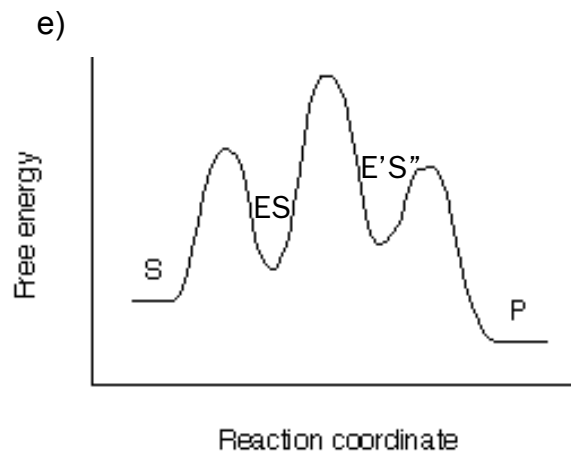
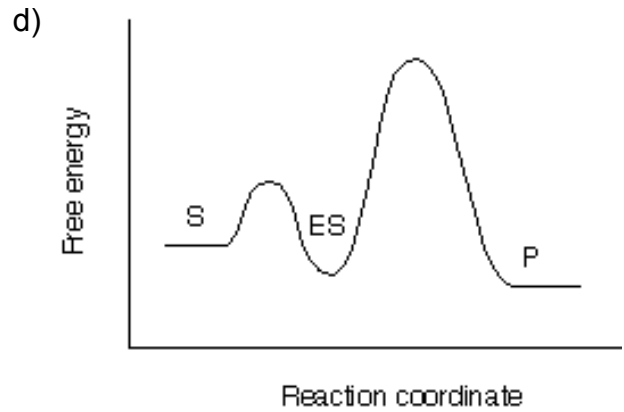
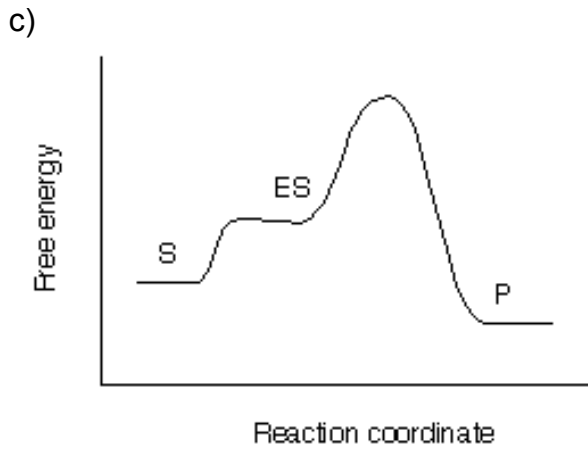
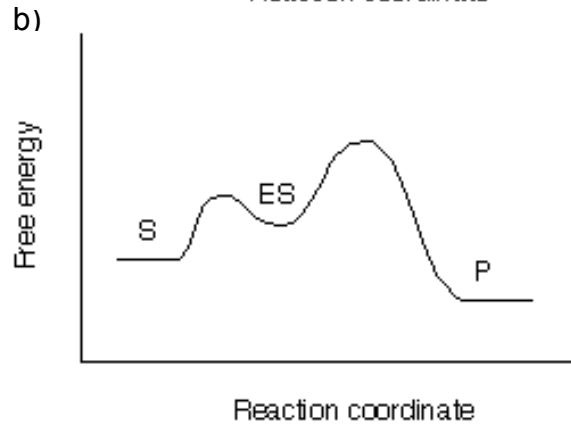
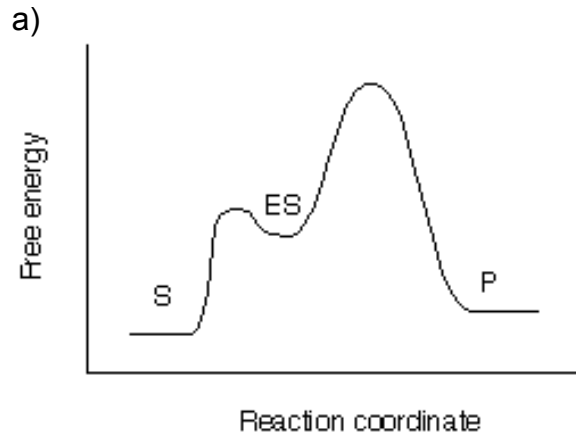
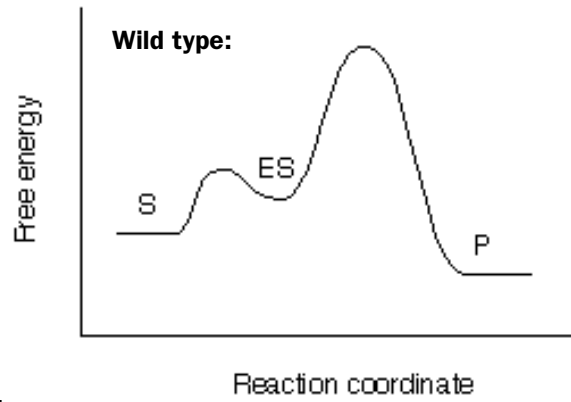


