

Biochemistry I - Spring 2001 - Final Exam

This exam has a total of 166 points and is divided into two sections. To do well, you should do all of the questions in sections A and B.

There are a total of 12 pages in this exam, including this one. Please write your name, or at least your initials, on each page before you begin.

Please be as succinct as possible in your answers!

Grade:

Part A: _____

Part B:

B1 _____

B2 _____

B3 _____

B4 _____

B5 _____

B6 _____

B7 _____

B8 _____

B9 _____

B10 _____

B11 _____

B12 _____

B13 _____

Total: _____

Section A: Multiple Choice. Please circle the BEST answer (20 questions, 40 points total, 2 pts each).

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
- the pK_a is near neutrality.
 - protonated imidazole has twice the number of protons.
 - unprotonated imidazole is invisible in the spectrum.
 - the chemical shift of His differs from His^+ .
10. The membrane proteins that catalyze active transport reactions differ from soluble enzymes in that
- they do not enhance the rates of reaction.
 - the product(s) of the reaction move in a specific direction.
 - the substrate(s) of the reaction are all outside the cell.
 - they are permanently changed during the reaction.
11. Non-alcoholic beer is obtained by
- fermenting yeast under anaerobic conditions.
 - fermenting yeast under aerobic conditions.
 - adding pyruvate to the yeast growth media.
 - adding acetic acid to the yeast growth media..
12. DNA differs from RNA in the following features
- DNA residues are linked by 3'-->5' phosphodiester bonds; RNA is 2'-->5' linked.
 - DNA has deoxyribose residues; RNA has ribose residues.
 - DNA contains the A, C, G and T bases; RNA contains A, C, G, and U.
 - All but the first choice are correct differences.
13. The force, or energetic term, that is most stabilizing for nucleic acids is:
- hydrogen bonds
 - electrostatics
 - van der Waals (base stacking)
 - conformational entropy
14. DNA Gel Electrophoresis is similar to SDS-PAGE of proteins because
- In both cases a plot of $\log(\text{Molecular weight})$ versus distance migrated is linear.
 - Both techniques rely on a constant charge to mass ratio.
 - Both techniques utilize the sieving properties of gels.
 - 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:
- it requires a sigma factor, which is normally not present in HIV infected cells
 - it lacks a 5'→3' exonuclease.
 - it has low affinity for the correct dNTP's.
 - it lacks a 3'→5' exonuclease.
16. The differences between polII and polIII are:
- one has a 3'→5' exonuclease activity, the other does not.
 - one has a 5'→3' exonuclease activity, the other does not.
 - one utilizes RNA primers the other does not.
 - PolII and PolIII are different names that describe the same enzyme.

17. During replication, overwinding or overtightening of DNA is caused by ____ and removed by ____:
- DnaA, Gyrase
 - DnaA, Single Stranded Binding Protein
 - DnaB, Gyrase
 - 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?
- Presence of glucose in the growth media.
 - Presence of arabinose in the growth media.
 - Presence of IPTG in the growth media.
 - Presence of ribose in the growth media.
19. Loss of the 3' CCA from a tRNA molecule would
- cause it to unfold, making it non-functional.
 - prevent the tRNA from binding to the mRNA.
 - prevent charging of the tRNA with its amino acid.
 - 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
- Trypsin
 - fMet aminopeptidase
 - HIV protease
 - 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:

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

B3:(11 pts)

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

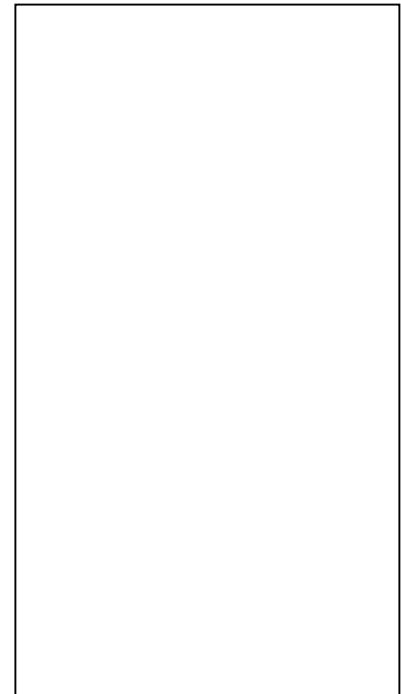
GGCCGGATACCGTA

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)

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)

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)

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



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.

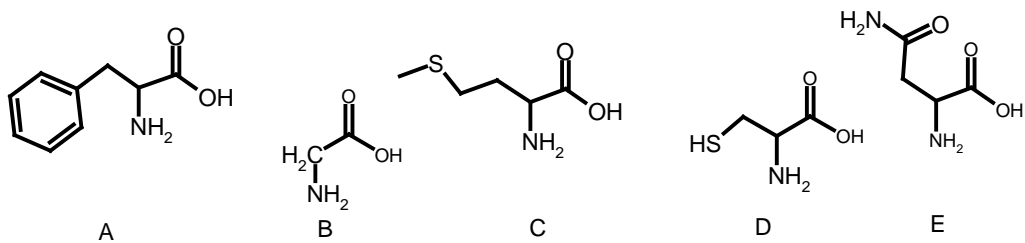
- i) Only proteins can catalyze enzymatic reactions.

