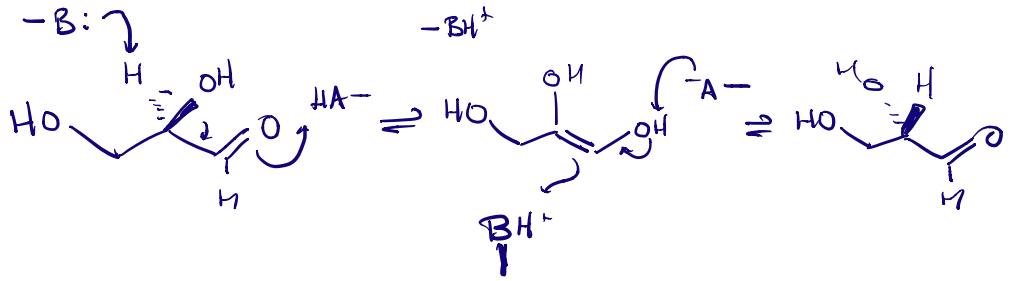
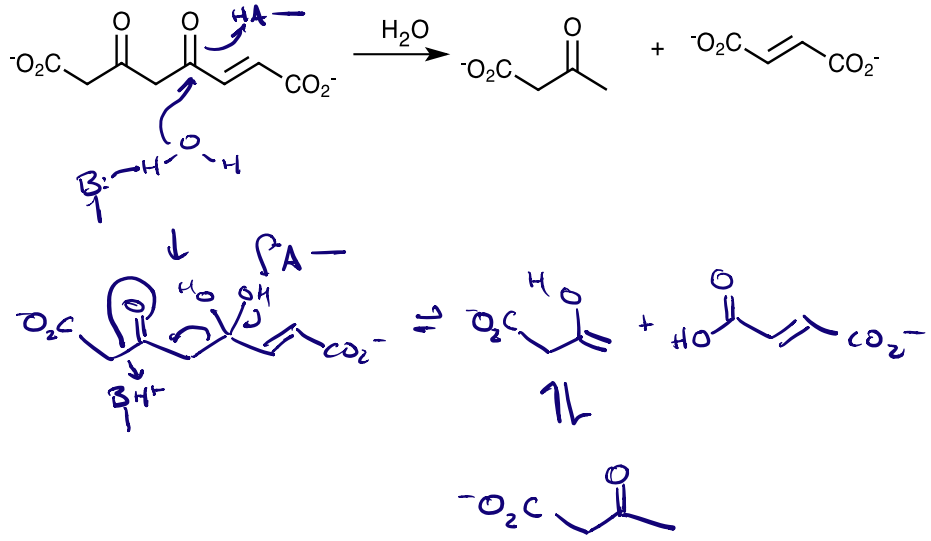
Problem Set #8 – Solutions **Name**_____

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.

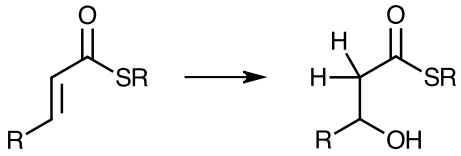
a. The isomerization of D-glyceraldehyde to L-glyceraldehyde

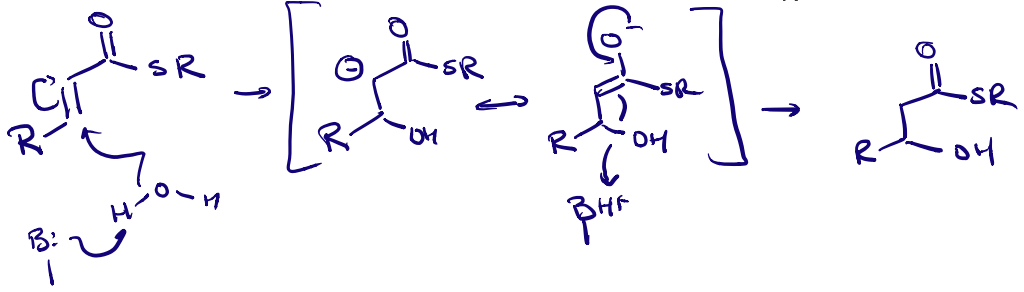


b. The reaction catalyzed by fumarylacetoacetate hydrolase, a wonder to me.

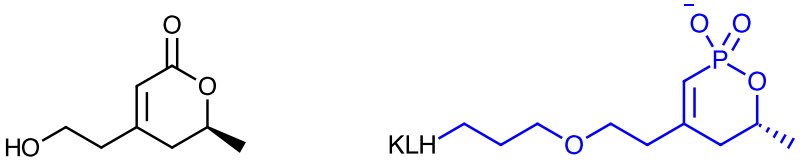


c. The hydration of an α,β-unsaturated thioester to create a β-hydroxy thioester as shown at right. There will be one intermediate in the reaction pathway. Show that it is resonance stabilized.