5. Types of physiological regulation of enzyme activity include all of the following EXCEPT:
- a) phosphorylation
 - b) allosteric activation
 - c) increased synthesis
 - d) suicide inhibition
 - e) competitive inhibition
6. Lysozyme mutants that contain positively charged, rather than nonpolar, residues near Glu-35 would have a pH optimum:
- a) greater than the wild-type enzyme
 - b) lower than the wild-type enzyme
 - c) identical to the wild-type enzyme
 - d) shifted to low pH and would now have a second optimum at high pH
 - e) This is a trick question: these mutants would have no activity.
7. A common feature of serine proteases is:
- a) cleavage of proteins on the carboxyl side of serine residues
 - b) multiple active sites per molecule, each containing a serine residue
 - c) synthesis as zymogens
 - d) the presence of the catalytic duo (Glu and Asp) at the active site
 - e) lack of substrate cleavage site specificity
8. Allosteric enzymes:
- a) respond slowly to their effectors
 - b) are regulated primarily by covalent modification
 - c) have more than one polypeptide chain
 - d) display Michaelis-Menten kinetics
 - e) include hemoglobin
9. Which of the following triggers the T to R state of hemoglobin?
- a) binding of CO₂
 - b) binding of CO
 - c) binding of O₂
 - d) binding of BPG
 - e) more than one of these

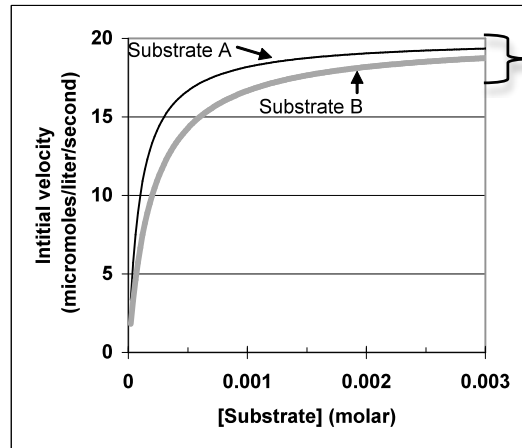
10. Select the energy diagram that best depicts a mutant enzyme that has a K_M and a k_{cat} lower than the wild-type enzyme, given that the wild-type enzyme looks like this:



Part II. Short Answer- Please show work and limit your answers to the space provided.

11. (15 pts) The enzyme trypsin hydrolyzes peptides at the carboxyl side of Arg and Lys residues. However, the amino acid residue that follows the Arg or Lys can influence either the K_M or the k_{cat} of the reaction. Suppose that the adjacent curves were generated by comparing two substrates for trypsin:

- Substrate **A** = Ser-Val-Arg-Pro
- Substrate **B** = Ser-Val-Arg-Phe



Assume that these curves do **not** reach the same limit.

a) The K_M of the enzyme is higher for (circle):

- A** **B** **Neither** **Can't tell from this information**

b) The enzyme binds (circle):

- More tightly to A** **More tightly to B** **Same to both** **Can't tell**

c) The approximate K_M for Substrate **A** is:

- 0.0001 M** **0.0005 M** **0.001 M** **>0.002 M**

d) The k_{cat} of the enzyme is higher for (circle):

- A** **B** **Neither** **Can't tell from this information**

e) Suppose that a similar experiment using the same enzyme and Substrate **A** was also carried out by another laboratory, but the V_{max} was only one-tenth of that found in the experiment shown above. This difference could be explained by the other laboratory's use of a lower amount of:

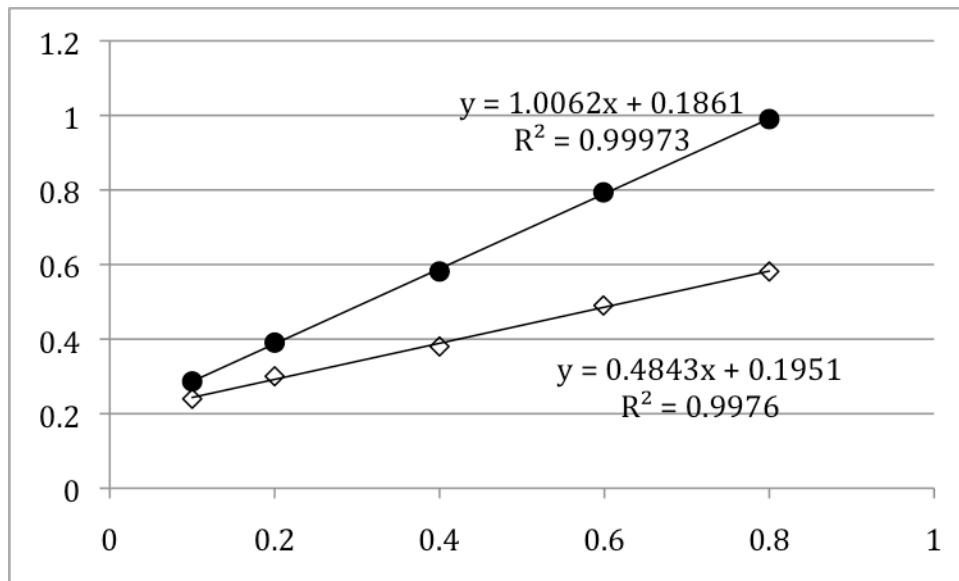
- Substrate** **Time** **Enzyme** **None of these**

12. (12 pts) An enzyme follows Michaelis-Menten kinetics. Indicate (with an "x") which kinetic parameter(s) would appear to be altered by the following factors.

K_M	V_{max}	Neither K_M nor V_{max}	Factor
			Competitive inhibitor
			Mixed inhibitor
			Uncompetitive inhibitor
			Irreversible inhibitor
			6 M urea
			Doubling [S]

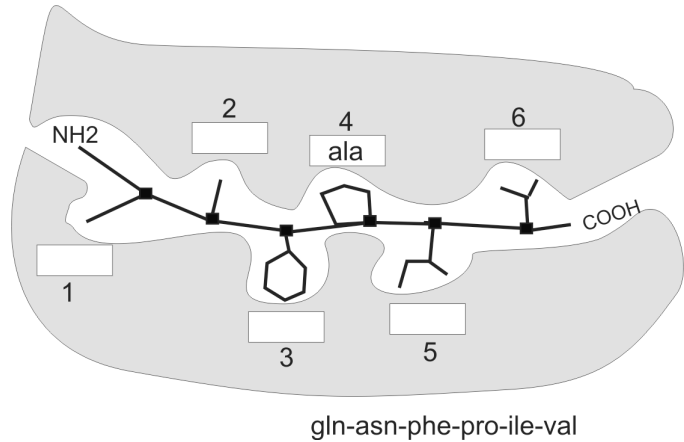
13. (24 pts) HIV protease is an enzyme that hydrolyzes a long viral polypeptide into smaller polypeptides that then fold and perform various functions in the viral life cycle. There are only a few peptide bonds that are hydrolyzed, all involving cleavage between two amino acid residues with hydrophobic side groups. Suppose one such cleavage occurs between the Phe and Pro in the sequence Gln-Asn-Phe-Pro-Ile-Val (peptide **A**). This isolated hexapeptide can serve as a substrate for the HIV protease in the laboratory.

Suppose that a similar hexapeptide, Gln-Asn-Phe-Trp-Ile-Val (peptide **B**), binds to the enzyme but is not hydrolyzed. Data for the reaction of peptide **A** in the presence of peptide **B** (at 5 mM) are presented in the following Lineweaver-Burk plot:



- Label the axes of the above plot appropriately. (Units of concentration are mM; units of time are minutes.)
- Calculate the V_{\max} and K_M for peptide **A**.
- Based on this data, what kind of inhibitor is peptide **B**? _____
- Calculate the dissociation constant for peptide **B**.

