Instructions: This exam contains 260 points in 29 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. (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)

   Imidazole would be the best since the pKa of the buffer should be within on pH unit of the desired pH.

   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.

   The fraction deprotonated is f_{A-}= R/(1+R), where R=10^{pH-pKa} = $10^{7.0-6.8}$=1.58, f_{A-}=.603

   You would need to add 0.603 equivalents of base, of 0.0603 moles of base.

   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?

   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-}=.603

   The fraction deprotonated @6 is f_{A-}= R/(1+R), where R=10^{pH-pKa} = $10^{6.0-6.8}$=1.58, f_{A-}=.136

   The difference, 0.467 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, and distinguish between mainchain and sidechain atoms.

   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. (10 pts) The amino acid sequence for secondary structures that are found at the exterior of water soluble proteins have a well defined distribution, or order, of polar and non-polar residues.
   i) What is that distribution, for **both** α-helices and β-sheets. Briefly justify your answer with reference to the structure of helices and sheets (6 pts).
   ii) Which groups face the inside of the protein? Polar or non-polar? (2 pts).
   iii) How will the arrangement of polar and non-polar residues change if you were discussing an integral membrane protein? (2 pts).

i) **Helices** – The periodicity of a helix is 3.6 residues per turn, so every 3rd or 4th residue would be on the same side of the helix, these would be non-polar. (3 pts)

Sheets – the sidechains alternate up and down for every pair of residues along the stand, the first would be polar, the second non-polar, etc., making one side of the sheet polar and the other side non-polar. (3 pts)

ii) In both cases the non-polar side of the helix or sheet would face the inside of the protein. (2 pts)

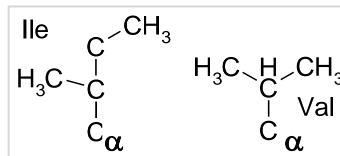
iii) The periodicity would not change, but the non-polar sides of the helix or sheet would now face the lipids. (2 pts)

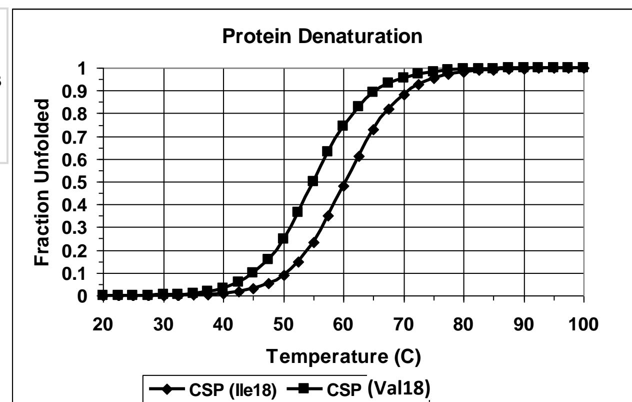
4. (8 pts)
   i) The peptide bond is fixed in one configuration, planer. Why is this so? (4 pts).
   ii) Imagine that the peptide bond is as freely rotatable as the other mainchain bonds in proteins, how would this affect protein stability? Increase or decrease it? You should justify your answer with 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" = 3 pts)

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)

5. (10 pts) The denaturation curves for wild type cold shock protein (CSP, Ile at position 18) and an Ile18 to Val substitution (mutation) 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. You should assume that the Ile is buried in the core of the protein.

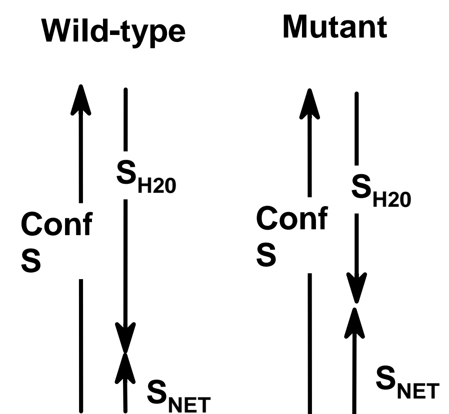




   i) How will the enthalpy of unfolding be affected by this mutation? Will it increase or decrease? Briefly justify your answer (5 pts).
   ii) How will the entropy of unfolding be affected by this mutation? Will it increase or decrease? Briefly justify your answer (5 pts).

i) 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.

ii) 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.



