Instructions: This exam contains 250 points in 31 questions on 12 pages. Please use the space provided, or the back of the previous page if necessary. On questions with choices, all of your attempts will be graded and you will receive the best grade for that question.

1. (5 pts) Please do **one** of the following choices:

Choice A: Why are weak acids buffers within one pH unit of their pKa?

Choice B: Briefly describe how to make a buffer solution, given that the pH of the solution should be equal to the pKa of a monoprotic buffer.

Choice A: If base is added then the weak acid dissociates to neutralize the base. If acid is added, the ionized weak acid becomes protonated. (4 pts for one, 5 points for two)

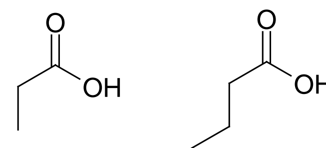
Choice B: Since the pH = pKa the fraction protonated = fraction deprotonated =0.5. (3 pts). There are three ways to make the buffer.

a) mix equal moles of the acid and its salt.

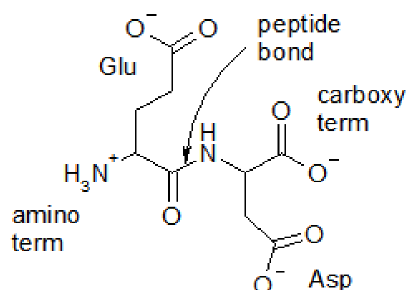
b) start with the fully protonated acid and add 0.5 eq of NaOH

c) start with the fully deprotonated acid and add 0.5 eq of HCl.

2. (14 pts) The dipeptide, Glu-Asp, is a substrate for a protease. The sidechains of Asp and Glu are shown on the right. *(Part iii of this question is on the next page.)*

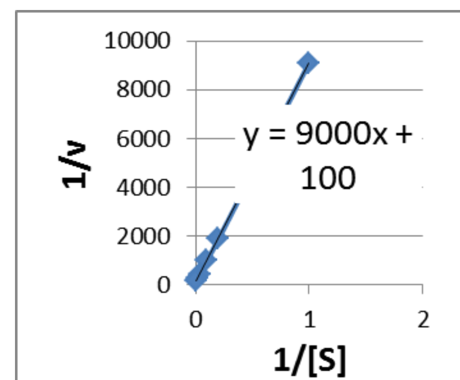


i) (6 pts) Draw the substrate (i.e. Glu-Asp) and label the following features on your diagram: a) amino terminus, b) carboxy terminus, c) peptide bond.



+3 for correct structure, +3 for labels.

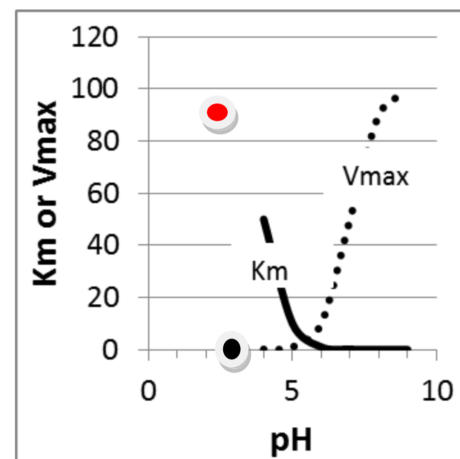
ii) (4 pts) The activity of the enzyme against the above substrate was measured at different pH values and the steady-state enzyme kinetic data obtained at pH 3 is are shown on the right. Obtain the Km and Vmax values at pH 3 and plot these values on the graph that shows the effect of pH on Km and Vmax. Please show how you obtained these values.



Vmax: 1/y-intercept = 1/100 = 0.01 (black dot)

Slope= Km/Vmax = 9000

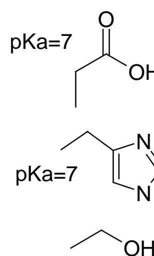
Km = 9000 x Vmax = 90 (red dot)

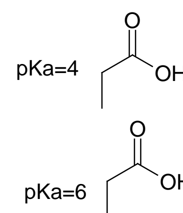


iii) Please do **one** of the following two choices: (4 pts)

Choice A: Based on the dependence of Km/Vmax on pH, what functional groups on the enzyme are most likely to be involved in **binding** of this substrate? Why?

Choice B: The active site residues in serine and aspartate proteases are shown on the right. Is the pH dependence of the Vmax consistent with the mechanism of either a serine protease (e.g. trypsin) or an aspartyl protease (e.g. HIV protease).





Choice A; Since the Km increased at lower pH, the binding is getting weaker due to protonation of a group on either the substrate or the enzyme. The substrate contains two acidic sidechains, and when these get protonated they will become neutral. Therefore the enzyme probably has positively charged resides in its active site.

