

Instructions: This exam has 5 pages. Total points=100. Use the space provided, or the back of the previous page if necessary. On questions with choices, your answer that gives the highest grade will be used.

A. Multiple Choice and fill in the blanks (15 points)

1. The increase in the rate of an enzyme catalyzed reaction at higher temperatures is principally due to:

- a) a higher concentration of transition states at the higher temperature
- b) a larger number of folded (active) enzymes at the high temperature.
- c) Faster motion of functional groups in the enzyme.

2. HIV Protease cleaves after phenylalanine residues because of interactions with (select **all** that apply):

- a) non-polar sidechains in the active site of the enzyme.
- b) hydrogen bonding sidechains in the active site of the enzyme.
- c) positively charged residues in the active site of the enzyme.
- d) negatively charged residues in the active site of the enzyme.

Phe is non-polar, so it can only interact with the enzyme via van der Waals and the hydrophobic effect, i.e. good contact with non-polar residues in the active site (e.g. Val82).

3. Place in correct order the events that happen during replication of the HIV virus by writing the letter in the blank spaces.

 b c d e a

- a. virus buds out of cell.
- b. Virus binds to the cell
- c. RNA is converted to DNA
- d. DNA integrates into chromosome
- e. Viral RNA and proteins are made.

4. The high mutation rate of the HIV virus is due to mistakes by which of the following?

- a) HIV Integrase
- b) HIV Protease
- c) DNA polymerases in the cell
- d) HIV Reverse Transcriptase
- e) RNA polymerases in the cell.

5. Which of the following amino acids is used by HIV protease to cleave (hydrolyze) the peptide bond:

- a) Aspartic acid (Asp25)
- b) Valine (Val82)

6. Most DNA polymerases will elongate the chain in the 5'→3' direction and remove incorrect bases in the 3'→5' direction (fill in the blanks)

7. The base on the right is a purine or a pyrimidine (circle correct answer)?

(Angels are pure and have two rings)

8. Which of the following activities can be accomplished by the HIV reverse transcriptase enzyme (select **all** that apply)?

- a) DNA polymerase, using RNA as the template.
- b) DNA polymerase, using DNA as the template.
- c) Ribonuclease activity, digestion of RNA.
- d) 3' to 5' exonuclease activity.

9. Circle the sequence(s) that are **not** a restriction site (only the DNA sequence of the top strand is shown):

 TTTT

GATC

TTAA

GCGC

The remaining three sequences are the same on both strands, so they could be restriction sites.

10. DNA polymerases add the tri phosphate form of the nucleotide to the 3' end of the primer (Fill in the blanks).



B. Short answer.

1. (8 pts) Please do **one** of the following choices:

Choice A: Describe how antibodies can be used to treat a drug overdose **or** cancer.

Choice B: Briefly describe the production of antibodies by the immune system; list the cell types and their role in the process.

Choice A:

- Drug overdose - the antibodies are specific for the drug. By binding the drug they prevent it from causing harm because the drug that is bound to the antibody can no longer affect the patient.
- Cancer - Antibodies are specific for proteins on the surface of the cancer cell that can only be found on the cancer cell. The antibodies can either carry drugs directly to the cancer cells, or cause the cell to be killed by NK (natural killer cells).

Choice B:

- B-cells have antibodies on their surface
- When they bind to their antigen they are activated by T_H cells.
- The activated B-cells become plasma cells which secrete soluble antibodies, which are the biologically active form.

2. (3 pts) What is the **key** difference between passive and active immunization.

Passive immunization provides antibodies to treat the current condition, but does not cause the production of memory cells, so the effect of the vaccination is sort lived.

3. (6 pts) Please do **one** of the following choices:

Choice A: Why is it necessary to produce a new flu vaccine every year?

Choice B: What are the key considerations for selecting which disease to develop a vaccine for?

Choice C: What is herd immunity and why is it important for protection of the population?

Choice A: Because the virus changes each year due to mixing of the RNA chromosomes in the virus by infection of the same cell by two different flu viruses. The virus produced from this cell will have a mixture of the chromosomes, which will change the properties of the virus.

