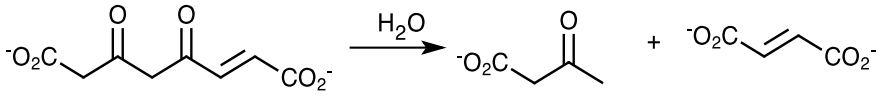
Problem Set #8 – Chem 391 **Name**_____

Due in class on Thursday, November 8th

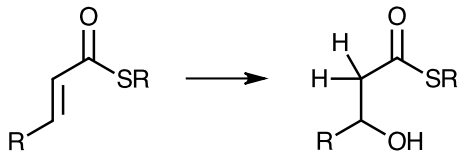
1. Please provide arrow-pushing mechanisms involving general acid/base catalysis for the following reactions. In each case include an "-AH" and "-B" in your scheme.

a. The isomerization of D-glyceraldehyde to L-glyceraldehyde

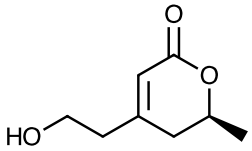
b. The reaction catalyzed by fumarylacetoacetate hydrolase, a wonder to me.



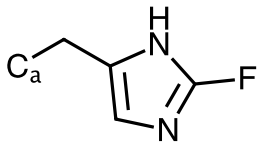
c. The hydration of an α,β-unsaturated thioester to create a β-hydroxy thioester as shown at right. There will be one intermediate in the reaction pathway. Show that it is resonance stabilized.



2. One of the great things about proteins is that they are chiral molecules and therefore substrate binding sites can distinguish between enantiomers (just as your right hand can distinguish between left and right-handed gloves). Let's say I want to purify the compound below from its enantiomer in a racemic mixture. Explain how you could create a catalytic antibody that would remove the unwanted enantiomer from solution (this will include designing the appropriate transition state analog for ester hydrolysis).



3. An enzyme contains two active site His residues at positions 20 and 40. It is proposed that they take part in general acid/base catalysis. To investigate this hypothesis, the unnatural residue fHis (shown at right) is used to replace the natural residues at each position. Based on the plot below, provide estimates of the pK_a of His20, fHis20, His40 and fHis40 and identify which residue is acting as **general acid**, and explain how you know.



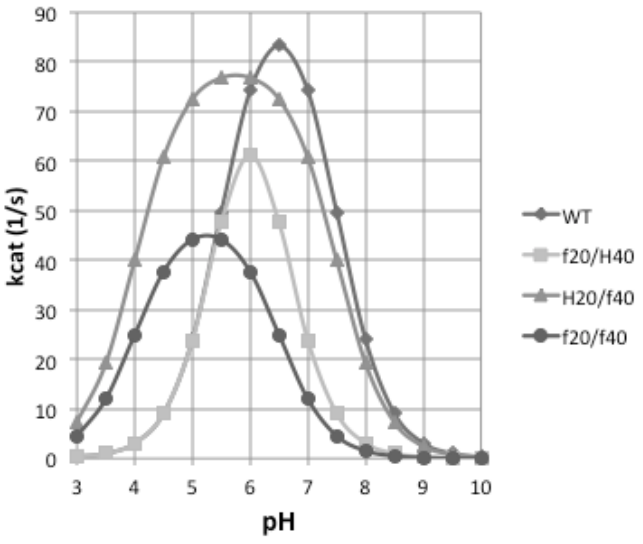
pK_a of His20=_____

pK_a of **f**His20=_____

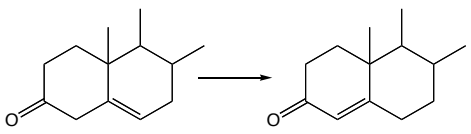
pK_a of His40=_____

pK_a of **f**His40=_____

Which residue (His20 or His40) is the general acid and what is the evidence?



4. Ketosteroid isomerase (KSI) catalyzes the transformation at right via formation of an enol intermediate. The kinetic parameters at 25°C are k_{cat} = 3.8 x 10^4 s^{-1} and K_m = 1.3 x 10^{-4} M.