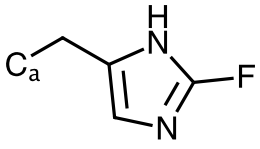


2. One of the great things about proteins is that they are chiral molecules and therefore substrate binding sites can distinguish between enantiomers (just as your right hand can distinguish between left and right-handed gloves). Let's say I want to purify the compound below from its enantiomer in a 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).



The trick is to develop an antibody that will hydrolyze the enantiomer that you don't want. The compound above, in blue, is a transition state analog for the hydrolysis of the unwanted enantiomer. An antibody elicited to the above compound, conjugated to keyhole limpet hemocyanin (KLH) will selectively bind the transition state of hydrolysis of the enantiomer we want rid of.

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.

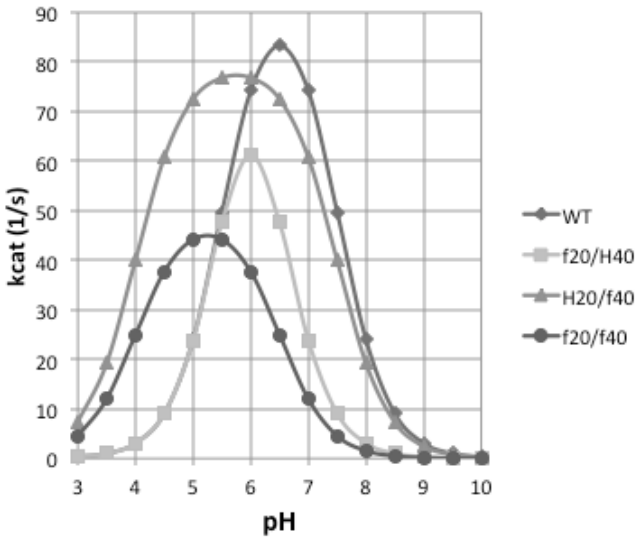


pK_a of His20= **7.5**

pK_a of **f**His20= **6.8**

pK_a of His40= **5.4**

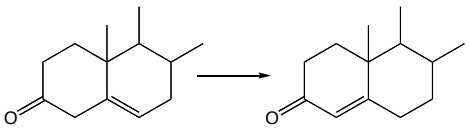
pK_a of **f**His40=**4.0**



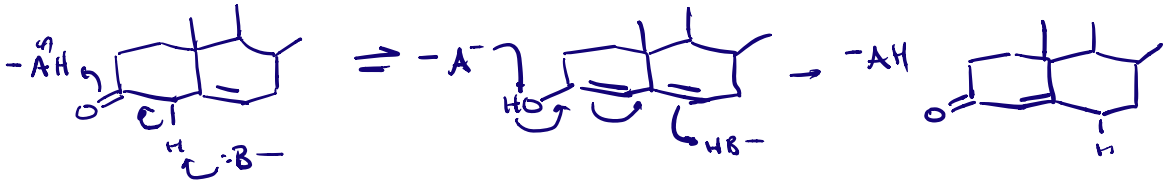
Which residue (His20 or His40) is the general acid and what is the evidence?

His20 is general acid. When it gets deprotonated (above pH7.5) kcat decreases.

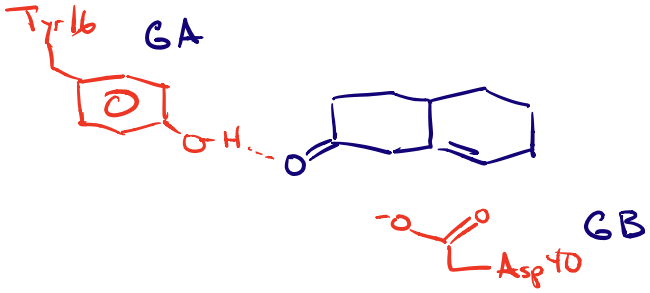
4. Ketosteroid isomerase (KSI) catalyzes the transformation at right via formation of an enol intermediate. The kinetic parameters at 25°C are k_{cat} = 3.8 x 10^4 s^{-1} and K_m = 1.3 x 10^{-4} M.



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?

