Section A:

1. A hydrophobic compound would dissolve easily in
   a**) hexane.**
   b) water.
   c) methanol.
   d) ethanol

2. Which of the following groups participate as hydrogen bond donors?
   a)NH.
   b)OH.
   c)All of the above.
   d)None of the above.

3. The isoelectric point of a protein is defined as
   a) the pH when there is no mobility in an electric field.
   b) the pH where the carboxyl group is uncharged.
   c) the pH where the amino group is uncharged.
   d) the pH of maximum electrolytic mobility.

4. Which of the following 'forces' is unfavorable for both protein and RNA folding?
   a) Conformational Entropy.
   b) Hydrophobic Interactions.
   c) Van der Waals Interactions.
   d) Hydrogen Bonds

5. Disulfide bonds most often stabilize the native structure of:
   a) extracellular proteins.
   b) dimeric proteins.
   c) hydrophobic proteins.
   d) intracellular proteins.

6. Positive cooperative binding can be identified by
   a) a non-hyperbolic binding curve.
   b) a Hill plot with a slope less than one.
   c) a Hill plot with a slope greater than one.
   e) part a) and c).

7. In comparing Myoglobin and Cytochrome C, which of the following statement is true?
   a) One contains iron, the other magnesium.
   b) One is α-helical, the other is β-sheet.
   c) One transports oxygen, the other electrons.
   d) One can be found in humans, the other only in bacteria.

8. The nucleophile in serine proteases is ___ and it is activated by ___.
   a) Ser, Ser
   b) Ser, His
   c) Water, His
   d) His, Ser

9. The pK_a of an ionizable side chain, such as imidazole in histidine, can be determined with NMR because
   a) the pK_a is near neutrality.
   b) protonated imidazole has twice the number of protons.
   c) unprotonated imidazole is invisible in the spectrum.
   d) the chemical shift of His differs from His^+.

10. The membrane proteins that catalyze active transport reactions differ from soluble enzymes in that
   a) they do not enhance the rates of reaction.
   b) the product(s) of the reaction move in a specific direction.
   c) the substrate(s) of the reaction are all outside the cell.
   e) they are permanently changed during the reaction.

11. Non-alchoholic beer is obtained by
   a) fermenting yeast under anaerobic conditions.
   b) fermenting yeast under aerobic conditions.
   c) adding pyruvate to the yeast growth media.
   d) adding acetic acid to the yeast growth media..

12. DNA differs from RNA in the following features
   a) DNA residues are linked by 3'-->;5' phosphodiester bonds; RNA is 2'-->5' linked.
   b) DNA has deoxyribose residues; RNA has ribose residues.
   c) DNA contains the A, C, G and T bases; RNA contains A, C, G, and U.
   d) All but the first choice are correct differences.

13. The force, or energetic term, that is most stabilizing for nucleic acids is:
   a) hydrogen bonds
   b) electrostatics
   c) van der Waals (base stacking)
   d) conformational entropy

14. DNA Gel Electrophoresis is similar to SDS-PAGE of proteins because
   a) In both cases a plot of log(Molecular weight) versus distance migrated is linear.
   b) Both techniques rely on a constant charge to mass ratio.

c) Both techniques utilize the sieving properties of gels.
d) All of the above are correct.

15. The rapid appearance of HIV-1 strains that are resistant to AID drugs is due in part to this property of its reverse transcriptase:
a) it requires a sigma factor, which is normally not present in HIV infected cells
b) it lacks a 5'→3' exonuclease.
c) it has low affinity for the correct dNTP's.
d) it lacks a 3'→5' exonuclease.

16. The differences between polI and polIII are:
a) one has a 3'→5' exonuclease activity, the other does not.
b) one has a 5'→3' exonuclease activity, the other does not.
c) one utilizes RNA primers the other does not.
d) PolI and PolIII are different names that describe the same enzyme.