6. (14 pts) Select one of the following enzymes: Trypsin, Chymotrypsin, Elastase, HIV protease, or the Potassium channel 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 interacts with Lys and Arg + charge.	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.
The lower the energy of the transition state, the more of it, the faster the rate. (1 pt)

7. (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 useful in the regulation of protein expression from expression vectors.

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.

8. (12 pts) What is the hydrophobic effect and what is its role in protein folding, lipid bilayer formation, and the stability of double stranded DNA?
    - Reduction in the entropy of water on exposure of non-polar groups to water (3 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).
    - DNA – has very little effect on the overall stability of DNA (3 pts).

9. (12 pts) The phosphate group on DNA has a pK_a of 1.0.
   i) What is the charge on DNA at pH 7.0? (1 pt)
   ii) Sketch the titration curve for a segment of double stranded DNA that is 10 nucleotides long. Be sure to indicate the number of equivalents on the x-axis. (5 pts)
   iii) Sketch a graph of the T_M for double stranded DNA as a function of pH. Justify your answer with a discussion which molecular force/interaction would be most affected by changing the pH. (6 pts)

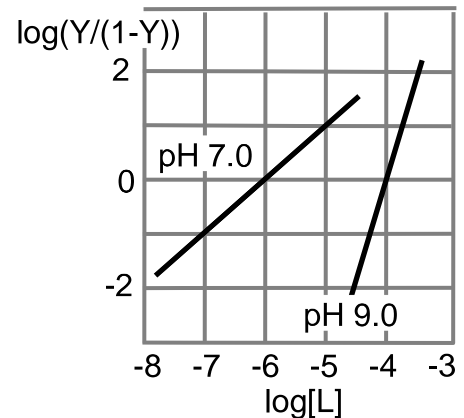
i) One negative charge/residue, so overall negative. (1 pt)

ii) There at 20 phosphate groups, so the x-axis should go from 0 to 20 equivalents (10 is also an acceptable answer). The infection point will be at a pH=1.0. (2 pts for labeled axis, 3 pts for curve)

iii) As the pH is lowered, the phosphates will be come protonated. This will stabilize the DNA due to neutralization of the repulsive forces between the phosphates on each strand. Thus TM will increase as the pH is lowered. The curve will be similar in shape to the fraction protonated curve. (6 pts)

Alternate answer: The increase in protons will shield the charges on the phosphate, decreasing electrostatic repulsion, raising Tm. (6 pts)

10. (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? (3 pts)
   iv) Explain the effect of pH on cooperativity by postulating the nature of the interaction between the individual proteins while bound to the DNA (2 pts).

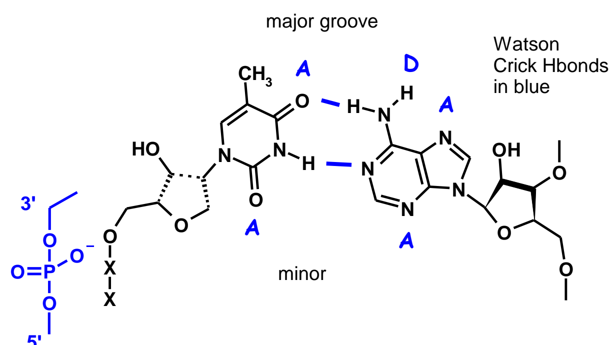
i) Kd is 10^{-6} M. Point were curve crosses x-axis is log Kd.

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.

iv) 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 (2 pts for a sensible model).

11. (9 pts) A TA basepair in DNA shown on the right. The "T" is the first base in a longer sequence, i.e. TXXXX.
    i) Although the relative position of the TA bases is correct, the diagram contains at least three errors. Identify and correct three of these errors (3 pts).
    ii) Sketch the phosphate linkage that you would observe linking the T to the next base (2 pts)
    ii) Indicate the "Watson-Crick' hydrogen bonds involved in AT basepairing (1 pt).
    iii) Indicate the major and minor groove (1 pt).
    iv) Label all hydrogen bond donor and acceptors in both grooves (2 pts)



i) Errors are:
   a) T (left base) is attached to 2' carbon, not 1' carbon
   b) The T is at the 3' end, it should be at the 5' end.
   c) The ribose on the A should be deoxy at position 2.
ii) Should be –O-P(OO)-O-, as shown on the diagram on the right.
ii) Between the C=O on T and NH2 on A, between NH on T and N on A
iii) Major groove is on top, minor on the bottom.
iv) Two acceptors in minor groove, A, D, A in major groove.