Choice B: The Vmax curve shows that the reaction proceeds more efficiently when the groups are deprotonated, with a pKa of the functional group = 7. This is consistent with the serine proteases, in which the His and Asp should be deprotonated to activate the serine.

3. (5 pts) The peptide bond is restricted to two conformations, cis and trans, with the trans the preferred lower energy state. Why does it have these properties?

It is in the planer cis or trans conformation because the nitrogen hybridizes to sp2. This allows its pz orbital to participate in the delocalized electrons in the C-O pi bond. Only two conformations of the peptide bond would allow the overlap of the orbitals, cis or trans. (3 pts)

The trans is more favored because it avoids unfavorable van der Waals interactions between the alpha protons. (2 pts)

4. (12 pts) The balance between the folded and unfolded state of a protein depends on a number of competing factors, i.e. those that stabilize the folded state and those that destabilize it.

i) What are these competing factors and are they related to enthalpic (ΔH^o) or entropic terms (ΔS^o) (6 pts).

ii) How do these factors "shape" the folded form of a protein, i.e. what are the common characteristics of all folded proteins and what thermodynamic forces are responsible for those characteristics? (6 pts)?

i)

The folded form is stabilized by H-bonds and van der Waals, both enthalpic terms. It is also stabilized by the hydrophobic effect which is the entropy of water.

The folded form is destabilized by conformational entropy, i.e. the unfolded state has more conformations than the folded state, which gives the unfolded state a higher entropy.

ii)

Proteins contain a well packed core that optimizes van der Waals interactions.

The core is non-polar, due to the hydrophobic effect. Burial of the non-polar groups increases the entropy of the water.

5. (6 pts) Compare and contrast the thermodynamic forces that affect the relative stability of folded proteins versus double stranded DNA, **highlight** those forces that play a dominate role in DNA structure. In particular discuss how the concentration of NaCl affects the stability of DNA.

Van der Waals plays a more important role in stabilizing dsDNA due to the polar nature of the bases. (1 1/2 pts)

The hydrophobic effect is less important for DNA since the bases are polar and largely solvent exposed. (1 1/2 pts)

Electrostatic interactions, which have little effect on protein stability, have a large effect on the stability of dsDNA due to charge repulsion between the phosphate groups on each strand. This destabilizes dsDNA. (2 pts)

The addition of NaCl, reduces the charge repulsion, so it would stabilize dsDNA, i.e. the melting temperature increases as the salt concentration increases. (1 pt)

6. (10 pts) What are the characteristics of allosteric systems and how are these characteristics used to control biochemical behavior? Give **one** example from the course where a biochemical process is **controlled** by allosteric behavior.

Allosteric systems can exist in two states; Relaxed and Tense. The relaxed form is the active form, the tense form inactive (4 pts)

The equilibrium between the two states is controlled by activators (increase R) or inhibitors (increase T) (4 pts)

Examples (2 pts)

Control of PFK in glycolysis by AMP, ADP, ATP, F26P

Control of bisphosphatase in gluconeogenesis by AMP, F26P

Control of glycogen synthase/glycogen phosphorylase by protein phosphorylation

Control of mRNA production by the lac repressor.

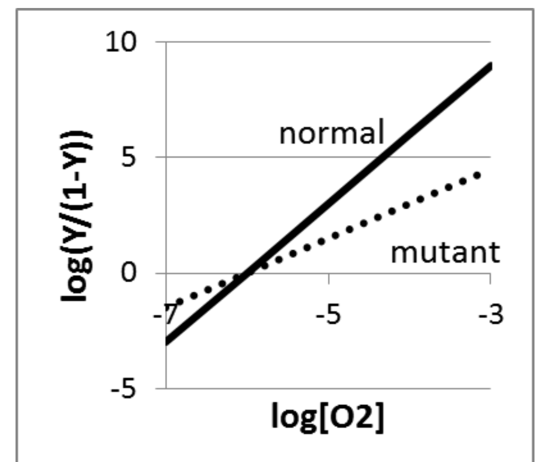
7. (7 pts) The Hill plot on the right shows the oxygen binding properties of normal hemoglobin and a mutant hemoglobin.

i) The individual with the mutant hemoglobin has difficulty providing oxygen to their tissues during exercise, why? (4 pts)

ii) In addition to low oxygen levels, individuals with the mutant hemoglobin also show high levels of lactic acid in their blood during exercise. Why? What is the source of the lactate (3 pts).

i) The mutant hemoglobin has lower cooperativity, so there would be less oxygen delivered because the change in fractional saturation between the lungs and the tissue would be smaller.

ii) They are undergoing more extensive anaerobic metabolism in their muscles. To regenerate NAD+ for glycolysis they are converting pyruvate to lactate.



8. (10 pts) How do **all** enzymes enhance the rate of chemical reactions?

