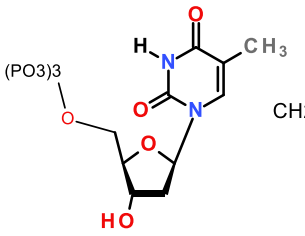
Problem Set 7 - Due Sunday October **Time required ~ 85 min.**

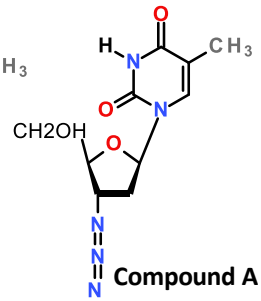
1. (10 pts, 10 min) Chymotrypsin binds Phe as a substrate based on the nonpolar Met192 and Val213 in its specificity pocket. View the JMol structure of chymotrypsin with different substrates to determine what additional interactions stabilize Tyr and Trp as bound substrates.

In both cases there is a hydrogen bond between the -OH on Tyr, or the NH on Trp with the mainchain C=O of Ser217. Take home message is that it is possible to have multifunctional specificity pockets.

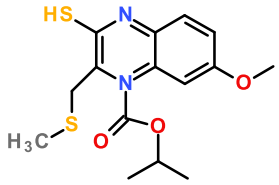
2. (10 pts, 20 min). You work for a drug company and you are trying to determine which two drugs would be better to use in clinical trials to inhibit HIV reverse transcriptase. The structure of the normal substrate and the two drugs are shown to the right.



Substrate



Compound A



Compound B

i) What is the role of this enzyme in the HIV lifecycle and indicate why it would be a particularly good drug target (2 pts).

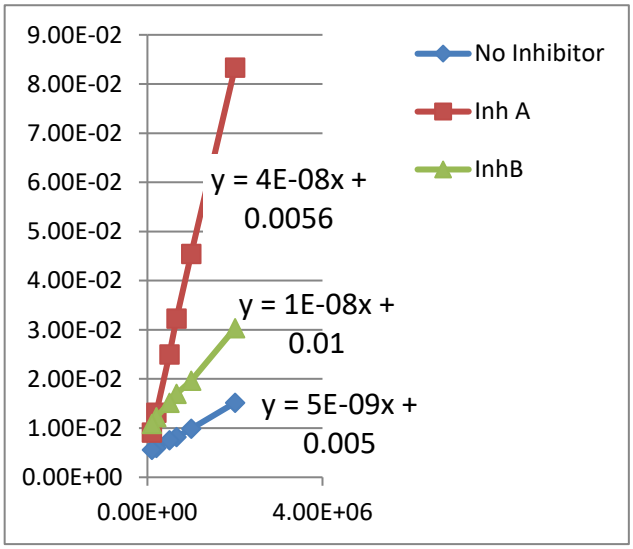
This enzyme converts the genetic material of the HIV virus (RNA) to double stranded DNA. It is a good drug target because:

a) It is an essential step in the life cycle of the virus; inhibition of this enzyme will prevent viral growth.

b) There is no human counterpart, reducing the possibility that the inhibitor will inhibit the human enzyme.

ii) The table on the right gives the steady state velocities acquired with no inhibitor and with 10 uM of inhibitor A or 10 uM of inhibitor B.

[S] M	*V* (μM/sec) No drug	*V* (μM/sec) [A]=10μM	*V* (μM/sec) [B]=10μM
0	0	0	0
0.5×10^{-6}	66	12	33
1.0×10^{-6}	101	22	51
1.5×10^{-6}	122	31	59
2.0×10^{-6}	133	40	66
5.0×10^{-6}	167	76	82
10.0×10^{-6}	180	110	92



Determine the appropriate inhibition constants (K_I or K_I and K_I') from the steady-state data using a double reciprocal plot. You should briefly discuss how you decided to determine K_I versus K_I and K_I', i.e. why a compound is a competitive or mixed type inhibitor. Note that there is some experimental error in the data, so the y-intercept on the double reciprocal plot for the competitive inhibitor may not be exactly the same as the value obtained in the absence of the inhibitor. Submit the double reciprocal plot with your problem set (5 pts).