12. (10 pts) A protein binds to the following DNA sequence with a K_D of 1 nM

TTTTTT
AAAAAA

and it binds with a lower affinity to

AAATAA
TTTATT

   i) Is this protein likely a homodimer or a heterodimer? Why? (2 pts)
   ii) Is this protein binding via the major or the minor grove? Justify your answer (5 pts).
   iii) Estimate the affinity for binding to TTTATT. Be sure to state any assumptions that you make (e.g. the strength of any bonds) in your answer. Assume T=300K. (3 pts)

i) Heterodimer. A homodimer would recognize the same sequence on both strands. This protein recognizes Ts on the top strand, As on the bottom.
ii) Major groove. It is not possible to distinguish a T from an A using minor groove binding because there two H-bond acceptors are in the same location for both a TA and an AT basepair.
iii) $\Delta G° = -RT \ln K_{EQ} = -8.3 \times 300 \ln 10^9 = -57{,}300$ J/mol = -57 kJ/mol. There are three H bonds in the major groove, assuming all three cannot be made when the TA is switched to AT, the standard energy will drop 15 kJ/mol (5 kJ/hbond), giving: $\Delta G° = -42$ kJ/mol. Keq = 2.1×10^{7}.
(Full credit for correct approach, -1 if H bond strength is stated to be 20 kJ/mol)

13. (8 pts) Does the T_M increase or decrease with respect to GC content? Why?

Increases (3 pts) due to:

i) 3 hydrogen bonds instead of two (3 pts)

ii) Stronger van der Waals interactions between GC base pairs (2 pts)

14. (8 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).

iii) What do most DNA polymerases do if they do make an error and incorporate the wrong base? (2 pts)

i) Factor are:

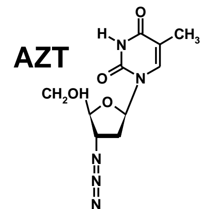
a) Watson-Crick hydrogen bonds, A pairs with T, G with C.

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.

iii) The remove it, using their 3'->5' exonuclease activity.

15. (5 pts) The drug ATZ is shown on the right. This drug is a competitive 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?



This is a substrate for reverse transcriptase - it can replace T in the polymerization reaction.

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.

Note: The next questions (16-19) are related.

16. (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
CGCGATGTATGTTCAT---GCGGGCCCGACCAGCCGCAAAGCGATTGAA---AGTAGTTACTTTTAA
GCGCTACATACAAGTA---CGCCCGGGCTGGTCGGCGTTTCGCTAACTT---TCATCAATGAAAATT
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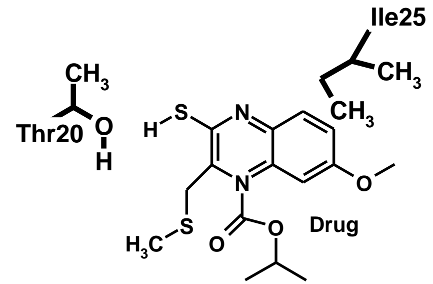


i) What thermodynamic forces or interactions are responsible for the stabilizing the bound form of this drug. Justify your answer with reference to functional groups on both the drug and the enzyme (6 pts).

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)

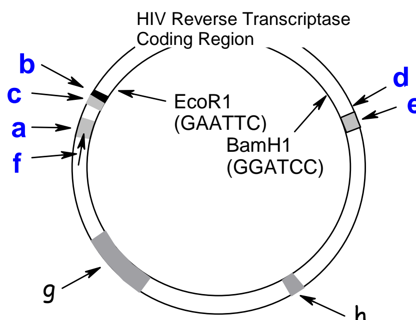
17. (23 pts) A mutation has arisen in the gene for reverse transcriptase that has reduced the drug binding, producing a drug resistant strain.

i) 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 a bacterial lysate (6 pts).

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)

ii) 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 intra-cellularly. 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

(Note: this question continues on the next page).

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

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.

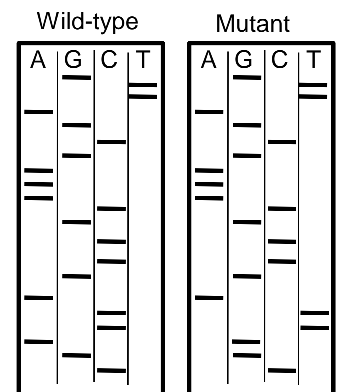
h) origin of replication. Allows plasmid to be replicated in bacteria.

