

This exam consists of 260 points on 11 pages. Use the space provided or the back of the preceding page.

1. (4 pts) Briefly explain how weak acids can act as pH buffers. Your answer should provide a clear explanation of the buffer region.

The buffer region is the range of pH values within one unit of the pKa of the weak acid (2 pts)

In this region the pH is resistant to change because the weak acid dissociates to neutralize added base or accepts a proton from added acid (2 pts).

2. (12 pts) Please do one of the following two questions. Please indicate your choice.

Choice A: Describe how you would make a one liter of a 0.1 M buffer solution of acetic acid at pH=5.0. You may assume that the pK_a of acetic acid is the same as the sidechain of aspartic acid. Make sure you state the pK_a value for acetic acid that you are using for this problem.

Choice B: A RNA binding protein binds to RNA solely via lysine-phosphate interactions. Sketch the dissociation constant, K_D, as a function of pH. Please state the pK_a value for the lysine sidechain that you are using for this problem.

Choice A:

Assume a pK_a of 4.0 (range from 3 to 5 is OK) (3 pts)

Calculate fraction protonated: $R=10^{\text{pH}-\text{pK}_a}$, $R=10^{5-4} = 10$.

$$f_{\text{HA}}=1/(1+R) = 1/11 \approx 0.1 \quad f_{\text{A}^-} \approx 0.9$$

Calculate total moles, A_T: 0.1M × 1L = 0.1 moles

Method 1: Mix 0.01 moles protonated acid (A_T × f_{HA}) and 0.09 moles of salt (A_T × f_{A⁻})

Method 2: Start with 0.1 moles of protonated acid, add 0.09 moles of base (sodium hydroxide)

Method 3: Start with 0.1 moles of the sodium salt, add 0.01 moles of acid (hydrochloric acid).

Choice B:

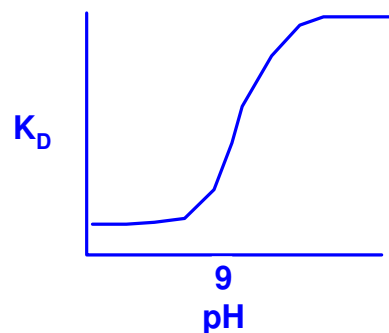
Assume a pK_a of 9.0 for the lysine side chain (range from 8 to 10 is OK) (3 pts)

The interaction will be strongest when the lysine is positively charged, at pH values below its pK_a. The K_D will be low.

The interaction will be weakest when the lysine is deprotonated, at pH values above its pK_a. The K_D will be high.

The midway point between these two extremes will be the pK_a.

Curve is shown to the right. (The level K_D at high pH indicates binding due to some other interaction, such as VdW.)



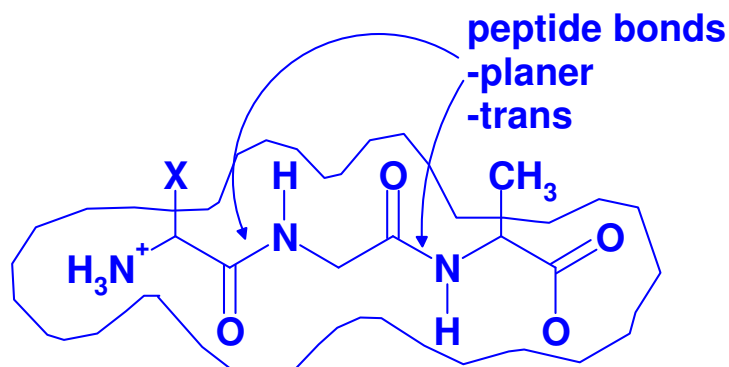
3. (14 pts)

i) Draw the structure of a tri-peptide whose sequence is X-Gly-Ala, where X represents your choice of a polar but non-ionizing amino acid (please do not use threonine). Assume a pH of 2.0 (6 pts).

ii) Please label all peptide bonds on your diagram and state two important features of this bond (2 pts).

iii) Circle the “mainchain” or “backbone” atoms in your drawing (2 pts).

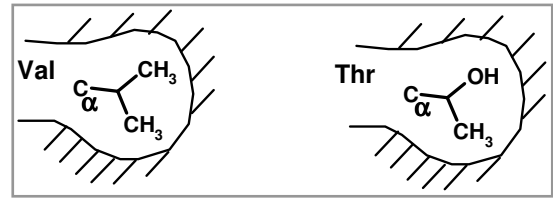
iv) Will this peptide bind to a cation exchange column at pH 2? Why or why not? (4 pts)



At pH 2.0 the amino terminus will be fully protonated (pK_a=9), the carboxy terminus will be $\frac{1}{2}$ protonated (assuming a pK_a=2). Therefore the net charge will be +1/2 and the peptide will bind because it is a cation.