Choice B: It should be used to protect against a lethal disease. The disease symptoms have to be relatively slow to allow the memory cells to become reactivated to produce antibody. For example, neither the common cold (not lethal) nor ebola (too fast) are good vaccine candidates, but the flu is (fairly lethal and slow).

Choice C: Herd immunity refers to the fact that most of the individuals in a population are vaccinated, but not all. Because most are vaccinated it is difficult for the pathogen to move from one person to another - they are more likely to encounter a vaccinated individual. Thus the ones that are not vaccinated are also protected.

4. (8 pts) "Enzymes lower the energy of the transition state."

i) What is the transition state?

A high energy intermediate in the reaction from substrate to products.

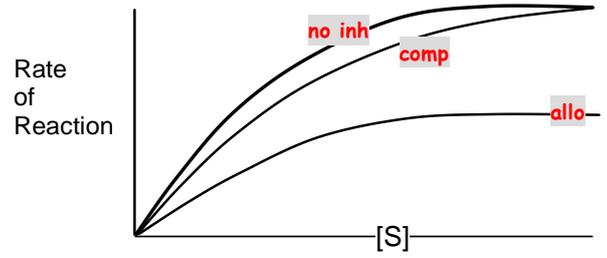
ii) Why does lowering its energy lead to a faster reaction?

1. It is easier for substrates to turn into transition states since the energy barrier is lower.

2. The concentration of transition states is higher.

3. Since the rate depends on the concentration of the transition state, the rate is faster.

5. (12 pts) The curve on the right shows the rate of reaction versus substrate for an uninhibited reaction, a reaction in the presence of a competitive inhibitor and a reaction in the presences of an allosteric inhibitor.



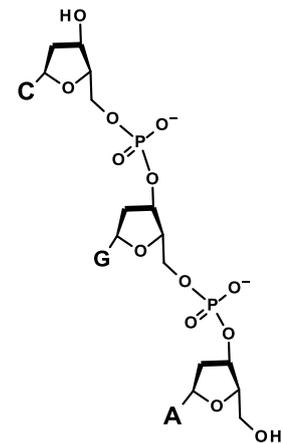
- i) Label the correct curve using the labels: “no inh”, “comp”, “allo”. (2 pts)
- ii) Justify your answer; be sure to discuss the binding site of the inhibitor on the enzyme in your answer (8 pts).

- A competitive inhibitor binds to the active site, so it can only inhibit at low substrate concentrations. At high substrate concentrations the substrate will prevent the inhibitor from binding since they are both binding at the same site. Since the inhibitor can't bind, the rate should be as fast as the reaction with no inhibitor.
- An allosteric inhibitor binds at a different site than the active site, so it will remain bound no matter how high the substrate concentration becomes.

iii) What is the most significant difference between a covalent inhibitor and a competitive inhibitor (2 pts).

One binds reversibly (competitive) the other is permanent (covalent)

6. (7 pts) For the nucleic acid on the right:



- i) Label the 5' and 3' ends (2 pts).
 - ii) Give the sequence (1 pt)
 - iii) Is this DNA or RNA? Briefly justify your answer (4 pts).
- i) The top is the 3' end.
 ii) 5' AGC
 iii) DNA, there is no -OH on the 2' carbon

7. (9 pts) You want to amplify the DNA sequence for HIV reverse transcriptase so that you can insert it into a plasmid for production of the enzyme in bacteria. In order to insert the PCR product into the plasmid you will need to add sites for EcoR1 on the left side and HindIII on the right side. The recognition sequence for EcoR1 is GAATTC and for HindIII is AGGCCT. Since the plasmid already provides a start and stop codon you only need to amplify codons for the 2nd residue (Leu) to the last (Val) residue, and add the restriction sites.