- The rate of the reaction is proportional to the transition state concentration: $v \propto [X]$. (3 pts)
- The amount of the transition state depends on the energy difference between the reactant (substrate) and the transition state. The lower the energy, the larger amount of transition state, the faster the rate. (3 pts)
- The energy of the transition state in enzymes is lowered because enzymes pre-order the functional groups needed for activity. In the uncatalyzed reaction, it is necessary to order these groups, which is entropically unfavorable. (4 pts)

9. (6 pts) Please do <u>**one**</u> of the following choices:

Choice A: Describe how the tertiary structure of a protein is determined by X-ray diffraction.

Choice B: Describe how the quaternary structure of a protein is determined by gel filtration and gel electrophoresis techniques.

Choice C: What is specific activity and why is it a useful parameter to monitor during protein purification?

Choice A:

The electrons scatter X-rays and the scattering depends on the relative positions of the electrons, leading to interference between the scattered X-rays. The intensity of the scattered x-rays is measured, and combined with the phase information to give the electron density map. The atomic positions are obtained after fitting the atoms into regions of high electron density.

Choice B:

The overall molecular weight is determined by gel filtration (size exclusion) chromatography.

The molecular weight of individual subunits, and their relative ratio, are determined by SDS-PAGE, giving the smallest possible overall molecular weight.

A multiple of the smallest overall molecular weight from SDS-Page will equal the overall molecular weight from gel-filtration.

Choice C:

Specific activity is the ratio of the activity of the target protein divided by the total protein. It is useful because after each purification step the total protein decreases, therefore the specific activity increases.

10. (12 pts) Briefly describe the structural features of either and alpha helix or a β-barrel. Why are these two structures found in integral membrane proteins? For the secondary structure you selected, how often would non-polar amino acids be found in the sequence, assuming one face of your secondary structure faced the lipid bilayer.

Structural Features (6 pts)

- Alpha helix: 3.6 residue/turn. Sidechains project out from the helix, H-bond parallel to helix axis.
- Beta-barrel: Multiple beta strands forming a closed barrel. Sidechains project into the barrel or out. H-bonds perpendicular to strand direction.

Why in integral membrane proteins (4 pts)

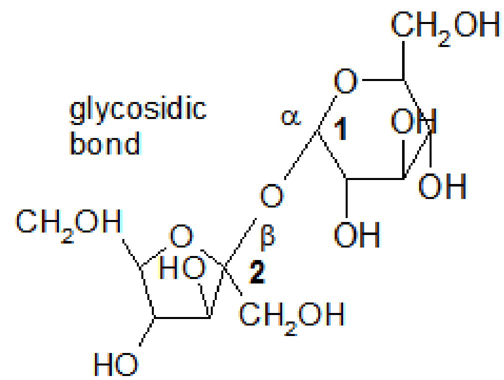
- Because there are no hydrogen bond donors or acceptors in the membrane, the protein must satisfy all of its H-bonds by forming them with itself. This is only possible with an alpha helix or a beta-barrel.

Frequency of non-polar residues (2 pts):

- Every 3-4 for a helix, every second for the beta-strands in the beta-barrel.

11. (6 pts) Sucrose is β-fructofuranosyl-(2→1)-α-glucopyranoside. Draw this structure. What is the name of the bond that joins the two monosaccharides? Can't draw sucrose? Then draw any disaccharide that contains only glucose, and give its name. [Hint: glucose is dud]

+5 for drawing (note glucose is flipped from it normal orientatin)
+1 for labeling glycosidic bond.



b-fructofuranosy (2-1) -a-glucopyranoside

12. (6 pts) Briefly discuss how sucrose would be metabolized after hydrolysis into the individual monosaccharides, i.e. through which pathway would the carbon atoms from these sugars flow?

Glucose and fructose would enter glycolysis (2 pts).
Glucose at the beginning and fructose below PFK (1 pt)
They would be converted to pyruvate, then to acetyl-CoA, and then the TCA cycle (3 pts).

13. (10 pts) A liver cell has high levels of AMP and ADP, but the blood glucose levels in that individual are low.
i) From the perspective of energy sensing, which pathway should be active, glycolysis or gluconeogenesis, and why. What regulatory features of PFK and bisphosphatase ensure that this would occur (5 pts)?
ii) From the perspective of the liver's role as a glucose bank, which of those two pathways should be active? How is their activity controlled by hormones (5 pts) ?

i)
AMP and ADP signal that the cell needs to make ATP (2 pts)
Therefore glycolysis, which produces ATP should be on, and gluconeogenesis should be off (2 pts)
This is accomplished by the fact that AMP/ADP activate PFK, the controlling enzyme in glycolysis, and AMP inhibits bisphosphatase the controlling enzyme in gluconeogenesis (1 pt)
ii)
Since the liver cell should be producing glucose to raise the blood glucose levels, glycolysis should be off because it consumes glucose and gluconeogenesis should be on, since that pathway produces glucose (2 pts)
Low blood sugar will cause the production of the hormone glucagon (1 pt)
Activation of its receptor on the surface of the liver cell will cause protein phosphorylation
The ultimate result of the phosphorylation is lowering the levels of F26P (1 pt)
This will turn off glycolysis because F26P is required to activate PFK (1 pt)
Bisphosphatase would no longer be inhibited by F26P, so gluconeogenesis would be on.