4. (10 pts) Please do **one** of the following choices. Please indicate your choice.

Choice A: Describe how van der Waals forces and hydrogen bonds work together to stabilize protein secondary structures. You should illustrate your answer with a sketch of *one* secondary structure. In this sketch indicate the location of hydrogen bonds and sidechains.



Choice B: A globular soluble protein contains a *buried* valine (Val) residue in its core. If this residue is replaced by threonine (Thr) will the melting temperature increase or decrease? Why? You should consider discussing both enthalpic and entropic terms in your answer.

Choice A:

The conformation of secondary structures optimizes VdW interactions, any other conformations will cause repulsive vdw forces (High energy regions of Ramachandran plot) (3 pts)

The conformation is further restricted by geometries that allow mainchain hydrogen bonds. Only certain pairs of phi and psi angles position donors and acceptors in the right position for hydrogen bond formation. (3 pts)

Sketch of helix should indicate hydrogen bonds || to helix axis, and sidechains projecting outward. The sketch need not show all of the atoms - just the mainchain tracing, but NH and O=C should be indicated.

Sketch of a β -sheet should show hydrogen bonds \perp to strand direction, with sidechains alternating up and down. Either parallel or antiparallel are acceptable. (4 pts)

Choice B:

The melting temperature will decrease, indicating a less stable mutant protein (3 pts).

The principal reason is that the buried Thr residue does not have an H-bond donor/acceptor since it is in the hydrophobic core. Since this Thr would form an H-bond to water in the unfolded state, it will cost about 20 kJ/mole of instability since a hydrogen bond to water is broken during folding, but not reformed. (6 pts)

Additional factors (1 pt for either, unless the main reason was missed and these are discussed at length.):

The mutant protein will be stabilized by increased vdw interactions with the core - the -OH group has a permanent dipole. This will be a relatively small effect.

The mutant protein will be less stable from a hydrophobic effect, since the exposure of the more non-polar valine will order more water molecules. Again, this will be a relatively small effect.

5. (4 pts) Assume that a secondary structure is found on the surface of a soluble globular protein, with one side facing the interior of the protein and the other side solvent exposed. How frequent (e.g. every fifth residue) would you expect to find amino acids with non-polar sidechains? How does this periodicity depend on the secondary structure?

Non-polar amino acids would face the non-polar interior. (2 pts)

For an α -helix every 3.4 (e.g. 3 to 4th) residue would be non-polar and face the interior.

For a β -strand, every second residue would be non-polar and face the interior. (2 pts)

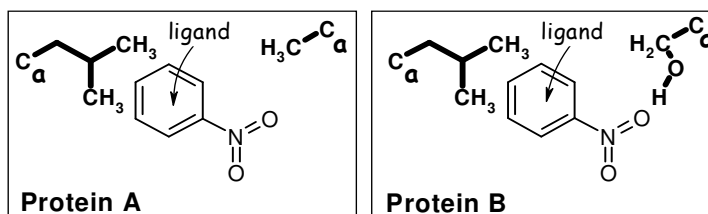
6. (16 pts) What thermodynamic feature of protein unfolding or DNA melting *greatly* destabilizes the folded state? Describe what force or thermodynamic feature is most important for opposing this force and stabilizing the folded protein and double stranded DNA. (Note, you should discuss **both** protein and double stranded DNA stability. The features causing destabilization and stabilization may, or may not be, the same for both.)

Both proteins and double stranded DNA are destabilized by conformational entropy (4 pts). The large number of possible conformations of the unfolded (single stranded) state increase the entropy of the system, which favors the unfolded state (4 pts).

Protein: The hydrophobic effect (2 pts), or the decrease in the entropy of the solvent (1 pt) is larger from proteins as they have a large non-polar core (1 pt).

DNA: van der Waals interactions between the faces of the bases stabilizes the double stranded for of the DNA (4 pt)

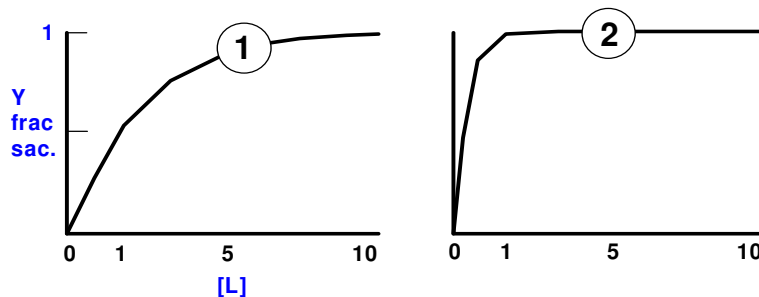
7. (15 pts) Two different homodimeric proteins bind the same ligand, nitrobenzene. The structure of the protein-ligand complex in one of the subunits on each protein is shown on the right. The two sidechains from the protein that contact the ligand are in bold.



