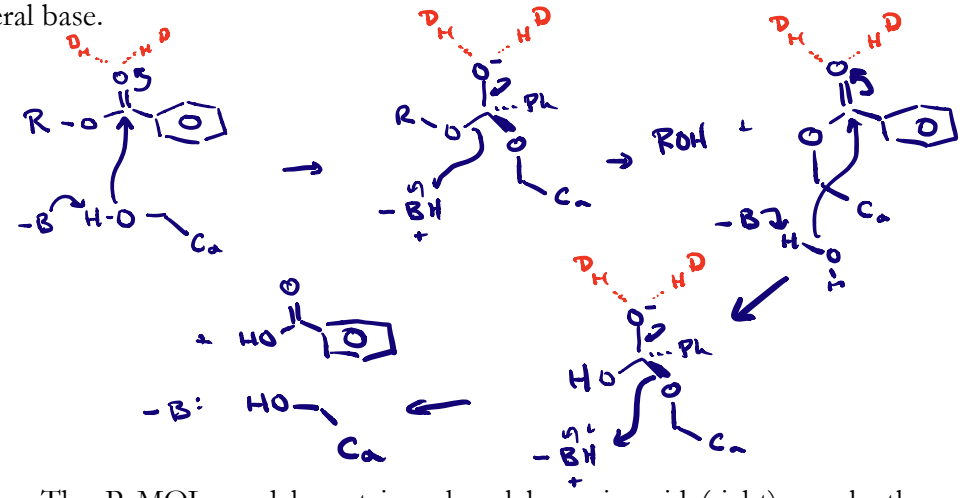
Chemistry 391 - Problem Set #9 **Name** Solutions

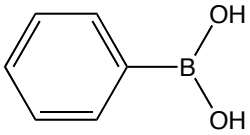
Due on Thursday 11/15/18 in class.

1. There is a real enzyme called "cocaine esterase" that is produced in bacteria that live at the base of the coca plant. The enzyme employs nucleophilic catalysis to hydrolyze cocaine. Download and open the file, **esterase.pse**, and examine the active site of the enzyme.

a. To start, draw a full arrow-pushing mechanism for the hydrolysis of a benzoic acid ester (you don't need to draw the entire cocaine molecule) assuming a nucleophilic serine residue activated by a general base.



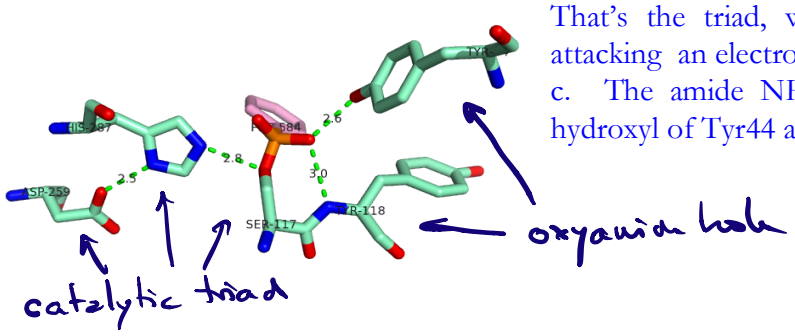
b. The PyMOL model contains phenyl boronic acid (right) covalently bound to a serine residue in the active site. The boron is electron deficient and therefore a great electrophile. ID the residues of the catalytic triad found in cocaine esterase and sketch the geometry, with distances here.



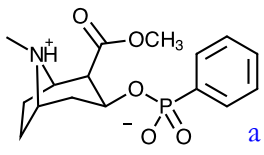
c. Identify the two groups that comprise the oxyanion hole in cocaine esterase and give relevant distances between H-bonding groups (no sketch necessary). Note that only one of the two hydroxyls on boron occupy the hole. The other is pointed to where the leaving group would be.

b. Note that Asp 259 H-bonds to His287, which in turn H-bonds to the sidechain oxygen of Ser117. That's the triad, with the serine caught flagrantly attacking an electrophile.

c. The amide NH of Tyr118 and the side chain hydroxyl of Tyr44 are doing the oxyanion hole thing.

