Instructions: This exam contains 220 points on 11 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. The last page of the exam provides a codon table.

1. (10 pts) This question involves a 0.1 M buffer solution with a volume of one(1) L whose pH is 7.0.
   i) Which of the following monoprotic weak acids would you use for this buffer: Acetic acid (pKa=4.8), Tris (pKa=8.2), Imidazole (pKa=6.8). Why? (2 pts)
   ii) Do one of the following two choices (8 pts):
   Choice A: Describe how you make this buffer using the fully protonated form of the weak acid.
   Choice B: The reaction that you are buffering produces protons, dropping the pH to 6.0. How many equivalents of NaOH do you need to add to restore the pH to 7.0?

   Imidazole would be the best since the pKa of the buffer should be within on pH unit of the desired pH.
   Choice A: The fraction deprotonated is f_{A^-}= R/(1+R), where R=10^{pH-pKa} = $10^{7.0-6.8}$=1.58, f_{A^-}=.613
   You would need to add 0.613 equivalents of base, or a total of 0.0613 moles of base (moles = eq x vol x concentration).
   Choice B: Need to calculate the difference in the fraction protonated between the two pH values.
   The fraction deprotonated @7 is f_{A^-}= R/(1+R), where R=10^{pH-pKa} = $10^{7.0-6.8}$=1.58, f_{A^-}=.613
   The fraction deprotonated @6 is f_{A^-}= R/(1+R), where R=10^{pH-pKa} = $10^{6.0-6.8}$=.158, f_{A^-}=.136
   The difference, 0.477 is the number of equivalents of base you would have to add.

2. (8 pts) Draw the structure of a tripeptide using three **different** amino acids. You can pick any amino acids that you like, with the exception of Ile, Thr, and Val. You should indicate the sequence of your peptide, the location of peptide bonds, distinguish between mainchain and sidechain atoms, and indicate any chiral centers.

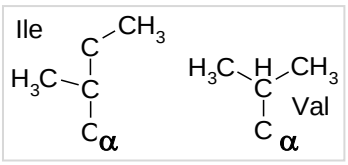
   Correct chemical drawing with minor errors in sidechain – 5 pts.
   Correct amino acid sequence – 1pt
   Peptide bond locations 1 pt
   Mainchain and sidechain atoms - 1 pt.

3. (8 pts)
   i) The peptide bond is fixed in one configuration, planer. Why is this so? (4 pts).
   ii) Imagine that the peptide bond is as freely rotatable as the other mainchain bonds in proteins, how would this affect protein stability? Increase or decrease it? You should justify your answer with a **quantitative** estimate of how rotation of the peptide bond would affect stability [Hint: S=RlnW] (4 pts).

i) It is planer because the carbon and nitrogen are both sp_2 hybridized, allowing energetically favorable overlap of the p_z orbitals on each atom. The orbital overlap constrains the bond in one of two orientations (cis or trans). The trans is more stable due to reduced vdw interactions (Simply saying "partial double bond" = -1 pt)
ii) This would destabilize the folded form, or stabilize the unfolded form, due to an increase in entropy due to unfolding (3 pts). The increase in entropy per residue would be S=Rln3, where 3 would be the number of possible conformations of the freely rotatable peptide bond. (1 pt)

Points on Page:_____

4. (6 pts) An Ile residue in the core of a protein is replaced by a valine residue. You should assume that changing the Ile to Val does not change the overall structure of the protein. Please do **one** of the following choices.

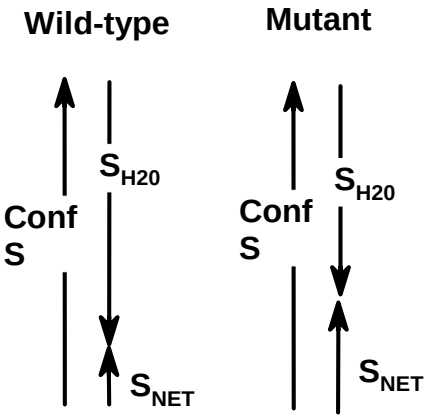


Choice A: How will the enthalpy of unfolding be affected by this mutation? Will it increase or decrease? Briefly justify your answer (6 pts).

Choice B: How will the entropy of unfolding be affected by this mutation? Will it increase or decrease? Briefly justify your answer (6 pts).

