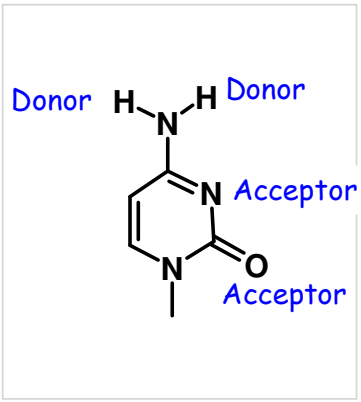
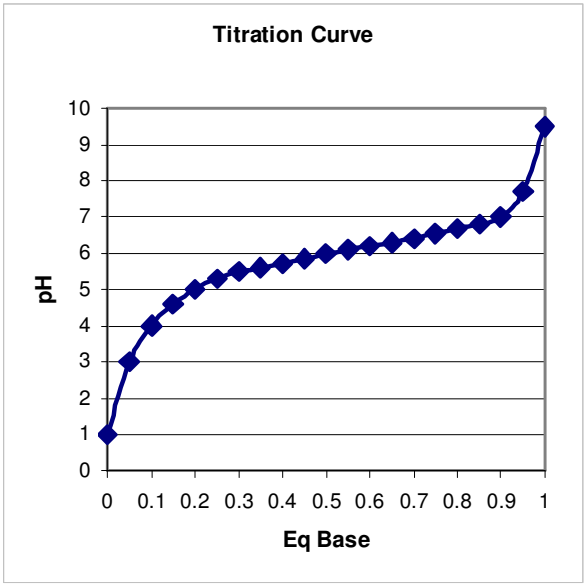
This exam has **14 pages** and is out of **300 points**. On questions with choices all of your attempts will be graded and you will receive the best grade. Use the space provided, or the back of the preceding page.



1. (6 pts) Provide a definition for a hydrogen bond. Identify and label a hydrogen bond donor and a hydrogen bond acceptor on the nucleobase shown on the right.

A hydrogen bond is formed when a hydrogen on an electron negative atom interacts with another electronegative atom, i.e. X-H Y (4 pts)

2. (15 pts) The diagram to the right is a titration curve of the side-chain of an amino acid. Please answer the following questions:



i) What is the pK_a for this ionizable group (2 pts)?

pKa is 6.0. This is the pH at the ½ equivalent point, also the inflection point of the curve.

ii) What pH range could this amino acid be used as a buffer? (2 pts)

pH ranges from 5 to 7 (in general pH ranges +/- from the pKa)

iii) How does the side-chain buffer the solution if a strong acid or a strong base were added (5 pts)?

If strong acid is added, it absorbs protons, i.e.

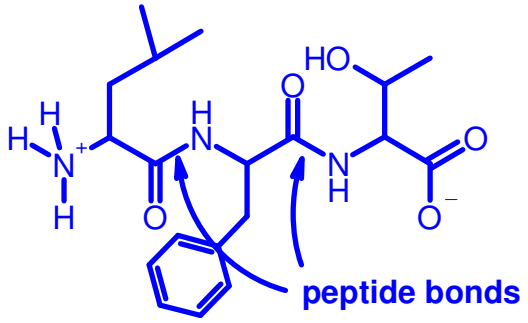
A- + H+ → HA

If strong base is added, it releases protons to neutralize the base, i.e. AH +OH- → A- + H_2O

iv) Approximately how many equivalents of a strong base would be required to make a buffer solution at pH=7.0, assuming you would be starting with the fully protonated compound (consider only ionization of the sidechain) (6 pts).

A total of 0.9 equivalents of base will be required, this will bring the pH to 7.0, as shown on the titration curve.

3. (25 pts) Draw a tri-peptide using any three non-ionizable amino acids (except Ser, Ile, and Val), one of which absorbs UV light. Assume pH=7.0 for your diagram. (6 pts for diagram).



Amino terminus should be protonated (+1/2 pt)

Carboxy terminus should be deprotonated. (+1/2 pt)

i) Give the primary structure (sequence of your peptide) (3 pts)

Leu – Phe - Thr

ii) label one peptide bond and briefly discuss how its features differ from other non-peptide bonds in the peptide (6 pts).

The peptide bond is planer because of orbital overlap (partial double bond)

The peptide bond is trans since this reduces unfavorable van der Waals interactions.

iii) What type of ion exchange column could you use to separate this peptide from a mixture of other peptides, assuming a pH = 2.0. Briefly justify your answer (6 pts).

At pH 2.0 this peptide will have a charge of +1/2, since the carboxyl group will be ½ protonated (3 pts)

Therefore the peptide will bind to a cation exchange column (2 pts), which has negatively charged beads (1 pt)

iv) How would you determine the concentration of the peptide after purification (2 pts)?

Using Beer's law: $A=c\varepsilon l$, the aromatic amino acid will absorb UV light (Trp would give the largest absorbance, then Tyrosine, then Phenylalanine)

v) What is "specific activity" and how would it differ before and after purification of your peptide (2 pts).

Specific activity is the amount of the peptide/total protein.

It should increase after the purification.

4. (12 pts) Describe or sketch one secondary structure that is commonly found in membrane proteins. What features of the amino acid sequence would you expect to observe in a membrane protein if it adopted the secondary structure you selected? What role do hydrogen bonds play in this structure? +6 pts for structural overview, +3 for Hbonds, +3 for periodicity of sidechains.