14. (6 pts) How do the structure of glycogen and cellulose differ? In what way are they the same? Which of these is used for energy storage in animals?
    - Both contain glucose (1 pt)
    - Glycogen contains alpha(1-4) linear chains with alpha(1-6) branches (2 pts)
    - Cellulose contains beta(1-4) linear chains, with no branching. (2 pts)
    - Glycogen is used for energy (glucose) storage. (1 pt)

15. (5 pts) In catabolic biochemical pathways, such as glycolysis, energy is released by the <u>oxidation</u> (type of reaction) of organic compounds. This energy is stored directly on <u>NADH</u> or <u>FADH2</u>. The stored energy is then used to generate a <u>proton gradient</u> during <u>electron transport</u> (a pathway), which is ultimately used to generate ATP.

16. (4 pts) Compare and contrast the structural properties of any **two** of the following lipids (you may sketch a chemical structure if you like): a) waxes b) triglycerides, c) phospholipid, d) cholesterol.

Waxes: fatty acid esterified to an alcohol

Triglyceride: three fatty acids esterified to glycerol.

Phospholipid: two fatty acids esterified to glycerol, phosphate + additional head group on C3 of glycerol

Cholesterol: Fused rigid rings with –OH group. Short flexible non-polar tail attached to the other end.

17. (10 pts) Describe, or sketch, the structure of a biological membrane. What are the properties of the membrane in terms of permeability and fluidity? What are the general functions of the proteins found in the membrane?

Sketch should show:

Phospholipid bilayer containing i) cholesterol, ii) transmembrane proteins, iii) proteins embedded in membrane (5 pts)

Transmembrane proteins function as channels/transporter to move things across membrane (1 pts)

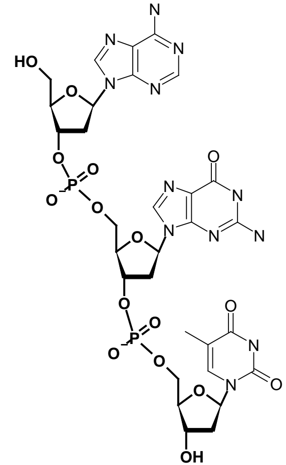
Transmembrane proteins also function as signaling molecules, bind something outside cell, transmit signal inside (1 pts)

Embedded proteins are enzymes (½ pt)

Membrane is fluid (1 pts)

Non-polar molecules, such as oxygen, freely cross membrane. Small polar, such as water more slowly. Large polar and ions cannot cross the membrane (1 ½ pt)

18. (10 pts) The following is a short segment of single stranded DNA, that contains A, G, and T.



i) Identify the 5' end. This is the top of the diagram (2 pts)

ii) Circle the purine base(s) Both of the top bases are purines (2 pts)

iii) What changes to this diagram would you make to convert this DNA to RNA?

Add OH to 2' carbon on ribose (2 pts)

Replace T with U (2 pts)

iv) If this were double stranded DNA, what is the orientation of the complementary strand, parallel or anti-parallel?

Anti-parallel (2 pts)

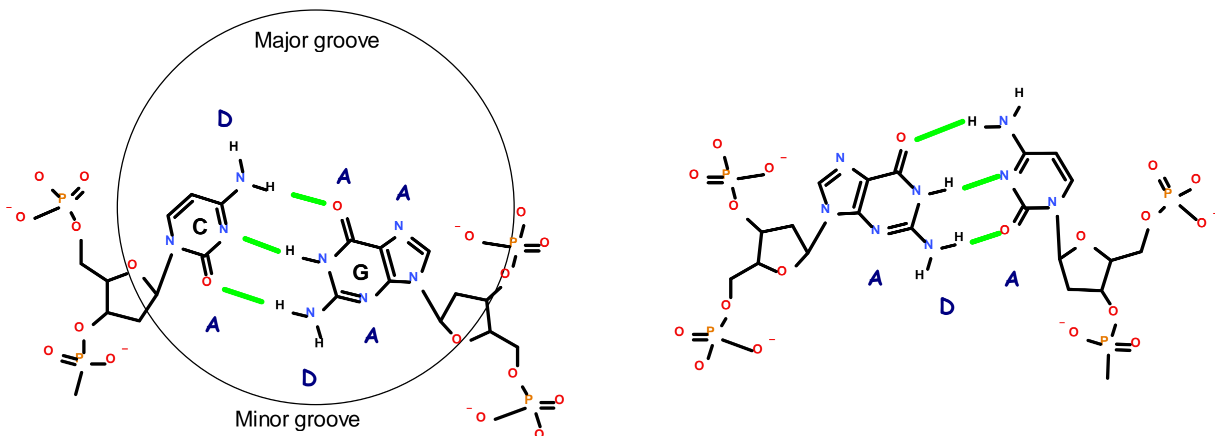
19. (9 pts) A CG basepair in DNA shown on the right.