Choice A: The positive enthalpy of unfolding will be decreased due to a reduction in the vdw effects because the Valine is one methyl group smaller than the Ile

Choice B: The entropy of unfolding will increase because the smaller Val will order less water in the unfolded state, thus the entropy decrease due to the hydrophobic effect will be smaller. Assuming that the overall conformation positive conformational entropy is about the same, the net increase in entropy will be larger.



5. (14 pts) Select one of the following enzymes: Trypsin, Chymotrypsin, Elastase, HIV protease, or the Potassium channel (or another one discussed in the course) and answer the following questions. Use the same enzyme for i and ii, you can use a different enzyme for iii if you like.
   i) Give the substrate and products of the reaction (1 pt).
   ii) Describe the role of functional groups in catalyzing the reaction (4 pts).
   iii) Discuss the basis of substrate specificity for the enzyme (4 pts).
   iv) Discuss the principal reason why **all** enzymes enhance the rate of reactions (5 pts).

Trypsin	Chymotrypsin	Elastase	HIV protease	K Channel
Product is cleaved peptide bond				Substrate is K ion on one side of membrane, product is K ion on the otherside.
Catalytic triad: Ser-nucleophil, attacks peptide bond. His – activated Ser. Asp – stabilizes pos charge on his			Catalytic Dyad (2 Asp) Asp25 - activates H2O as nucleophil. H2O attacks peptide bond. Second Asp provides proton for new amino terminus.	Carbonyl groups of the main chain atoms are responsible for desolvation of ion so that it can pass through the membrane.
2nd Asp residue in active site interacts with Lys and Arg + charge on substrate.	Large non-polar specificity pocket binds Tyr, Trp, Phe	Small non-polar pocket binds Ala	Several non-polar residues (Val 81) bind Phe	Distance between C=O groups provide specificity. Na is too small to be stabilized in the desolvated state.

iv)
The rate of the reaction depends on the concentration of the transition state (+1 pt)
Enzymes lower the energy of the transition state by: (3 pts)
a) pre-ordering functional groups so there is no decrease in entropy in going from (ES) to (EP)
b) forming energetic interactions with just the transition state. (-1 if they only list this)
The lower the energy of the transition state, the more of it, the faster the rate. (1 pt)

Points on Page:_____

6. (12 pts) Describe the important features of allosteric systems. Then select one allosteric system and describe why its allosteric features are important for biological function or the expression of proteins in E. coli.

Allosteric Systems:

- Two different conformations of the protein (1 pt)
- T-state low activity (1 pt)
- R- state high activity (1 pt)
- T and R are in equilibrium with each other. (1 pt)
- Allosteric activators increase R state (1 pt)
- Allosteric inhibtors increase T state (1 pt)
- Homotropic/heterotropic affect binding of same (homo) or different (hetero) ligand (1 pt)

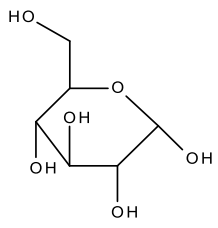
Examples (5 pts)

- Hb – oxygen binding, homotropic activator causes positive cooperativity that enhances oxygen delivery.
- Hb – BPG, heterotropic inhibitor, conc. Increases at high altitudes, changes shape of O2 binding curve to enhance deliver at high altitudes.
- Enzyme Phosphorylation – Under hormonal control. Used to control glycogen synthesis and degradation, controls F26P levels to control glycolysis/gluconeogenesis.
- ATP/AMP, ADP control glycolysis through PFK, gluconeogenesis through bisphosphatase
- Lac repressor – turns genes off by preventing mRNA production, addition of inducer (lactose, IPTG) cause release from the DNA and allows mRNA to be made.

7. (10 pts) What is the hydrophobic effect and describe is its role in **both** protein folding **and** lipid bilayer formation.

- Reduction in the entropy of water on exposure of non-polar groups to water (4 pts).
- Protein folding – stabilizes folded state, causing core to be composed of non-polar residues. (3 pts).
- Lipid bilayer –stabilizes bilayer, causes non-polar fatty acyl chains to be in the middle of the bilayer, phosphate groups on the outside. (3 pts).

8. (5 pts) Glycogen and cellulose are composed of the same monomeric unit. Draw the cyclic structure of that monosaccharide, give its name, and indicate how it is linked together in **either** glycogen or cellulose. Which of these two polymers, glycogen or cellulose, is used for energy storage?



