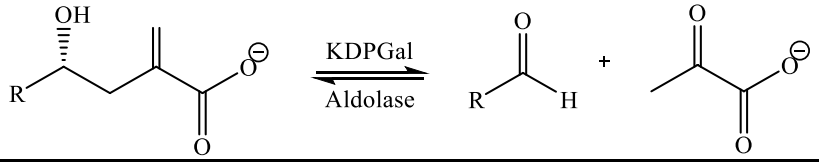
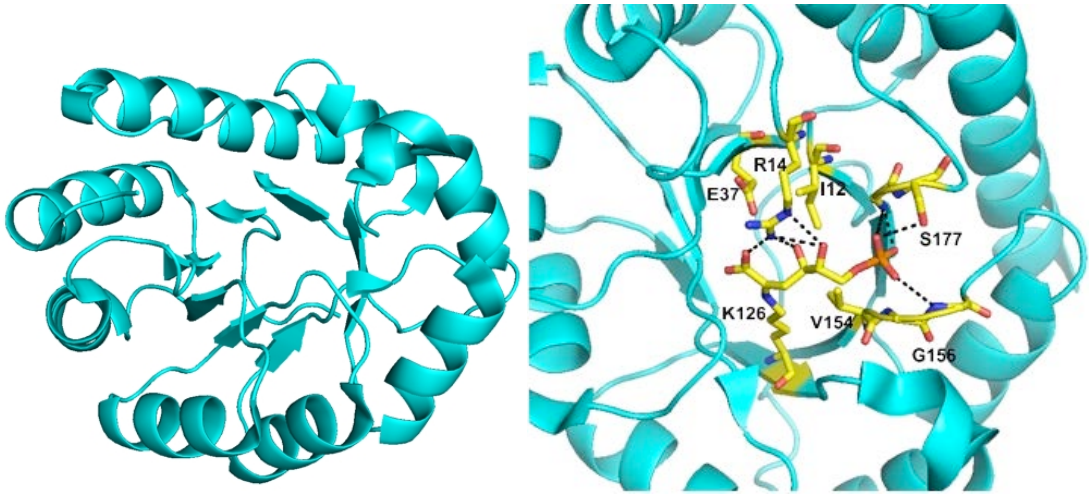
Problem Set 4 (C-C bond formation, phosphoryl transfer reactions and the role of ATP)

1. Chemists can use the same strategies as nature to make new carbon-carbon bonds stereospecifically using enzymes such as aldolases. One particularly useful enzyme catalyzes the reaction shown in Eq 1, where R (electrophile) can be highly diversified. This enzyme uses 2-keto-3-deoxy-6-phosphogalactonate as a substrate. The enzymes have a TIM barrel (α8β8) structure shown in Fig 1A. A blow up of a product in the active site of this barrel with a constellation of residues that might be involved in catalysis is shown in Fig 1B.

Eq 1



Electrophile	Nucleophile	KDPGal aldolase V_{rel}
D-Glyceraldehyde	Pyruvate	100
2-Pyridinecarboxaldehyde	Pyruvate	+++
D-Ribose	Pyruvate	+++
Chloroacetaldehyde	Pyruvate	++
D-Erithrose	Pyruvate	+++
L-Erithrose	Pyruvate	+
D-Threose	Pyruvate	+
L-Threose	Pyruvate	+++
Glycoaldehyde	Pyruvate	++++



A B

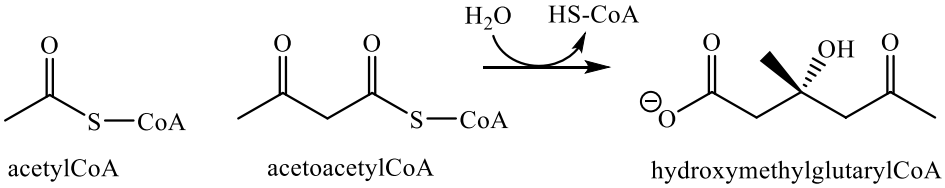
Figure 1. A ribbon diagram of the aldolase (A) and a close up of the active site (B) including the bound substrate.

Questions:

1) What does the structure tell you about the type of aldolase involved in the reaction in Eq1? What does it tell you about the residues that might be involved in catalysis?
2) Using the structural information, draw a mechanism (curly arrows using not AH or B for acids and bases, but amino acid side chains) by which the C-C bond can be made or cleaved.
3) Looking at the structure in Fig 1B and the bound substrate, can you rationalize the specificity of the enzyme suggested by the data in the Table above?