- i) Label both the x- and y-axis of one binding curve. Give the correct scale for the y-axis (1 pt)

x-axis is free ligand

y-axis is fractional saturation.



- ii) Match the protein (A or B) to the correct binding curve (1 or 2). Justify your answer in terms of the molecular interactions between the protein and ligand (8 pts).

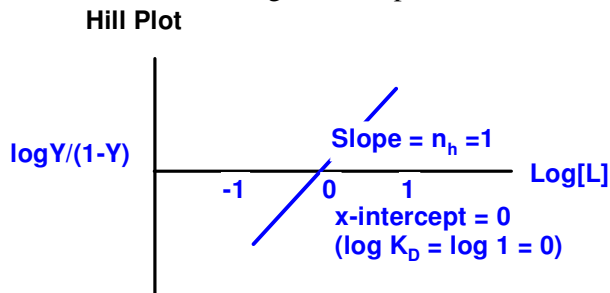
1=A: Curve 1 has the higher K_D (≈ 1) indicating fewer interactions with the ligand, Protein A lacks a hydrogen bond.

2=B: Curve 2 has the lower K_D (≈ 0.2) indicating more interactions with the ligand, Protein B has an additional hydrogen bond.

- iii) What does the shape of the binding curve suggest about the nature of the binding, is it cooperative or non-cooperative? Why? (2 pts)

Both curves are hyperbolic and not S-shaped, indicating that the binding is non-cooperative.

- iv) Based on your answer to part iii, sketch the Hill plot that you would expect to obtain from the data plotted in curve 1. Use the space to the right. Label the axis of this plot and indicate the important features of the plot (4 pts).



8. (12 pts) Describe the general features of an allosteric protein. Illustrate your answer using either oxygen transport, regulation of glycolysis or gluconeogenesis, or genetic regulation of protein expression, to demonstrate the importance of allosteric effects in biological systems.

- An allosteric protein is found in two different conformations, a tense (T) and an relaxed state (R) (2 pts)
- The R state is active, T state is inactive. (2 pt)
- R and T interconvert, with R state prevalent when an activator binds, T state when an inhibitor binds. (2 pts)

3 pts for example 3 pts for biological importance. Only one example necessary - e.g. PFK+F26P
Oxygen transport:

O₂ increases the affinity of hemoglobin (T to R), causing a higher affinity in the lungs, so that the hemoglobin becomes fully saturated with oxygen. As oxygen leaves, hemoglobin goes back to T state, enhancing the release of oxygen.

BPG, whose concentration is increased at high altitudes, stabilizes the T state of hemoglobin. The change in the shape of the binding curve enhances delivery when the oxygen concentration is low in the lungs.

Glycolysis - PFK:

F26P is an allosteric activator. High levels of this compound indicate high blood sugar, glycolysis is turned on.

ATP is an allosteric inhibitor. High levels of ATP turn off glycolysis since there is sufficient ATP.

AMP is an allosteric activator. High levels of AMP turn on glycolysis because ATP is needed.

Gluconeogenesis - fructose bisphosphatase

F26P is an allosteric inhibitor - turning off gluconeogenesis when there is plenty of glucose.

AMP is an allosteric inhibitor - turning off glyconeogenesis because there is no ATP to make glucose.

Gene Regulation - lac repressor. Lactose or IPTG is an allosteric inhibitor, converting the lac repressor protein from a high DNA affinity (R) state to a low DNA affinity T state, this causes the production of enzymes to digest lactose.

9. (15 pts) What features of the active site of an enzyme leads to an increase in the rate of the chemical reaction *and* specificity towards substrates. Illustrate your answer by providing details for one enzyme that was discussed in this course.

Rate enhancement: Pre-organization of functional groups on amino acid sidechains lowers the energy of the transition state because there is no decrease in entropy when going from the ES complex to the transition state. (4 pts). Increasing the concentration of the transition state increases the rate of the reaction. (3 pts)

Examples (2 pts):

- catalytic triad in serine proteases Ser, His, Asp
- Catalytic dyad in HIV protease Asp, Asp

It is OK to use different enzymes for each part of the question.

Substrate specificity: Functional groups on amino acid sidechains interact more so with preferred substrate so that it binds more tightly (3 pts)

Examples (3 pts)

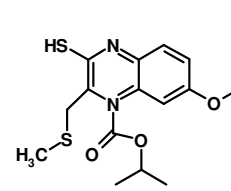
- Trypsin cleaves after Lys/Arg due to neg charge (Asp) in specificity pocket.
- Chymotrypsin cleaves after Phe/Trp/Tyr due to large non-polar specificity pocket.
- HIV protease cleaves after Phe due to interaction with Val82
- Restriction endonucleases recognize specific base sequences by h-bonding in major groove.

