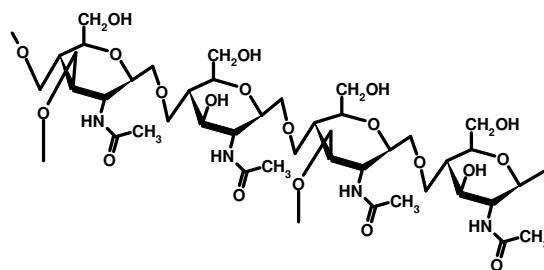
Choice B: Briefly describe how *any* dietary disaccharide or polysaccharide enters glycolysis.

Starch (or glycogen) is composed of glucose. The glucose is released and enters glycolysis as the first compound.

Lactose contains glucose and galactose. The galactose is converted to glucose by inversion of a chiral center.

Sucrose contains glucose and fructose. The fructose enters a fructose-6-P after phosphorylation.

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.



Both cellulose and the bacterial polysaccharide have $\beta(1-4)$ linkages and both have glucose (cellulose) or modified glucose as the residue.

Lysozyme must recognize the N-acetyl groups, allowing the enzyme to bind and hydrolyze the $\beta(1-4)$ linkage.

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.

- **The non-polar center of the bilayer prevents ions, such as the proton, from crossing.**
- **This is essential since ATP synthesis relies on a proton gradient across the membrane. If the membrane were permeable to protons, they would simply flow back across the membrane instead of through the ATP synthase.**

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

The surface of globular proteins contains polar, charge, and non-polar residues.

The surface of membrane proteins that contact the lipid are completely non-polar.

Since all mainchain hydrogen bonds have to be made, membrane proteins tend to be all α -helical or β -barrel.

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.

Oxidations generate energy (4 pts)

G-3-P to G-1,3-P

Pyr to acetyl CoA

NAD⁺ or FAD as cofactors/cosubstrates (2 pts), generic name is dehydrogenase (2 pts)

+2 pts for example:

Alkane to alkene

Alcohol to ketone.

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.

Protein phosphorylation: Enzyme in pathways can be substrates for kinases. The phosphorylated form of the protein can be active or inactive. Dephosphorylation would reverse this, causing an inactive protein to become active.

In glycogen storage, the phosphorylation of proteins that occurs when glucagon or epinephrine are present causes glycogen phosphorylase to become active.

Feedback inhibition: An enzyme is regulated by some compound that is further down the pathway, i.e. not the product of the enzymes. PFK in glycolysis is inhibited by ATP, which is produced by glycolysis. Pyruvate dehydrogenase and citrate synthase are inhibited by NADH, which is produced by the TCA cycle.

Product inhibition: An enzyme is inhibited by its product, i.e. pyruvate dehydrogenase is inhibited by acetyl CoA, citrate synthase by citrate.

Changes in the levels of enzymes: e.g. lactose metabolism genes.

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

The monosaccharides in these two compounds enter glycolysis. In the absence of oxygen as an electron acceptor, it is necessary to regenerate NAD⁺. This is done by reducing pyr to ethanol in yeast.

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

- **During vigorous exercise most of the energy comes from glycolysis.**
- **Glycogen is used to provide glucose for glycolysis.**
- **Since amino acids cannot be efficiently converted to glucose, glycogen levels will be low.**

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° ? Which provides a more useful description of energy changes in metabolic pathways? Why?

- **The Gibbs energy is the energy released when going from a non-equilibrium state to equilibrium.**
- **The standard energy is the energy released when one mole of reactant is converted to one mole of product. It describes the properties of the system at equilibrium.**
- **The Gibbs energy, because metabolic pathways are not at equilibrium.**

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

- **For a pathway to flow, all steps must show a negative Gibbs energy.**
- **To give unfavorable steps a negative Gibbs energy, they are coupled to favorable reactions. The energy from the favorable reaction is used to reduce the energy of the unfavorable reaction.**

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 |

K_D , K_I , and K_I' all refer to the dissociation constant for:

- a ligand (K_D),
- an inhibitor from the free enzyme (K_I),
- or an mixed-type inhibitor from the ES complex (K_I')

K_M is almost the K_D for an enzyme substrate reaction, but contains k_{CAT} as well.