Glucose (3 pts for name (1 pt) and correct structure (2 pts))
β(1-4) linkages in cellulose (1 pt)
or
α(1-4) linkages and α(1-6) branches in glycogen (1 pt)

Glycogen is used for energy storage (1 pt)

Points on Page:_____

9. (12 pts)

i) Outline the major metabolic pathways in yeast cells that are responsible for the complete oxidation of carbohydrates, beginning with monosaccharides and ending with the reduction of water. You answer should focus on the fate of carbon as well how the energy released by these oxidations is captured for ATP formation (8 pts).

ii) How would your answer change if cells were cultured under low oxygen conditions? (4 pts).

i)

Carbon: Glucose -> glycolysis -> Pyruvate -> Acetyl CoA -> TCA cycle. 1 CO2 released Pyr-Acetyl CoA, 2 CO2 in TCA cycle. (4 pts).

Energy: Organic oxidations produce NADH and FADH2, these enter electron transport and produce a proton gradient due to their oxidation. The proton gradient is utilized as an energy source by ATP synthase to make ATP (4 pts).

ii) The carbon in pyruvate is converted to ethanol (3 pts) because NAD+ cannot be regenerated by electron transport (1 pt)

10. (12 pts) Please do **one** of the following two choices:

Choice A: You haven't eaten in a while and your liver has been actively metabolizing, consuming ATP. You then have a large influx of glucose due to eating lunch.

i) What will happen to glycogen levels in the liver cell after your lunch? Describe the regulatory events that cause this effect to happen (6 pts).

ii) What will happen to ATP levels in the liver cell, initially, and some time later (i.e. you need to discuss both hormonal and energy regulation of the appropriate pathways (6 pts).

Choice B: You had an enormous breakfast and your liver hasn't been too busy, so it has adequate ATP levels. After some time your blood glucose levels begin to drop.

i) How will your liver respond to the drop in blood glucose? What will happen to glycogen levels in the liver cell? Describe the regulatory events that cause this effect to happen (6 pts).

ii) What will happen to ATP levels in the liver cell, initially, and some time later (i.e. you need to discuss both hormonal and energy regulation of the appropriate pathways (6 pts).

Choice A:

Insulin binds to its receptor, causing dephosphorylation of enzymes.
Glycogen synthase is activated, excess glucose is stored in glycogen (6 pts)
F26P levels rise, activating PFK in glycolysis.
Glycolysis is on because ADP/AMP are high (ATP was converted to ADP/AMP).
Glycolysis produces ATP, until ATP levels become high, and then glycolysis is off (6 pts)

Choice B:

Low blood glucose will cause glucagon to be released, causing dephosphorylation of enzymes.
Glycogen phosphorylase is activated, glucose is released from glycogen (6 pts)
F26P levels drop, glycolysis will be off (no matter what else is happening) (6 pts)
The high ATP levels will also shut glycolysis off.
Gluconeogenesis is on because: i)no longer inhibited by F26P), ii) ATP available (AMP is low).

Points on Page:_____

11. (8 pts) Please do both parts of this question.
i) Draw the chemical structure of any triglyceride or any phospholipid, be sure to indicate your choice.
ii) A closed phospholipid vesicle is placed in a solution of glucose. What happens to the size of the vesicle, and why?

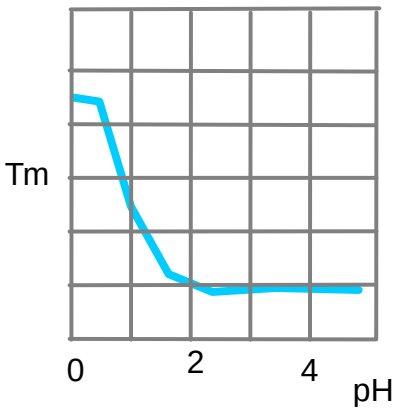
i) Triglyceride – three fatty acids esterified to glycerol.
or
Phospholipid – two fatty acids esterified to carbon 1 and 2 of glycerol, phosphate group on carbon 3. (4 pts)

ii) Glucose cannot cross the membrane, but water can. There is a higher concentration of water inside the vesicle, so water will leave and the vesicle will shrink. The internal volume can get quite small since there is no glucose inside the vesicle – consequently the concentration of water inside is always greater than outside. (4 pts)

12. (6 pts) How would you determine whether a protein had a quaternary structure (a definition of a quaternary structure would be a good way to start your answer).

