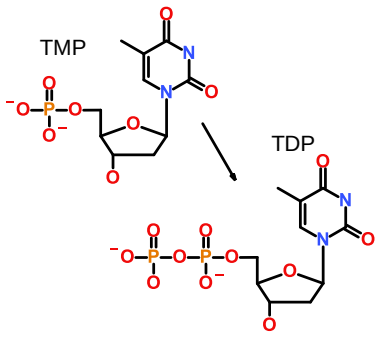
**Instructions**: this exam consists of 11 questions on 6 pages, for a total of 100 points. On questions with choices, all of your attempts will be graded and you will be awarded the highest grade. Please use the space provided or the back of the preceding page. **Note Q10 on page 5 is worth 18 points.**

**1. (10 pts)** Thymidylate kinases are enzymes that add a phosphate group to the nucleobase TMP (thymidine monophosphate) to generate TDP (thymine diphosphate). You want to measure the binding of TMP to a thymidylate kinase. Please answer the following questions for **one** of the following choices:




- **i)** What is the fractional saturation (5 pts)?
- **ii)** Briefly discuss how you would obtain $K_D$ from a series of measurements at different TMP concentrations (4 pts)
- **iii)** Although you have measured $K_D$, what other parameter is your measurement similar to? $K_M$, $K_I$, or $K_I$'? (1 pt)

**Choice A:** You place 10 uM of the enzyme inside a dialysis bag and add TMP on the outside. After equilibrium is reached you find the concentration of TMP outside the bag is 100 uM and the concentration inside the bag is 109 uM.

**Choice B:** The UV absorption of a 10uM solution of enzyme is 0.1 in the absence of TMP and 0.4 when saturated with TMP. You measure the absorbance when the TMP concentration is 100 uM and the measured absorbance is 0.37.

**2. (10 pts)** Discuss the general framework for allosteric effects on ligand binding (e.g. T and R states). Use any **one** feature of oxygen transport (by hemoglobin) to illustrate your answer (e.g. oxygen binding, altitude adaptation).



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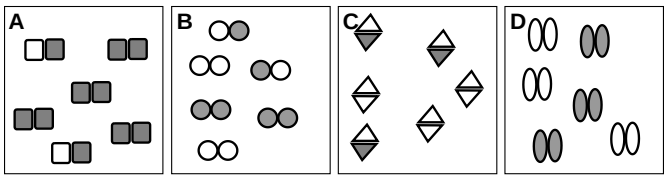
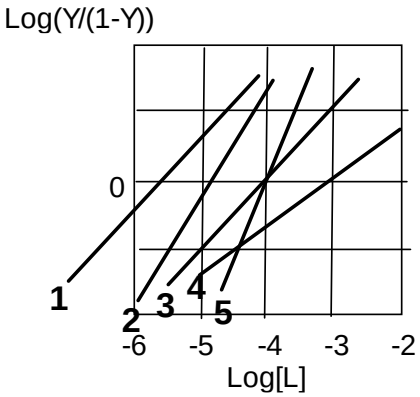
**3. (14 pts)** You are measuring the binding of a ligand to four different dimeric proteins. You have a magic camera that allows you to take a snapshot of the distribution of bound ligands at equilibrium. In all cases the ligand concentration is **$10^{-4}$ Molar**. Free ligand is not shown and subunits with ligand bound are shaded. You will find it useful to determine Y for these four cases, a column has been provided on the table to enter these values if you like.




i) Match the distribution of bound ligands to the curve on the Hill plot and indicate the correct match in the table below. Note that there are five curves on the Hill plot. *You must justify your answer with a discussion of $K_D$ and cooperativity for each protein (10 pts).*

| Protein | Hill Curve (1-5) | Y |
|---|---|---|
| A | | |
| B | | |
| C | | |
| D | | |

ii) Sketch the binding curve (Y versus [L]) that you would expect to see for proteins B and D on the graph on the right. Be sure to label the axis and provide a scale (4 pts). *Briefly justify your answer.*

**4. (8 pts)** Why do enzymes increase the rates of reaction? Discuss the key feature that is applicable to <u>all</u> enzymes.



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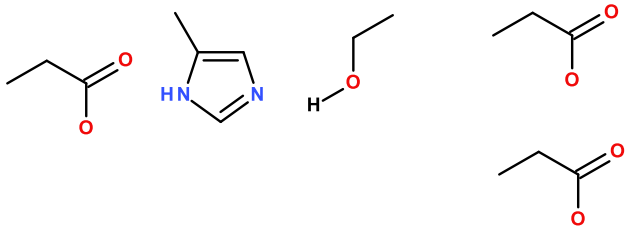
**5. (8 pts)** Please do **one** of the following choices:

**Choice A:** Select either serine proteases or HIV protease and briefly discuss the role of active site residues in the mechanism. The structures on the right may be helpful.

**Choice B:** Why is trypsin specific for Lys and Arg substrates while Chymotrypsin is not?




**6. (6 pts)**

i) Sketch a curve of [ES] versus time, beginning at t=0 when enzyme is mixed with substrate.

ii) What time period on your plot would be suitable for determining the velocity of the reaction? Why?