α-helix:

Right handed helix with hydrogen bonds parallel to the direction of the helix.

It is important that all hydrogen bonds are made since there are no donors or acceptors in the membrane.

Side chains project out, non-polar sidechains will contact non-polar acyl groups in the lipid. If the face of the helix faces the lipid, every 3rd or 4th residue will be non-polar.

β-barrel.

β-strands adjacent to each other, forming a barrel that satisfies all mainchain hydrogen bonds, this is important since there are no donors or acceptors in the membrane.

The outside of the barrel will be non-polar, meaning every 2nd residue in the strand will be non-polar.

5. (12 pts) Briefly describe the hydrophobic effect and discuss its role in **two** of the following:

a) Structure of globular proteins.

b) Membrane stability

c) Stability of double stranded DNA.

The hydrophobic effect is a change in the ordering of water molecules due to interaction with non-polar groups. Non-polar groups order water molecules, decreasing their entropy (6 pts)

(3 pts each)

a) Globular proteins have a non-polar core, when these residues become buried they release their ordered water molecules, increasing the entropy of the systems, favoring the folded state.

b) The membrane has a non-polar core. When this forms, any ordered water molecules surrounding the non-polar acyl chains are released, increasing the entropy of the systems, favoring the bilayer.

c) Since the bases are mostly polar, and very little area is buried with ds DNA forms, there is little influence of the hydrophobic effect on DNA structure.

6. (8 pts) Briefly describe conformational entropy and discuss its role in **either** protein folding **or** the formation of double stranded (ds) DNA.

Conformation entropy is the entropy of the protein or DNA polymer due to the number of conformations: S=RlnW. (4 pts)

In the case of proteins or DNA, the conformational entropy is low in the folded (protein) or double stranded (DNA) state, lowering the entropy. (4 pts)

7. (10 pts) Briefly describe the role of van der Waals interaction in the **both** of the following and indicate the relative importance of this interaction in stabilizing one structure over another.
   a) Folding of globular proteins
   b) Formation of double stranded DNA.

Van der Waals forces are optimized in the well packed core of proteins (4 pts)

Van der Waals forces between the basepairs stabilize dsDNA (4 pts)

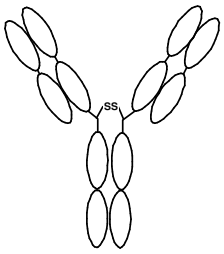
They are more important in DNA (2 pts)

8. (6 pts) What other significant interaction, besides the hydrophobic effect, conformational entropy, or van der Waals, plays an important role in the stability of double stranded DNA, but not protein stability. Why is this interaction more important for DNA?

Electrostatic interactions (not H-bonds, these are also important in stabilizing the folded protein). The phosphate groups on DNA (and RNA) are negatively charged and close to each other when dsDNA forms, giving a large repulsive energy.

9. (10 pts) Please do one of the following two choices.

Choice A: The quaternary structure of the immunoglobulin was determined by simple techniques 20 years before the X-rays structure of an immunoglobulin was determined. What techniques would you use to determine the quaternary structure? Briefly describe the techniques, the data you would obtain, and how you would use this data to substantiate the structure shown on the right.



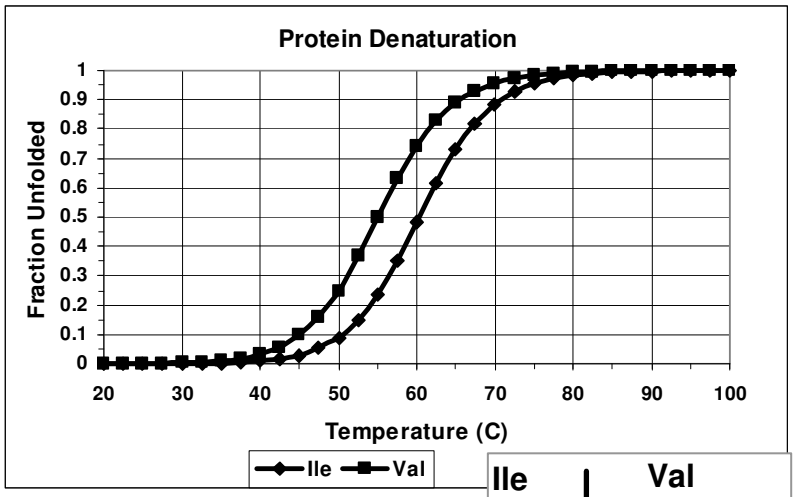
The overall native molecular weight would be obtained by Gel filtration, this would be the sum of the four chains (2 ½ pts)

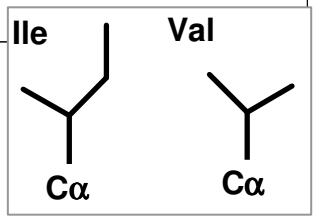
The molecular weight of the individual chains would be obtained by SDS-PAGE in the presence of a compound that breaks disulfide bond (BME) showing that there was a light chain and a heavy chains. (2 ½ pts)

The location of disulfide bonds would be determined by SDS-PAGE in the absence of BME (2 ½ pts)

There would have to be two light and two heavy chains to account for the overall molecular weight. (2 ½ pts)

Choice B: The denaturation curves for a wild type protein and a mutant protein with Ile replaced by Val are shown to the right. The thermodynamic parameters for unfolding of the wild-type protein are: ΔH^o = +200 kJ/mol, ΔS^o= +600 J/mol-deg.