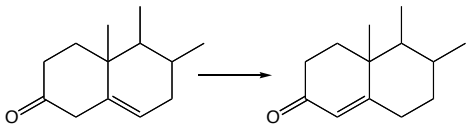


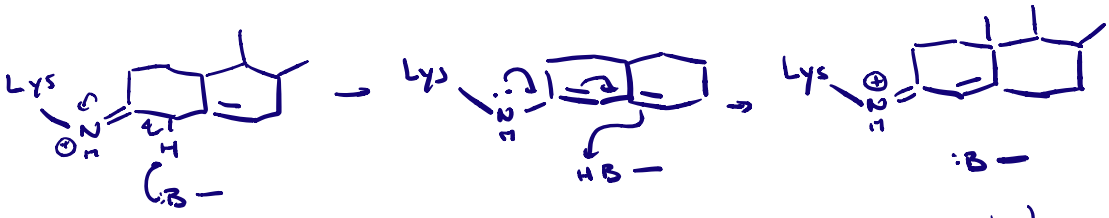
d. Why wouldn't a phosphonate transition state analog (such as the one used in generating a catalytic antibody) work as an inhibitor for this enzyme?



The phosphonate is a tetrahedral species that does not include the active site serine, so that's not going to work. If the serine does attack it, the phosphorus goes penta-coordinate, which is not the shape of the transition state. So, not a good TS analog.

2. A different KSI enzyme than the one from last week has an active site lysine, in which the side chain amino group forms an imininum ion. Explain how this covalent enzyme-intermediate complex might enhance the rate of reaction via *both* enthalpic **and** entropic stabilization of the transition state vs. the ground state. *Ref.* Lin et al. (1997) *PNAS* **94**, 11773. (Hint: why isn't a general acid needed here?).



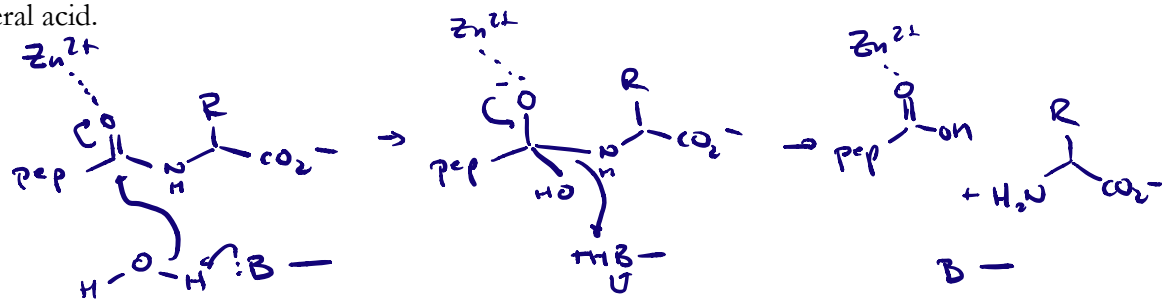


$\Delta H^{\ddagger}$: Charge is quenched in $X^{\ddagger}$ leading to a reduction in the enthalpic barrier.

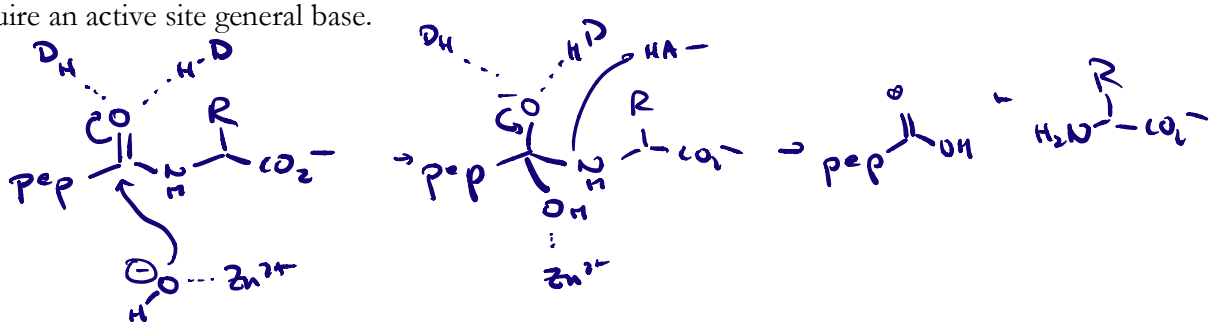
$\Delta S^{\ddagger}$: Covalent linkage significantly reduces entropy in ES complex, making $X^{\ddagger}$ more accessible w/ less loss of entropy.