2. Under physiological conditions, HMG-CoA synthase catalyzes the conversion of acetyl-CoA and acetoacetyl-CoA to 3-hydroxy-3-methylglutaryl-CoA shown in (Eq 2). The back reaction (conversion of HMG-CoA back to acetylCoA and acetoacetyl-CoA) can occur in the test tube. HMG-CoA synthase is the second step in the pathway that makes the 5-carbon fragments required to make lanosterol and cholesterol. In patients with type I diabetes, this enzyme is overactivated and the excess HMG-CoA produced is shuttled into ketone bodies (acetoacetate and acetone) which will be discussed in the second half of the course. Studies have shown that the synthase that catalyzes this reaction has an essential cysteine (C111) in its active site. In Figure 2 is a diagram depicting the x-ray crystal structure of the synthase incubated with HMG-CoA.

Eq 2



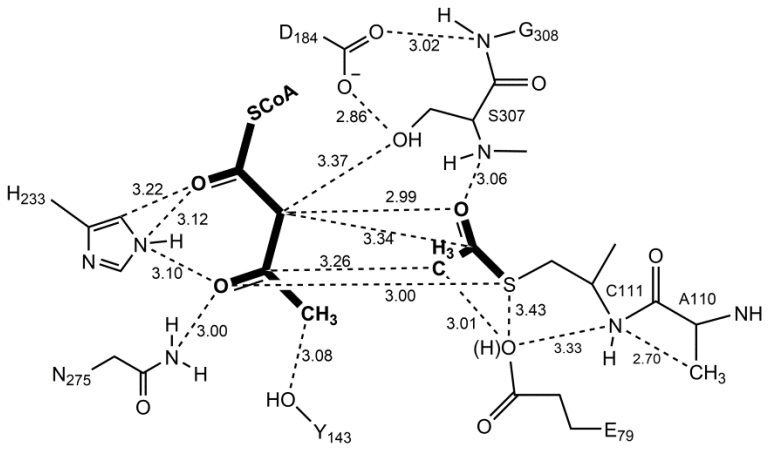


Figure 2. The structure (with distances in Å) resulting from incubation of HMG-CoA with synthase which took several weeks to crystallize prior to the solution of the structure.

Questions:

1) The acetylCoA ends up as the $-CH_2CO_2^-$ moiety in HMG-CoA. Propose a mechanism (curly arrows and if possible specific residues that might be involved) for C-C bond formation and CoA hydrolysis in this reaction that uses the insight you can obtain from the structure (Figure 2).
2) You have now encountered carbonyl chemistry in the mechanism of peptide bond formation by serine proteases and in the mechanisms of aldol and Claisen reactions. In each case you have learned that these reactions involve tetrahedral intermediates (high energy) or transitions states. Include these states in your mechanism in part 1, if you have not already done so. How would this type of intermediate be stabilized by the enzyme?
3) In class we discussed that enzymes are able to catalyze rates of reactions by 10^6 to 10^{15} times the rates of the uncatalyzed reactions. We learned about three distinct types of mechanisms that can contribute to this acceleration. Given your mechanism and the structure in Figure 2, propose mechanisms for rate acceleration with HMG-CoA synthase.
4) In Eq 2 and in metabolism in general, thioesters are made from coenzymeA. What is the structure of CoA? What would you expect the difference in chemical reactivity to be between thioesters of CoA and $HSCH_2CH_2NHCOCH_3$? Why?
5) Rationalize why Nature uses thioesters rather than oxygen esters.

3. AcetylCoA has a large free energy of hydrolysis:

Eq 3. $CH_3COSCoA + H_2O \rightarrow CH_3CO_2^{\ominus} + HS\text{-}CoA \qquad \Delta G^{\circ\prime} = -31.5$ kj/mol

Nature uses ATP to make $CH_3COSCoA$.

Questions:

1) If the sulfur S in Acetyl-CoA structure is replaced by O to make an oxygen ester, what would you expect its free energy of hydrolysis to be in comparison with AcetylCoA: more exergonic, the same, or less exergonic? Provide a chemical rational for your choice.
2) Given the large free energy of hydrolysis for acetylCoA, its synthesis must use the energy currency in the cell, specifically ATP, to convert acetate into this molecule. Propose two distinct ways in which ATP could be used to activate acetate so that the thioester could be formed. Show the two mechanisms (curved arrows). Why is Mg^{2+} essential for this process given what you know about the properties of ATP?
3) In one of your mechanisms, one of the products of the reaction can be further hydrolyzed to help drive the reaction of thioester formation to the right. Show how and why this could occur. Hint: Look in your book Table 14-4 "Standard Free Energies of Phosphate Hydrolysis of some interesting biological molecules" to help you answer this question.

5.07SC Biological Chemistry I
Fall 2013