i) Assuming that ΔS^o of unfolding is the same for both the wild-type and the Valine containing protein, calculate the ΔH^0 for the unfolding for the Val mutant [Hint: No van't Hoff analysis is required, $\Delta H^o = T_M \Delta S^o$].

ii) Compare your ΔH^o value with the value obtained for the wild-type protein. Briefly discuss, with reference to the structure of the wild-type and Val containing protein, possible reasons for this difference in ΔH^o.

i) The melting temperature of the mutant (Val) is about 53 C = 326 K.
The enthalpy is 326 x 600 = 195.6 kJ/mol. (+5 pts)

ii) The lower entropy is due to a reduction in van der Waals interactions, the Val sidechain is smaller and interacts with the non-polar core via van der Waals by a lesser extent. (+5 pts)

10. (12 pts) Allosteric effects play a predominate role in many aspects of biochemistry. Briefly define the term allosteric, and discuss the role of allosteric effects in any **one** of the following.

a) Oxygen transport.
b) Regulation of glycolysis
c) Regulation of glycogen levels.
d) Regulation of mRNA synthesis.

1. Allosteric effects refer to the fact that a protein/enzyme has two structures.
2. One structure is "active" (R-state) the other structure is "inactive" (T-state).
3. The two states are in equilibrium with each other, so the system can be switched on (R-state) or off (T-state) by changing the equilibrium

(2 pts each item = 6 pts, 6 pts for one of the following examples).

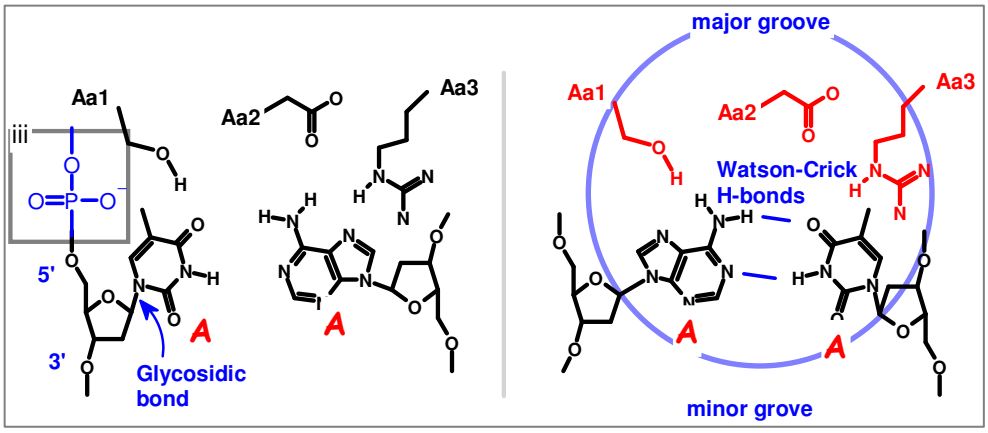
a) oxygen transport: O2 affects its own binding to Hemoglobin – positive cooperativity, as more binds the affinity increases (Kd decreases).
Hb becomes fully saturated in lungs since affinity is high.
As O2 is lost on the way to the tissues, affinity drops and more oxygen is released than normal. (Could also discuss BPG regulation as well).

b) glycolysis: F26P is an allosteric activator. When blood glucose levels are high, F26P is high, turning on glycolysis-allowing the cell to make ATP if it needs too (could also discuss ATP/AMP+ADP)

c) Glycogen levels are controlled by enzyme phosphorylation which switches enzymes from T to R state or R to T state. e.g. Phosphorylation of glycogen phosphorylase when blood glucose levels are low activates it (R state) and inhibits glycogen synthase (T state).

d) The lac repressor protein binds to DNA in the R state, binding of lactose or IPTG causes it to switch to the T state – and leave the DNA (allowing RNA polymerase to begin making mRNA).

11. (21 pts) A protein binds to a specific sequence of double stranded nucleic acid. Part of the interaction between the protein and the nucleic acid is shown on the left side of the diagram. The amino acid sidechains from the protein are labeled Aa1, Aa2, Aa3. The reversal of the two bases is shown on the right part of the diagram; this part of the diagram will be helpful for parts *viii* and *ix*.



i) Label the 5′ and 3′ carbons of left-most base (2 pt).

ii) Identify the glycosidic bond on the left-most base (1 pt).

iii) Place the appropriate missing atoms in the box labeled "iii" that would be required to connect this residue to the previous residue (2 pts).

iv) Indicate the "Watson-Crick" hydrogen bonds on the right-most base pair (2 pts).

v) Is this protein binding to DNA or RNA? Why? (3 pts).

DNA because: The 2′ is deoxy (and the pyrimidine is a T)

vi) Is this protein binding in the major groove or the minor groove? How did you determine this? (2 pts).

Major groove, I drew a circle through the C1′ on the ribose and the large arc is the major groove.

vii) What is the principle energetic interaction between the protein and the DNA, based on this diagram? (1 pt)

Hydrogen bonding.

viii) How would the binding affinity change if the protein bound to the reversed basepair (shown on the right)? You should assume that the structure of the protein does not change. (4 pts)

The binding will become weaker since the hydrogen bonds can't form properly. For example Aa1 donates a hydrogen bond to the TA pair on the left, but faces a hydrogen bond donor in the right AT basepair.