Quaternary structure consists of multiple chains.
Run SDS-Page to get sizes of individual chains – if more than one chain is seen, then there is a quaternary structure. If one chain is seen, then there might be a quaternary structure.
Run gel filtration to get overall size, if the size is the same as the single chain from SDS-page, there is no quaternary structure.

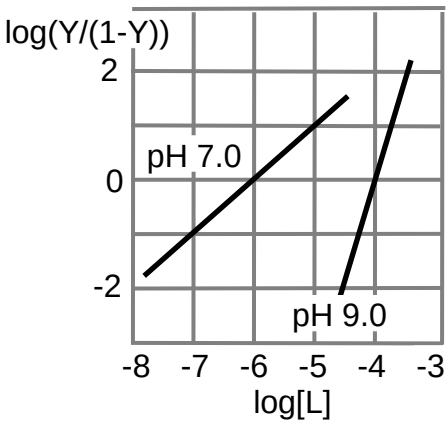
13. (8 pts) The phosphate group on DNA has a pK_a of 1.0.
i) What is the charge on DNA at pH 7.0? (2 pt)
ii) Sketch a graph of the T_M for double stranded DNA as a function of pH in the space to the right. Justify your answer with a discussion which molecular force/interaction would be most affected by changing the pH. Hint: you may find it useful to also plot the fraction protonated versus pH (6 pts)



i) Negative (2 pts)
ii) See diagram on right. As the pH is lowered, the phosphates will become protonated. This will stabilize the DNA due to neutralization of the repulsive forces between the phosphates on each strand. Thus T_M will increase as the pH is lowered. The curve will be similar in shape to the fraction protonated curve, with an inflection point at pH of 1 (= pKa). (6 pts)

Points on Page:_____

14. (12 pts) Single stranded binding protein binds to single stranded DNA. In this question, you should consider the protein to be the ligand (L) and the DNA to be the macromolecule (M). Many proteins can bind to one DNA molecule, i.e. possible liganded species are ML, ML_2, ML_3, …, and it is possible to have cooperative protein-protein interactions between adjacent bound proteins. The Hill plot for this system is shown on the right, obtained under two different pH values, pH 7.0 and pH 9.0.



i) What is the K_D for binding at pH 7.0? Justify your approach (2 pts).

ii) Explain the effect of pH on the K_D for the protein-DNA interaction, i.e. what interaction between the protein and the DNA is most likely being affected by pH? State the most likely functional groups (5 pts).

iii) How does pH affect the cooperativity? Provide a possible explanation for the effect (5 pts)

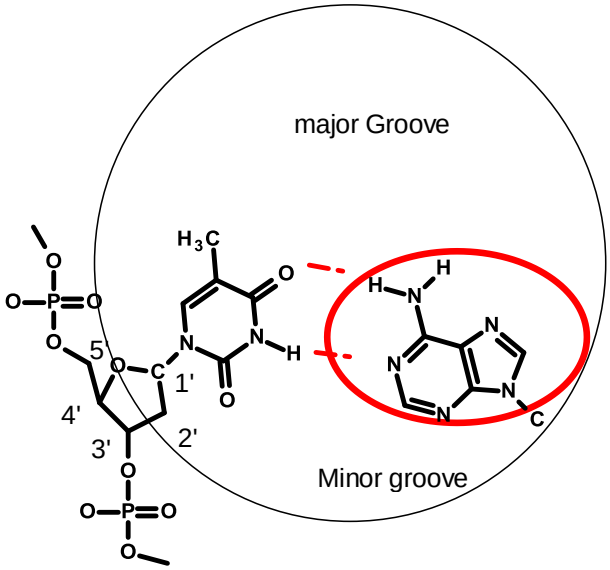
i) Kd is 10^{-6} M. Point were curve crosses x-axis is log Kd. (2 pts)

ii) The Kd increases at the pH is raised from 7 to 9. What is most likely happening is that lysine residues on the protein are being deprotonated, reducing the interaction with the phosphate, decreasing the binding and raising the Kd (3 pts for saying a decrease in affinity occurs, 2 pts for a plausible explanation).

iii) The cooperativity is increased as pH is increased. The binding is non-cooperative at pH 7.0 and positive coop at pH 9, based on the slope of the Hill plot. (4 pts)

Possible explantion: The protein-protein interactions are weaker at pH 7.0 and become stronger at pH 9.0. You could imagine charge repulsion between lysine groups on the protein that is reduced when the pH is raised, allowing the proteins to interact with each other (1 pt for a sensible model).