GGTGTG**ATG**TTGGTTGTT..... . AGCGGTGTG**TAA**TTCA
 Met**LeuValVal**..... . Ser**GlyVal**

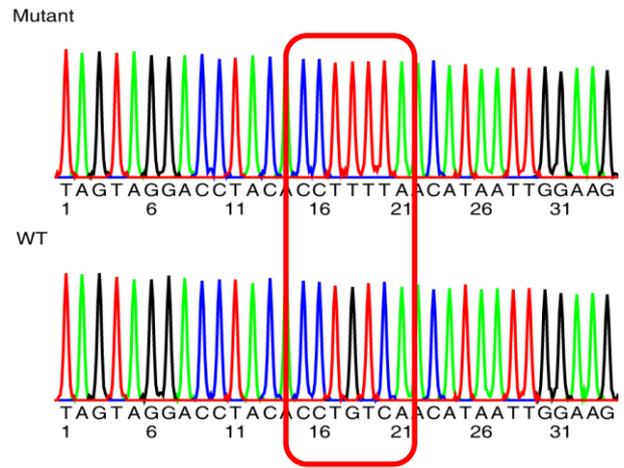
i) Give the sequences of the left and right primers, assuming the primers are a total of 10 bases long (7 pts). Following these steps:

1. Define target region and boundary points.
2. Left primer = sequence of top strand at left boundary. Add any restriction sites to its 5' end.
 Left Primer: **GAATTC**TTGG (EcoR1 sequence in bold, boundary sequence underlined)
3. Right primer = sequence of lower strand at right boundary. Add any restriction sites to its 5' end.
 Right Primer: **AGGCCT**CACA (HindIII sequence in bold, boundary sequence underlined)

ii) Write the sequence of the PCR product, the top strand is sufficient (2 pts).

GAATTC**TTGGTTGTT**..... . AGCGGTGTG**AGGCCT**

8. (17 pts) An HIV infected individual no longer responds to the normal HIV protease inhibitor. You obtain the genetic information from the virus and determine a portion of the sequence of the HIV protease gene. The sequencing data is shown on the right. The complete sequence of the HIV protease and a codon table are provided on your formula sheet.



i) Identify the change in the DNA sequence. There are two changes that are close to each other, within one codon. You can indicate the location of the changes on the data on the left, or give the residue numbers below (2 pts).

The changes occur between 16 and 21.

ii) Determine the reading frame and the change in the amino acid. This sequencing data is found within the HIV protease sequence at the line beginning with base 238 (amino acid residue 68). The sequence data corresponds to the non-grey highlights (4 pts). Starting at the two Cs:

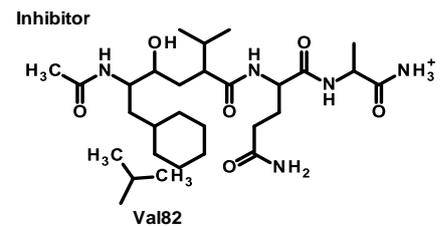
Mutant : CCT TTT A

Wildtype: CCT GTC A

Amino acid: Pro Val Asn

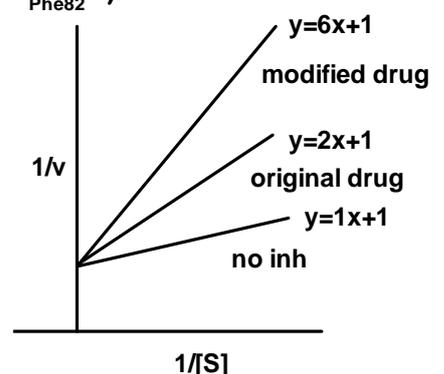
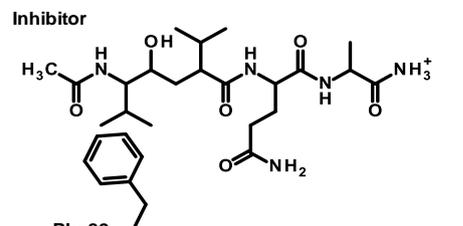
The mutant codon is TTT, so Val82 has been changed to Phe82.

iii) Suggest a modification of the current drug that would bind more effectively to the mutant HIV protease. The structure of the current drug interacting with Val82 is shown on the right (4 pts).



