This exam consists of 90 points on 5 pages. **Please allot 1 min/2 pts**. On questions with choices, all of your attempts will be graded and you will receive the highest scoring attempt.

1. (10 pts) Please do **one** of the following choices:

Choice A: Describe, in very general terms, the active site of an enzyme. Provide some detail about the role of residues in catalysis and substrate binding using HIV protease as an example.

Choice B: Enzymes increase the reaction rate by lowering the energy of the transition state. What is the "transition state" and how do enzymes lower the energy of the transition state?

Choice A: The active site of an enzyme is the part of the enzyme that is responsible for catalysis (+3 pts)

It consists of residues whose sidechains perform two functions:

i) binding of specific substrates (3 pts) – e.g. Val82 in HIV protease is why the enzyme prefers to cleave after phenylalanine (1/2 pt)

ii) involved in the chemical process that the enzyme catalyzes. (3 pts) In HIV protease the aspartic acid residues at position 25 are responsible for cleaving the bond (1/2 pt)

Choice B:

The transition state is a high energy intermediate that is found between the substrate and the products of a reaction (3 pts).

In uncatalyzed reactions, the reactants have to become ordered before they go to the transition state. Ordering is unfavorable, so there is a high energy barrier.

An enzyme pre-orders the reactants prior to going from the substrate to the transition state, because the pre-ordering occurs before going to the transition state, the barrier is smaller.

2. (8 pts) Please do **one** of the following choices:

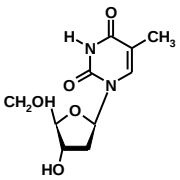
Choice A: How does the rate of an enzyme catalyzed reaction change with temperature? What happens to the rate as the temperature is increased?

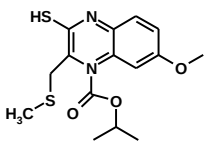
Choice B: How does the rate of an enzyme catalyzed reaction change with substrate concentration? What happens to the rate as the substrate concentration is increased?

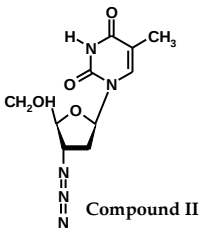
Choice A: As the temperature is raised, the reactants have more energy and will be more likely to go to the transition state. Therefore the rate will increase (6 pts), until the enzyme denatures at which point the rate will drop to zero. (2 pts).

Choice B: As more substrate binds, the rate will increase because increasing the substrate will increase the concentration of the transition state. Once all of the enzyme molecules have substrate bound, the rate will stay constant since all of the enzymes will be occupied converting substrate to product and it is not possible to go faster.

3. (10 pts) Compare and contrast a competitive inhibitor to an allosteric inhibitor. You may use the compounds shown on the right of this page to illustrate your answer. Your answer should include a brief discussion of:
   i) Where each type of inhibitor binds on the enzyme
   ii) Whether the inhibitor is similar or different than the substrate.
   iii) How effective the inhibitor is at low and high <u>substrate</u> concentration.


Substrate


Compound I


Compound II

A competitive inhibitor looks similar to the substrate and binds at the active site. It is not effective at high substrate because the substrate can displace the inhibitor from the active site. Compound II is a competitive inhibitor (5 pts)

An allosteric inhibitor binds elsewhere, not at the active site, and is usually different in structure than the substrate. For example compound I is an allosteric inhibitor. This inhibitor is equally effective at high and low substrate since the substrate cannot displace the inhibitor (5 pts).

4. (8 pts) Select **<u>one</u>** of the following enzymes and describe its role in the life cycle of the HIV virus. Why are either of these enzymes particularly good targets to inhibit with anti-viral drugs?
   Choice A: HIV reverse transcriptase
   Choice B: HIV integrase

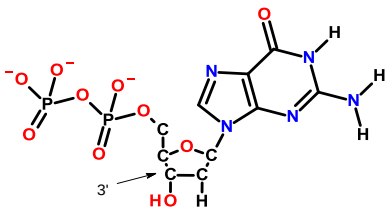
Choice A: This enzyme converts the viral RNA to double stranded DNA (4 pts). It is a good drug target because this step is essential to the lifecycle of the virus, and if inhibited the virus cannot replicate (3 pts). There are also no equivalent human enzymes, so the side-effects of the drug should be small (1 pts).

Choice B: This enzyme inserts the double stranded DNA, made by reverse transcriptase, into the chromosome of the host cell (4 pts). It is a good drug target because this step is essential to the lifecycle of the virus, and if inhibited the virus cannot replicate (3 pts). There are also no equivalent human enzymes, so the side-effects of the drug should be small (1 pts).

5. (4 pts) Why do individuals who are infected with HIV develop AIDS (acquired immunodeficiency syndrome)?

The HIV virus infects T-helper cells. T-helper cells are required for the activation of B-cells to produce antibodies. If the T-helper cells are non-functional, no antibodies of any type can be produced – the individual's immune system is deficient.