17. During replication, overwinding or overtightening of DNA is caused by ____ and removed by ____:
a) DnaA, Gyrase
b) DnaA, Single Stranded Binding Protein
c) DnaB, Gyrase
d) DnaB, Hu protein

18. Which of the following conditions would cause the release of the Lac repressor protein from the lac operator site on DNA?
a) Presence of glucose in the growth media.
b) Presence of arabinose in the growth media.
c) Presence of IPTG in the growth media.
d) Presence of ribose in the growth media

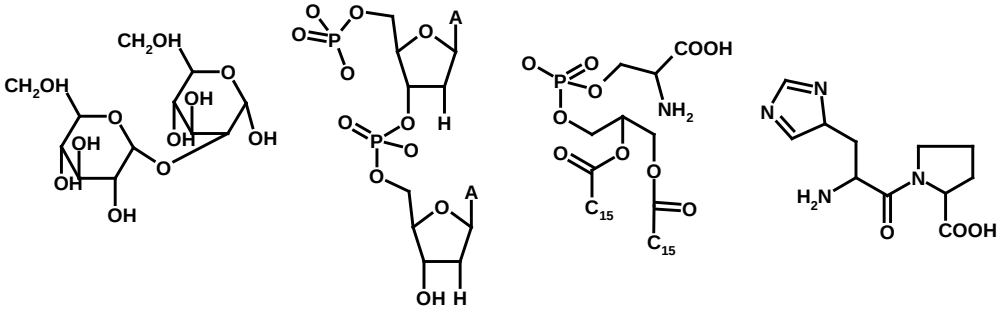
19. Loss of the 3'CCA from a tRNA molecule would
a) cause it to unfold, making it non-functional.
b) prevent the tRNA from binding to the mRNA.
c) prevent charging of the tRNA with its amino acid.
d) prevent the charged tRNA from binding to the 50S subunit.

20. Removal of the signal peptide from a protein that is translocated across a membrane is accomplished by
a) Trypsin
b) fMet aminopeptidase
c) HIV protease
d) None of the above

Part B: Please do All of the following Problems.

B1(6 pts): Draw the molecular structure for **TWO of the following four** molecules:

i) O-α-D-glucopyranosyl α(1-2) glucopyranose.
ii) pdAA (Specific details about the adenine base are not required, the general structure of a purine will suffice)
iii)dipalmytoylphosphatidylserine
iv) Histidine-Proline



B2 (10 pts): Discuss two key features of enzyme catalyzed reactions that are commonly found in all enzymes. Use any of the following enzymes as a source of examples to support your discussion.
i) Trypsin
ii) HIV protease
iii) Lysozyme

1. Stabilization of the transition state by directly forming bonds with the transition state. This occurs in trypsin, two hydrogen bonds are formed between mainchain amides in trypsin and the oxy-anion in the tetrahydral intermediate. Enzymes also enhance reaction rates by holding chemically reactive amino acid sidechains in the correct position for chemistry. This reduces the entropy that would be required to organize the active site

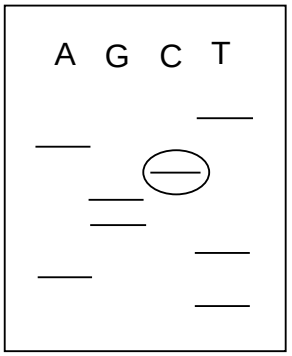
residues around the substrate. This occurs for any enzyme. In the case of HIV protease, two Asp residues (Asp25 and Asp25') are held close to the bond to be cleaved.

2. Enzymes are specific for their substrates. For example, l,ysozyme cleaves after N-acetylglucosamine residues due to the formation of specific hydrogen bonds between the N-acetyl group on the NAG residue and amino acid sidechains in the protein. Trypsin cuts after Lys and Arg because it contains a negatively charged Asp that interacts with the positive charges on these substrates. Hiv Protease interacts with hydrophobic substrates via Val82 in its active site.

B3:(11 pts)

The following is a short segment of DNA that is used as a template in a DNA sequence reaction:

GGCCGGATACCGTA



i) You first attempt to obtain the sequence of this fragment using the following primer: CCGGCC. However, there was no polymerase activity because the primer did not hydrogen bond to the template. How might you adjust the solution conditions of your reaction to increase the binding to the primer to the template? Briefly justify your answer (3 pts)

Lower the temperature below Tm, this would make the ΔG^o for the annealing of the primer to the template favourable. Add more salt, e.g. NaCl. This would raise the Tm because it would reduce the unfavourable electrostatic repulsion between the primer and the template.

ii) Assuming that you were successful at getting this primer to work, sketch the DNA sequencing gel that you would expect to obtain from this sample. Please draw your gel in the space provided to the right (3 pts)