15. (8 pts) The nucleotide base portion of a basepair is shown on the right.



i) Draw the Watson-Crick hydrogen bonds between the bases (1 pt) Dotted red lines between bases.

ii) Circle the purine base (1 pt) Red circle

iii) Draw the complete structure of one of the bases, i.e. draw the ribose (or deoxyribose) and show the correct linkage to the nucleotide that would be above and below the base. The C1' carbon on the sugar is drawn for you (5 pts) (Q18 may be helpful).

-completing deoxyribose or ribose

-5' phosphate

-3' phosphate

iv) Which edge, top or bottom, is the major groove (1 pt)?

Top, larger arc of circle through riboses

Points on Page:_____

16. (4 pts) Please do **one** of the following choices.

Choice A: A protein binds to the following DNA sequence: TTTAAA
AAATTT

Is this protein likely a heterodimer or a homodimer? Why?

Choice B: A protein that binds in the minor groove can bind to double stranded C or G with equal affinity, Why?

Choice C: DNA protein interactions are often affected by salt, why?

Choice A: Since the same sequence is being recognized on the top and the bottom strand, it is likely a homodimer with two-fold symmetry. Each subunit recognizes TTTAAA.

Choice B: The pattern of hydrogen bond acceptors in the minor groove is symmetric, so it doesn't matter which side the G or C is on.

Choice C: Non-specific recognition of DNA is by electrostatic interactions between + charges on the Lys or Arg residues and the negative charge on the phosphate. Ions would reduce the strength of this interaction by screening the charges.

17. (6 pts) DNA polymerases usually insert the correct residue in the newly synthesized strand.

i) What are the factor(s) the affect the selection of the new base? (3 pts)

ii) How is the new base added? Give a brief description of the reaction (3 pts).

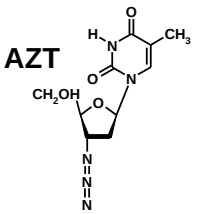
i) Factor are:

a) Watson-Crick hydrogen bonds, A pairs with T via two H bonds, G with C via 3 Hbonds.

b) Size of the basepair, purine (two rings) always paired with pyrimidine (one ring)

ii) The 3'OH on the existing DNA attacks the phosphate on the incoming base, releasing pyrophosphate.

18. (6 pts) The drug ATZ is shown on the right. This drug is an inhibitor of HIV reverse transcriptase, the enzyme involved in copying the viral RNA to DNA. AZT is very effective at preventing HIV replication, even at relatively low concentrations. Why is this drug very effective at interfering with the life cycle of the HIV virus (Hint: a similar concept is exploited in dideoxy sequencing of DNA).



This is a substrate for reverse transcriptase – it can replace T in the polymerization reaction (AZT is converted to the triphosphate first).

It lacks a 3'-OH, so once it is incorporated it terminates the chain, thus the enzyme cannot copy the entire genetic material of the virus. This is equivalent to chain termination by a dideoxy base in DNA sequencing.

Note: The next questions (19-23) are related.