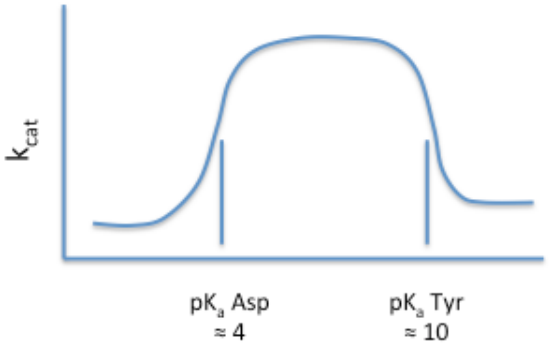
Compare k_{cat}/K_m to $k_{acetate}$: Ratio is (3.8 x 10^4 s^{-1}/1.3 x 10^{-4} M)/(1.8 $M^{-1}s^{-1}$) = 1.6 x 10^8

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

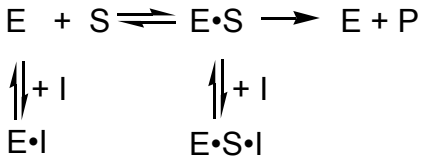
$\Delta H^{\neq}$: Arguably Tyr is a superior general acid to water or acetic acid because phenols are better matched in pK_a to the protonated intermediate.

$\Delta S^{\neq}$: Pre-organized relationship of GA & GB in active site removes entropic loss suffered by acetate in solution.

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.

$$\frac{v}{V_{max}} = \frac{k_{cat}[ES]}{k_{cat}[E]_{tot}} = \frac{[E\cdot S]}{[E]_{tot}}$$

$$= \frac{[E\cdot S]}{[E] + [E\cdot S] + [E\cdot I] + [E\cdot S\cdot I]}$$

$$= \frac{[E][S]/K_m}{[E] + [E][S]/K_m + [E][I]/K_i + [E][S][I]/K_m K_i} \cdot \frac{K_m}{K_m}$$

$$= \frac{[S]}{K_m + [S] + \frac{K_m[I]}{K_i} + \frac{[S][I]}{K_i}}$$

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

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

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

K_m negligible

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

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

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).

If the electric field is oriented antiparallel to the bond dipole of C=O its energy of interaction will be stronger when the carbon-oxygen bond is polarized in the transition state so there is a build up of negative charge on the oxygen.

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

The vibrational Stark effect results from the influence of an electric field on the frequency of a bond vibration. A field aligned antiparallel to the bond dipole can weaken the bond and lead to lower frequency vibrations.

The authors hypothesize that KSI uses a fixed electric field to selectively stabilize the transition state in the reaction, because the C=O bond will be more highly polarized in the transition state and will receive more substantial stabilization from the electric field.

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

Note that the frequency of the C=O vibration is lowered in more polar solvents, indicating the power of a polar solvent to interact favorably with the bond dipole as described above.

By showing a linear correlation of C=O frequency to the electric field created by the solvent, the authors show that they have a ready method of interrogating the electric field of the enzyme active site.

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?

The effect of the mutations on the C=O vibrational frequency range over 60 cm^{-1} and can be placed in the context of solvent fields, providing an absolute value for the value of the active site field. When $\Delta G^{\neq}$ is plotted against the field strength, a linear correlation is observed indicating a $\Delta G^{\neq}$ of 18.8 kcal/mol in zero field and a 1 kcal/mol stabilization of $X^{\neq}$ per 1 kcal/mol•D increase in strength.

The Y16S mutant is interesting because it has a large field despite removing a big side chain. The authors suggest that bound water replacing the phenol ring leads to the field.

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.

The authors note a profound difference in k_{cat} for Asp40 mutants but only a modest change in electric field. They argue that Asp40 serves a different purpose than Tyr16 (not related to electric field stabilization, hence orthogonal) and therefore does not impact C=O stretching.

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?

Starting at the 18.8 kJ/mol in a zero field, the wild type enzyme gives $\Delta G^{\neq}$ of about 11.2 kcal/mol. So the authors are using hexanes as a model non-polarizing environment.

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?

The Tyr16Phe mutant replaces an H-bonding residue with a non-polar residue, introducing a more non-polar environment around the carbonyl than it would experience in water. That is evident in Figure 3A of the original paper in which the C=O stretchin the Y16F active site is a little blueshifted compared to the stretch seen in water.

"Ablated" mutants have small residues compared to Phe16 and see activity about 100x stronger than with the Y16F mutant suggesting that the "18.8 kcal/mol" barrier is over estimated, and maybe something closer to 14.5 kcal/mol is more appropriate. That means the general base, Asp40, is responsible for something more like 7.5 kcal/mole of catalysis, if we pick a field of -80 MV/cm to describe bulk waters contribution to catalysis.