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 and BamH1 sites. You need not worry about the annealing temperature; make your primers 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).

- Since the vector has both a start codon and a stop codon, they can be omitted from the PCR product (& primers).
- The left primer has to generate a Eco R1 site on the PCR product. The remainder of its sequence is identical to the top strand of the gene, so the complete sequence is: 5′ GAATTCTATGTT
- The right primer has to generate a Bam H1 site on the right end of the PCR product. The remainder of its sequence identical to the bottom strand of the gene, so the complete sequence is: 5′ GGATCCAAAGTA

v) How could you use gel electrophoresis & restriction digests to confirm that your final expression vector was correct? (4 pts)

Digest the plasmid with EcoR1 and Bam and run the digest on a gel. You should have two fragments, on the size of the original plasmid (not stated in the problem), and the second fragment the size of the reverse transcriptase gene, about 600 bases.



18. (14 pts) A section of the sequencing gel of the 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 (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
		CG	GTT	AGC	CGC	AAA	GCG	ATT	G

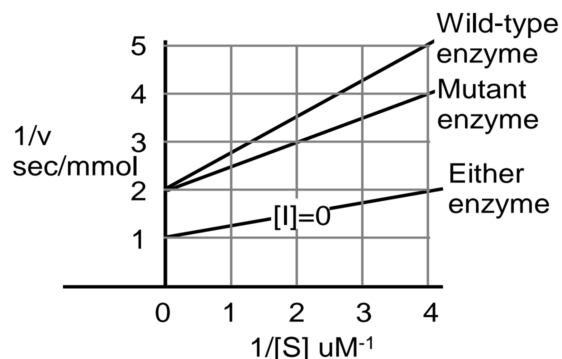
The sequence of the mutant was read from the bottom of the gel, giving the sequence in the 5′ ->3′ direction. It was matched to the wild-type sequence, this indicates that the first two bases on the gel correspond to the last two bases of the codon for Pro19. The sequence is identical except for Thr20. Its codon has been changed to GTT, which encodes valine, i.e. the mutation has replaced Thr20 with Val20.

ii) How would you alter the drug to increase its affinity to the mutant enzyme? (4 pts)

The –OH on serine has been replaced by a $-CH_3$ group. The $-CH_3$ group cannot form an H-bond with the SH group on the drug. The SH group could be replaced by a methyl, giving a good hydrophobic and vdw interaction with the valine residue in the mutant enzyme.

(Note: this question continues on the next page).

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:

It is obtained from the ratio of the slopes of the lines in the double reciprocal plot: $K_I = [I]/(\alpha-1)$, where α 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, α = 0.50/0.25 =2, K_I = 1 nM/(2-1) = 1 nM.
- The slope for the wild-type enzyme is 0.75, α = 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.

19. (5 pts) Please do **one** of the following three choices.

Choice A: why is it advantageous to use different restriction endonucleases (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.

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.

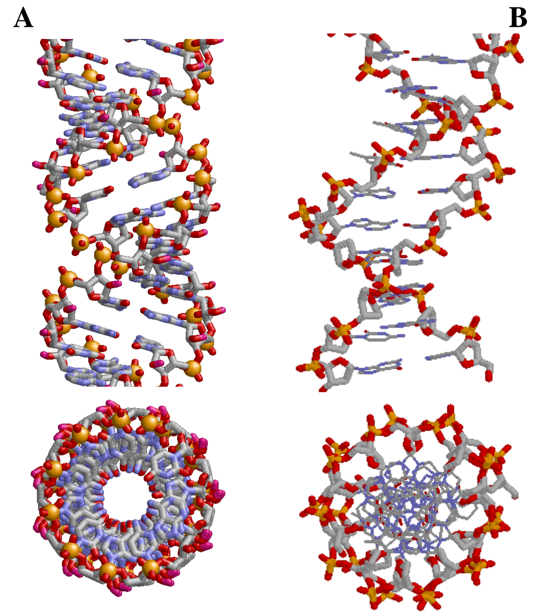
Choice B: Add codons at the beginning of the gene that represent the leader sequence. The protein will have the leader sequence as part of its primary structure. This will cause the protein to be exported out of the cell, the leader sequence is cleaved off as part of this process.