19. (6 pts) A mixed-type inhibitor of reverse transcriptase is shown on the right. Residues from the reverse transcriptase (Thr20, Ile25) are shown in bold. The beginning, middle, and ending sequence of the 600 basepair reverse transcripase gene is shown below:

```
     1  2  3  4     17 18 19 20 21 22 23 24 25 26            200
     MetTyrValHis---AlaGlyProThrSerArgLysAlaIleGlu---SerSerTyrPhe
CGCG ATG TATGTTCAT---GCGGGCCCGACCAGCCGCAAAGCGATTGAA---AGTAGTTACTTT TAA
GCGCTACATACAAGTA---CGCCCGGGCTGGTCGGCGTTTCGCTAACTT---TCATCAATGAAAATT
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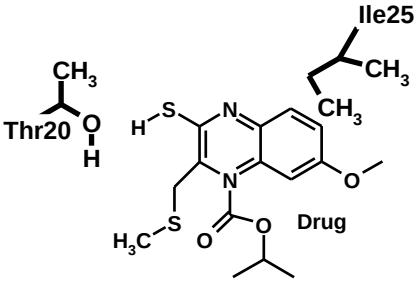
What thermodynamic forces or interactions are responsible for the stabilizing the bound form of this drug. Justify your answer with reference to functional groups on both the drug and the enzyme.



Thr 20: Hydrogen bond to the thiol group (2 pts)

Ile25: van der Waals since the Ile sidechain is in contact with the drug (2 pts). Also both the sidechain and the drug are non-polar, so the hydrophobic effect will enhance binding (2 pts)

Points on Page:_____

20. (22 pts) A mutation has arisen in the gene for reverse transcriptase that has reduced the drug binding, producing a drug resistant strain of HIV virus.

i) Why are high levels of mutations found in the HIV virus (1 pt)?

ii) Describe in general, 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 bacteria (6 pts).

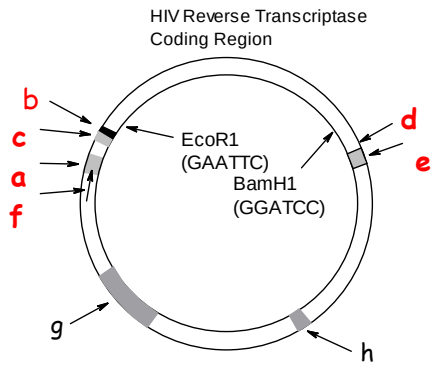
i) HIV reverse transcriptase lacks a 3'-5' exonuclease proofreading function, so there is high mutation rate.

ii)

1. Copy vRNA to dsDNA using reverse transcriptase.
2. Use PCR to amplify gene, placing restriction sites on the end of the PCR product.
3. Cut PCR product and expression plasmid with restriction enzymes, mix, ligate.
4. Check plasmid to make sure it is right.
5. Place plasmid in bacterial cell (transformation)
6. Grow up cells, add inducer (IPTG)
7. Harvest cells, purify protein.

(full credit for most of the steps in a logical order)

iii) An image of the expression vector is shown on the right. The arrows on this diagram indicate the location of all of the control elements that will be required to produce the reverse transcriptase intracellularly. These elements are listed on the right, labeled a-h. Place the label in the correct position. The last two (g, h) have been done for you **(3 pts).**



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

iv) Select two (2) of elements from the above list (a-g) from part ii and provide a brief description of their function **(4 pts). One of your selections must be choice a) (lac operator).**

a) lac operator: binds lac repressor, used to control production of mRNA
b) start codon: Initiates protein synthesis.
c) ribosome binding site: Binds mRNA to ribosome (30 s subunit)
d) stop codon: Terminates protein synthesis.
e) mRNA termination: Terminates mRNA synthesis, recruiting rho factor.
f) promoter: Binds RNA polymerase, begins mRNA production.
g) antibiotic resistance gene: Bacteria with the plasmid will be resistant to the antibiotic, allowing selection for those bacterial that contain the plasmid.
h) origin of replication. Allows plasmid to be replicated in bacteria.

iv) 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. Make your primers a total of 12 bases in length. Pay close attention to control elements that are already present on the vector and therefore do not need to be part of the PCR product (6 pts).

Left Primer: G A A T T C T A T G T T – no need to include the start codon (-1 if included)
Right Primer: G G A T C C A A A G T A – no need to include the stop codon (-1 if included)

v) Suggest an annealing temperature for PCR, based on the melting temperature of the left primer (2 pts).

Tm = 81.5 +(3/12)*100*0.41 – 625/12 = 39.67
T_{ANNEAL} = 39.67 – 5 = 34.67

Points on Page:_____

21. (16 pts) A section of the sequencing reaction from wild-type and mutant DNA is shown on the right.

i) 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 the structure of the enzyme-drug complex is also redrawn below (4 pts).

```
17   18   19   20   21   22   23   24   25   26
Ala  Gly  Pro  Thr  Ser  Arg  Lys  Ala  Ile  Glu
GCG  GGC  CCG  ACC  AGC  CGC  AAA  GCG  ATT  GAA
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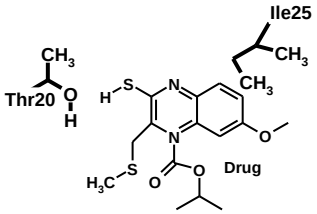
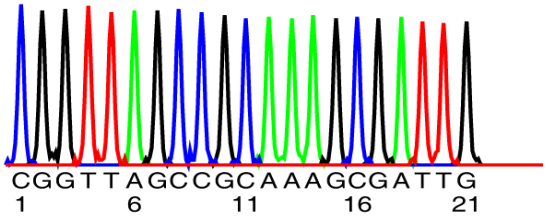