The template-primer has the following structure:

CCGGCC

GGCCGGATACCGTA

Therefore the sequence should read 5'-TATGGCAT-3', beginning at the bottom of the gel

iii) Pick one of the bands on your gel (*circle it please*) and answer the following:

a) What components were in the reaction to produce the DNA samples in this particular lane of the gel.(3 pts)

dATP, dGTP, dCTP, dTTP, dideoxyCTP, DNA polymerase, primer, template.

b) Give the sequence and nature of the 3' end of the band you circled.(2 pts)

5'-CCGGCCTATGGC-3', ending with a 3' hydrogen, not hydroxyl.

B4 (8 pts): Alter the following statements to make them correct. If you alter the word 'all' to 'most' provide an example of the exception [Note: there is more than one correct answer]

i) Only proteins can catalyze enzymatic reactions.

Protein and RNA can catalyze enzymatic reactions.

ii) Oxygen is used by all organisms in oxidative phosphorylation.

Oxygen is used by most organisms in oxidative phosphorylation. Some organism can use other electron acceptors in oxidative phosphorylation.

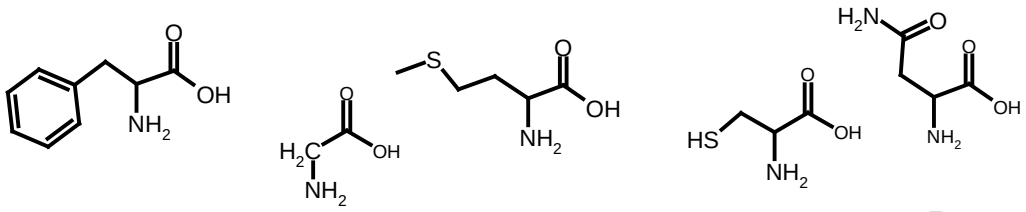
iii) Electron transport is used to generate a proton gradient in all organisms.

Electron transport is used to generate a proton gradient across a sealed membrane in all organisms.

iv) All proteins have a uniform non-polar exterior surface.

All integral membrane proteins have a uniform non-polar exterior surface.

B5: (10 pts) The following are the structure of five amino acids.:



A Phe

B Asn

C Met

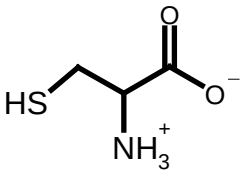
D Cys

E Gln

i) Write, underneath **two(2)** of the structures, the name of the amino acid (three letter code is quite acceptable)(2 pts)

ii) Pick one of the above (*indicate your choice*), and write the correct ionic form of the amino acid that you would expect to find at pH 7.0.(2 pts).

With the exception of Cys, none of these have an ionizable sidechain. The structure of Cys at pH7.0 is shown to the right. The -SH group is 90% protonated (pKa=8.0). All amino acids have a charged amino and carboxy terminus at this pH.



Cys

iii) For the following statements, write the letter of the amino acid to which the statement best applies. Note, you may want to use the same amino acid more than once. If two (or more) are equally acceptable, write all of them. In some cases none of these may apply. If so, just write 'none'.(1 pt each)

a) Sidechain is hydrophobic and found buried in the core of protein: ___A___

b) Sidechain absorbs UV light strongly: __A____

c) Sidechain found at active sites of some enzymes: __D, E__

d) Sidechain can form disulfide bonds : __D____

e) Sidechain can form hydrogen bonds with nucleotide bases: __B, E__

f) A modified form of this amino acid is the 1st one used by the ribosome ___C___

B6. (9 pts) Compare and contrast the role and importance of hydrophobic interactions in protein folding, the formation of biological membranes, and the formation of double stranded DNA from single strands.

In all cases hydrophobic interaction drive the burial of non-polar groups because the ordered water that is bound to the non-polar groups is released, greatly increasing the entropy of the system. In order of importance:

lipid bilayers> protein folding > DNA.

This order is based roughly on the non-polar surface area that is buried during assembly of the structure.