14. (15 pts) Pictured schematically here is a view of the substrate-binding site of the HIV protease with peptide **A** from the last question bound. The alpha carbon of each amino acid residue is indicated with a small filled square and each side group is depicted in a skeletal form. The sequence of peptide **A** is reproduced below this cartoon.



a) For amino acid residues 1-3 of peptide **A**, identify an amino acid in the HIV protease whose side chain could interact with that residue and name the type of interaction involved:

HIV amino acid:	Interaction:
Peptide A residue 1. _____	_____
Peptide A residue 2. _____	_____
Peptide A residue 3. _____	_____

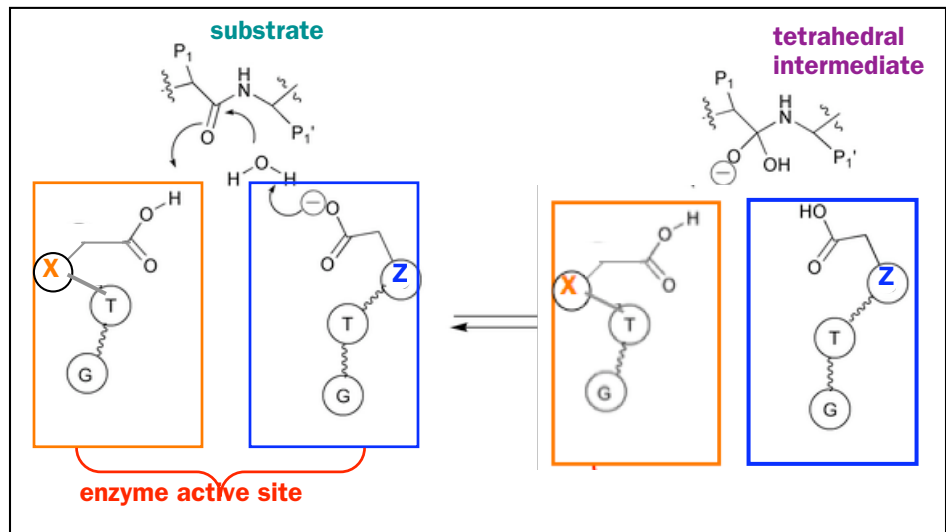
b) Suppose that a strain of HIV were found that produced a protease with a greatly reduced affinity for peptide **B** (Gln-Asn-Phe-Trp-Ile-Val). This HIV protease variant was found to differ from the original strain by a single amino acid substitution for alanine at position #4 in the protein as marked in the diagram above. Among the following choices, the new amino acid at position #4 that could best explain its different affinity is (circle):

- Arg Asp Asn Gly Ser

15. (5 pts) An enzyme-catalyzed reaction was carried out with the substrate concentration initially a thousand times greater than the K_M for that substrate. After 9 minutes, 1% of the substrate had been converted to product, and the amount of product formed in the reaction mixture was 12 mmol. If, in a separate experiment, one-third as much enzyme and twice as much substrate had been combined, how long would it take for the same amount (12 mmol) of product to be formed?

16. (24 pts) The first step in the mechanism of HIV protease is believed to be as shown here:

a) Two amino acids act as a catalytic dyad in this mechanism, **residue X** and **residue Z** in this diagram.



- What is the identity of **residue X**?

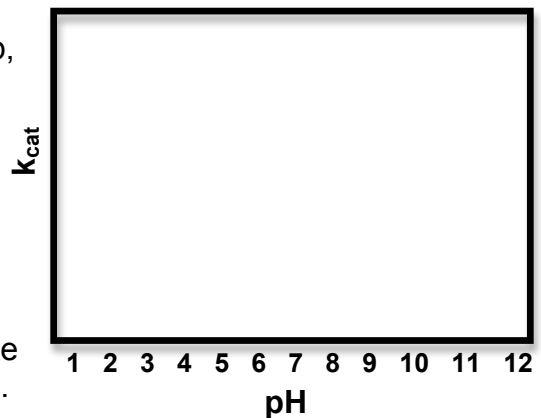
- What is the identity of **residue Z**?