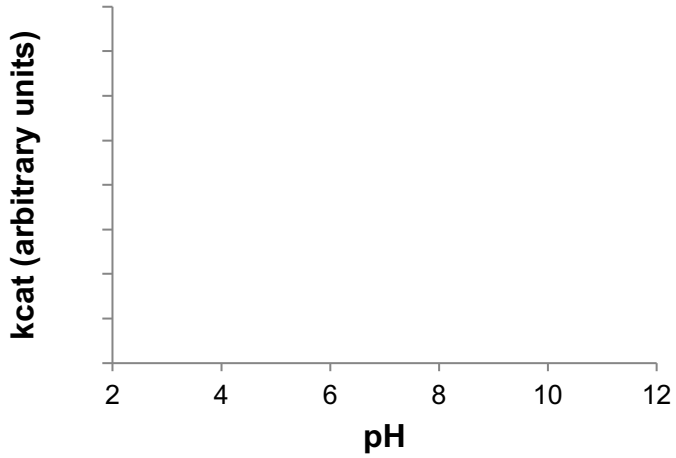
a. Write out a complete arrow-pushing mechanism for the transformation. Take care to explicitly draw any intermediates in the reaction.

b. The enzyme has two important active site residues, Tyr16 and Asp40, that contribute to general acid/base catalysis. Identify the roles of each residue based on position in the active site. A PyMOL file, KSI.pse, shows the positions of Y16 and D40 as green residues near a pink inhibitor.

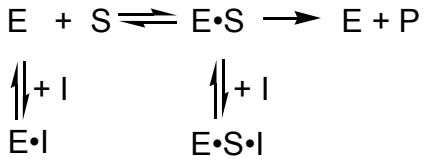
c. Acetate can catalyze the reaction above with a rate constant of 1.8 $M^{-1}s^{-1}$ at pH 7. For the acetate-catalyzed reaction, $\Delta H^{\neq}$ is 16 kcal/mol and $\Delta S^{\neq}$ is –17 cal/mol•K. Compare the efficiency of KSI as a catalyst to acetate ion. What is the rate enhancement achieved by using a protein catalyst vs. acetate?

d. Suggest why KSI is a better catalyst than acetate, invoking one entropic and one enthalpic rationale.

5. Given your mechanism for KSI in problem 3a, sketch the plot of k_{cat} vs. pH.



6. Non-competitive inhibition has the following mechanism. Let's assume that a non-competitive inhibitor complexes with both the E•S complex and free enzyme with the same dissociation constant, K_i.



$$v = \frac{V_{max}[S]}{(K_m + [S])\left(1 + \frac{[I]}{K_i}\right)}$$

a. Derive the above rate law, given the above mechanism and assumptions.

b. What is the maximum velocity of this reaction at any given concentration of inhibitor?

Questions related to Fried et al. (2014) Extreme electric fields power catalysis in the active site of ketosteroid isomerase. *Science* **346**, 1510.

1. The authors hypothesis that an electric field will to contribute to catalysis by KSI. In figure 1 the authors indicate that the dot product of $-\vec{F} \cdot \vec{\mu}$ may serve to promote catalysis. Explain why the electric field might selectively stabilize the transition state (text on p. 1512 may assist and I'll post a summary of the dot product on-line).

2. In one or two sentences, describe the vibrational Stark effect and how might it be used address the hypothesis of this work?

3. Explain the information in Figures 2C & D and what value is provided by these data.

4. A series of mutations to Tyr16 and Asp103 are made and the results are described in Figure 4A and B. Generally what conclusions are drawn? Why is it that the impact of Y16S is less than Y16F, according to the authors?

5. Describe the results obtained when Asp40 is mutated. How are those results different from mutations to 16 and 103? Provide an explanation for that difference. Brownie points if you can use the word orthogonal in a convincing manner.

6. The take home lesson of this paper appears to be summarized in Figure 3C, where solution is a baseline given a maximum $\Delta G^{\neq}$ of 22 kcal/mol and Asp40 contributes about 3 kcal/mol stabilization. How was the 7+ kcal/mol additional stabilization by the electric field obtained from Figure 3 B?

Oops – rebutted! Natarajan et al. (2014) *Science* **349**, 936-a
The Herschlag lab objects. They argue that the "zero field" from Figure 3C of the original paper is an unrealistic starting point for evaluating catalysis by KSI.

7. What evidence do they cite regarding ablation in the "oxyanion hole" to indicate that the "zero-field" is not an appropriate starting point to consider the contribution to catalysis made by an electric field (see Figure 1A as well). Attempt to redraw Figure 3C from the original paper incorporating the ideas of this rebuttal. How does this new evaluation affect the estimation of the importance of Asp40 to catalysis?