i) Indicate the "Watson-Crick' hydrogen bonds involved in this basepair (1 pt). (shown in green)

ii) Indicate the major and minor groove (1 pt). As indicated on the diagram.

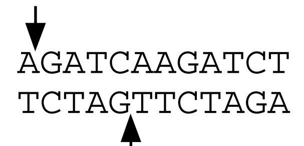
iii) Label all hydrogen bond donor and acceptors in both grooves (2 pts) (labeled with "A" and "D")

iv) Is it possible for a protein to distinguish a CG basepair from a GC basepair (i.e. interchanging the bases) if it binds in the minor groove? Justify your answer (5 pts).



iv) **No**, it is not possible to distinguish a CG from a GC, The pattern of hydrogen bond donors and acceptors is the same for both the CG and GC basepairs, i.e. A-D-A.

20. (6 pts) A new restriction endonuclease, CmuII recognizes the sequence shown on the right, and cuts at the arrows.



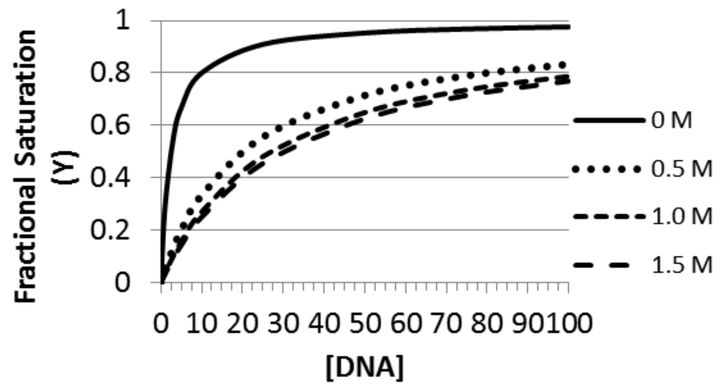
i) Is this enzyme a homo- or heterodimer? Why? (2 pts)

ii) Could you ligate DNA fragments generated by CmuII to DNA fragments generated by BamHI (G^GATCC) [Hint: Draw the products after digestion with each enzyme.] (4 pts). Why?

i) Heterodimer, since the sequence of the top and bottom strand, and cut sites, are non-symmetric. A homodimeric enzyme would recognize the same sequence on the top and bottom strands.

ii) Yes, they have the same sticky ends: GATC

21. (8 pts) The binding of the lac repressor protein to DNA was measured as a function of salt concentration and the binding curves are shown on the right. Based on these data, what type(s) of interactions are used by the lac repressor to bind to DNA [Hint: It may be useful to sketch a plot of K_D versus [NaCl]]



The Kd increases as salt increases, indicating weaker binding (3 1/2 pts).

The interaction that is being disrupted by the salt is electrostatic, positively charged Lys and/or Arg residues on the protein are interacting with negatively charged phosphate groups on the DNA (3 1/2 pts).

The protein still binds with high salt, so there must be additional interactions, i.e. hydrogen bond to the bases or the deoxy ribose (1 pts).

22. (12 pts) DNA and RNA Polymerases:

i) What are the factor(s) the affect the selection of the new base that is added to the growing chain?

ii) How is the new base added? Give a brief description of the reaction.

iii) The standard energy change between the reaction and product (addition of a base) is approximately zero, yet polymerization is spontaneous. What feature of the reaction ensures that it is spontaneous?

iv) What is the most significant difference between DNA polymerases and RNA polymerases, besides the fact that one generates DNA and the other RNA.