- What type of catalysis is **residue X** inducing as shown here? _____

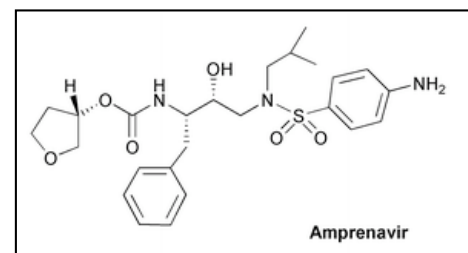
- What type of catalysis is **residue Z** inducing as shown here? _____

b) What is unusual about the ionization state of **residue X**? How can you explain this?

c) Assuming that this step is the rate-determining step, sketch the pH-dependence of the reaction by plotting k_{cat} vs. pH in the adjacent box.



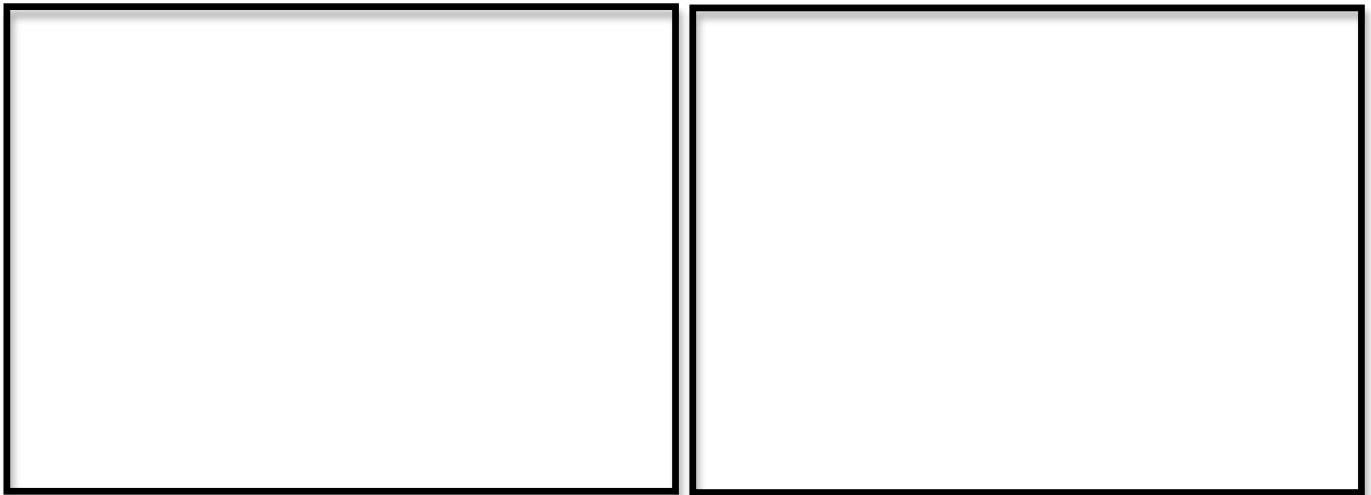
d) HIV protease inhibitors such as the one shown at the right are effective anti-HIV drugs. Predict what type of inhibitor amprenavir is, briefly justifying your choice.



17. (26 pts) Following a disastrous trip to K2, you are organizing another mountaineering expedition to the Himalayas. Your goal is a first ascent of the west face of Annapurna I (the 5th highest peak in the world). All of your final candidates demonstrate equal ability under conditions of low exertion at sea level, but extensive biochemical testing demonstrates that each contains a different mutant hemoglobin:

- Kenny- mutant hemoglobin of (α_2, γ_2) composition.
- Kirby- mutant hemoglobin of (α_4) composition.
- Jen- mutant hemoglobin such that her Hill coefficient for O_2 binding is 3.8.
- Zach- mutant hemoglobin such that His 146 β has been replaced by a Lys.

a) Sketch (and label) the predicted oxygen saturation curves under physiological conditions for Kenny, Kirby, and Jen in the left-hand box below. Label the axes, and include a “normal” hemoglobin curve as a reference point.



b) In one sentence, rationalize each binding curve:

- Kenny: _____
- Kirby: _____
- Jen: _____

c) Sketch the predicted oxygen saturation curves for Zach at pH 7.4 and pH 7.0 in the right-hand box. Clearly distinguish which curve is which. In one sentence, rationalize these binding curves.

d) Based on the information provided in this problem, who is the best candidate for your expedition? Explain briefly.

total points	_____
of 151	_____
final %	_____