The K_I values are obtained from the ratio of the slopes:

A: 4e-8/0.5e-8 = 8, K_I = 10 uM/(8-1) = 1.42 uM

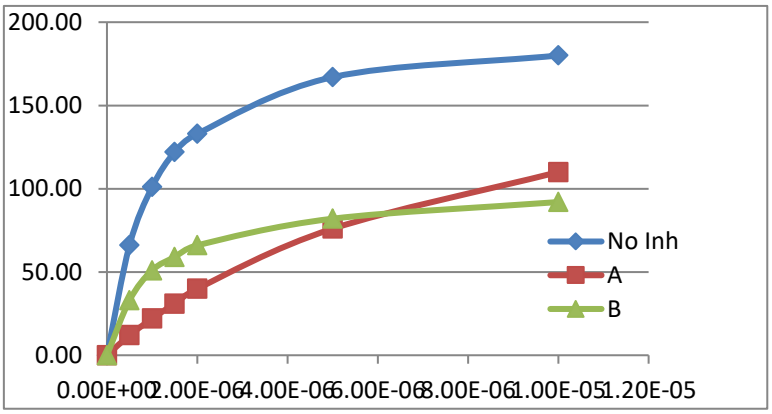
B: 1e-8/0.5e-8 = 2, K_I = 10 uM/(2-1) = 10 uM

The K_I' values are obtained from the ratio of the y-intercepts. It looks like InhA has the same y-intercept as the data without inhibitor (as you would expect since it is a comp. inhibitor). In this case K_I' is undefined.

Inh B: Since this is a mixed-type inhibitor, we also have a K_D 0.01/.005 = 2, K_I' = 10 uM/(2-1)=10uM

iv) Can you determine from these data which inhibitor will make the best drug? If not, what additional information beyond the binding affinities will be required? Many factors come into play in making a successful drug besides how well it may bind to its target enzyme in a laboratory setting. Think about where drugs have to function and what might be unknown in that environment. [Hint: A plot of *v* versus [S] in the absence and the presence of each inhibitor may give you an idea.] (3 pts)

The drug with the lower K_I will, in general be the better drug because it binds to the enzyme more efficiently. However, the situation is a bit more complicated here since one drug acts as a competitive inhibitor while the other acts as a non-competitive inhibitor. The ability of Drug A to inhibit will depend on the substrate concentration. Drug A is the better inhibitor at low substrate (because it is a competitive inhibitor and won't be displaced by the substrate AND has a lower K_I than the other drug), while drug B is the better inhibitor at high substrate. Therefore, you need to know the substrate level in the average person.



3. (21 pts, 25 min) The Jmol page associated with this problem set shows wild-type and a mutant HIV protease in complex with a number of different HIV drugs. One of these drugs is the same as the one presented in class. This particular drug contains a cyclohexane ring at one end and it binds to the wild-type enzyme with high affinity. Three different drugs, with alteration in the cyclohexane ring, have been developed for the purpose of inactivating the *mutant* HIV protease (structures given at the end of question).

i) What feature of the HIV life-cycle leads to a high level of mutations in the HIV genetic material (2 pts)?

Error prone conversion of viral RNA to DNA by HIV reverse transcriptase

ii) Based on the Jmol page, which residue is altered in the mutant HIV protease? How has it been changed? (1 pt)

Val82 has been changed to Threonine, replacing one non-polar methyl with an polar -OH group.

iii) Does this residue contribute mostly to catalysis or specificity? Justify your answer. (2 pts)

Specificity, Val82 is one of the residues in the specificity pocket of HIV protease. The two Asp residues at position 25 are responsible for cleavage of the peptide bond, these have not been altered. So, the enzyme is still functional.

