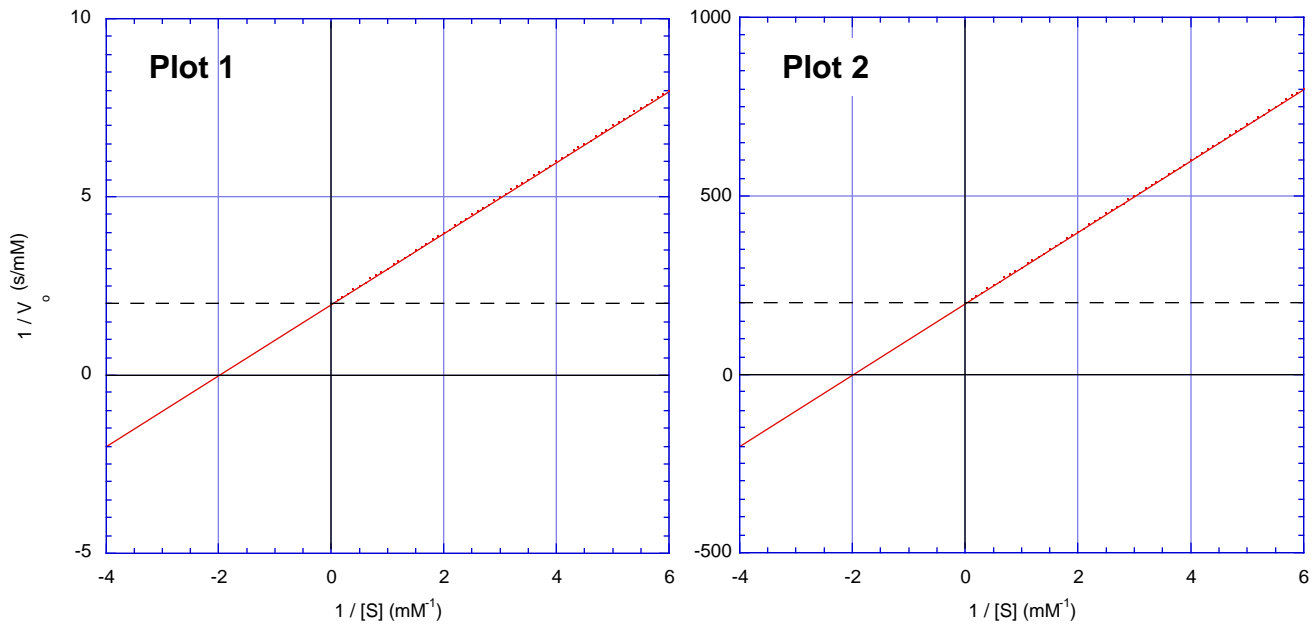


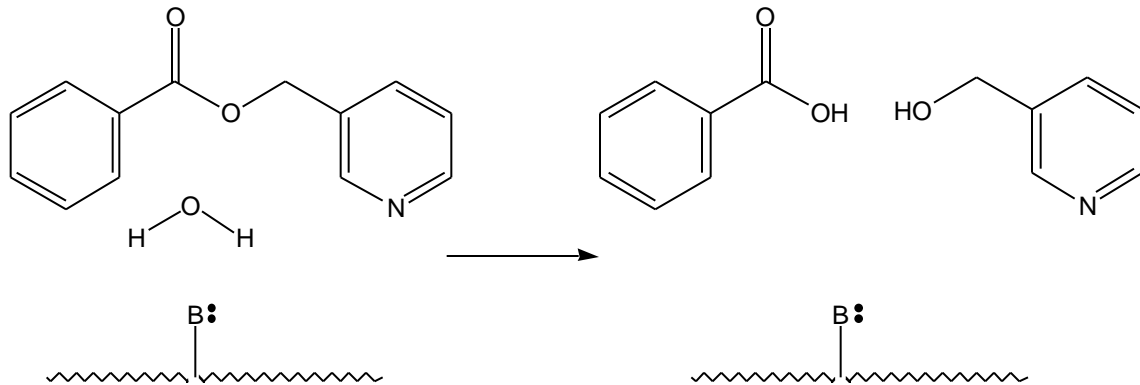
Problem Set VII - Due Friday, November 30th

- 1) (15 points) As you know, serine proteases (SPs) and cysteine proteases (CPs) are pretty much the same, except that the aspartate residue present in SPs is absent in CPs. You isolated a SP, **SP-VII**, and a CP, **CP-VII**, and found that, apart from the aspartate residue difference, both enzymes have remarkably similar primary and tertiary structures. Not having anything better to do, you collected steady-state kinetic data for both enzymes, which is presented as Lineweaver-Burke plots below. As you've done countless times, you lost the labels for the graphs.