6. (6 pts) Please answer the following with reference to the structure on the right.



i) Is the nitrogenous base a **purine** or a pyrimidine?

ii) Label the 3' carbon atom.

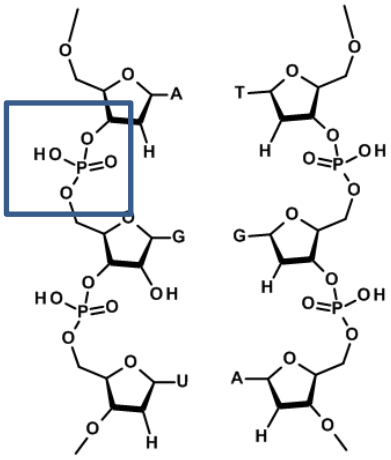
iii) Is this DNA or RNA? Why? (2 pts)

DNA, since it lacks a 2' OH.

iv) Given that the base is guanine (G), what is the short hand name for this compound?

dGDP – deoxy guanosine diphosphate.

7. (8 pts) Please answer the following questions with reference to the molecule shown on the right.



i) The following DNA molecule has several errors associated with it, please identify at least **two** (2) errors.

ii) Indicate the location of the 5' end of the **left** strand

iii) Indicate the location of a phosphodiester bond.

iv) Write the sequence of the left strand, from 5' to 3'.

i) Errors are:

- Middle base on the left has a 2'-OH, should be 2'-H
- Lower left base should be T instead of U
- Phosphates are protonated, should be deprotonated
- Strands are parallel, should be anti-parallel.
- Middle basepair is G-G, should be C-G

ii) The top is the 5' end

iii) joining the bases (on is indicated by a box)

iv) AGT (or AGU)

~~8. (4 pts) The following is a list of the steps in the construction of a plasmid (expression vector) that could be used to produce HIV reverse transcriptase in bacteria, beginning with the virus and ending with the plasmid that has the HIV reverse transcriptase coding region inserted into it. Indicate the correct order of the central four steps by writing "a" (first step), "b", "c", or "d" next to each step. The first and last steps are given for you.~~

~~Isolation of genetic material from virus~~

~~__b__PCR amplification of DNA~~

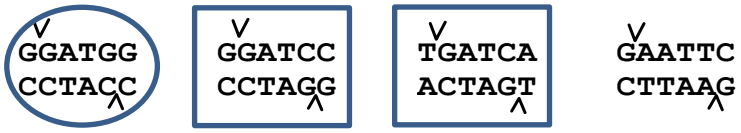
~~__d__Ligation~~

~~__a__Conversion of RNA to DNA~~

~~__c__Digestion of PCR product and plasmid with restriction enzymes~~

~~Transformation of plasmid into bacteria.~~

9. (4 pts) Given the following sequences:



i) Circle the sequence that **cannot** be a restriction enzyme site. The sequence of the top and bottom strands are different top: GGATGG, bottom CCATCC.

ii) Put a box around the two sequences that could be efficiently ligated to each other. Both of these enzymes will produce the same "sticky ends" GATC

10. (2 pts) DNA polymerases always add the new **_____dNTP_____** to the **____3'-OH_____** of the primer.

~~11. (2 pts) When proteins recognize DNA **non-specifically**, they are most likely interacting with the (circle best answer): **(-1 ½ for ribose)**~~

~~nucleotide base ribose phosphate.~~

12. (8 pts) Please do **one** of the following choices.

Choice A: The following primer/template was incubated with DNA polymerase, dNTPs, and ddCTP. List all possible fragments that would be produced.

```
          5'G-C-A
3'A-T-G-C-A-G-A-C-G-T-G-C-G 5'
```

Choice B: What property of HIV reverse transcriptase leads to a high level of mutations in the HIV genetic material, leading to drug resistant HIV viruses.

Choice C: When DNA polymerases are adding bases, they usually add A opposite T and G opposite C. What are the two reasons why this basepairing rule is followed?

Choice A: The ddCTP will cause termination of the growing chain anytime a C is incorporated. So the fragments will be.

GCAC and GCACGC

Choice B: The enzyme lacks the 3'-5' exonuclease activity that would remove a mispaired base. If the wrong base is inserted it stays there, potentially causing a mutation.

Choice C:

i) Watson-Crick hydrogen bonds between A &T and between G & C.

ii) Purine-pyrimidine matching gives the right size, a purine-purine pair is too large, a pyrimidine-pyrimidine pair is too small.

13. (8 pts) The following is the sequence of the HIV reverse transcriptase coding region:

```
5'-GCGATGGTGGCGCAATCGCTA---ATATGCAGCTCGCTACACTAACGCG-3'
3'-CGCTACCACCGCGTTAGCGAT---TATACGTCGAGCGATGTGATTGCGC-5'
     MetValAlaGlnSerLeu---IleCysSerSerLeuHisStp
```

Please do the following two questions:

i) Give the sequence of the right and left PCR primers that you would need to amplify the codons from the Methionine (Met) codon (ATG) to the stop codon (TAA). Your primers should **also** add the restriction sites for EcoR1 (G^AATTC) and BamHI (G^GATCC) to the left and right end of the PCR product, to give the following PCR product:

```
5'-GAATTCATGGTGGCGCAATCGCTA---ATATGCAGCTCGCTACACTAAGGATCC-3'
3'-CTTAAGTACCACCGCGTTAGCGAT---TATACGTCGAGCGATGTGATTCCTAGG-5'
```

Your primers should be 12 bases in length.