10. (14 pts) The following two compounds are currently used to treat AIDS patients. Both of these drugs inhibit the *same* enzyme. This enzyme is used by the virus in one of the first steps in its life-cycle.

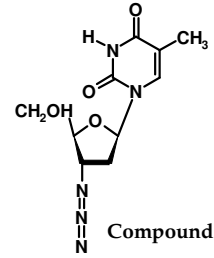
i) Which is the name (or general nature) of the enzyme that is inhibited and which compound is more likely to be a competitive inhibitor and which is more likely to be the mixed-type inhibitor? Briefly justify your answer (6 pts).

ii) Sketch the double reciprocal curves ($1/v_i$ versus $1/[S]$) that you would expect to find for data acquired without inhibitor (curve 1) and data acquired in the presence of the mixed-type inhibitor (curve 2). Briefly justify your answer (6 pts).

iii) How would your plot (curve 2) change if you doubled the inhibitor concentration? (2 pts).



Compound I

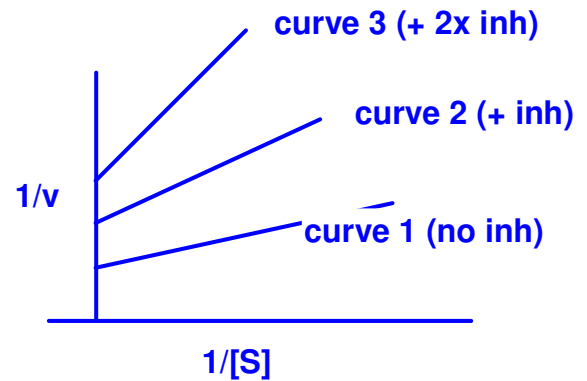


Compound II

i) Compound II is a nucleotide analog - 3'OH is replaced by an Azide. This is likely a competitive inhibitor of HIV reverse transcriptase (a polymerase). Therefore compound I is likely a mixed-type inhibitor (6 pts).

ii) The mixed-type inhibitor will show changes in the slope and the y-intercept. The y-intercept will be higher (lower velocity). (6 pts).

iii) The slope and intercepts are affected in the same way: $\alpha=1+[I]/K_i$. You cannot answer this question quantitatively unless you know $[I]$ and K_i , but both will certainly increase. (2 pts).



11. (6 pts) Please do one of the following choices. Clearly indicate your choice.

Choice A: Compare and contrast any **one** of the following pairs of methods of protein purification by column chromatography methods. What is similar about the methods? How do they differ? What is the principle of separation? How is the protein eluted from the column?

a) Cation versus anion exchange chromatography.

Both bind charged proteins.

Bound proteins are eluted by changes in pH or salt.

Cation binds + charged proteins, anion - charged proteins.

b) Affinity versus ion exchange chromatography.

Affinity binds by specific ligand-protein interaction, eluted by free ligand.

Ion exchange by charge, eluted by changes in pH or salt.

c) Gel filtration versus affinity chromatography.

Gel filtration separates by size, proteins are eluted by flow.

Affinity binds by specific ligand-protein interaction, eluted by free ligand.

Choice B: Compare and contrast SDS-PAGE (SDS polyacrylamide gel electrophoresis) of proteins versus agarose gel electrophoresis of DNA. How are they similar? In what way do they differ?

Both separate molecules by size using electrophoresis through a gel. (4 pts)

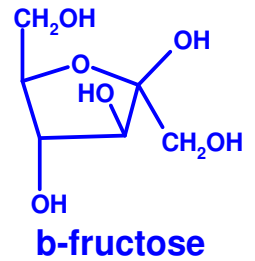
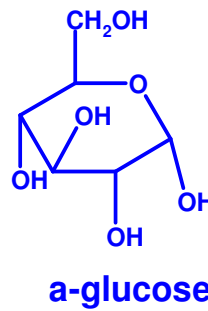
SDS-PAGE it is necessary to denature the proteins with SDS to give them a negative charge. (2 pts)

Choice C: Define specific activity and briefly discuss its importance in protein purification.

Specific activity is the ratio of the activity of the target protein/total mass of all proteins in the sample.

It should increase during purification because the total mass of all proteins decreases as impurities are removed.

12. (8 pts) Draw the ring form (Haworth representation) of either α -glucose or β -fructose. Be sure to indicate your choice. (+4 pts) Which of these two monosaccharides is found in both glycogen and cellulose? How does the linkage between the monosaccharides differ in glycogen versus cellulose?