B7 (10 pts). A new bacteria has been discovered whose ATP synthase can generate one ATP from the pumping of a single proton (clearly a hypothetical protein). Please answer the following questions.

i) This organism maintains a pH gradient across its membrane of 2 units. Calculate the free energy stored in this gradient, assuming a membrane potential of 0 volts. Is this free energy sufficient to synthesize ATP (ΔG^0 for synthesis is +30 kJ/mol) (4 pts)

$\Delta G = -RT\ln[100/1] = -2.5$ kJ/mol (2.3) (2) = -11.5 kJ/mol at 300 K. No, this is insufficient to generate an ATP since 11.5 < 30.

ii) To insure that the free energy associated with the transfer of a proton is sufficient to generate ATP this organism pumps Cl^- ions into the cell to generate a voltage difference that is sufficiently large such that one ATP can be produced by the transfer of one proton. Calculate the minimum voltage required to do so.(4 pts)

An additional 19 kJ/mol is required, $\Delta G = Z F \Delta\Psi$, $\Delta\Psi$=19/96 = 0.2 volts (inside positive)

iii) Can the organism achieve the same goal (increasing the free energy of the proton gradient) by pumping Na^+ ions out of the cell? Briefly justify your answer.(2 pts)

Yes, this would place a + voltage outside the cell, essentially raising the energy of the protons due to the repulsion of like charges, such that additional energy would be produced when the protons went from the outside to the inside.

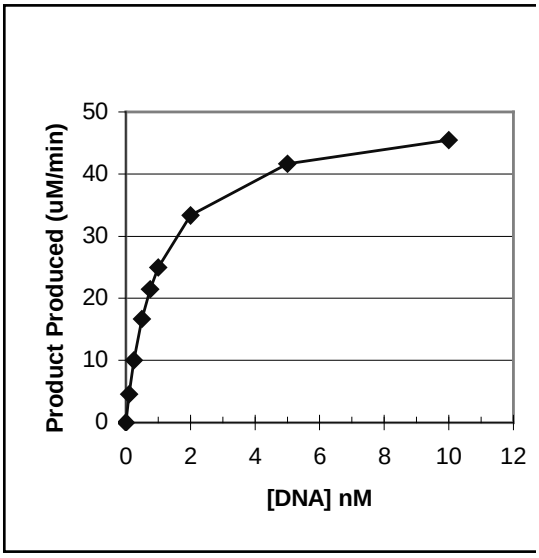
B8:(20 pts) Enzyme Kinetics and Protein-DNA Interactions.

i) The binding of a double stranded oligonucleotide to BamHI was measured using equilibrium dialysis. The sequence of the oligonucleotide is CGGATCCG.

[DNA]	Y (fractional Saturation)
0.1 nM	0.1
1.0 nM	0.5
10 nM	0.9

What is the dissociation constant (K_D) for this oligonucleotide to BamHI? Briefly explain your approach. (Hint: a Scatchard plot is not necessary) (2 pts).
When Y=0.5 the [L]=K_D. This occurs at [DNA]=1 nM, so K_D=1 nM

ii) The plot above shows the initial velocity of BamHI activity as a function of DNA concentration (in nM). The same DNA was used for this reaction as for the binding studies.