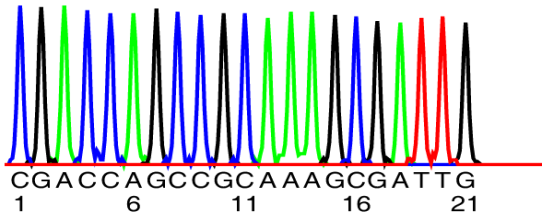
Mutant



Mutant Sequnce: CG GTT AGC
Wildtype Seque: CG ACC AGC
Reading frame Pro Thr Ser

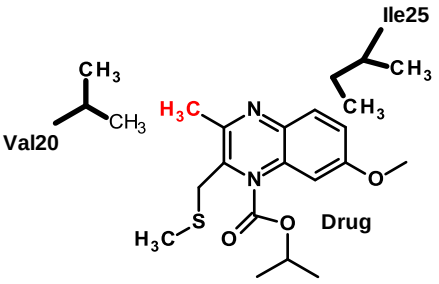
The Thr codon is change to GTT = Val.

Wildtype

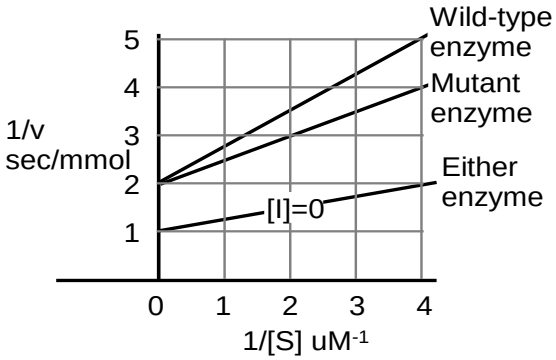


ii) Draw the structure of the changed residue, and indicate how would you **alter the drug** to increase its affinity to the mutant enzyme? (4 pts)

The sidechain of Val is drawn, showing its interaction with the drug. To increase the drug's affinity you could change the thiol (SH) group to a methyl.



iii) Steady state enzyme kinetics was performed using the wild-type and mutant enzyme in the presence of 1 nM of the inhibitor. Did the mutation increase or decrease the affinity of the drug for the free enzyme (K_I)? By how much? (6 pts)



K_I is a measure of the affinity of the drug to the free enzyme:

$$E + I \rightarrow (EI).$$

It is obtained from the ratio of the slopes of the lines in the double reciprocal plot: $K_I = [I]/(a-1)$, where a is the ratio of the slopes.

- The slope in the absence of inhibitor is 0.25.
- The slope for the mutant enzyme is 0.50, a = 0.50/0.25 =2, K_I = 1 nM/(2-1) = 1 nM.
- The slope for the wild-type enzyme is 0.75, a = 0.75/0.25 = 3, K_I = 1 nM/(3-1) =0.5 nM.

The K_I is lower for the wild-type enzyme, so the drug binds with higher affinity to the wild type. The mutation decreased the binding affinity of the drug.

iv) Please do one of the following two choices (2 pts):

Choice A: What sequencing primer was used to obtain this sequencing data?

Choice B: Briefly explain how the second peak in the data, corresponding to a G, was generated.

Choice A: GCGGGCC (underlined above). With this primer, the first base to be added would be C, followed by G, etc.

Choice B: The polymerase added a ddGTP instead of a dGTP, causing termination of the chain.

Points on Page:_____

22. (5 pts) Please do **one** of the following three choices.

Choice A: why is it advantageous to use different restriction endonucleases (e.g. EcoR1 & Bam H1) for insertion of the PCR product into the vector, rather than a single restriction endonuclease?

Choice B: how might you modify the expression vector to export the HIV reverse transcriptase out of the cell?

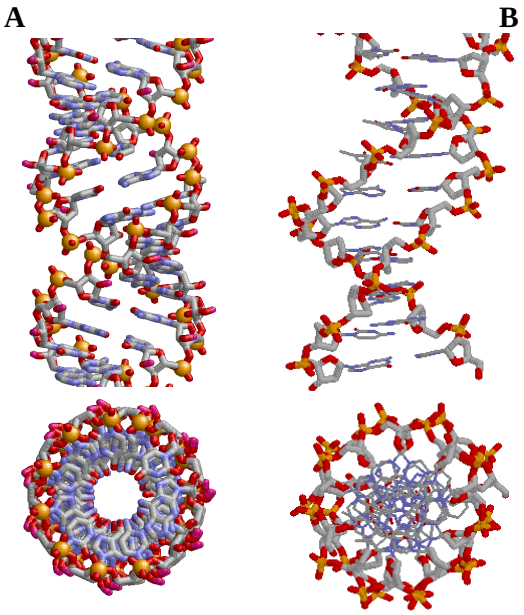
Choice C: how might you modify the expression vector to facilitate purification of the HIV reverse transcriptase by affinity chromatography of beads containing nickel ions?