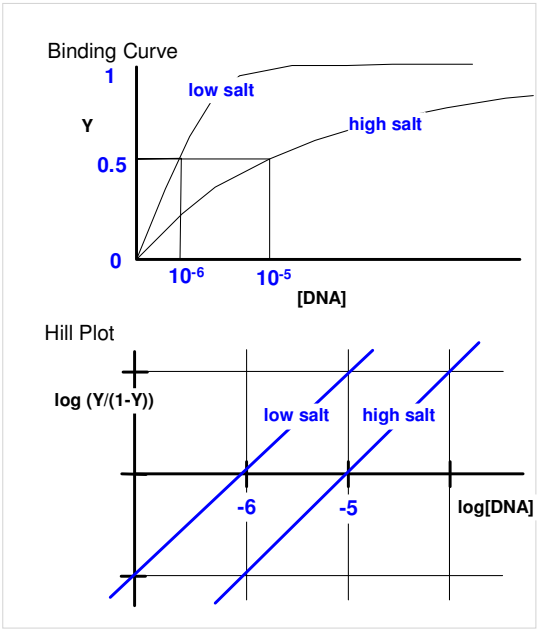
ix) Would your answer to part viii change if the protein used a similar type of interaction in the other groove? (4 pts)

If it bound in the minor groove it would have to present two hydrogen bond donors to the two accepts in the minor groove (labeled with red As). The same pattern of acceptors is in the minor groove, so it could bind equally well to both.

12. (19 pts) A protein binds to DNA and the binding is sensitive to the concentration of NaCl in solution.

i) What functional groups are likely to be involved on both the protein and the DNA? (5 pts)

Positively charged residues (3 pts), such as Lysine or Arginine (1 pt) are interacting with the negatively charged phosphate (1 pt) on the DNA.



ii) Sketch, in the space to the right, the binding curve and the Hill plot that you would expect to observe if you measured binding at low salt and at high salt. Assume that the K_D at low salt is 10^{-6} M and the K_D is changed by a factor of 10 at high salt (i.e. 10 fold lower or 10 fold higher). Be sure to justify your answer, in particular whether you feel the K_D is increased or decreased at the higher salt concentration (5 pts).

Adding salt would reduce the electrostatic interaction, lowering the affinity, and thus the Kd would increase to 10^{-5} M.

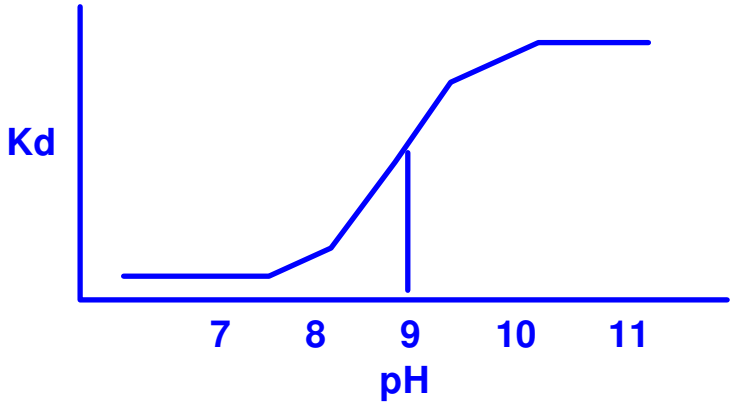
iii) What assumption did you make about the type of binding when you sketched these curves? (2 pts).

The hyperbolic curves in the binding curve, and the slope of 1 in the Hill plot indicate non-cooperative binding

iv) Which kinetic parameter, the on-rate or the off-rate, would you expect to change as the salt concentration is changed? Why? (3 pts).

The off rate will increase more than the on-rate (2 pts) since it is more sensitive to interactions between the DNA and protein. The on-rate reflects the rate of collisions between the protein and DNA (1pt)

v) Based on your answer to part *i*, how would you expect pH to affect the binding? You can answer this question with either a discussion or a sketch of K_D versus pH. Be sure to explain the major features of your sketch (4 pts).



Since the amino acid from the protein is ionizable and has a + charge, when it is deprotonated the electrostatic interaction will disappear. For Lysine the ½ point will be pH=9, and for Arginine, pH~11, the pKa values of the sidechains.

The Kd will be low for pH values < pKa (group is protonated)

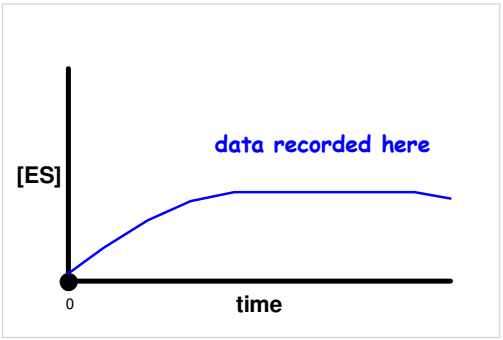
The Kd will be higher for pH values > pKa (group will be deprotonated)

13. (8 pts) Please do **one** of the following three choices.

Choice A: Proteases (e.g. serine protease, HIV protease) and the Potassium channel both increase the rate of a reaction. What are the common features of the mechanism of rate enhancement? How do the enzymes differ?