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

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

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

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



- 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)
- 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: _____
 - b) Sidechain absorbs UV light strongly: _____
 - c) Sidechain found at active sites of some enzymes: _____
 - d) Sidechain can form disulfide bonds : _____
 - e) Sidechain can form hydrogen bonds with nucleotide bases: _____
 - f) A modified form of this amino acid is the 1st one used by the ribosome _____

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.

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)

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)

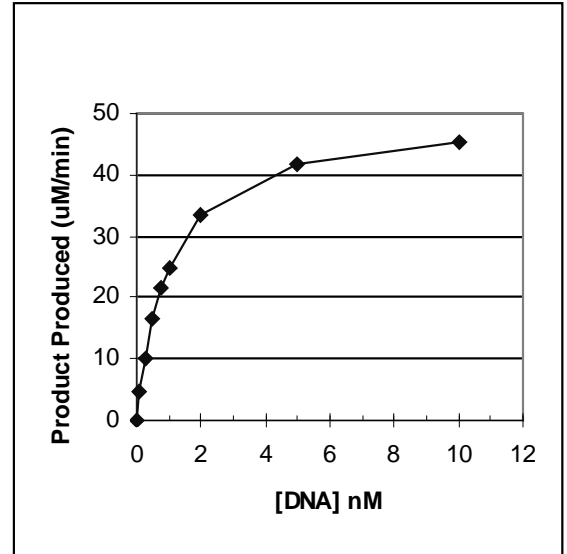
iii) Can the organism achieve the same goal (increasing the free energy of the proton gradient) by pumping Na^+ ions out of the cell? Briefly justify your answer.(2 pts)

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).

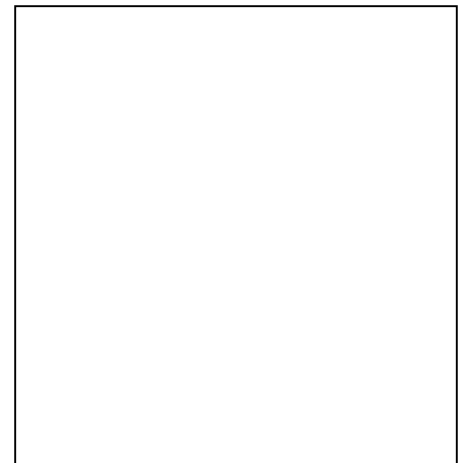


ii) The plot above shows the initial velocity of BamHI activity as a function of DNA concentration (in nM). The same DNA was used for this reaction as for the binding studies.

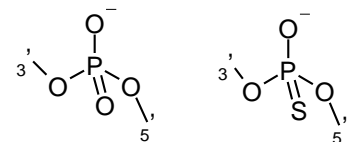
a) Draw the structure of the product of this reaction (Hint, this enzyme makes sticky ends that are 4 bases long) (2 pts)

b) Estimate K_M from this graph. You may assume V_{MAX} is 50 uM/min. Briefly explain your approach.(3 pts)

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)



e) 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.



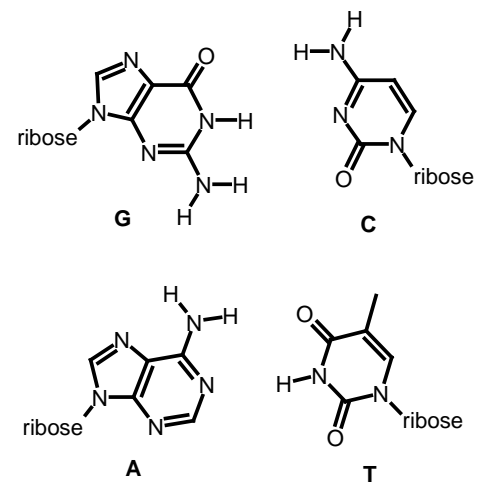
(Question Continued on Next Page)

B8, Continued.

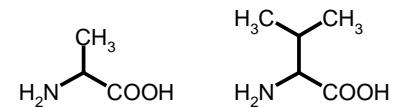
If the same DNA sequence that was used in the binding and enzyme kinetics studies was synthesized with phosphosulfur bonds and used as an inhibitor in the above enzymatic reaction how would the enzyme kinetics change (i.e. what type of inhibitor is this compound?)(2pts).

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).

f) This enzyme forms a hydrogen bond with the second G in the recognition sequence (GGATCC). 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).

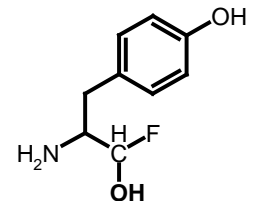


B9 (Choice!). (4 pts)How does the cell avoid charging tRNA^{Val} with Ala? Give a *plausible* description of the active site of the enzyme that charges tRNA^{Val} (i.e. indicate the amino acids that might be present in the active site of the enzyme). Your description should discuss *both* activities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



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?



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

- Reverse transcriptase

- DNA ligase.

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)

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) | 5. AUG codon |
| 2. Signal Peptide | 6. Origin of DNA replication |
| 3. -35 and -10 region | 7. Termination codon |
| 4. lac repressor binding site (lac operator) | 8. Antibiotic resistance marker |

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

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.)

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)

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

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

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.

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.