3. Carboxypeptidase A is a Zn-dependent protease that catalyzes the hydrolysis of the C-terminal residue from a protein with an active site zinc. Although it has been studied for years, there is some disagreement about the mechanism of the enzyme. In general, hydrolysis of an amide, as for an ester, requires a general base to deprotonate the nucleophilic water, and an active site general acid to protonate the carbonyl oxygen.

a. Suggest a mechanism for zinc-catalyzed peptide bond hydrolysis that does not require an active site general acid.

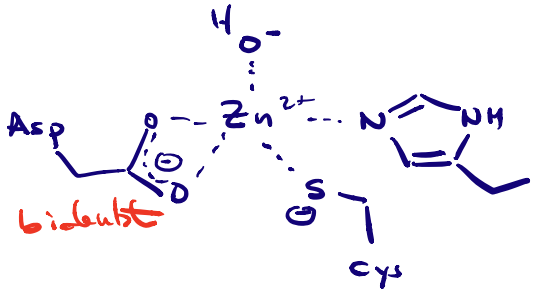


b. Now suggest an alternate mechanism for zinc-catalyzed peptide bond hydrolysis that does not require an active site general base.



4. An enzyme active site employs a Zn^{2+} hydroxide as an active site base. A PyMOL file, ZnSite.pse, is available on the website.

a. In addition to the HO^-, the Zn^{2+} is bound by three active site residues: His, Asp and Cys. Using the PyMOL file, draw the binding site of the Zn^{2+} ion, taking care to draw each residue side chain in the correct protonation state. Also ID the residue that is acting as a bidentate ligand to zinc.



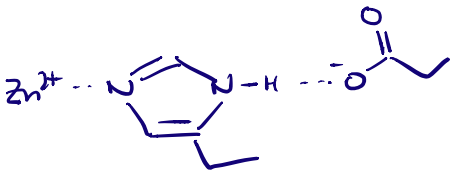
b. The pK_a of a water molecule bound to this zinc ion is 8.0. How would the following substitutions of metal binding residues affect the pK_a (give a once sentence explanation)?

His to Asp: Adds e⁻ density to Zn^{2+}, weakens Lewis acidity
pK_a bound H_2O ↑

Asp to Asn: Remove e⁻ density for Zn^{2+}, enhances Lewis acidity
pK_a bound H_2O ↓

c. It is observed that the His forms a hydrogen bond to an Asp residue via the nitrogen not employed in metal binding. Sketch the interaction and then predict the impact of the following mutations on the pK_a of the active site water, along with a brief explanation.

Sketch:

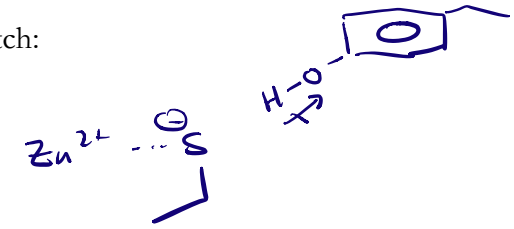


Asp to Ala: pK_a ↓ Removes e⁻ density

Asp to Asn: pK_a ↓ Removes e⁻ density (but not as much as above)

c. It is observed that the Cys forms an ion-dipole interaction with a tyrosine. Sketch the interaction and then predict the impact of the following mutations on the pK_a of the active site water, along with a brief explanation.