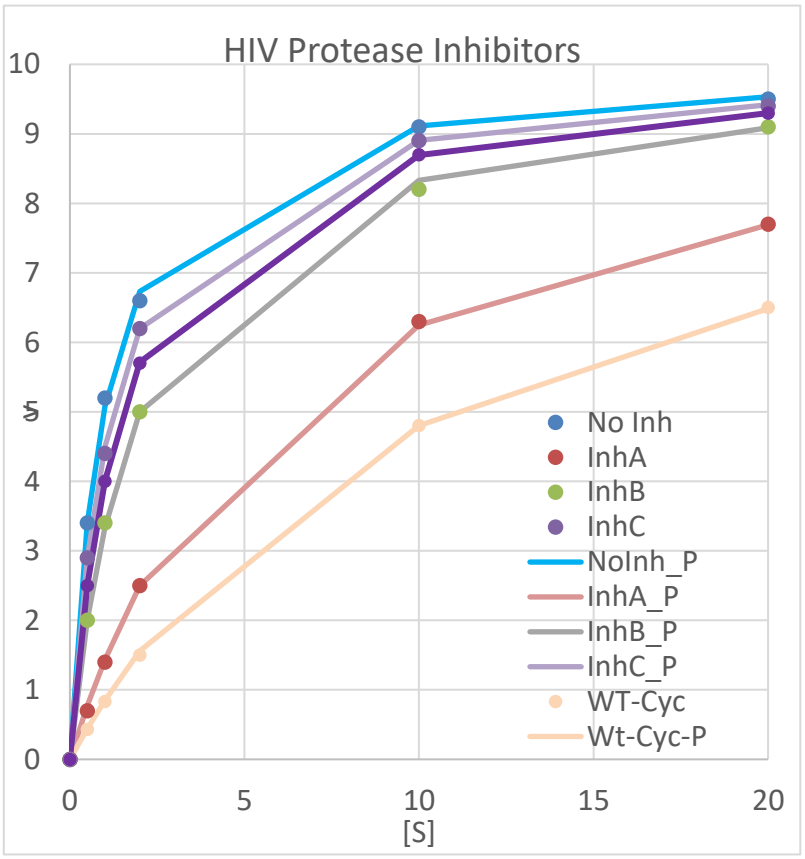
iv) Determine the K_I values using solver. Download the spreadsheet for this problem set (do not use the one from recitation). It is all set up for you, except for column K (predicted values for WT-Cyc). You will have to generate the correct formula for those cells, using the following (K_M = W1, V_{MAX} = W2, alpha = W3). You have to adjust V_{MAX} to get solver to fit the data; adjust the value of W2 to do so. Note that we have assumed the same V_{MAX} and K_M for the wild-type and the mutant. Include a graph that shows the best fit to all of the data. The concentration of the drug is 10 nM (5 pts).

The correct formula for column K is:

$$v_{predicted} = \frac{V_{MAX}[S]}{\alpha K_M + [S]} == \$W\$2 * A2/(\$W\$3 * \$W\$1 + A2)$$

Enzyme	Drug	α	KI = 10 nM/(α-1)
Wild-type (WT)	Cyclohexane (Cyc)	11.16	0.98
Mutant	Cyclohexane	1.55	18.33
Mutant	Drug 1 (amide)	6.18	1.93
Mutant	Drug 2 (alcohol)	2.06	9.43
Mutant	Drug 3 (methyl)	1.26	38.11

v) Explain, with reference to the change in the K_I values and the structure of the two enzymes, why the affinity to the original cyclohexane drug has been affected by this mutation (e.g. compare lines 1 and 2 in the above table as well as the interaction between the protein and the drug.) (3 pts)



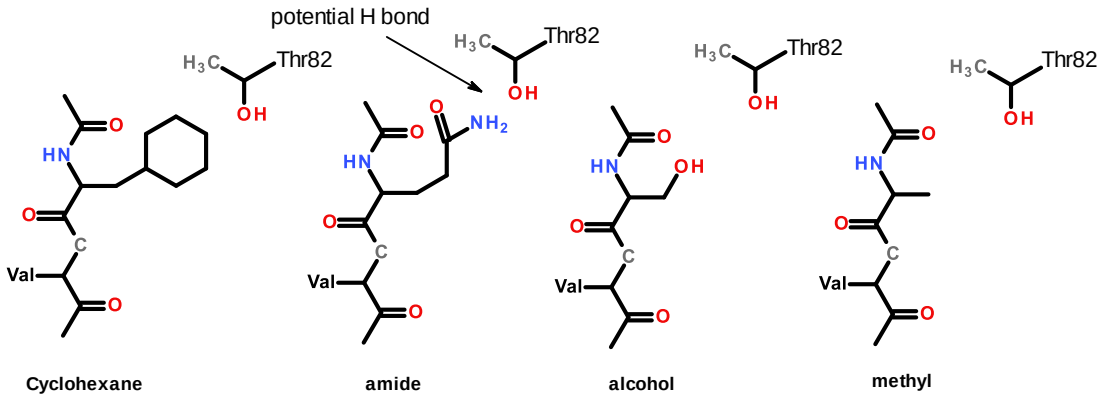
The K_I increased due to the mutation, so the cyclohexane containing drug binds less well to the threonine containing enzyme. The valine in the wildtype enzyme had good van der Waals contact with the cyclohexane ring, as well as a non-polar interaction. The size of threonine is the same as valine, so vdw would be about the same (perhaps larger because the -OH on Thr is polar, giving stronger dipole-induced dipole interactions), but the hydrophobic effect between the cyclohexane and the Thr would be smaller, reducing the affinity (increasing K_I).

vi) Which of the three drugs would be the *worst* inhibitor of the mutant protease? Justify your answer with reference to the K_I values as well as the interaction between the drug and the mutant enzyme. Please include a simple sketch of the interaction between the drug and the inhibitor (4 pts).