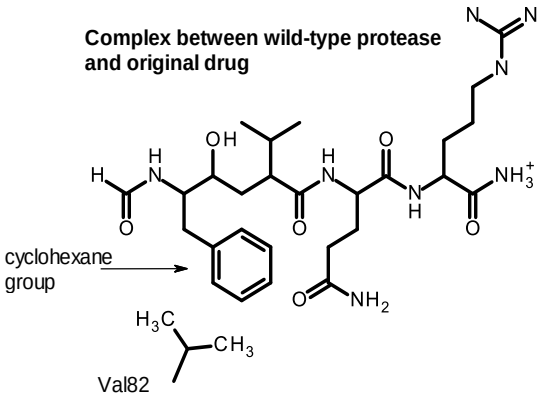
Sequence of left primer: GAATTCATGGTG (2 pts)

Sequence of right primer: GGATCCTTAGTG (2 pts)

ii) Why have the restriction sites EcoR1 and BamH1 been added to the ends of the reverse transcriptase coding region, i.e. why is this useful?

Treatment of the PCR product with these restriction enzymes will produce sticky ends that can be used to insert the coding region into an expression vector (4 pts)

14. (8 pts) The last page of this exam shows DNA sequencing information for a mutant and wild-type HIV protease. The mutation occurs in the codon for Val82 in the enzyme. The structure of the wild-type (Val82) enzyme with the normal cyclohexane drug bound to it is shown on the right. Please answer the following questions.

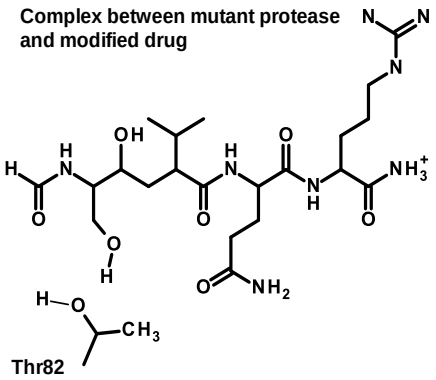


i) (3 pts) Use the sequencing data to identify the residue that has replaced Val82. **The mutation occurs near position 51 on the sequencing data.** For reference, the complete sequence from the wild-type data is:

```
GAAATCTGTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTG
GluIleCysGlyHisLysAlaIleGlyThrValLeuValGlyProThrProValAsnIleIleGly
Mutant sequence                                   CCTACTAA
```

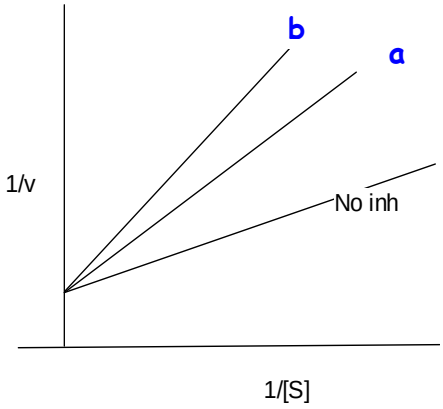
The sequence of the mutant, in the region of Val82 is shown above. The codon for Val82 has been changed to ACT, which is Threonine (Thr).

ii) (3 pts) How would you modify the cyclohexane part of the drug to increase its binding to the mutant enzyme? You can draw your modification using the diagram on the right.



The threonine has a polar –OH group that could form hydrogen bonds with the inhibitor (drug). Therefore adding a hydrogen bond donor/acceptor to the drug would make it bind better.

iii) (2 pts) The double reciprocal plot shown on the right has three lines. The one line that is labeled is for the reaction with the mutant enzyme with no inhibitor present. Please label the other two lines with the letters "a" and "b", as follows:



a) the reaction with the mutant enzyme with the original cyclohexane drug.

Since this drug should bind poorly to the mutant enzyme, the slope of the line will be small, indicating weak inhibition.

b) the reaction with the mutant enzyme with the redesigned drug that binds more effectively to the mutant enzyme.

This should inhibit more strongly because of the favorable interaction (hbond), giving a line with a steeper slope, indicating a greater reduction in the rate of the reaction.

Be sure to justify your answer.

Bonus (4 pts):

What is sickle cell anemia? How is it caused? It is a mutation in the human hemoglobin gene that changes a polar residue to valine, causing the hemoglobin molecule to form rods due to non-polar interactions between adjacent molecules. These rods distort the shape of the red blood cell, making it difficult for the cell to get through narrow capillaries.