Sketch:

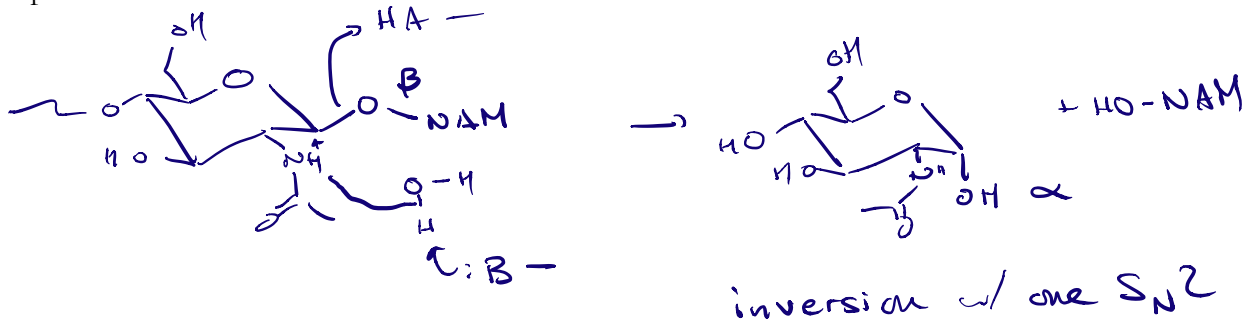


Tyr to Phe: pK_a ↑ Puts more e⁻ density on Zn^{2+}

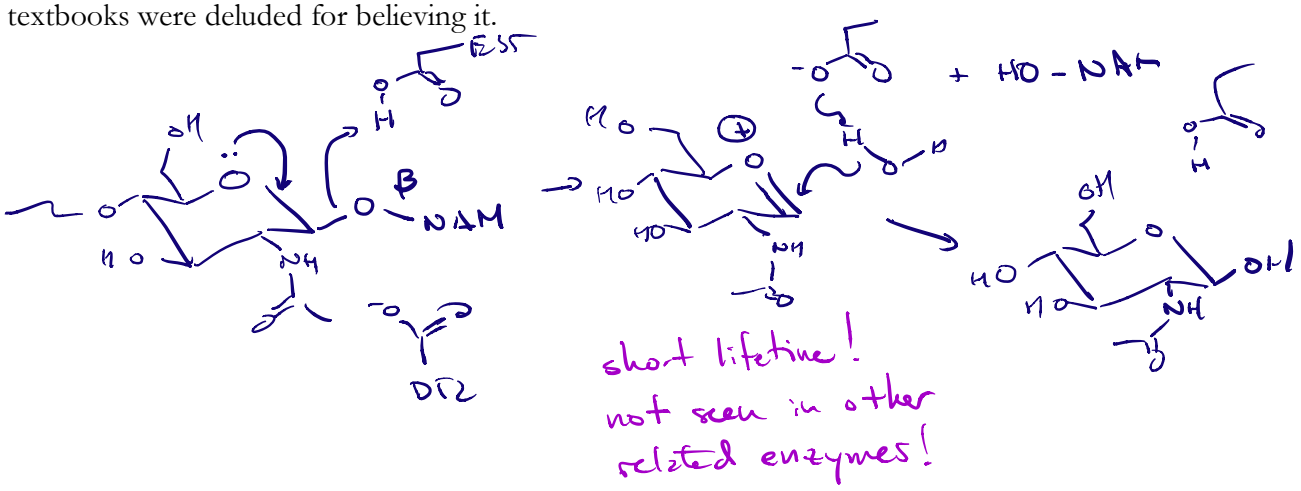
Tyr to Lys: pK_a ↓ Removes e⁻ density

Vocadlo et al. (2001) Catalysis by hen egg-white lysozyme proceeds via a covalent intermediate. *Nature* **412**, 835-8. The **lysozyme.pse** file on the web page contains the structure reported in this paper.