The methyl containing drug is the worst based on K_I. It is small and non-polar, giving poor vdw interaction with the Thr sidechain on the mutant enzyme, the methyl group is too far away from residue 82.

vi) Which of the three drugs would be the *best* inhibitor of the mutant protease? Justify your answer with reference to the K_I values as well as the interaction between the drug and the mutant enzyme. A simple sketch of the interaction between the drug and the enzyme would be useful (4 pts).

The drug containing the amide group is the best based on K_I values. It could form a hydrogen bond with the Thr residue in the mutant protease.



4. (20 pts, 30 min) You are given an equal mixture of all the proteins listed in the table below. The size of the protein, its solubility in ammonium sulfate, and the number of charged residues are presented in this table. Using the on-line protein purification simulator, develop a purification scheme that will result in the purification of one of the proteins in their native, non-denatured, form. The protein that you need to purify depends on your last name. Enter the value in the 'Key" column on the first page of the simulator, where it asks for your "first letter last name:". The correct target protein should be assigned to you.

Name	Key
Moza, Ayah A., Mohammed A.	D
Asma, Amna, Lulwa	H
Alreem, Sara, Maryam	J
Laila, Mahnoor, Maher	M
Mohammad H, Syeda, Thamanna	Q
Ayah S, Mohammed S	T

In developing your purification scheme, you may use ammonium sulfate precipitation, size exclusion (gel filtration), anion exchange, and cation exchange chromatography.

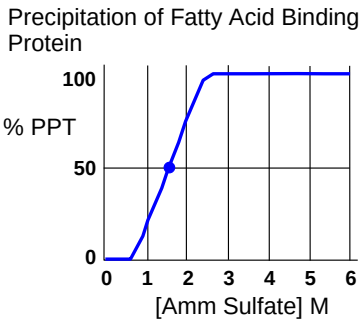
After completing the purification of your protein, you must click on the submit button to send your work to the server. Feel free to make as many practice runs as you like, but only hit submit when you are satisfied with your purification scheme and want to submit it for grading.

Starting amount and Yield: The initial number of enzymatic units of each protein is 100, therefore your % yield = number of final units.

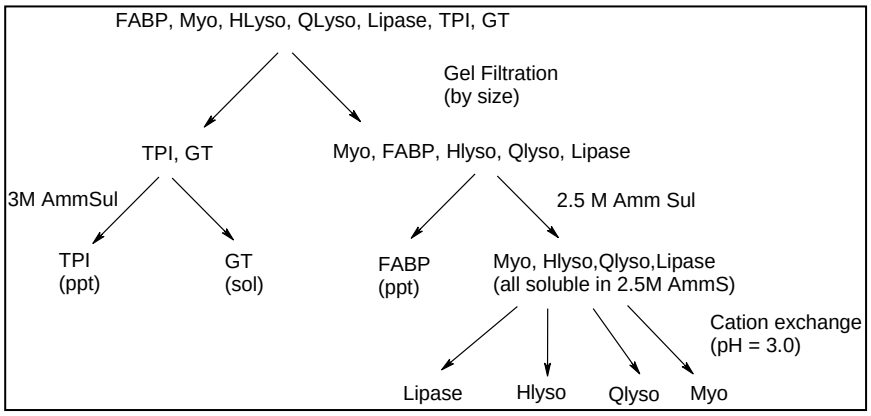
How to evaluate purity? The specific activity of each protein can be found on the "Handbook" tab and it is also listed below. Due to round off error, your final specific activity may be ~5% lower, this will be considered as a pure protein. The specific activities of the pure protein are also listed in the table.

Protein	**Sol. Amm Sulf***	**# Residues**	**Number of Charged Residues**				**Spec. Act. (pure protein)**	**Key: Enter as "*first letter of last name*"**
			Acidic Asp+Glu	**His**	**Lys**	**Arg**		
Fatty Acid Binding Protein	1.5	131	10	1	10	5	69.3	Recitation
Myoglobin	3.5	153	10	10	15	5	59.4	D
Hen Lysozyme	5.0	130	5	1	5	15	69.9	H
Quail Lysozyme	6.0	129	5	5	5	15	70.4	J
Phospholipase	5.5	133	10	1	5	5	68.3	M
Triose-phosphate isom.	2.0	240	10	0	15	10	37.8	Q
Glutathione Transferase	6.0	230	15	0	20	10	39.5	T