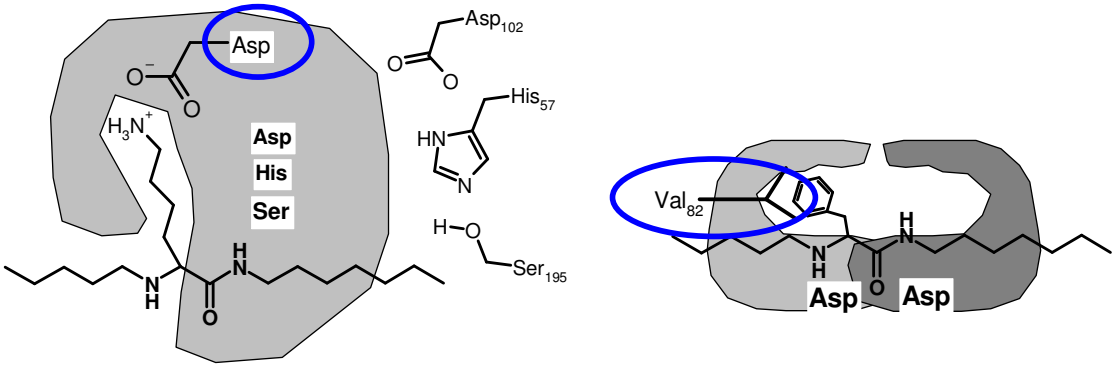
Choice B: Sketch a graph of [ES] versus time, beginning with t=0 when the substrate is mixed with the enzyme. Which time period on your sketch would measurements of product formation allow you to represent your data using the formula to the right.

$$v = \frac{[E_t]k_{CAT}[S]}{K_M + [S]}$$



Choice C: The diagram below shows a "cartoon" diagram of either trypsin (left) or HIV protease (right). You can choose either enzyme to answer the following questions.

i) Which residue would be altered in chymotrypsin (left) or altered in HIV protease mutations



(right) that affect drug binding . Circle the residue and briefly justify your answer (2 pts).

ii) Select one residue that is responsible for catalytic activity and describe how replacement of that residue with glycine (i.e. removal of the sidechain) would affect the catalytic mechanism (4 pts)

iii) Which steady-state kinetic parameter would be altered due to the change you made in the protein in part ii? Briefly justify your answer (2 pts).

Choice A:

Both lower the activation energy of the transition state, thus increasing the concentration of the transition state and therefore the rate of the reaction (+4 pts)

Serine proteases do so by pre-ordering the catalytic residues, thus there is no entropy loss in reaching the transition state. (+2 pts)

K channel provides a completely different mechanism, i.e. the ion goes through the channel, not the membrane. (+2 pts)

Choice B: The curve starts at zero, and then reaches a steady-state level, as substrate is consumed the steady-state levels drop. (+6 pts)The data should be measured when [ES] is not changing, as indicated in the plot above. (+2 pts)

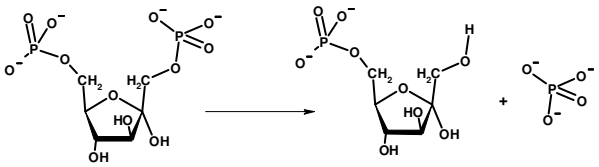
Choice C:

i) The upper Asp would be come non-polar in chymotrypsin, Val 82 would be altered in protease mutants. (2pts)

ii) Trypsin: Ser – no nucleophile, His, Asp – no activation of nucleophil. HIV Prot. Asp – no activation of water as the nucleophile. (3 pts)

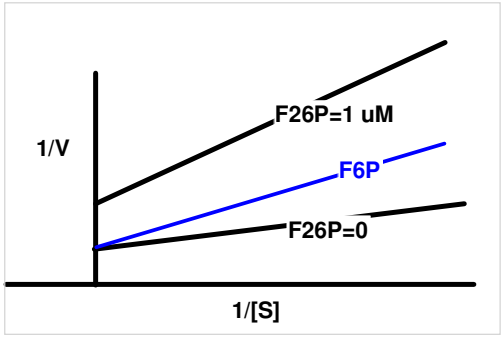
iii) These changes, since the affect the ability of the enzyme to do chemistry, would affect k_{CAT}. (3 pts)

14. (18 pts) The enzyme kinetics of fructose 1,6 bisphosphatase were measured in the presence of different levels of F26P and these data are present on the right. The reaction catalyzed is also shown on the right.



i) Is F26P acting as a competitive or mixed type inhibitor? Briefly justify your answer (5 pts).

The double reciprocal plot shows that it is a mixed type inhibitor since Vmax is different.



ii)Based on your answer to ii, where on the enzyme is the F26P binding (active site or elsewhere?) (2 pts).

A mixed type inhibitor binds elsewhere.

iii) Briefly describe how you would obtain the K_I (and if appropriate, K_I') values from these data? (4 pts).

K_I and K_I' are obtained from the degree of inhibition, α and α': $K_I = [I]/(\alpha-1)$, where [I] is the inhibitor concentration.

α is the ratio of the slopes.

α' is the ratio of the y-intercepts.

iv) Sketch, on the graph, the effect you would expect to observe if the reaction was performed in the presence of high levels of Fructose-6-phosphate. Justify your answer, using the back of the previous page if necessary. (4 pts).

Fructose-6-phosphate is the **product** of the reaction. It would bind to the active site and act as a competitive inhibitor.