The phenylalanine the replaced the Valine is large and non-polar, so you would want to just reduce the size of cyclohexane ring, but keep it non-polar. The easiest thing to do would be to change the cyclohexane ring to something that is similar to valine.

iv) (6 pts) You measure the enzyme kinetic data using the original drug and your modified drug (using the mutant enzyme) and plot the data on a double reciprocal plot (shown on the right). You used a drug concentration of 1 nM for both of your assays.



a) Determine K_I for both the original drug and your modified drug from these data. (2 pts).

b) Based on the K_I value, is your modified drug more, or less, effective at inhibiting the mutant enzyme. Why? (4 pts)

Original drug: $K_I = 1 \text{ nM} / (2 - 1) = 1 \text{ nM}$

Modified drug: $K_I = 1 \text{ nM} / (6 - 1) = 0.2 \text{ nM}$

The lower K_I for the modified drug indicates that it binds better.

v) Give the sequence of the sequencing primer that was used to generate this sequencing data. Assume the primer is 6 bases long (1 pt).

It would be the 6 bases before the start of the sequence data: CAGTAT, i.e. the primer-template would be the following, and the first dd terminated fragment would be CAGTATT (first colored peak in the sequencing data).

5'-CAGTAT

TTTCGATATCCATGTCATAATCATCCTGGATGTGGACAGTTGTATTAACCTTCT

9. (8 pts) Please do **one** of the following choices:

Choice A: Describe a PCR cycle and briefly explain what occurs in each step of the cycle (you **do not** have to discuss multiple cycles, etc.)

Choice B: What is a ddNTP and why are they important for DNA sequencing?

Choice A:

DNA is heated to high temperature (98 C), becomes single stranded.

Solution is cooled to allow primers to anneal to the template, via Watson-Crick hydrogen bonding

Temperature is raised to the optimal temperature for polymerase, polymerase synthesizes a copy of the template by adding bases to the primer.

Choice B:

ddNTP is a di-deoxy nucleotide tri phosphate. It has three phosphates on its 5' carbon so it can be incorporated into the growing DNA, but since it lacks a 3'OH it terminates the chain.

In DNA sequencing each ddNTP is a different color, allowing us to determine which base was added at the end of strand.

10. (6 pts) Please do **one** of the following choices:

Choice A: What are tandem repeats and how are the number of repeats measured?

Choice B: Briefly explain how tandem repeats can be used to **exclude** suspects in criminal cases. Feel free to use a diagram to illustrate your answer.

Choice A:

Tandem repeats are the same DNA sequence repeated over and over again, e.g. ACTACTACT... They can be measured by using PCR primers that are unique sequences outside of the repeat area.

The number of repeats is proportional to the length of the PCR product.

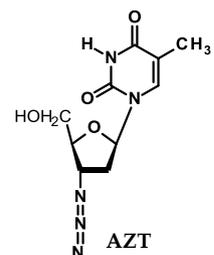
Choice B:

Individuals have different numbers of repeats on each chromosome and the number of repeats can be measured by PCR.

If the number of repeats in a DNA sample at a crime scene is different than the number of repeats in the DNA sample from a suspect, the person is no longer a suspect because the number of repeats do not match.

(Many different people have the same number of repeats, so if the number of repeats match, the person may not be guilty).

Bonus: (4 pts) A number of antiviral drugs that inhibit polymerases from viruses are "pro-drugs", which means they have to be converted to something else before they can be incorporated by the polymerase in the growing polynucleotide chain. What is added to the drug and why are the modified drugs inhibitors? AZT is an example of a pro-drug and its structure is shown on the right.



Three phosphates have to be added to 5' OH group. This then allows the triphosphate to be incorporated into the growing chain by the polymerase.

However, the chain will then terminate because there is no 3'OH for the next base to be added to. Thus the virus cannot replicate its entire genetic material and it cannot replicate.