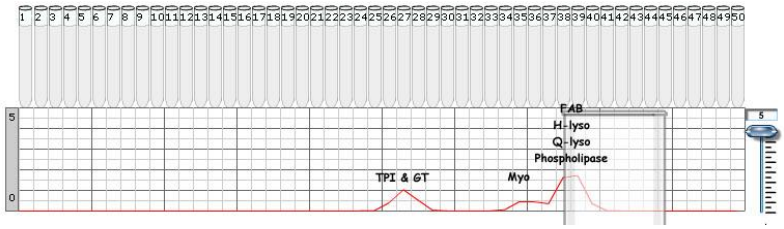
*Ammonium sulfate concentration at which 50% of the protein will precipitate. A concentration that is 0.5 M below and above this concentration will precipitate 25% and 75% of the protein, respectively. A concentration that is 1.0M below or 1.0M above will precipitate 0% and 100% of the protein, respectively



In developing a purification scheme you have to look at how the properties of the various proteins differ and then use the appropriate separation scheme to take advantage of that difference. The overall purification scheme for any of the 7 proteins is diagrammed on the right, details are below. This scheme is not necessarily unique or optimized for any single protein.



Triose phosphate isomerase and Glutathione Transferase: First, looking at the molecular weight (# residues) the last two proteins are cleanly separated from the first 5 by gel filtration (step 1). They can be separated from each other because one is not very soluble in Ammonium sulfate (Triose-phosphate isomerase) while the other, Glutathione transferase, is. Therefore, a second step of ammonium sulfate fractionation will produce pure TPI and GT. The elution profile from the gel-filtration column is shown on the right. (Note: that these two proteins also differ in charge at pH 9.0 and cation exchange chromatography could also be used as a second step to separate them).



Fatty acid binding protein can be separated from the other four proteins (after the gel filtration step) due to its low solubility in ammonium sulfate. An ammonium sulfate conc. of 2.5M will ppte all the fatty acid binding protein, leaving the other four in solution.

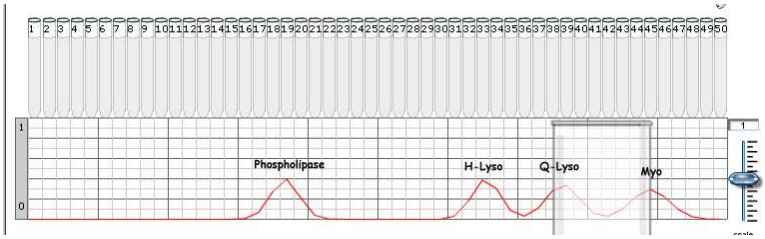
The remaining four proteins (**Myo, HLyso, QLyso, Lipase**) must be separated by charge. All four can be separated from each other at pH 3.0 using cation exchange, based on their charge difference:

Myo: -10(0.1) +10(1) +15(+1)+5(+1) = +29

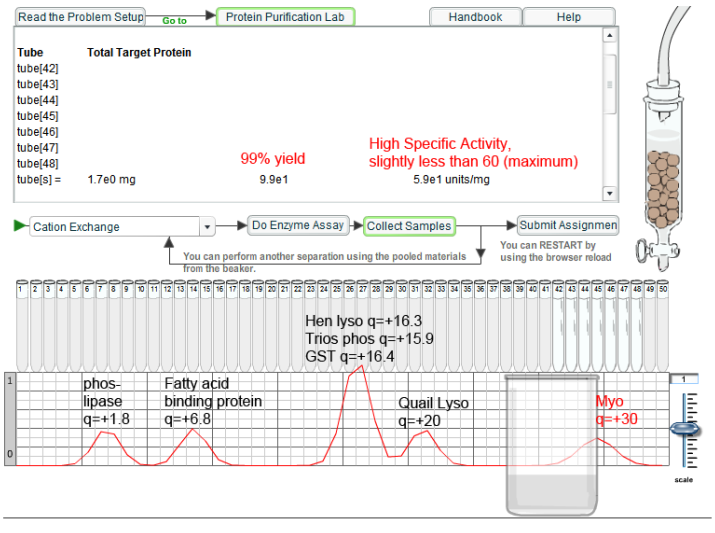
HLyso: -5(0.1) +1(+1) +5(+1) +15(+1) = +20.5

QLyso: 5(0.1) +5(+1) +5(+1) +15(+1) = +24.5

Phos.lip.:10(0.1) +1(+1) +5(+1) +5(+1) = +10



The elution profile of the final cation exchange column @pH 3.0 that separates phospholipase, Hen lysozyme, quail lysozyme, and myoglobin from each other is shown on the right.



Note: This scheme is not optimal for myoglobin, requiring three steps. Myoglobin, phospholipase, and fatty acid binding protein can be purified in one step by simply running a cation exchange at pH 9.0. The screenshot to the right shows the results for myoglobin.