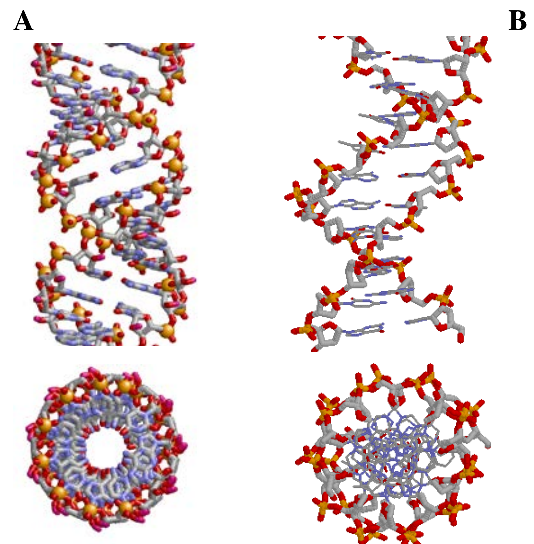
i) Watson-Crick hydrogen bonds (A-T, G-C) (2 pts), plus size matching purine:pyrimidine (1 pt)

ii) The new base, as a triphosphate, is attacked by the 3'OH of the growing strand, forming the new phosphodiester linkage and releasing pyrophosphate. (3 pts)

iii) The Gibbs energy is negative because of indirect coupling. Conversion of pyrophosphate to inorganic phosphate is very favourable, thus the concentration of pyrophosphate is kept below its equilibrium level. (3 pts)

iv) RNA polymerases do not require a primer (2 pts), and do not have error correction. (1 pt)

23. (5 pts) The side view and top view of an RNA molecule and a DNA molecule are shown on the right. Which is which? Briefly justify your answer.



A is RNA because it has shallow and deep grooves with uniform spacing of the phosphate. The bases are also off of the helix axis.

24. (5 pts) Please do one of the following choices:
Choice A: Explain the role of the sigma factor in RNA polymerase activity.
Choice B: Explain why the first amino acid is N-formyl methionine instead of methionine.

Choice A: The sigma factor is part of the holoenzyme and recognizes the -35 and -10 regions of the promoter.

Choice B: This amino acid is found in the P-site at the start of protein synthesis. Normally a peptide is found in the P-site. The N-formyl group looks like a peptide bond.

25. Please do **one** of the following choices (8 pts):
Choice A: Describe the steps associated with the elongation step of protein synthesis.
Choice B: What events occur during termination of protein synthesis?
Choice C: The codon table shows that many amino acids are encoded by more than one codon. However, there is usually only one tRNA per amino acid. How can a single tRNA interact with multiple codons?

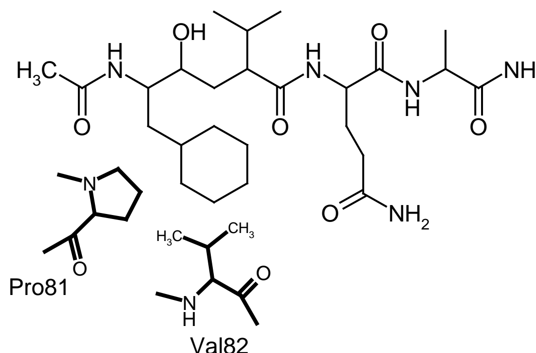
Choice A:
The peptide chain, attached to a tRNA is in the P-site
The next tRNA, carrying the correct amino acid binds to the A-site, by virtue of the codon-anticodon interactions.
Peptide bond formation occurs, transferring the peptide to the A site.
The ribosome shifts, and the elongated peptide is now in the P-site, and the uncharged tRNA exits.

Choice B:
The completed protein, attached to the last tRNA is in the P site.
A stop codon is at the A site.
A protein, release factor or termination factor binds
It catalyzes the hydrolysis of the protein off of the tRNA, releasing the protein

Choice C: The first two bases of the codon-anticodon interaction are typically Watson-Crick. The third base of the codon forms an alternative basepair with the anticodon, often called a wobble basepair.

Note: The remaining questions on this exam are related.

26. (6 pts) The structure of an inhibitor of HIV protease is shown on the right. Residues from the enzyme are shown in bold. What thermodynamic forces or interactions are responsible for the stabilizing the bound form of this drug. Justify your answer with reference to functional groups on both the drug and the enzyme.



There is close contact between Pro81, Val82 and the cyclohexane ring on the drug. Suggesting van der Waals interactions (3 pts)

Both Pro81 and Val82 are non-polar, as is the cyclohexane ring, so the hydrophobic effect would also stabilize the bound state. (3 pts)

27. (16 pts) A mutation has arisen in the gene for HIV protease that has reduced the drug binding, producing a drug resistant strain.

i) Why are mutations in the viral genetic information quite prevalent (3 pts)?