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

- The Hill coefficient is the slope of the Hill plot when it crosses the x-axis.
- It is a measure of cooperativity of binding, i.e. if equal to 1 the binding is not cooperative, if greater than one, positively cooperative.

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:

- both 5' ends
- a single glycosidic bond
- a single ribose (or deoxyribose)
- a single phosphodiester bond
- a single purine

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

- indicate the edge of the basepair that represents the major groove,
- the edge that represents the minor groove,
- draw all Watson-Crick H-bonds.
- Indicate additional donor/acceptors in the minor groove.

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

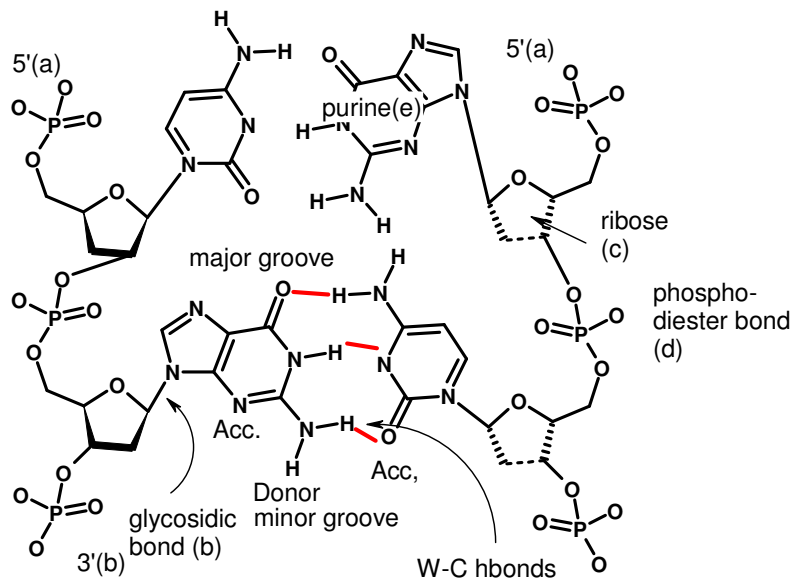
DNA, or deoxyribonucleic acid, since the 2' OH is missing.

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

- Strands are parallel.
- One sugar is linked to the 2'OH, not the 3'OH, the top left one.

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

- | | |
|-------------------------------|---------------------------------------|
| a) Hydrogen bonds (+1) | c) van der Waals interactions (+2pts) |
| 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.

A GC basepair has three hydrogen bonds instead of the two in AT basepairs, therefore more energy is required to break the strands apart.

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

The double stranded form is destabilized by charge repulsion between the phosphates. This repulsion is reduced in solutions with higher salt.

19. (12 pts) Please do one of the following three choices. Please indicate your choice. Use the back of the previous page if you require more room.

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 (8 pts)

- **Enzyme requires a primer hydrogen bonded to the template.**
- **Bases added to 3'-OH of primer.**
- **Base that is added is selected based on H-bonding to template, and size (purine-pyrimidine).**
- **Have a error checking 3'-5' exonuclease.**

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

- **NTP added to primer, using DNA as template.**
- **no primer needed**

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

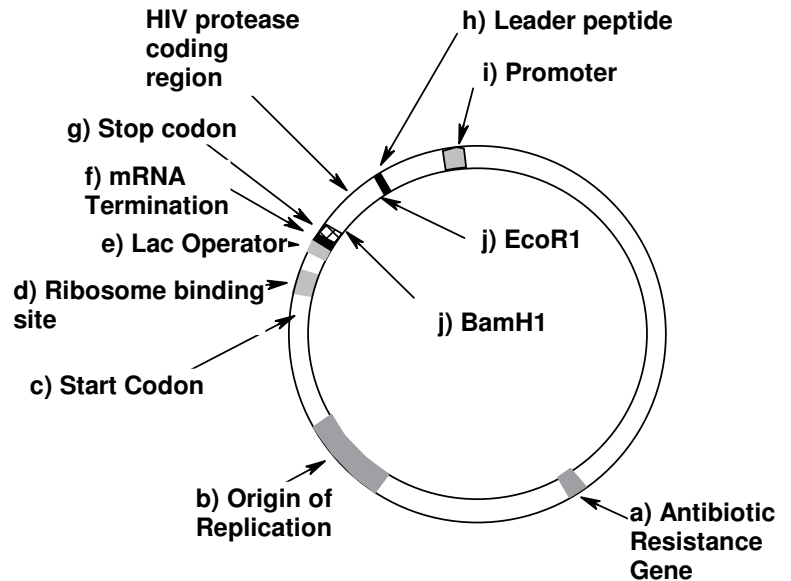
- **In PCR two primers are selected that point toward each other. The left primer has the same sequence as the top strand. The right primer has the same sequence as the bottom strand.**
- **Denaturation of the template, followed by annealing of the primers.**
- **DNA polymerase activity will extend from each primer, copying the template between the two primers.**
- **After several cycles the DNA between the two primers becomes selectively amplified.**

