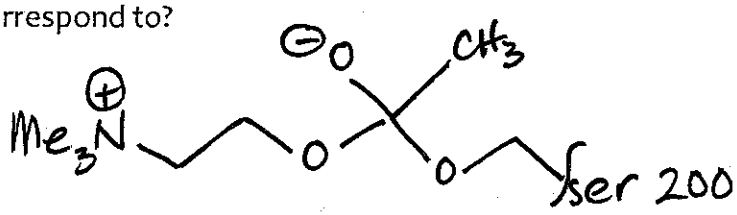
Study Question 6

Use Chimera file AChEv1.py to answer these questions (derived from 2ace.pdb)

What substrate is in the active site? Draw its structure. What mechanistic intermediate does this correspond to?



tetrahedral intermediate

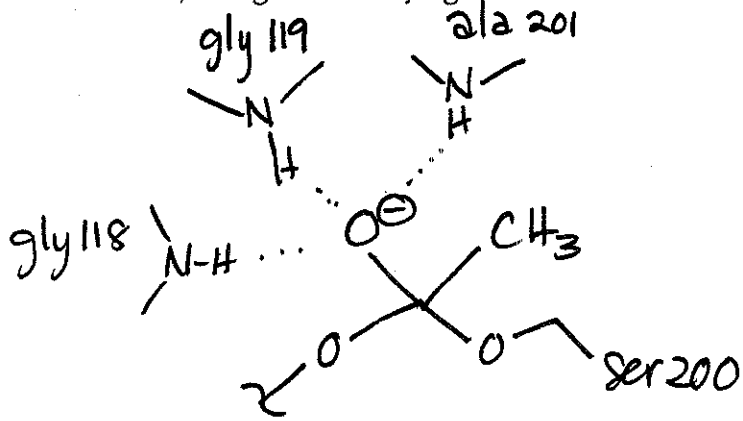
Locate the catalytic triad, and give the identifiers/numbers for each residue.

ser 200 his 440 glu 327

What residue(s), and what intermolecular force(s), appear to stabilize the quaternary nitrogen?

phe 330 + trp 84 cation - pi interactions
glu 199 electrostatic attractions

There are several backbone pieces which stabilize the so-called "oxyanion" hole. Draw a picture of these interactions below, and give identifying names and numbers.



same intermediate as above

Describe the location of the active site relative to the entire structure.

at the bottom of a large canyon

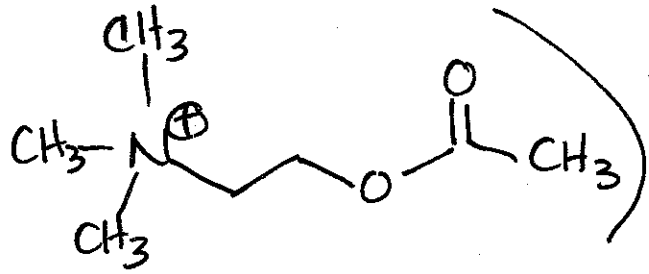
Switch to Chimera file AChEv2.py (also derived from 2ace.pdb)

Four additional amino acid side chains have been included in this view (relative to the previous structure). What are they (name and number), what do they have in common, and what role do they appear to be playing?

phe 331, phe 288, phe 290, tyr 23

all non-polar, they appear to use steric hinderance + London forces to block the area around $-CH_3$

With your answer above in mind, what limits are placed on the design of an inhibitor for AChE? Answer by drawing and annotating the structure of acetylcholine.

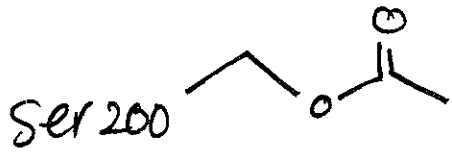


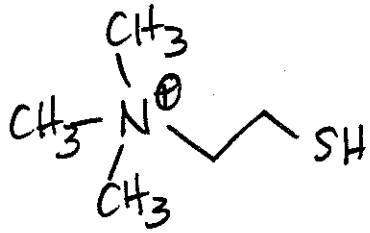
they surround this $-CH_3$

it would appear that the CH_3 cannot be made much larger

Switch to Chimera file AChEv3.py (derived from 2c58.pdb)

This file has most of the active site side chains stripped out for clarity. What molecule(s) is(are) at the active site? Draw the structures here.





This structure is part-way along the reaction pathway. Describe "where" is it stuck. What about the structures you identified just above is mechanistically consistent with where the reaction is stuck?

Compared to the 2-page handout on serine proteases, this structure is ½ way through the cycle, analogous to the state at the bottom of page 1

Switch to Chimera file AChEv4.pdb (derived from 1amn.pdb)

This structure has an inhibitor bound to AChE.

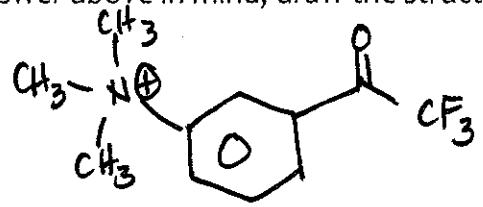
Examine the side chains near the inhibitor. What is different about these particular side chains?

The hydrogens are shown on N's + O's

Examine serine 200 which is the active site serine. What's "wrong" with it?

It looks like the -OH is missing. In fact, the bond between the $-CH_2-$ and the O was not drawn automatically

With your answer above in mind, draw the structure of the inhibitor below.



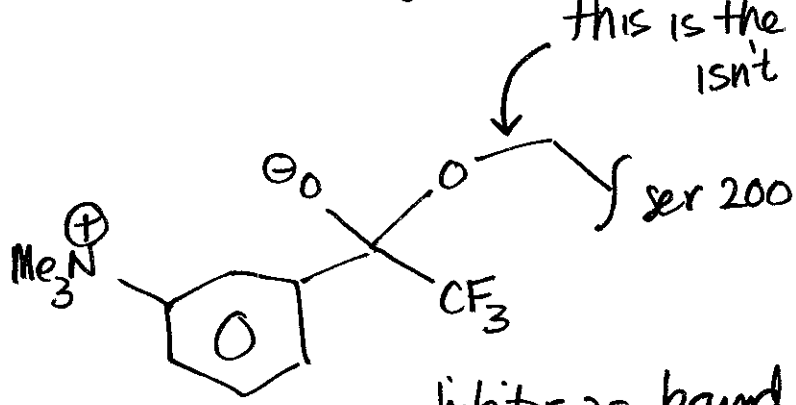
See also below

This inhibitor is "non-hydrolyzable." What does this mean? Explain mechanistically.

There is no LG, so the tetrahedral intermediate cannot collapse. The structure is stuck @ addition

Why is this inhibitor reactive? Explain

The CF_3 is strongly e^- withdrawing, making the adjacent C=O very electrophilic.



Inhibitor as bound @ active site.