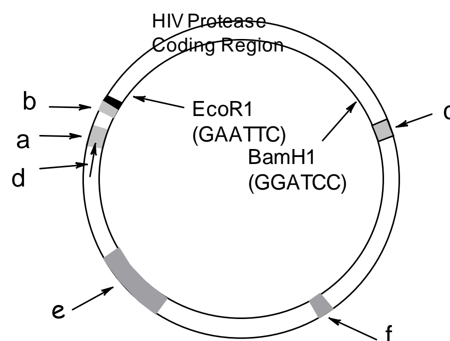
ii) Describe in general (e.g. with a flowchart), all of the steps that you would need to take to produce this mutant protein in E. coli, beginning with the viral RNA and ending with purification of the protein from a bacterial lysate (6 pts).

i) HIV reverse transcriptase, the polymerase that converts the viral RNA to DNA is error prone since it has no 3'-5' exonuclease activity.

ii)The steps are (their answer can be missing one or two)

Conversion of vRNA to DNA
PCR to amplify the region containing the HIV protease
Ligation of the HIV protease PCR product into the expression vector
Transformation of the expression plasmid into cells
Growth of cells in the presence of antibiotic
Induction of HIV protease by IPTG
Harvest cells, lyse, purify protein.

iii) An image of the expression vector is shown on the right. The arrows on this diagram indicate the location of the control elements that are present on this plasmid. These elements are listed on the right, labeled a-f. Place the label in the correct position. The last two (e, f) have been done for you (3 pts).



a) lac operator
b) ribosome binding site
c) mRNA termination
d) promoter
e) antibiotic resistance gene
f) origin of replication

iv) Select any **4** of elements a-f from part iii and provide a brief description of their function (4 pts).

a) lac operator
Bind lac repressor, preventing synthesis of mRNA until induced with IPTG.

b) ribosome binding site
Binds mRNA to the 30s (small) ribosomal subunit.

c) mRNA termination
Terminates mRNA production.

d) promoter
RNA polymerase binds, initiating mRNA production

e) antibiotic resistance gene
Allows bacteria that contain the plasmid to grow in the presence of antibiotic, making sure plasmid is present.

f) origin of replication
So that the plasmid will replicate in the bacteria.

28. Provide the sequences of the PCR primers that would be necessary to generate the desired segment of DNA to insert into the vector using EcoR1 (GAATTC) and BamH1 (GGATCC) sites. You need not worry about the annealing temperature; make your primers 15 bases in length. Pay close attention to control elements that may, or may not, be present on the vector and whether they should be included in the PCR product (6 pts). The sequence of the HIV gene is shown below:

```
 1  2  3  4  5  6          79 80 81 82 83 84 85 86         94 95 96 97 98 99
ProGlnIleThrLeuTrp--------ProThrProValAsnIleIleGly-------GlyCysThrLeuAsnPhe
CCTCAGATCACTCTTTGG--------CCTACACCTGTCAACATAATTGGA-------GGTTGCACTTTAAATTTTTAA
GGAGTCTAGTGAGAAACC--------GGATGTGGACAGTTGTATTAACCT-------CCAACGTGAAATTTAAAAATT
```

Rules are:

1. Left primer = sequence of top strand Right primer = sequence of bottom strand.
2. Are start and stop codons required? **Yes** in this case since they are not found on the vector.
3. What restriction sites are needed on the ends of the PCR fragment? R1 on the left, Bam on the right.

Left Primer: 5'GAATTCATGCCTCAG Right Primer: 5' GGATCCTTAAAAATT

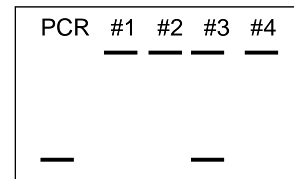
EcoR1 fMetProGln Bam STP

Other possibilities for the right primer are (different stop codons)

5' GGATCCCTAAAAATT

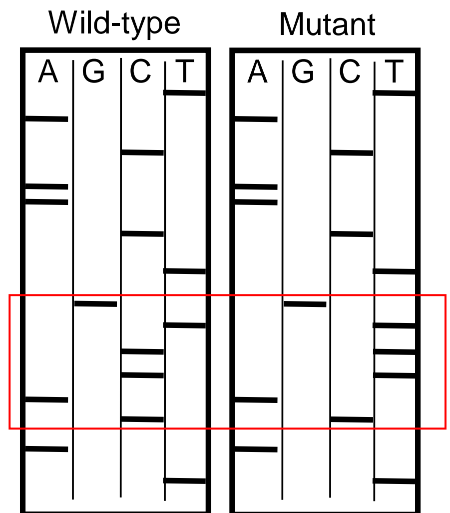
5' GGATCCTCAAAAATT

29. (4 pts) After construction of the completed expression vector, you tested four different plasmids to determine which one was correct. The plasmid DNA was digested with EcoR1 and BamH1 and electrophoresis was performed. The gel contained the original PCR product, and the digestion product of the four plasmids. Which plasmid is correct, and why.



Only number 3, since it produces a band the same size as the PCR product after digestion.

30. (16 pts) After determining which plasmid is correct, you sequence the DNA to determine the location of the mutation. A section of the sequencing gel of the wild-type and mutant DNA is shown on the right.



i) What would the "A" lane of the gel look like if the ddATP was accidentally omitted from the reaction? Briefly justify your answer (2 pts)

There would not be any bands since no termination would have been possible when A is inserted into the newly synthesized DNA. Chain termination occurs with ddATP because the 3'OH is missing and new bases are added to the 3'OH.

ii) Identify changes in the DNA sequence and determine which changes occurred in the protein sequence. A portion of the wild-type sequence is given below and a codon table is given with the formula sheet (4 pts).