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

- **In DNA sequencing, it is necessary to generate molecules that begin at the same location and end with a known base.**
- **Using a primer, and a polymerase, produces molecules that begin at the same location.**
- **The use of a dideoxy nucleotide causes chain termination, giving molecules that end in known bases.**
- **The position of the dideoxy base is "measured" by size separation using gel electrophoresis.**

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.



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

- The origin of replication and antibiotic resistance gene can be anywhere.
- Changing the order of the restriction sites can be accommodated in the design of the PCR primer.

- 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: **Provides a method to insure the plasmid remains in the bacteria. Only those bacteria with the plasmid will grow in the presence of the antibiotic.**
- b) Origin of replication: **Insures that the plasmid will be replicated in the bacteria.**
- c) Leader peptide: **Causes export of the HIV protease out of the cell.**
- d) Promotor: **Site where RNA polymerase binds to initiate the synthesis of mRNA.**
- e) Lac operator: **On/off switch for mRNA production. The lac repressor binds at this site, turning off transcription. Addition of IPTG turns transcription on.**

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

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

1. The ribosome has 3 tRNA binding sites, one for the exiting uncharged tRNA (E), one that holds the tRNA-peptide (P) and one that hold the incoming charged tRNA (A)
2. tRNA-met is in the P-site.
3. next tRNA-AA binds to the A-site
4. amino group of AA in A-site attacks peptide-tRNA ester, transferring growing chain to tRNA in the A-site.
5. Ribosome shifts, the uncharged tRNA in the P-site goes to the E(exit) site and leaves. The elongated protein is in the P-site.

Choice B: Discuss initiation and elongation of mRNA synthesis.

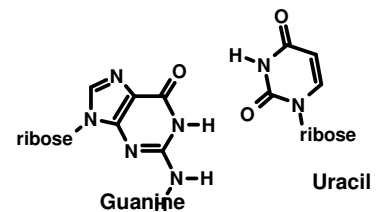
1. RNA polymerase binds to the promoter via sigma factor.
2. DNA opens, forming open complex.
3. NTPs are laid down, without a primer
4. Additional NTPs are added, according to the template.
5. Sigma leaves as core enzyme moves down DNA.

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. The structure of the bases are given to the right.

The two tyrosine codons are UAU and UAC. These pair with the anticodon loop as follows:

3'-AUG 3'-AUG
5'-UAU 5'-UAC

Note the anti-parallel nature of the codon-anticodon interaction. The second codon pairs with perfect Watson-Crick basepairs. The first codon uses a wobble GU basepair, making two hydrogen bonds.



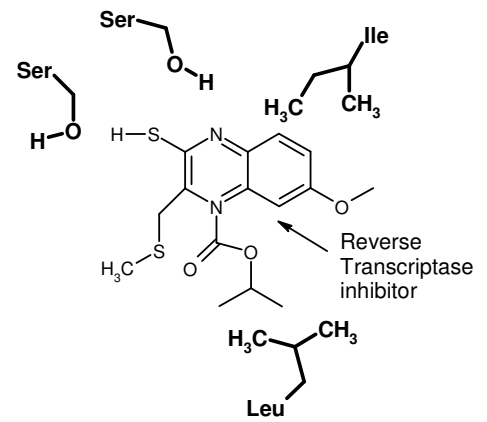
Choice D: Briefly discuss the role of the ribosome binding (Shine-Delgarno) site on the selection of the correct start codon during protein synthesis. Your answer should include a brief description of the structure of the ribosome and the initiation complex.