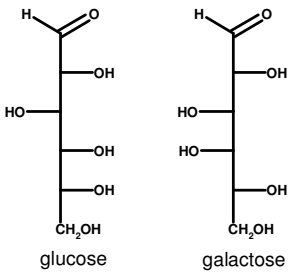
Therefore the double reciprocal plot would have the same y-intercept, but a larger slope.

v) What regulatory method would best describe how the enzyme is regulated by fructose-6-phosphate? (3 pts)

Product inhibition.

15. (10 pts) Please do **one** of the following choices.

Choice A: The structure of glucose and galactose are shown on the right.



i) Are these sugars aldoses or a ketoses? (2 pts)

ii) Draw β-glucose in its pyranose form using the reduced Haworth representation. (2 pts)

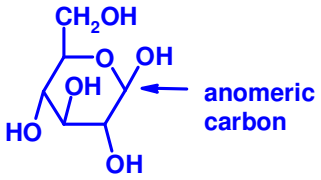
iii) Indicate the location of the new chiral centers on the pyranose ring of glucose, what is this new center called? (2 pts)

iv) Sketch, or describe, any **one** of the following carbohydrates (4 pts)

a) lactose
b) sucrose
c) glycogen
d) cellulose

i) aldoses (+2 pts)

ii) Diagram +1, β pointing up +1



iii) the new chiral center is the anomeric carbon(+2 pts)

iv)

lactose: β(1-4) linkage between galactose and glucose – ok to omit β

sucrose: (1-2) linkage between glucose(α) and fructose(β) – ok to omit α, β

Glycogen: α(1-4) and α(1-6) linked glucose.

Cellulose: β(1-4) linked glucose.

Choice B: In what way is a biological membrane similar to a membrane composed of only phospholipids, i.e. what is a general description of their structure? In what way do they differ? List the additional components that are found in a biological membrane and briefly state their role in the function of the membrane.

General Similarities: Polar head groups on outside of **bilayer**. Two acyl chains form non-polar core (+5 pts)

Differences: Biological membranes have proteins (transport, enzyme, signaling +3 pts), cholesterol (maintain fluidity) + 2 pts.

16. (12 pts) Please do **one** of the following questions.

Choice A: Pretend it's next Sunday and you just finished the Pittsburgh marathon. As a consequence, your glycogen levels and ATP levels in the liver are quite low. Discuss the process, with the major focus on regulation in your answer, by which your glycogen levels and ATP levels are restored as you eat lots of carbohydrates after the race.

Choice B: Pretend it's next Sunday and you just finished the Pittsburgh marathon by sprinting the last ½ mile. As a consequence of low oxygen in your leg muscles considerable lactic acid was produced. Why was the lactic acid produced in the muscle? After it leaves the muscle, where does it go? When it gets there, discuss the process by which it is converted back to glucose.

Choice A:

Lots of carbs → high blood glucose.

Insulin released → enzymes become dephosphorylated. (+3 pts)

Glycogen synthase is activated, glucose stored in glycogen. (+3 pts)

F26P levels become high(+3 pts)

PFK in glycolysis becomes active, regenerating ATP. (+3 pts)

Choice B:

Pyruvate converted to lactate to replenish NAD+ for glycolysis under low oxygen conditions (6 pts).

Lactate goes to liver (2 pts)

When oxygen is plentiful (after you stop running), lactate in liver is returned to Pyruvate.

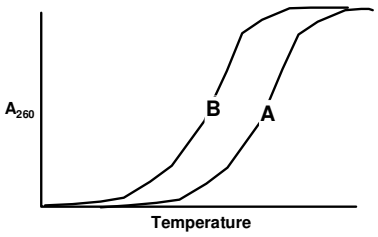
Pyruvate synthesized to glucose by gluconeogenesis.(4 pts)

17. (8 pts) The plot on the right show two melting curves, labeled A and B. What kind of difference in **either** the DNA **or** the solution conditions would cause the melting temperature to shift from A to B. Briefly justify your choice.

Curve B has the lower melting temperature, reduction could be due to:

a) An increase in the number of AT bases, two hydrogen bonds instead of three, and weaker vdw interactions lower Tm

b) The salt concentration has decreased, increasing the repulsion between the phosphates on each strand, destabilizing the double stranded DNA.



18. (10 pts) The following DNA sequence: GCGAATTCCCG
CGCTTAAGGGC

was treated with the restriction endonuclease EcoR1 (G^AATTC).

i) Draw the expected products of the reaction (4 pts)

GC**GAATTC**CCG → GC**G** **AATTC**CCG
CG**CTTAAG**GGC CG**CTTAA** **G**GGC

ii) Explain why the recognition sequence is the same on both the upper and lower strand (6 pts).

The same sequence on the top and bottom represent two fold symmetry in the DNA. Restriction endonucleases are homodimers which have two fold symmetry. Thus they recognized DNA sequences with two fold symmetry. (6 pts)

19.(12 pts) Please do **one** of the following choices.

Choice A: Describe the basic reaction mechanism for a typical DNA polymerase. Discuss why the Gibbs energy for the overall reaction is negative and also comment on the fidelity of the reaction, or why the polymerase is more likely to incorporate the correct base (Note: do not discuss removal of an incorrect base, see following question).

Choice B: Describe the mechanism by which tRNA molecules are charged and then briefly discuss how the same tRNA can be used to translate more than one codon. The diagram to the right may be helpful.