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.

20. (3 pts) Although expression of proteins in bacteria has many advantages, it is not the method of choice for proteins that are to be used as drugs, such as insulin or human growth hormone. Why?

Bacterially expressed proteins can contain toxic impurities (that are difficult to remove). Thus they can harm the individual if administered.

21. (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.



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 deep and shallow grooves, DNA has narrow minor groove and wide major groove.
- The left structure has ribose, right deoxy)

(+3 pts is students use U/T.)

22. (6 pts) 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?

The third base of the codon can form non-Watson Crick H-bonds with the first base of the anticodon. This is called a wobble base pair. So a tRNA can recognize at least two codons, one with a normal W-C basepair at the third position and another codon using wobble basepairing.

23. (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 C: Explain how indirect coupling is used to make nucleic acid polymerization and tRNA charging spontaneous.

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

Choice B: The first amino acid/tRNA sits in the "P" site that usually binds the growing peptide chain. The P-site interacts with the peptide bond. The N-formyl groups mimics the peptide bond.

Choice C: 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.

24. (6 pts) Glycogen and cellulose are composed of the same monomeric unit. Draw the structure of that monosaccharide, give its name, and indicate how it is linked together in **either** glycogen or cellulose.

Glucose – 6 membered ring with C1 the anomeric. C2 down, C3 up, C4 down. (3 pts)

Glycogen – linear chains linked a(1-4) with a(1-6) branches. (3 pts)

OR

Cellulose – linear chains linked β(1-4). (3 pts)

25. (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 CO_2 released Pyr-Acetyl CoA, 2 CO_2 in TCA cycle. (4 pts).

Energy: Organic oxidations produce NADH and $FADH_2$, 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)

26. (12 pts) Please do **one** of the following two choices:

Choice A: You haven't eaten in a while but 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? 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).

25. (6 pts) The concentration of potassium ions outside a membrane is 0.1 M and the concentration inside is 0.05 M. The membrane potential is +100 mV, with the inside positive. What direction will the potassium ions flow? Briefly justify your answer (assume T=300K).

Assume direction of reaction is from outside to inside.

$$\Delta G = RTln\frac{[K^+_{IN}]}{[K^+_{OUT}]} + NF\Delta V$$
$$= 8.3 \times 300\, ln\frac{0.05M}{0.1M} + (+1) \times 96000 \times 0.1$$
$$= -1{,}725 + 9{,}600$$
$$= +7{,}875\, J/mole$$

Since the Gibbs free energy is positive, the reverse direction is spontaneous; the ions will flow from the inside to the outside.

28. (2 pts) Define specific activity as it relates to protein purification.

It is the ratio of the activity of the target protein (the one that you are trying to purify) to the total mass of protein in the sample (1 pt).

After a successful purification step, the total protein mass should decrease, thus the specific activity should increase (1 pt)

29. (7 pts) You are measuring the binding of a DNA fragment to a protein. The off-rate of the protein from the DNA is very, very, slow such that it is possible to separate the free protein and DNA from the protein-DNA complex using standard purification schemes.

i) Describe how you might separate the three different components, i.e. the free DNA, free protein, and the protein DNA-complex. (5 pts)

ii) Explain how you could measure the fractional saturation using UV absorbance of your three separated samples. You should give a clear explanation of how you would obtain the relevant extinction coefficients (2 pts).

Gel Filtration: If the DNA is long enough the size of the Protein DNA complex would be larger than the protein alone, and the DNA would be smaller. Thus the protein-DNA complex would elute first, then the protein, and then the complex.

Cation Exchange: Assume that the protein is positively charged, then the protein-DNA complex will have less of a positive charge, and the DNA will be negatively charged. The order of elution would be DNA, Protein-DNA, and then protein alone.

Anion Exchange: Order of elution would be Protein, Protein-DNA, and then DNA.

The concentration of the free protein and free DNA can be obtained using Beer's law: $A=C\varepsilon l$. The extinction coefficient, ε, is obtained by summing the extinction coefficients of the individual monomers (Tyr, Trp, Phe for the protein; A, G, C, T for the DNA). (+2 pts for a correct description of how to get ε). Since you know the total protein and total DNA, you can then calculate the amount of the complex, and then calculate the fractional saturation.

Bonus (4 pts) 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.