- The ribosome has 3 tRNA binding sites, one for the exiting uncharged tRNA (E), one that holds the tRNA-peptide (P) and one that hold the incoming charged tRNA (A)
- The ribosome binding site is a short segment in the mRNA about 6 bases from the start codon.
- When the ribosome binding site binds to the 30s subunit, the AUG codon is placed in the P-site by virtue of the distance between the RBS and the AUG codon.

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).

Reverse transcriptase is a polymerase that uses dNTPs as substrates to copy the viral RNA to DNA. Since the drug does not look like the substrate, and therefore does not bind to the active site - it is a mixed-type inhibitor.



- ii) Resistance to this drug is caused by a mutation where the leucine (Leu) is changed to glutamic acid (Glu = -CH₂-CH₂-COOH)
- a) Why do mutations arise frequently in the HIV virus? (1 pts).

The reverse transcriptase makes errors when it copies the viral RNA to DNA.

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

The leu interacts with favorably with the isopropyl sidechain of the drug by hydrophobic interactions. The Glu is a polar residue, thus the hydrophobic interaction would be lost.

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

Change the isopropyl group to a positively charged amine. The favorable electrostatic interaction would enhance binding.

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

Make an affinity column with the drug attached to the beads.

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 $1 \mu\text{M}$ ($[M_T]$). 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:** $\text{pK}_a = 2.0$ 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	10^{-9} M	10^{-8} M	10^{-8} M	10^{-7} M	10^{-7} M

(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).

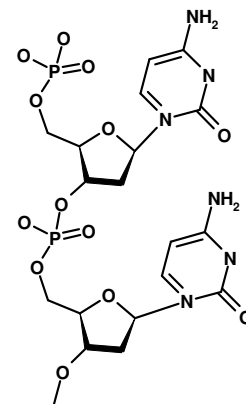
The K_D is the ligand concentration to give 1/2 fractional saturation, which would be $0.5 \mu\text{M}$.

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).

rC_6 at pH=7 and 1 mM salt, since it has the lowest K_D .

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).

- The pH dependence (column 1 versus column 2) indicates that a charged group on the protein is involved. It is likely a positive charge that is interacting with the neg charges on the phosphate. Base on the pH values, it is likely a Lysine residue, with a pK_a of ~ 9 . At $\text{pH}=7.0$, this group would have a positive charge (4 pts).
- The interaction with a phosphate is supported by the decrease in affinity at higher salt, suggesting an electrostatic interactions (2 pts).
- Although the protein binds to DNA as well, it has reduced affinity, suggesting a favourable interaction with the $2' \text{-OH}$ on the ribose (2 pts)

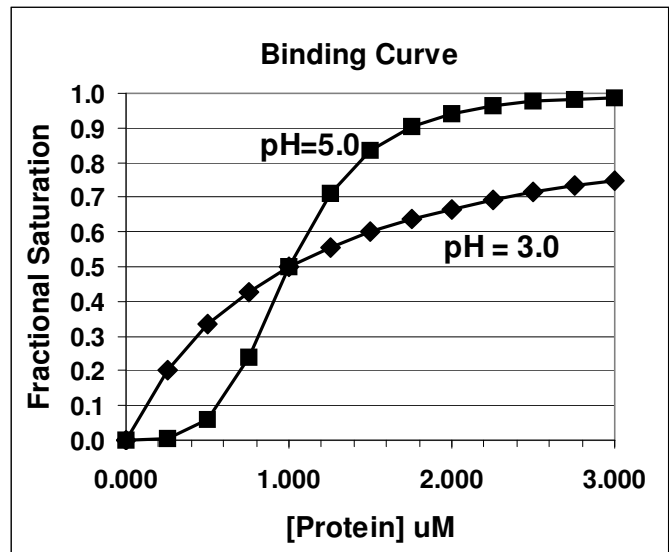


You can't say much about the base, since C was used in all cases (2 pts)