Choice A:

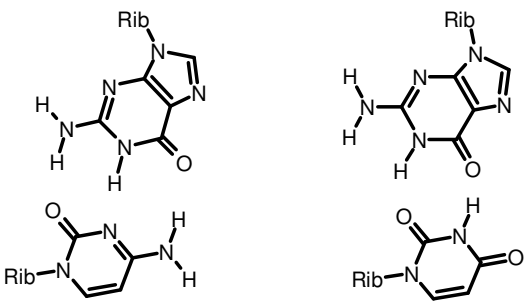
A template with an annealed primer with a 3'-OH is required (+ 3pts)

dNTP are added to the 3'-OH releasing pyrophosphate. (+3 pts)

The pyrophosphate is hydrolyzed to inorganic phosphate, this reaction has a large negative Gibbs energy, which drive the addition. (+3 pts)

The fidelity is based on correct Watson-Crick base pairs, as well as the pairing of a purine to a pyrimidine is the right size to allow rapid incorporation into the DNA (kinetic editing)(+3 pts)

Choice B:



ATP reacts with the amino acid to form AMP-phosphate ester to amino acid. (+4 pts)

AMP-amino acid reacts with tRNA, transferring the amino acid to the 3' OH of the tRNA. (+4 pts)

The three bases in the codon pairs with three bases in the anticodon loop of the tRNA. The first two pairings are standard Watson Crick (AT, GC). The third base of the codon can pair with one tRNA using Watson-Crick, but can also form a wobble base pair, which is shown in the right part of the diagram. (+4 pts)

20. (8 pts) Please do **one** of the following choices.

Choice A: Discuss the mechanism by which some DNA polymerases remove incorrectly incorporated bases. What is the consequence of lack of this function in a polymerase and how does it affect the treatment of HIV?

Choice B: Discuss how editing occurs during the charging of tRNAs by certain amino acids, such as Ile and Val.

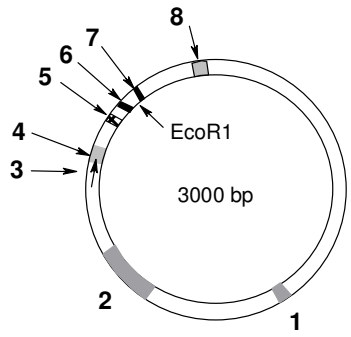
Choice A:

The mis-matched base is removed by hydrolysis due to a 3'-5' exonuclease activity.
A polymerase that lacks this function will have a high error rate. (+4 pts)
The HIV reverse transcriptase lacks this function, leading to mutations in the virus, leading to resistance to clinical drugs. (+4 pts)

Choice B:

The aminoacyl tRNA synthetase has two sites that recognize the amino acid, the activating site and the editing site (+4 pts for "editing site")
Both the large (Ile) and small (Val) amino acid can fit into the site that activates the amino acid by reacting it with ATP to form AA-AMP.
Only the smaller (incorrect) one can fit into the editing site, where the AMP is removed and free AA is released before it can be added to the tRNA. (+4 pts)

21. (12 pts) The diagram to the right shows an expression vector. The numbers refer to essential features that are contained in most expression vectors. Please do both parts of this question.



i) Complete the following table. The start and stop codons have been done for you as an example (6 pts for each correct labels & involvement, +2 pts each for correct descriptions.)

Number	Name & Role	Involved in mRNA synthesis (Yes/No)	Involved in protein synthesis (Yes/No)
1	Origin of replication – ensures that the plasmid is replicated in the cell.	NO	NO
2	Antibiotic resistance gene – produces a protein that makes the bacterial resistant to an antibiotic. Growth of the bacterial in the presence of the antibiotic ensures that the bacterial will keep the plasmid.	NO	NO
3	Promoter – RNA polymerse binds here to begin mRNA synthesis.	YES	NO
4	Lac operator sequence – the lac repressor protein binds here preventing mRNA synthesis. Addition of IPTG turns on mRNA synthesis by causing the lac repressor to leave the DNA.	YES	NO
5	Ribosome binding site:	NO	YES
6	Start codon:	No	Yes
7	Stop codon:	No	Yes
8	mRNA termination. Site at which RNA polymerase finishes mRNA synthesis.	YES	NO

ii) Pick any **three** of the above features, except 5, 6, and 7, and discuss their role in either maintenance of the plasmid in the cell, or the expression of protein (6 pts)

22. (10 pts) The following DNA contains a protein coding sequence that you would like to express in bacteria. The sequence, along with the protein translation, is given in bold:

```
          |-----------500 bases-----------|
AGCTGCTCATGCTCCCCACA......GTGAGGGGGAAATTAACCGCCGGCG
TCGACGAGTACGAGGGGTGT......CACTCCCCCTTTAATTGGCGGCCGC
          MetLeuProThr......ValArgGlyLysStop
```

The expression vector (complete diagram shown in the previous question) contains a single EcoR1 site between the start and stop codons that are contained in the vector, i.e. the vector sequence is:

```
--TTAGTAGGGCACCTCAATGGAATTCTTAA---,
```

consequently it is not necessary to amplify the start and stop codons from the DNA, just the sequence that codes for the remaining amino acids (LeuPro-----GlyLys).

Give the sequence of the left and right primers that would generate the desired PCR product. Make the total length of your primers 12 bases, i.e. don't worry about adjusting the length to generate the correct T_M. Write the sequence of the final PCR product.