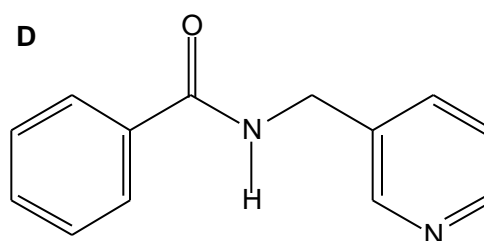
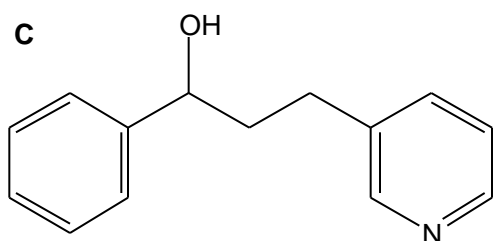
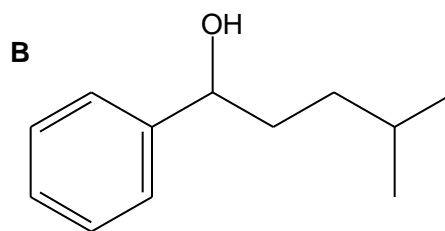
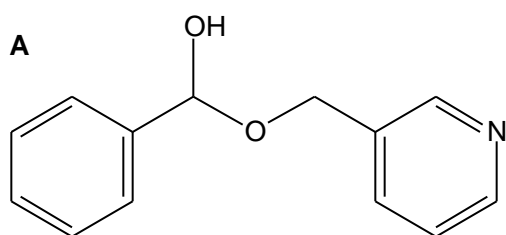
- Which plot corresponds to **SP-VII**, and which one to **CP-VII**? Justify your answer clearly.
- Using the data presented in the graphs, calculate V_{\max} and K_M for both enzymes (you may need to do this first to answer part (i)).
- Compare the values of K_M you obtained for both enzymes. Is this result consistent with the characteristics of **SP-VII** and **CP-VII**? Hint: Think of what process is associated with K_M , and think if this will change by having or not the Asp residue as part of the catalytic machinery...

- 2) (15 points) Lipases, like phospholipase A₁ and A₂, catalyze the hydrolysis of esters using almost the same mechanism than serine proteases. The only difference is that only two of the three amino acids of the Ser/His/Aps catalytic triad are present in lipases. The missing amino acid is replaced by a water molecule present in the active site. For the following ester:



- Write a detailed mechanism for the hydrolysis of the ester bond catalyzed by a lipase, accounting for all nucleophilic attacks and hydrogen abstractions ('push electrons wisely...')
- In the reaction mechanism, indicate which steps can be considered as general acid-base catalysis, and indicate all the transition states that form during the reaction.
- Which amino acid from serine proteases is replaced by water in the active site of lipases?

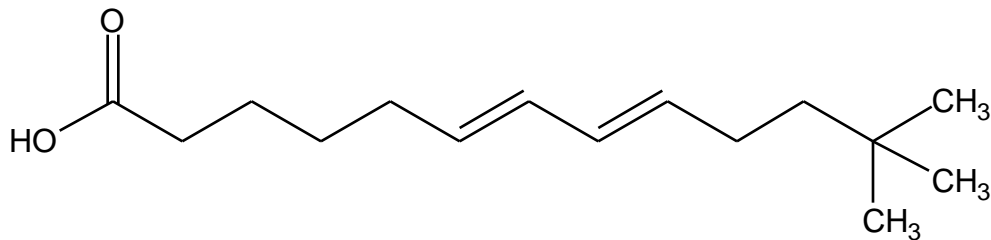
- 3) (15 points) You have a lipase just like the one above, which is specific for the ester from question (2). You now want to design a transition state analog for that particular lipase, and asked your organic chemist buddy to come up with something. He gave you the following compounds:



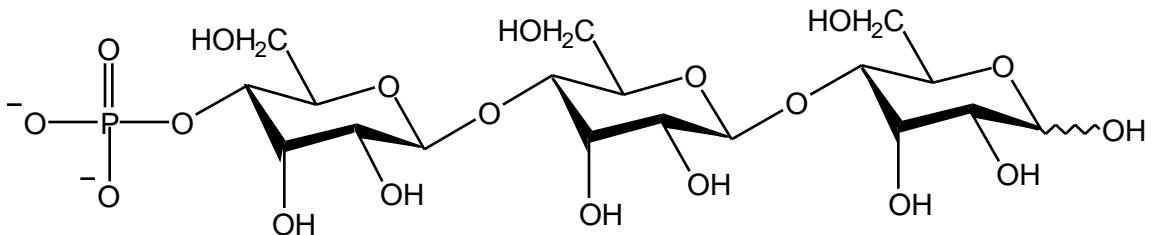
- Which compounds will you discard from the start, because they could either behave as substrates, or they look too similar to the transition state?
 - Based on your knowledge of the mechanism of this lipase, which of the compounds proposed will be transition state analogs?
 - From the compounds you picked in part (ii), which one will be the best transition state analog? Explain clearly why one is better than the other.
- 4) (15 points) The double bonds in unsaturated fatty acids are prevalently **cis**, and depending on their concentration, the physical characteristics of the bilipic membrane will change. Consider that you isolated the fatty acids from the membranes of a newly discovered bug, and found that all unsaturated fatty acids are **trans**.
- Based on your knowledge of non-covalent interactions and the flexibilities of different bond types, predict if the melting point of these fatty acids will be higher or lower than the melting points of saturated fatty acids and normal (**cis**) unsaturated fatty acids. Explain your reasoning clearly.
 - Taking into account your answer for part (i), will bilipidic membranes having high content of these **trans** fatty acids be fluid or rigid?
 - Do you think that this bug from which you isolated the fatty acids lives in the Artic tundra or in hot water springs? Be consistent with your answers for parts (i) and (ii).

5) (20 points) In the last mission that NASA did to Mars before they started smashing their spaceships against the ground, scientists found a new martian bacteria, and in another unexplainable move from the Space Agency, you were given a specimen. You analyzed the bacterial membrane, and found a new kind of phospholipid. In order to identify its chemical structure, you performed several enzymatic digestions of the compound with phospholipases:

- Treatment of the martian phospholipid with **phospholipase A₁** gives no products, only starting materials.
- Treatment of the phospholipid with **phospholipase A₂** gives no products, but treatment with a peptidase equivalent to **phospholipase A₂** gives you the following compound:

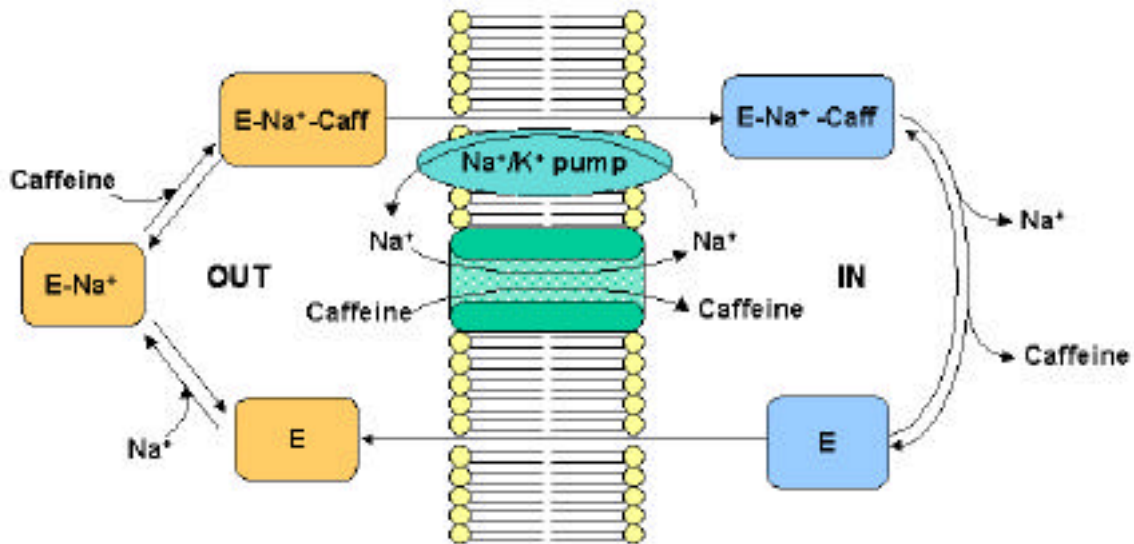


- Treatment with **phospholipase C** gives the following fragment:



- Considering which types of phospholipases were active and which ones were not, is the phospholipid a phosphoglyceride, a sphingophospholipid, or something not yet on the records?
- From the fragments produced by the different phospholipase treatments, reconstruct the chemical structure of this martian phospholipid.
- Considering your answer from (i), will this phospholipid be found in the outer or in the inner layer of the martian bacteria's membrane? Justify your answer.

6) (20 points) You found a transporter that promotes transport of Na^+ ions and caffeine across the cell membrane:



- Is this an example of passive transport, active transport, or secondary active transport? As usual, justify your answer to the best of your abilities.
- How many conformers will the membrane transporter adopt in order to accomplish this process?
- What type of cotransport can you identify for the Na^+ /caffeine pair?
- If this hypothetical membrane transporter existed, what would you increase or withdraw from the diet of a person who constantly suffers from caffeine jitters (apart from coffee, obviously)?