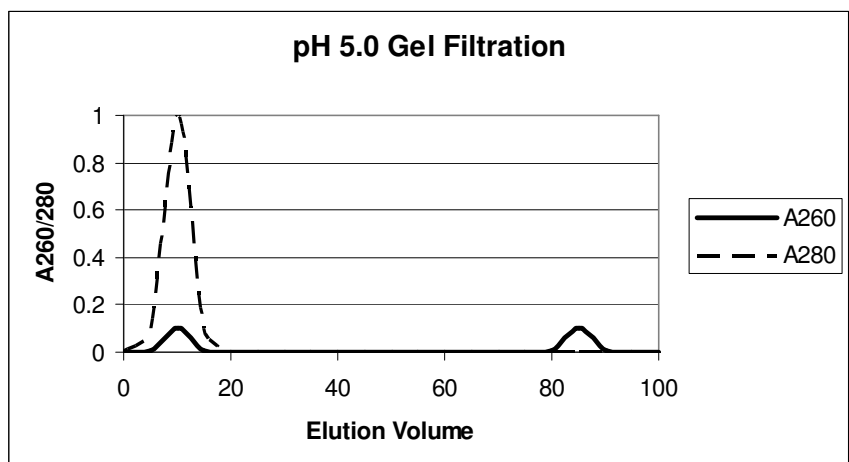
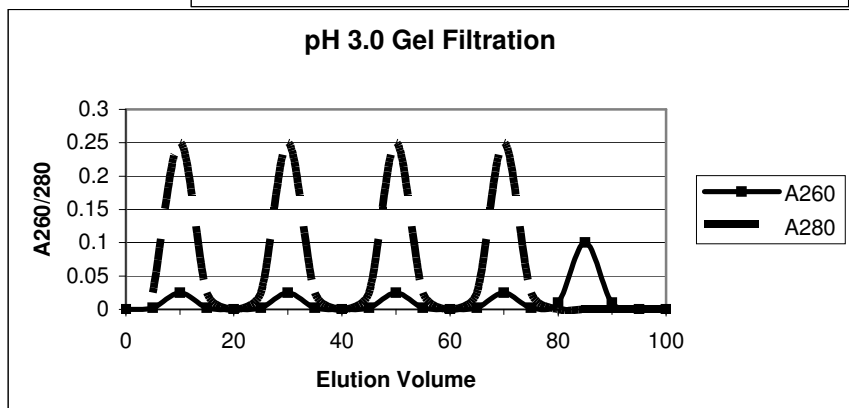
Choice B: The binding properties of a protein to a 100 basepair strand of DNA is measured. In this case the *protein* is considered the ligand (L) and the DNA is considered the macromolecule (M); more than one protein can bind to a single DNA molecule. The fractional saturation as a function of the protein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

The protein-DNA mixture, at a protein concentration of 1 μM , 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).



No, the average affinity is the same, the DNA is 1/2 saturated at the same protein concentration.



Question continues on next page:

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

- The binding at pH 3 appears to be non-cooperative, based on the shape of the curve.
- The binding at pH 5 appears to be positive in its cooperativity, based on the shape of the curve.

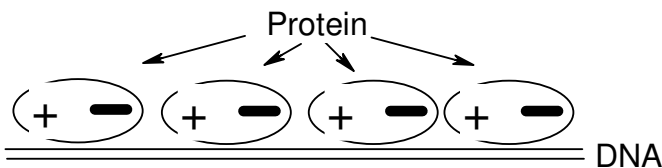
iii) Provide a simple model of the protein-DNA complex that would account for the binding and the gel filtration data at both pH values (8 pts).

The gel filtration data shows that a pH=3 DNA molecules with 1, 2, 3, or 4 proteins can be found. While at pH=5 the DNA has either no protein or four bound. Thus at pH=5 the protein binding shows infinite positive cooperativity with four proteins bound to one DNA.

Since the behavior of the binding changes between pH 3 and 5, the ionization of a carboxylic acid is likely the cause, with the cooperative behavior resulting from a negative charge.

There are several ways to explain the positive cooperativity.

- Binding of one protein somehow affects the structure of the DNA such that subsequent proteins bind with high affinity. It is difficult to imagine how the introduction of a negative charge on the protein could do this, but its possible due to interactions with the phosphate. You might expect this to change the binding affinity however.
- The protein has a positive residue that enhances the binding to already bound protein on the DNA, as shown to the right.