a) Draw the structure of the product of this reaction (Hint, this enzyme makes sticky ends that are 4 bases long) (2 pts)

```
CGGATCCG    ----> Product   CG        GATCCG
GCCTAGGC                    GCCTAG      GC
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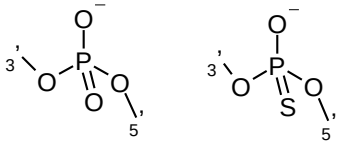
b) Estimate K_M from this graph. You may assume V_{MAX} is 50 uM/min. Briefly explain your approach.(3 pts)

K_M is the substrate concentration that gives $V_{MAX}/2.0$, in this case K_M=1nM because the reaction velocity = 25 uM/min at that substrate concentration.

c) Describe how you would obtain the K_M for this enzyme using a Lineweaver-Burk (double reciprocal) plot. Sketch the actual plot in the space provided in the lower right. Don't be concerned about the units on the axes.(3 pts)

1. Plot 1/V versus 1/S
2. Y-intercept gives 1/Vmax.
3. Slope gives K_M/V_{MAx}. Since V_{Max} is know from step 2, K_M can be calculated by multiplying the slope by $V_{MAX/}$

d) Normally DNA contains a phosphate ester joining the individual bases. If an oxygen in the phosphate ester is replaced by sulfur (see figure to the right), it is very difficult for restriction enzymes to hydrolyze the phosphate ester, thus DNA containing phosphosulfur linkages is an inhibitor.



If the same DNA sequence that was used in the binding and enzyme kinetics studies was synthesized with phosphosulfur bonds and used as an inhibitor in the above enzymatic reaction how would the enzyme kinetics change (i.e. what type of inhibitor is this compound?)(2pts).

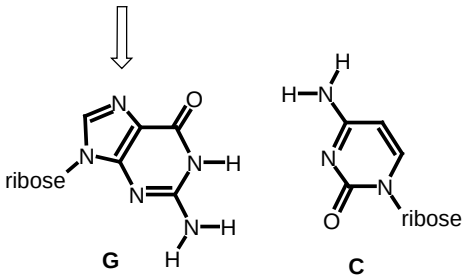
This would act as a competitive inhibitor, because the phosphsulfur containing DNA would bind in the active site.

f) Sketch the expected reaction velocity data you would expect to obtain on the same double reciprocal plot as you used for part c. You should assume that the concentration of the inhibitor DNA is 1nM in this enzymatic reaction. It is *very* important that you state, in the space below, your assumptions regarding how you estimated K_I for this inhibitor. Specifically, do you expect to observe a difference between the binding

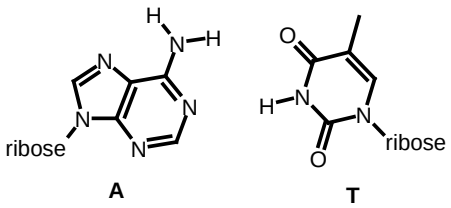
of the enzyme to the inhibitor versus its normal substrate? If so, how would you estimate that difference. If you do not expect a difference, why not? (4 pts).

Since the inhibitor has all of the same features as the true substrate, e.g. bases for BamH1 to recognize, negative charges on the backbone, etc, the binding of both DNA molecules should be the same. If K_I = 1 nM, and if [sDNA]=1nM in the reaction, then $\alpha=1+[I]/K_I = 2$. The slope of the double reciprocal plot would increase by 2 since α=ratio of slopes.

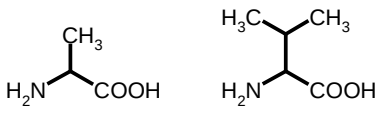
f) This enzyme forms a hydrogen bond with the second G in the recognition sequence (G**G**ATCC). However, replacement of this G with an A does not affect the binding affinity of the DNA to the enzyme. Based on the structures of an AT base pair versus a GC base pair (both shown to the right), indicate the most likely hydrogen bond between the enzyme and the G base (you can simply draw your answer on the sketch to the left. Provide a brief justification of your answer in the space below.(4 pts).



The enzyme must recognize a common feature of both A and G bases. Thus it must form hydrogen bonds with the -N- in the major groove. This atom is indicated by an arrow in the diagram. Note that recognition occurs in the major groove, not the minor groove.



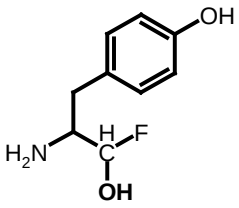
B9 (Choice!). (4 pts)How does the cell avoid charging $tRNA^{Val}$ with Ala? Give a *plausible* description of the active site of the enzyme that charges $tRNA^{Val}$ (i.e. indicate the amino acids that might be present in the active site of the enzyme). Your description should discuss *both* activities present in the active site (hint: hydrolytic editing). The structures of Ala and Val are given to the right.



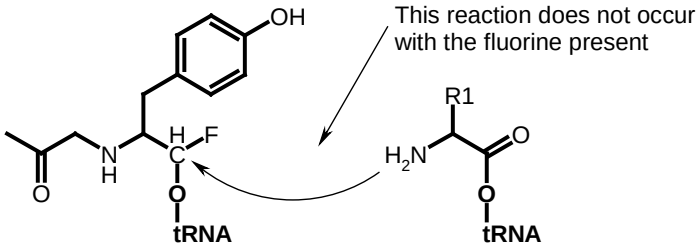
Both Ala and Val could be added to $tRNA^{Val}$, although the alanine may not bind as well. The second step of the reaction involves hydrolytic editing. the Ala-$tRNA^{Val}$ would fit into the editing site, and will be come cleaved. The Val-$tRNA^{Val}$ must be too large to fit into this site and thus remains attached to its tRNA [This is called hydroltic editing and was not covered in 2003]

OR