Choice A: It allows control of the direction that the fragment can be inserted into the vector. If a single restriction site is used the fragment can go in both ways, only one of which will give the correct sequence in the mRNA.

Choice B: Add codons at the beginning of the gene that represent the leader sequence. The protein will have the leader sequence as part of its primary structure. This will cause the protein to be exported out of the cell, the leader sequence is cleaved off as part of this process.

Choice C: Add codons that encode six histidine at the end (or beginning) of the gene. The histidine resides can bind to nickel ions on the column material. Thus the protein with the 6 his residues is bound to column and the impurities can be washed off.

23. (4 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.



RNA is A" and DNA is "B". Any one of the following is acceptable.

- The most obvious difference is that in RNA the bases on not on the central axis while in DNA they are.
- RNA the bases are not perp to the axis, in DNA the bases are perp to axis.
- RNA had evenly spaced phosphates, on eachs side of the deep and shallow grooves, DNA has narrow phosphate spacing with the minor groove and wide phosphate spacing associated with the major groove.
- The left structure has ribose, right deoxy (difficult to see)

24. (6 pts) Please do one of the following choices:

Choice A: Explain the role of the sigma factor in RNA polymerase activity.

Choice B: Explain how indirect coupling is used to make nucleic acid polymerization (or tRNA charging) spontaneous.

Choice A: the sigma factor recognizes the -35 and -10 regions of the promoter.

Choice B: Both reactions produce pyrophosphate. This is readily hydrolyzed to two molecules of inorganic phosphate. This reaction is so favorable that it keeps the concentration of pyrophosphate well below its equilibrium concentration, making the Gibbs energy for the addition of the base negative & therefore spontaneous.

Points on Page:_____

25. (6 pts) Please do **one** of the following choices:

Choice A: Briefly describe the role of the three tRNA binding sites on the ribosome in protein synthesis?

Choice B: How is the reading frame determined by the ribosome during protein synthesis?

Choice A:

E-site: the tRNA that has just added its amino acid to the peptide chain will exit from this site.

P-site: This one holds the growing peptide chain most of the time

A-Site: At the beginning of an addition cycle the charged tRNA, with the next amino acid to be added is bound. The correct tRNA-AA combination is based on codon-anticodon interactions. Right after peptide bond formation, the entire protein is attached to the tRNA in the A-site. After protein translocation, this tRNA-peptde complex is moved to the P-site.

Choice B:

The spacing between the ribosome binding site and the start codon.

The ribosome binding site binds to the rRNA in the 30s subunit, and positions the start codon in the P-site.

Bonus (2 pts each)

B1. If you ran in the Pittsburgh marathon and hope to run in another one 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 carbs to replenish your glycogen. You cannot make very much glucose from fats and amino acids since there is no way for you (humans) to convert acetyl CoA to pyruvate.

B2. If the ribosome is an apple, then the stem is the newly synthesized peptide.

5' Base	Middle Base				3'
	T	**C**	**A**	**G**	
T	Phe	Ser	Tyr	Cys	**T**
	Phe	Ser	Tyr	Cys	**C**
	Leu	Ser	**Term**	**Term**	**A**
	Leu	Ser	**Term**	Trp	**G**
C	Leu	Pro	His	Arg	**T**
	Leu	Pro	His	Arg	**C**
	Leu	Pro	Gln	Arg	**A**
	Leu	Pro	Gln	Arg	**G**
A	Ile	Thr	Asn	Ser	**T**
	Ile	Thr	Asn	Ser	**C**
	Ile	Thr	Lys	Arg	**A**
	Met	Thr	Lys	Arg	**G**
G	Val	Ala	Asp	Gly	**T**
	Val	Ala	Asp	Gly	**C**
	Val	Ala	Glu	Gly	**A**
	Val	Ala	Glu	Gly	**G**

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