Glucose is found in glycogen and cellulose. (2 pts)

In glycogen the linkages are $\alpha(1-4)$ and $\alpha(1-6)$ (2 pts)

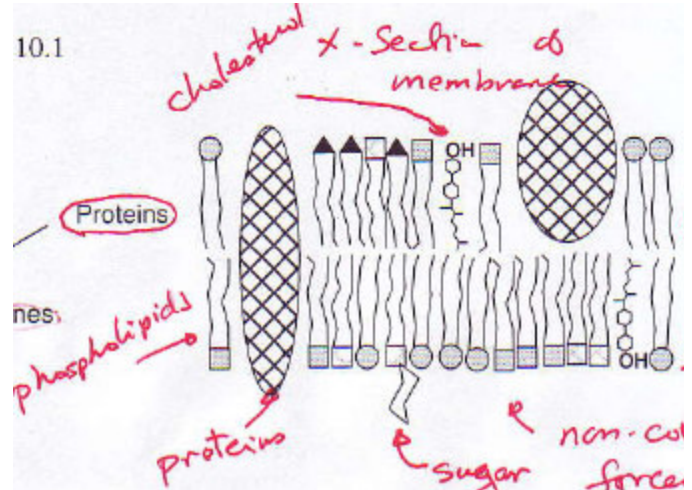
In cellulose the linkages are $\beta(1-4)$

13. (20 pts)

i) Draw a labeled "cartoon diagram" of a biological membrane, including typical components (4 pts)

Diagram should contain:

- Lipids
- Integral membrane proteins
- Cholesterol



ii) Briefly describe (or draw) the chemical features of **one** of the components in your membrane (4 pts).

Lipids have a polar head group (phosphate) and non-polar acyl chains. (4 pts)

Membrane proteins have a non-polar exterior, usually polar interior, either α -helix or β -barrel. (4 pts)

iii) What thermodynamic force or interaction causes the self-assembly of the membrane? Briefly describe the molecular basis of this force (6 pts).

The hydrophobic effect (2 pts)

Release of ordered water molecules from non-polar groups when the membrane forms. (4 pts)

iv) Which features of the electron transport chain would require that the membrane be fluid and not solid? How might an organism maintain a fluid membrane, even if it grows at low temperatures? (6 pts).

Coenzyme Q - the lipid soluble electron carrier must move between complexes - only possible in a fluid membrane (2 pts).

An organism could either (4 pts)

- increase the cholesterol content (membrane plasticizer)
- decrease the acyl chain length of the phospholipids.
- increase the number of cis double bonds.

14. (10 pts) Compare and contrast the standard energy change (ΔG°) to the Gibbs free energy change (ΔG) (5 pts) and then answer **one** of the following choices (5 pts).

The standard energy is the difference in the chemical potential of products and reactants under standard state. It is also the energy required to convert one mole of reactants to one mole of products (4 pts)

$$\Delta G^\circ = \mu_{\text{Products}}^0 - \mu_{\text{Reactants}}^0$$

The Gibbs free energy is the "potential" energy of the system. It is the amount of energy that can be released as a system goes from a non-equilibrium position to an equilibrium position. It is related to the standard energy as follows (4 pts)

$$\Delta G = \Delta G^\circ + RT \ln \frac{[\text{Products}]}{[\text{Reactants}]}$$

Choice A: The addition of a phosphate to a sugar (e.g. conversion of glucose to glucose-6-P) is unfavorable, with a standard energy change, ΔG° of +15 kJ/mol, yet reactions of this type in glycolysis occur spontaneously. How is this accomplished?

The energy released by the hydrolysis of ATP (30 kJ/mole) is used to reduce ΔG° from +15 to -15, making ΔG negative. [Direct coupling] (2 pts)

Choice B: The aldolase reaction in glycolysis converts fructose 1-6 biphosphate to two three-carbon sugars. This reaction is unfavorable, with a ΔG° of +30 kJ/mol, yet this reaction occurs spontaneously in glycolysis. How is this accomplished?

The concentration of the products are kept far below their equilibrium concentration due to a favorable downstream reaction (PEP \rightarrow Pyr). $\Delta G < 0$ because the second term in the formula for ΔG becomes more negative than the first. [Indirect coupling]. (2 pts)

15. (12 pts) Please do one of the following choices.

Choice A: Assume that you just ate a bagel, which is rich in carbohydrates. Trace the flow of carbon from glucose to CO_2 **and** the flow of energy, from glucose to ATP, in the metabolism of the bagel in muscle cells. You should list and briefly describe the pathways involved. How would your answer change if your muscles were operating under anaerobic (no oxygen) conditions?