The organic compound shown on the right can be used to charge $tRNA^{Tyr}$ by formation of a bond between the **OH** group and the 3'OH on the adenine of the CCA acceptor stem. However, when this charged tRNA is incorporated into the growing polypeptide chain on the ribosome it terminates elongation of the chain. Based on your knowledge of peptide bond formation in the ribosome, how does this compound cause termination?



During elongation, the peptide bond formation occurs when the amino group of the incoming amino acid attacks the ester of the peptidyl-tRNA in the P-site. Once the compound is incorporated, the fluorine atom prevents hydrolysis of the ester:



B10. (15 pts) On one of the problem sets you were asked to suggest alterations to lysozyme that would allow it to hydrolyze cellulose. The only way to test whether your suggestions are correct is to produce recombinant lysozyme containing your amino acid change(s) in bacteria and then to test the resultant enzymatic properties.

i) Briefly describe the role of the following three enzymes in the process of constructing the expression vector:(3 pts)

- Restriction endonucleases
  These would be used to cut DNA at specific sites and the cohesive, or sticky ends, would allow a DNA fragment containing lysozyme to pair with a DNA fragment from the vector (cut with the same enzymes, or enzymes that give the same sticky ends) via normal Watson-Crick hydrogen bonds. These fragments would then be joined using DNA ligase.
- Reverse transcriptase
  If the original source of genetic information was RNA, then reverse transcriptase would be used to copy the RNA to DNA in the first step of generating a double stranded DNA.

- DNA ligase.

Uses ATP to join basepaired DNA together by reformation of phosphodiester bond.

ii) Briefly discuss how you would decide on the length of the radioactive probe that you would use to screen the cDNA library.(2 pts)

It must be long enough such that the probablility of obtaining the same sequence by change is quite small. Specifically, $(1/4)^n \times l << 1$, where n is the length of the probe and l is the size of the genome.

iii) For 4 of the following 8 control elements, discuss the role of each of these elements in the expression of the recombinant lysozyme. Sketch a diagram showing the relative location on the plasmid DNA of the elements you elected to discuss.(8pts)

1. Ribosome binding site (SD Sequence)
   Must be to the 5' end of the start codon - binds mRNA to 30S subunit during initiation, positions start codon for initiation in correct reading frame.
2. Signal Peptide
   Must be part of the amino terminal sequence of the protein to be exported. The peptide sequence cause export of the protein across the membrane and out of the cell. The signal peptide is cleaved by signal peptidase during the export process.
3. -35 and -10 region
   These are regions of the promoter, to which RNA polymerase binds. These must be 5' to the ribosome binding site such that the RBS is included in the mRNA.
4. lac repressor binding site (lac operator)
   Must be between the promoter and the structural gene. Is an on/off switch for RNA polymerase function. Addition of IPTG causes lac repressor to fall off of the DNA, permitting the synthesis of mRNA.
5. AUG codon
   This is the start codon, coding for the first amino acid to be incorporated into the protein. When protein systhesis occurs the fMet-tRNA is loaded into the P-site in the initiation complex.
6. Origin of DNA replication
   This allows the plasmid to be replicated within the cell, it can be anywhere on the plasmid.
7. Termination codon
   This signals the end of the protein, causing release factors to bind to the ribosome. The release factors hydrolyze the peptide, releasing it from the ribosome.
8. Antibiotic resistance marker
   Allows for selection of the plasmid within bacterial cells. Only those cells with the plasmid will be resistant to the antibiotics.

iv) Briefly describe how you would purify this recombinant protein from bacteria *in a single step* (2pts).

Make an affinity column with N-acetylglucosamine on it, lysozyme will bind to NAG, elute by the addition of free NAG.

B11: (12 pts)

Select **ONE of the three** polymer synthesis reactions that were discussed in the course (DNA synthesis, RNA synthesis, Protein synthesis) and discuss *all* of the following aspects of the reaction.

i) Initiation of synthesis (e.g. what signals are present that indicate the start of synthesis, what accessory proteins are required, etc.)

mRNA Synthesis	Protein Synthesis	DNA Synthesis