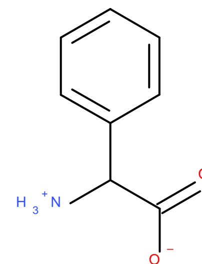
```
 79 80 81 82 83 84 85 86         94 95 96 97 98 99
--ProThrProValAsnIleIleGly-------GlyCysThrLeuAsnPhe
--CCTACACCTGTCAACATAATTGGA-------GGTTGCACTTTAAATTTT
--GGATGTGGACAGTTGTATTAACCT-------CCAACGTGAAATTTAAAA
```

The region of the gel showing the difference in sequence is boxed. The sequence and reading frame for the wild-type sequence is:

```
           ThrProVal
Wildtype:   CACCTG
Mutant:     CATTTG
```

Proline (CCT) has been changed to TTT which is Phe

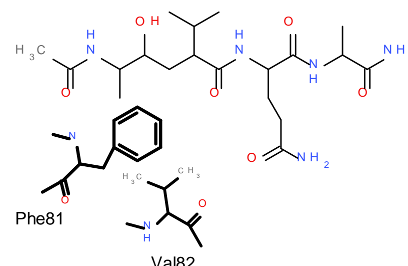
iii) Draw the structure of the altered amino acid. How would you alter the structure of the **drug** to increase its affinity to the mutant enzyme? Be sure to discuss any interactions that might be important for binding your modified drug to the mutant enzyme (4 pts)



Correct structure + 1 pt.

Correct description of change in drug, + 3pts

Phenylalanine is larger than the original proline. Therefore the cyclohexane group on the drug should be made smaller, but still non-polar.

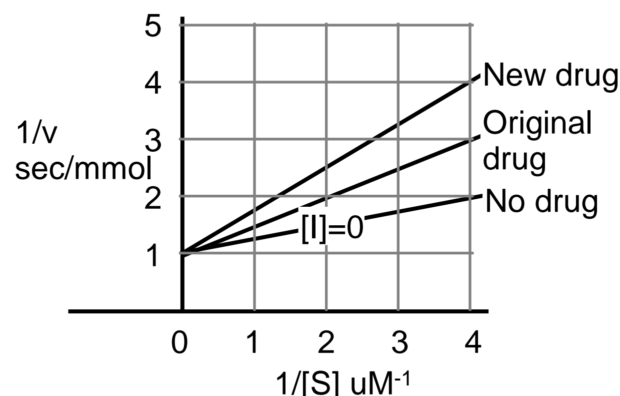


iv) Steady state enzyme kinetics was performed using the original drug and your modified drug, in the presence of 1 nM of the inhibitor. How much stronger does the new drug bind to the enzyme? [Hint: Determine K_I] (4 pts)

$K_I = [I]/(\alpha-1)$, α from ratio of slopes.

Original drug: $\alpha = (2/4) / (1/4) = 2$, $K_I = 1$ nM
New drug: $\alpha = (3/4) / (1/4) = 3$, $K_I = 0.5$ nM

The new drug binds with twice the affinity, since the K_I dropped by a factor of 2.



v) Is this a competitive or mixed type inhibitor? [Hint: how does the inhibitor affect the double reciprocal plot?](2 pt)

This is a competitive inhibitor because i) Vmax is unaffected, ii) the inhibitor resembles a peptide substrate.

31. (5 pts) Please do **one** of the following choices.

Choice A: How might you modify the expression vector to export the HIV protease out of the cell?

Choice B: If the following codons were appended to the end of the coding sequence, how could this facilitate the purification of the HIV protease? CACCACCACCACCACCAC

Choice A: Add codons that would code for a leader peptide at the amino terminus of the HIV protease.

Choice B: These code for six histidine residues, or a his tag. This would bind to a Ni affinity resin.

Bonus (3 pts each)(Use the back of the page to answer).

B1. If you ran in the Pittsburgh marathon and hope to run another within a week or two, would it be better to eat a high fat/protein diet, or a high carbohydrate diet, in preparation for your next race. Why?

High carbohydrate to replenish your glycogen. Fat and protein are difficult to convert to glycogen because the reaction of pyruvate to acetylCoA is one way in humans.

B2. In what way is the ribosome like an apple?

The stem coming out of the top is the new protein leaving the exit tunnel.

B3. In what way are peppermint patties like IPTG?

They cause the lac repressor student to let go of the lac operator sequence in the DNA to grab the patty, allowing the RNA polymerase student to make mRNA.