Carbon flow:	Energy flow
Glycolysis: Glucose \rightarrow Pyruvate (+1 pt) TCA cycle: Pyruvate \rightarrow CO_2 (+1 pt)	Glycolysis: ATP and NADH produced from organic oxidations. (+2 pts) TCA: NADH, FADH_2 ATP and NADH produced from organic oxidations. (+2 pts)
Anaerobic conditions (+2 pts): No O_2 , therefore: Carbon flow: to pyr only. Energy flow: only ATP from glycolysis.	Electron transport: Oxidation of NADH & FADH_2 produce a hydrogen ion gradient across the membrane. (+2 pts) ATP synthase: Energy in proton gradient used to make ATP. (+2 pts)

Choice B: I've just eaten a high carbohydrate meal and shortly afterward I get involved in an intense game of touch football. Describe changes in hormones, glucose-glycogen metabolism in the liver, and the regulation of glycolysis and gluconeogenesis in the liver that would occur, beginning *immediately* after the meal to the end of the football game, when I'm *completely* exhausted.

	Initial state (just after meal)	Transition (started)	Ending state (exhausted)
1 pt each	Insulin released (high blood sugar)	Glucagon (low blood sugar)	Glucagon (low blood sugar)
1 pt each	Glucose stored in glycogen	Glucose released from glycogen	No glycogen - (energy from fat)
2 pts each.	F26P high \rightarrow PFK in glycolysis until liver meets ATP needs.	F26P levels fall. Glucose made from gluconeogenesis since ATP levels are high (AMP low)	F26P still low, no glucose from gluconeogenesis since AMP levels are high.

16. (12 pts) The diagram to the right shows two nucleotides that represent a dinucleotide.

i) Connect the two nucleotides correctly, as you would find in DNA or RNA.

A phosphodiester bond would link the 3' and 5' OH of the ribose.

ii) Indicate the end that would accept additional bases during a DNA polymerase reaction.

The 3' OH

iii) Is this dinucleotide DNA or RNA? Why?

RNA, since the ribose has an -OH on the 2' position.

iv) Indicate an N-glycosidic bond.

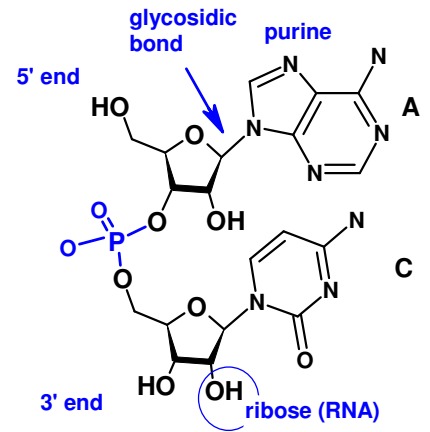
Between the sugar and the base.

v) Indicate the purine base.

A - two heterocyclic rings.

vi) What is the correct sequence of this dinucleotide, AC or CA? Justify your answer.

AC - naming convention is to begin at the 5' end.



17. (20 pts) The restriction endonuclease CmuI recognizes and cleaves a six base sequence after the first base. The *incorrect* recognition sequence for the enzyme is shown on the right.

i) Correct the error in the sequence and briefly justify your answer (Note: There may be more than one way to correct the error.) (4 pts).

Because restriction endonucleases are homodimers they must recognize the same sequence on both strands. Two possible correct sequences are shown on the right.

ii) Show the products that would occur when this DNA is treated with CmuI (2 pts).

iii) What enzyme could be used to rejoin the fragments? Which molecular interaction would serve to hold the two fragments together during the joining process? (4 pts)

DNA ligase would be used.

The fragments would be held together by hydrogen bonds.

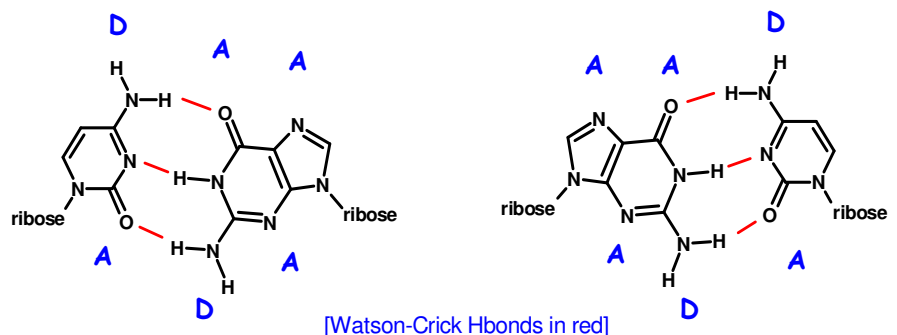
iv) The rate of cleavage of DNA by CmuI is insensitive to salt. Based on this observation, the enzyme interacts with which component of the DNA, the bases, ribose, or phosphate? Justify your answer (4 pts).