• RNA polymerase binds to promoter, recognizing DNA sequences at -35 and -10, forming closed complex. • Open complex forms.	• mRNA binds to 30S via interation between rRNA and SD sequence on mRNA. • tRNA-fMet loaded into P-site • 50S subunit binds to form the initation complex.	• DNA A and DNA C bind to origin of replication. • DNA B (Helicase) opens up strands • DNA prevented from reannealing by coating with single stranded binding protein. • Two RNA primers are added to the DNA strands

ii) Propagation of synthesis (e.g. what enzymes are involved, what is used as the template for information, what is the biochemistry of chain extension)

The key here is: template directed polymer synthesis.

mRNA Synthesis	Protein Synthesis	DNA Synthesis
• RNA polymerase reads the template strand, incorporating NTP (e.g. ribonucleotides) based on Watson-Crick base pairing with template. • No primer is needed.	• Next aa is bond to A-site by virtue of the interaction between the anticodon loop on the tRNA and the mRNA. • Peptide bond formation occurs, moving the peptide, now one unit longer, over to the A-site. • Translocation of the ribosome occurs, moving the peptide back to the P-site. Note that it is still attached to tRNA. • The uncharged tRNA is in the E site and leaves the ribosome.	• On the leading strand, pol III initiates synthesis from the RNA primer, continueous synthesis in the 5'-3' direction. Lagging strand synthesis: • Pol III lays down DNA until previous RNA primer is reached. • Pol I removes RNA primer • Pol I completes DNA synthesis • DNA ligase joins strands.

iii) Termination of synthesis (e.g. What signals and accessory proteins are required)

mRNA Synthesis	Protein Synthesis	DNA Synthesis
• rho factor binds to termination signal on mRNA, causing termination.	• Stop codon causes release factors to bind to ribosome, releasing final product.	• Specific DNA sequences cause termination of DNA synthesis.

B12. (3 pts) Compare and contrast the structural and hydrogen bonding features of an α-helix versus those of a β-sheet.

In an α-helix a single polypeptide strand forms a helical structure. The hydrogen bonds are parallel to the helical axis, between the i^{th} residue and the $i+4^{th}$ residue.

A β-sheet is composed of two or more strands, which can be either parallel or anti-parallel. The hydrogen bonds are between the strands, perpendicular to the direction of the strands.

B13 (Choice Question!). (8 pts)
Both hemoglobin and phosphofructokinase (PFK) are allosteric proteins. Discuss the necessary structural requirements of an allosteric protein and briefly explain how allosteric regulation in these two systems is essential for their normal biological function.

Allosteric proteins bind a ligand and the binding of the ligand at one site affects the binding/enzymatic properties at another site. A homotropic allosteric effector affects its own binding while a heterotropic allosteric affector affects the binding of a different ligand.

In the case of hemoglobin O_2 is an homotropic allosteric activator, increasing oxygen affinity as more is bound. This allows for efficient delivery of oxygen to the tissues as more is released as the oxygen concentration drops as the blood goes to the tissues. BPG is an example of a heterotropic allosteric inhibitor of hemoglobin. It reduces the oxygen affinity and changes the shape of the binding curve. In doing so, it allows for increased oxygen delivery at high altitudes.

PFK is activated by F2,6P, AMP, and ADP and inhibited by ATP. F2,6P levels are high when glucose is high, signaling the liver that it should convert glucose to ATP. AMP and ADP have the same effect. ATP inhibits PFK, turning off glycolysis because it is not necessary to make ATP when levels are already high.

OR

Discuss the consequences to glycogen metabolism of a defective receptor for the hormone glucagon (5 pts). Could you provide another hormone to affected individuals that would restore normal glycogen metabolism? Briefly describe how this would work.

If the glycogen receptor is defective, then glycogen will not induce phosphorylation of proteins involved in glycoen metabolism. Consequently glycogen synthase and glycogen phosphorylase would be dephosphorylated all of the time. This will keep glycogen synthase in an active state all of the time. Thus any glucose that this individual had would always go into glycogen and would never be released as free glucose since glycogen phosphorylase would never be activated.

You could by-pass this defect by giving the individual epinephrine. Because epinephrine shares the same signaling pathway as glycogen (both stimulate adenyl cyclase) they will have the same effect.