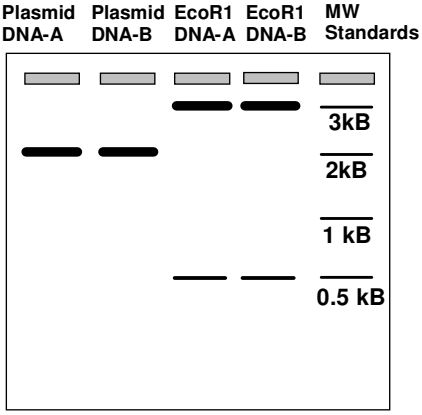
The left primer is; 5'-GAATTCCTCCCC [EcoR1 site + 6 bases of the top strand]

The right primer is: 5'-GAATTCTTTCCC [EcoR1 site + 6 bases of the bottom strand]

```
GAATTCCTCCCCACA......GTGAGGGGGAAAGAATTC
CTTAAGGAGGGGTGT......CACTCCCCCTTTCTTAAG
```

+3 pts each for correct primers, +4 points for PCR product.

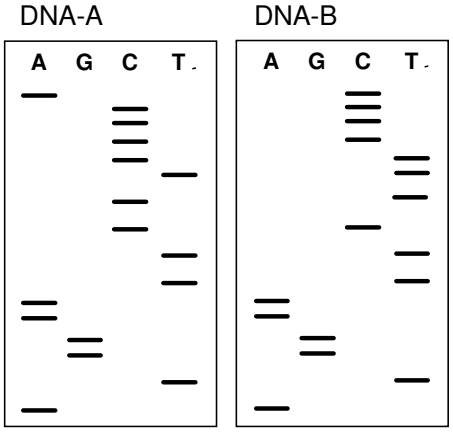
23. (10 pts) After cutting the PCR product and the vector, and ligating the mixture, you isolate two different plasmids. The undigested and digested DNAs are shown on the gel on the right, along with molecular weight standards. Plasmid A and plasmid B are identical based on this gel. The lower portion of the DNA sequencing gel of both of these plasmids, using the primer GGGCACCTCA, is also shown on the right.



i) Briefly explain why the migration distance of the uncut plasmid is different (faster) than the fragment generated after cutting the plasmid (4 pts).

The uncut plasmid is supercoiled and is smaller and migrates faster.

ii) Plasmid A generates the required protein after induction with IPTG, but plasmid B does not. Why? Use the back of the previous page to answer this question (6 pts).



The sequence gel begins at the start codon: ATG...

Plasmid A shows the predicted DNA sequence of the PCR product after insertion into the expression vector.

```
TTAGTAGGGCACCTCAATGGAATTCCTCCCCACA..GTGAGGGGGAAAGAATTCTTAA
AATCATCCCGTGGAGTTACCTTAAGGAGGGGTGT..CACTCCCCCTTTCTTAAGAATT.
```

Plasmid B contains the PCR product inserted backwards, which has the following sequence.

```
TTAGTAGGGCACCTCAATGGAATTCTTTCCCCCTCAC..TGTGGGGAGGAATTCTTAA
AATCATCCCGTGGAGTTACCTTAAGAAAGGGGGAGTG..ACACCCCTCCTTAAGAATT
```

The resultant mRNA would code for a different amino acid sequence.

24. (6 pts) Briefly explain how the different DNA molecules in the "A" lane in the above sequencing gels were generated.

The following template and primer were incubated with dNTPs and a small amount of ddATP, which acts as a chain terminator when incorporated when there is a T on the template. For example, the first band on the gel is the DNA fragment after the addition of a ddA.

```
Primer              5'GGGCACCTCAA-3'H
Template:  3'- AATCATCCCGTGGAGTTACCTTAAGGAGGGGTGT..CACTCCCCCTTTCTTAAGAATT.
```

The next A band occurs when the next T is reached on the template:

```
Primer              5'GGGCACCTCAATGGA-3'H
Template:  3'- AATCATCCCGTGGAGTTACCTTAAGGAGGGGTGT..CACTCCCCCTTTCTTAAGAATT.
```

25. (8 pts) Please do **one** of the following choices.

Choice A: How would you modify the protein produced in the previous question to cause it to be exported out of the cell? How does this modification lead to export of the protein?

Choice B: How would you modify the protein produced in the previous question to facilitate purification by affinity chromatography? What type of chromatography resin would you use?

Choice A:

Add a sequence of amino acids at the amino terminus of the protein that would code for the leader peptide.

When the leader peptide emerges from the ribosome the peptide binds to the export machinery which will push the peptide out of the cell, cleaving the leader sequence off.

Choice B:

Add 6 histidine residue on the end of the protein.

The protein can be purified with affinity chromatography with Ni ions attached to the resin (beads).

26. (12 pts) Discuss the roles of the ribosome binding site, the start codon, and the stop codon on the overall process of protein synthesis. (+3 pts for each)

Ribosome binding site on the mRNA binds to ribosomal RNA (rRNA) in the 30s subunit.

Start codon binds a tRNA that is charged with formyl-Methionine, the first amino acid in the protein.

Stop codon binds protein releasing factor that hydrolyzes the ester between the completed protein chain and the last tRNA.

Bonus questions (2 pts each).

1. In what way is the ribosome like an apple?
   Similar shape, stem is peptide emerging from exit tunnel.
2. In what way is a peppermint patty like IPTG?
   Can be used to cause lac repressor students to release the DNA (lac operator) that they are holding on to.