Only electrostatic interactions would be sensitive to salt, therefore the enzyme is NOT interacting with phosphate groups.

v) CmuI can distinguish between a GC and a CG basepair at the second position. To do so does it bind in the major groove or the minor groove? Briefly justify your answer with reference to the C-G and a G-C basepairs shown to the right (6 pts).

As with most restriction enzymes

- major groove binding is required to distinguish the two bases since the pattern of hydrogen bond donors (D) and acceptors (A) are different in the major groove and the same in the minor groove.



18. (8 pts) Please do one of the following choices. Please indicate your choice.

Choice A: In what way does double stranded RNA differ from double stranded DNA? In what way are they similar? Why is RNA more susceptible to cleavage under alkaline conditions?

Similarities:

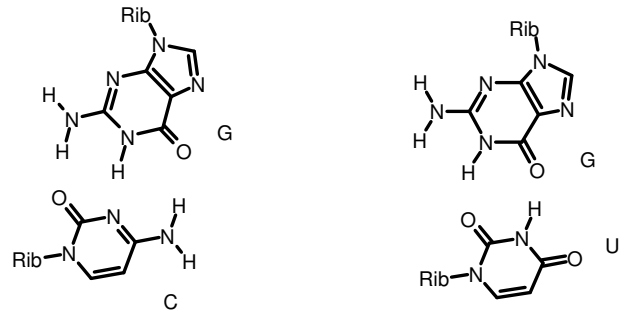
- Antiparallel chains (2 pts)
- Watson-Crick Hbonds between bases (1 pt)
- Bases in the center, ribose & phosphate on the outside. (2 pts)

Differences (1 pt)

- DNA: major and minor grooves
- RNA: deep and shallow grooves.

Base hydrolysis: RNA's 2'OH is activated to a nucleophile by base, attacking the phosphodiester linkage, causing cleavage (+2 pts).

Choice B: A number of amino acids are associated with more than one codon. For example, the amino acid Phe can be incorporated into a peptide chain whether the codon is UUU or UUC, yet there is only one tRNA molecule that is charged with Phe. Briefly explain how this occurs. The following bases may be helpful.



The codon-anticodon pairings are

Anti-codon 3'-AAG 3'-AAG

Codon: 5'-UUU 5'-UUC

Important to note that same anticodon recognizes both codons (+4 pts)

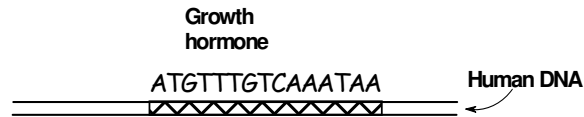
In the first case a G:U wobble basepair, with two hydrogen bonds is formed for the 3rd base of the codon (right hand basepair).(+2pts)

In the second case all three pairing are standard Watson Crick.(+2 pts)

19. (35 pts) The diagram below (next page) shows a segment of human DNA with the gene for a small, four amino acid, human growth hormone indicated as a stippled box. The start codon (ATG) and stop codon (TAA) are indicated. A diagram of an expression vector is also provided. The vector has a *single* BamHI site, whose recognition sequence is G[^]GATCC. You desire to produce recombinant human growth hormone that will remain inside the bacteria after induction of transcription. Please answer the following questions.

i) There are five numbers on the expression vector. The table below gives the names of three items. Please complete the missing entries (label, or position on the vector, and the function) for **all** three (7 pts).

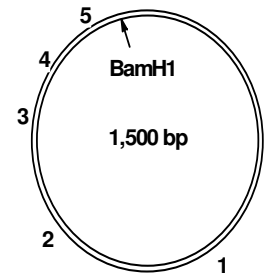
Label (1pt)	Name	Function (2 pts each)
4	Lac operator	On/off switch for mRNA production. The lac repressor binds at this site, turning off transcription.
5	Ribosome binding site	Sequence on mRNA that causes mRNA to bind to the 30s subunit on the ribosome.
3	Promoter	Site where RNA polymerase binds to initiate the synthesis of mRNA.



- ii) Briefly describe how you would generate PCR primers to amplify the DNA segment that codes for the growth hormone. You should include the start and stop codons in your final product because these are not present in the expression vector. Your primers should contain only 6 bases of the growth hormone sequence (8 pts).

+4 pts for correct sequence of growth hormone gene.

+4 pts for correct addition of BamHI sites.



The left primer would be the sequence of the top strand, beginning with ATG, with a BamHI site at its 5' end.