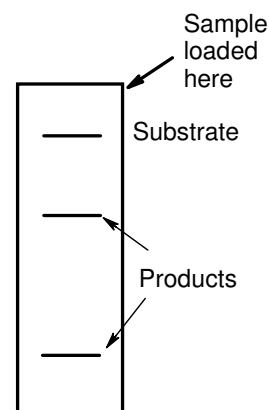
Choice C: Various concentrations of the following DNA molecule:

AACGTATGAGAAACAACGTTGACATGGAAGCTTGCGGCC
TTGCATACTCTTTGAAGCTTCTGTACCTTCGAACGCCGG

were treated with the restriction endonuclease E_zas3.14 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.*

See diagram. The substrate (intact DNA molecule) is larger than the two products.



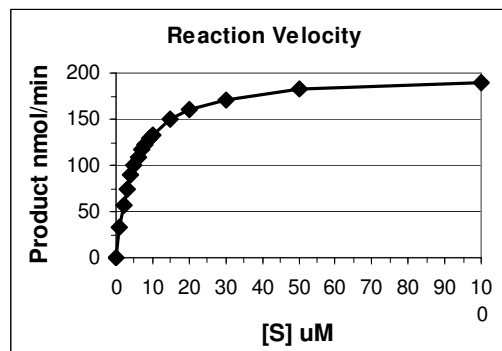
ii) What is the recognition sequence for E_zas3.14? *Briefly justify your answer.* (2 pts)

There are two sequences in the above DNA molecule that show two-fold symmetry in there sequence: AACGTT, near the middle, and AAGCTT towards the end. The latter is more likely because of the different sizes of the product bands.

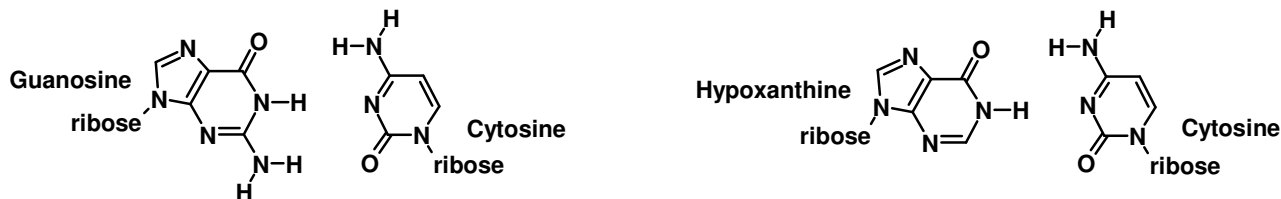
iii) Determine K_M and V_{MAX} for this substrate. Explain your approach. (6 pts)

$V_{max} = 200$ nmol/min - reaction velocity at high substrate.

$K_m = 5$ μ M, substrate concentration to give $v = V_{max}/2$.



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).



These two differ in the minor groove. Since most restriction enzyme bind in the major groove you would not expect to see much of a difference. Any difference would likely show up as K_M , since the same chemical cleavage would occur.

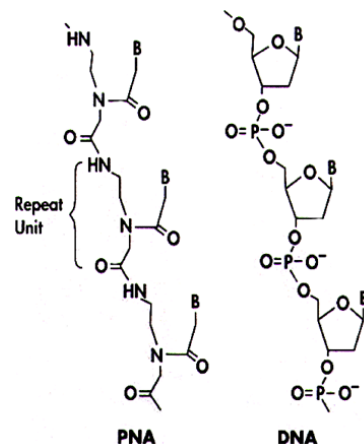
v) (5 pts) The substrate DNA can be modified by replacement of the phosphate-ribose groups in the DNA with a modified polypeptide chain to give a peptide-nucleic acid (PNA). The nucleotide bases (B) remain the same. The PNA could not be cleaved by the restriction endonuclease and is thus an inhibitor.

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

Competitive, since it looks like the substrate and will therefore bind at the active site.

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)

Just K_M , since high concentrations of DNA would displace all of the inhibitor.



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].

Both of these are chain-terminators, once incorporated into the growing polypeptide chain or nucleotide chain, another residue cannot be added. In the case of puromycin the next amino acid cannot break the amide linkage between the tyrosine and the tRNA (the linkage is usually an ester). In the case of AZT, there is no 3' hydroxyl for polymerase to add a base to.