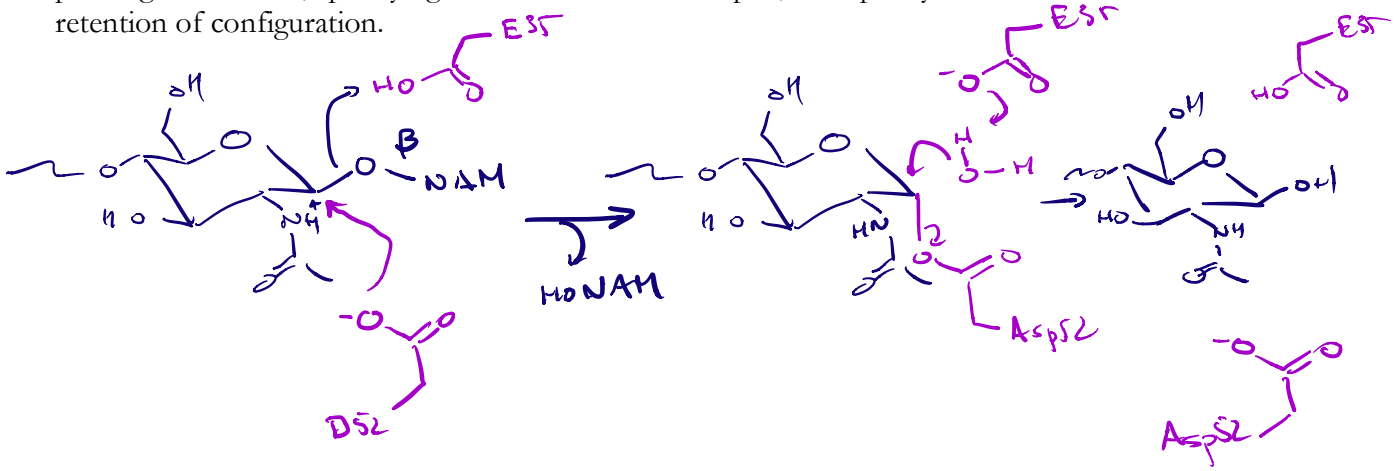
1. Lysozyme catalyzes the hydrolysis of a NAM β(1→4) NAG linkage with retention of configuration. What does that mean and why is a simple S_N2 attack of water on C1 of NAM a mechanistic impossibility given that stereochemical outcome? An arrow-pushing mechanism may help.



2. Prior to this publication, textbooks favored something called the "Phillips Mechanism" for lysozyme. Reproduce the arrow-pushing mechanism here and explain why the authors think textbooks were deluded for believing it.

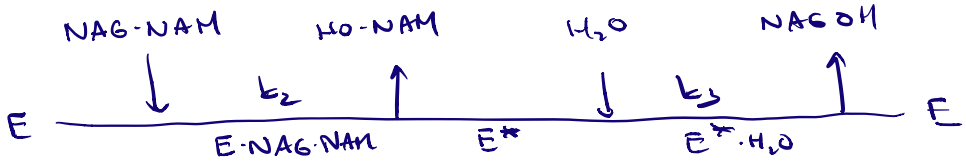


3. In contrast, the authors favor a mechanism invoking nucleophilic catalysis. Draw an arrow-pushing mechanism, specifying roles for Glu35 and Asp52, and specify how that mechanism leads to retention of configuration.



4. The authors cite an interest in reducing the rate constant, k_3, vs. k_2.

a. Sketch a ping pong mechanism "time line" for the reaction and ID where k_3 lies.



b. Which relationship gives burst phase kinetics: $k_2 << k_3$ or $k_3 << k_2$? Explain.

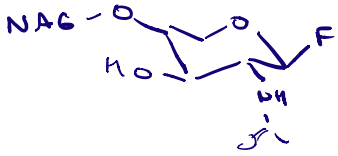
$k_3 << k_2$, which allows E*, the covalent intermediate to build up.

5. Figure 2 shows a series of results with each of the following modifications to lysozyme and its substrate. In each case, discuss why the authors believe might the modification would serve to slow k_3 relative to k_2, with a drawing to illustrate your argument if useful. Did it succeed?

a. E35Q mutation with native substrate.

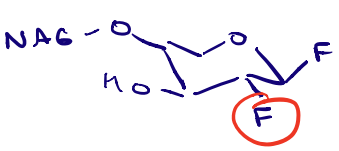
In #3 above Glu 35 plays essential role in attack of Asp 52 (k_2) & that of water (k_3) on anomeric carbon. Both steps are slowed.

b. Substrate modification NAG_2F with E35Q.



F^- is activated leaving group & doesn't need a general acid (k_2), but 2nd step, reactic w/ H_2O remains slow. So k_2 is resurrected, but not k_3

c. Additional substrate modification: NAG2FGlcF (here define the role of the "oxocarbenium-like transition state")



Circled F will destabilize S_N2 transition state in both steps so k_2 & k_3 both affected, but F^- leaving group leaves k_2 step slow while k_3 is nearly dead.