The right primer would be the sequence of the lower strand, beginning with TTA, with a BamHI site at its 5' end as well

The actual primer sequences (also an acceptable answer):

Left primer: **GGATCCATGTTA**

Right primer: **GGATCCTTATTT**

Original Sequence:

5' **ATGTTTGTCAAATAA**

3' **TACAAACAGTTTATT**

PCR Product:

5' **GGATCCATGTTTGTCAAATAAGGATCC**

3' **CCTAGGTACAAACAGTTTATTCCTAGG**

- iii) How would you verify that you inserted the PCR fragment correctly into the vector, without sequencing the DNA? (4 pts)

Cutting the DNA with Bam HI should produce two fragments, one that is 1500 basepairs in length, plus a short fragment (15 bases) that was inserted.

- iv) Briefly describe how you would induce the production of the growth hormone after placing (transforming) the vector into the bacteria. What compound would you use (4 pts)?

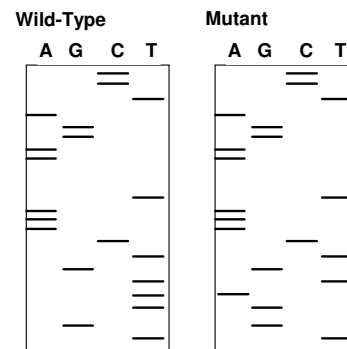
The addition of lactose or IPTG would cause the lac repressor to be released from the lac operator DNA sequence.

- v) How would you modify the expression vector to *either* cause export of the protein out of the cell *or* to purify the protein using affinity chromatography (2 pts).

Export: Add codons for the leader peptide after the initial AUG codon.

Affinity chromatography: Add codons for six histidine residues either after the AUG or before the stop codon.

vi) (10 pts) Certain individuals show slow growth due to a mutation in the growth hormone gene. You obtain DNA from one such individual, and amplify the growth hormone gene using your PCR primers. After inserting the mutant growth hormone gene into the vector you obtain the nucleotide sequence of both the normal (wild-type) and mutant gene. The sequencing gels are shown on the right.



- What DNA sequence would make a suitable primer in this case?
- What nucleotide change(s) have occurred in the mutant individual?
- How have these changes affected the amino acid sequence of the growth hormone?
- Why would this change affect the binding of the hormone to its receptor in the membrane?

a) The Primer must be to the 5' end (upstream) to the region that would be sequenced. A possible primer would be **GGATCC**. This sequence is would be common to both. [Note: This is actually too short and would anneal to two places on the vector (there are two Bam sites in the complete expression vector. In practice it would be necessary to include some additional bases to the 5' end)] (1 pt)

b) The sequences of both DNAs are listed below with the change indicated. (3 pts)

Protein: MetPheValLysEND
 Wild-type: TG**TT**TGTCAAATAAGGATCC
 Mutant: TG**GA**TGTCAAATAAGGATCC
 MetAspValLysEnd

- Before these changes can be interpreted correct, the reading frame must be established. The AUG is the start codon, so TTT must be the next codon, i.e. the A from the AUG codon is not seen on this gel. The amino acid changes are shown above: the second residue has been changed from Phe to Asp. (4 pts, 3 pts for wrong reading frame, but otherwise correct interpretation of codons.)
 - The phenylalanine in the original hormone has been changed to Asp. The original large non-polar group has been replaced by a small charged group. This should lead to a reduction in affinity; presumably the receptor has a complementary non-polar pocket for the Phe, which does not interact favorably with the Asp in the altered hormone. (2 pts)
20. (12 pts) Briefly describe the events that occur in *either* DNA transcription (mRNA synthesis) *or* protein synthesis. Your answer should include a description of the initiation events, how polymerization occurs, and termination. Feel free to use a well labeled diagram.

mRNA synthesis:

Initiation (3 pts):

- RNA polymerase binds to the promoter via sigma factor.
- DNA opens, forming open complex.
- NTPs are laid down, without a primer

Elongation (6 pts):

- Additional NTPs are added, according to the template, no error checking.
- Sigma leaves as core enzyme moves down DNA.

Termination (3 pts): Termination signal causes rho factor to bind to mRNA, when rho reaches RNAP, complex dissociates.

Protein Synthesis :

Initiation (3 pts): mRNA, 30S and 50S subunit + tRNA-fMet in the P site.

Elongation (6 pts):

- 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)
- tRNA-met is in the P-site.
- next tRNA-AA binds to the A-site
- amino group of AA in A-site attacks peptide-tRNA ester, transferring growing chain to tRNA in the A-site.
- 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.

Termination (3 pts): Stop codon recruits protein releasing factor to the A-site. Causes hydrolysis of peptide from peptide-tRNA bound in the P site.