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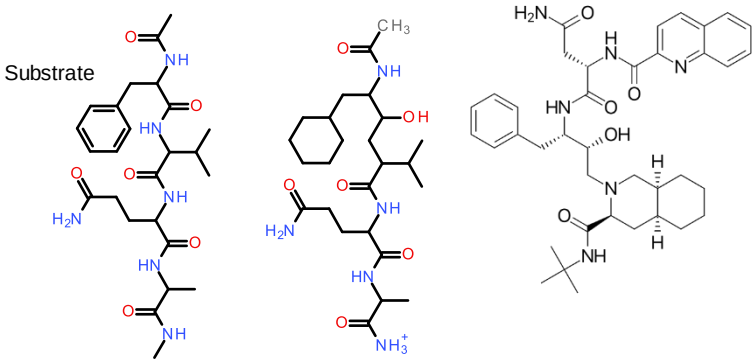
**7. (10 pts)** Compare and contrast competitive and mixed-type inhibitors. How are they similar and how do they differ. Your answer should explain their effect on kinetic parameters $k_{CAT}$ ($V_{MAX}$) and $K_M$.

**8. (2 pts)** The structure of the normal substrate for HIV protease, and the structure of two HIV protease inhibitors, are shown to the right. Why are both of these compounds inhibitors, what do they have in common?



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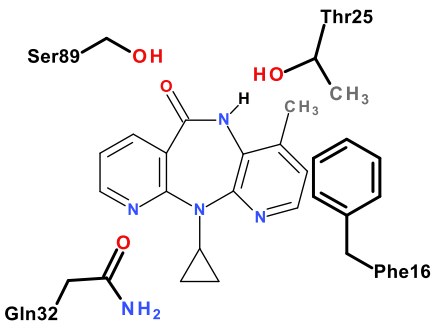
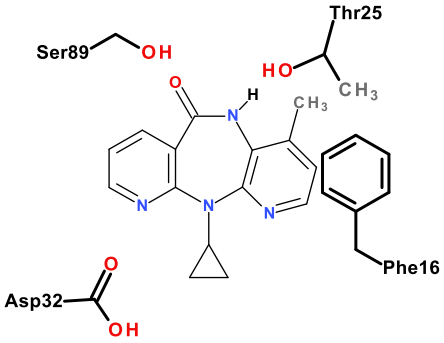
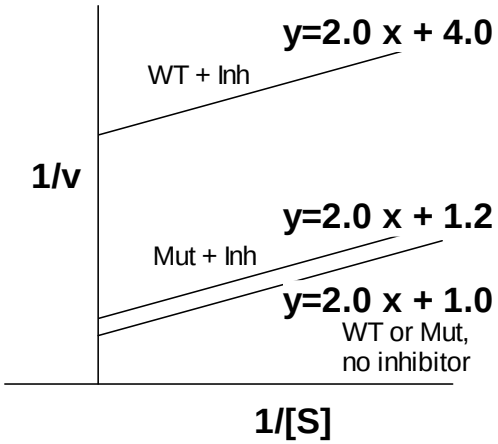
**9. (18 pts)** You have determined the structure of nevirapine bound to wild-type and a mutant HIV reverse transcriptase. The structure of the two complexes is shown on the right. The drug (thin lines) interacts with Gln32 (Asp in the mutant), Ser89, Thr25, and Phe16 in the wild-type enzyme. You also measure enzyme kinetic data using a concentration of **1 nM of nevirapine** with both enzyme and the resultant double reciprocal plots are shown on the right. The activity of the mutant and wild-type in the absence of the inhibitor was the same.

Wild-type Enzyme-Drug Complex




Mutant Enzyme-Drug Complex




i) Nevirapin is a mixed-type inhibitor. How would the double reciprocal plots change if it was a competitive inhibitor (you can use the plot on the right to illustrate your answer) (2 pts)?

ii) Determine the affinity of nevirapine to the wild-type and mutant enzymes from the double reciprocal plots, i.e. obtain $K_I$ and $K_I'$ (6 pts).

iii) Explain the change in affinity with reference to the interactions between the drug and the enzymes and the values you obtained in *part iii.* (4 pts).

iv) Suggest a modification to the drug that might restore affinity to the mutant enzyme (4 pts).

v) Based on your $K_I$ and $K_I'$ values that you obtained in part ii, is Nevirapin able to bind to the free reverse transcriptase (2 pts)? Why or why not?



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**10. (4 pts)** Typically, patients infected with HIV are treated with both nevirapin and a HIV protease inhibitor.

i) Why is this approach more effective at reducing replication of the virus than treating the patient with just one drug? (1 pt)

ii) Which drug would be more effective at higher substrate concentrations, nevirapine, or an HIV protease inhibitor, why? (3 pts)

**Bonus (2 pt):** Nevirapine is rapidly removed from the patient by the kidneys. In what general way could you modify nevirapine to increase its lifetime in the patient?

**11. (10 pts)** You are trying to purify thymidine kinase from a mixture of other kinases.

i) Suggest purification step(s) that will purify thymidylate kinase. *Briefly describe how the separation method works for any chromatography method that you use (8 pts).*

ii) What will happen to your specific activity after performing these steps (2 pts).

| | [Ammonium Sulfate] that precipitates 50% of protein* | # Residues (Mol Wt) | #Asp (pKa=4) | #Lys (pKa=9) |
|---|---|---|---|---|
| Thymidylate kinase(TK) | 1.0 M | 120 (13,200 Da) | 6 | 4 |
| Adenosine kinase(AK) | 1.5 M | 120 (13,200 Da) | 6 | 6 |
| Pyruvate kinase(PK) | 4.0 M | 120 (13,200 Da) | 8 | 5 |
| Hexose kinase(HK) | 6.0 M | 240 (26,400 Da) | 10 | 12 |
| *Concentration 1M above will precipitate 100% of the protein, 1 M below will precipitate none of the protein. | | | | |



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