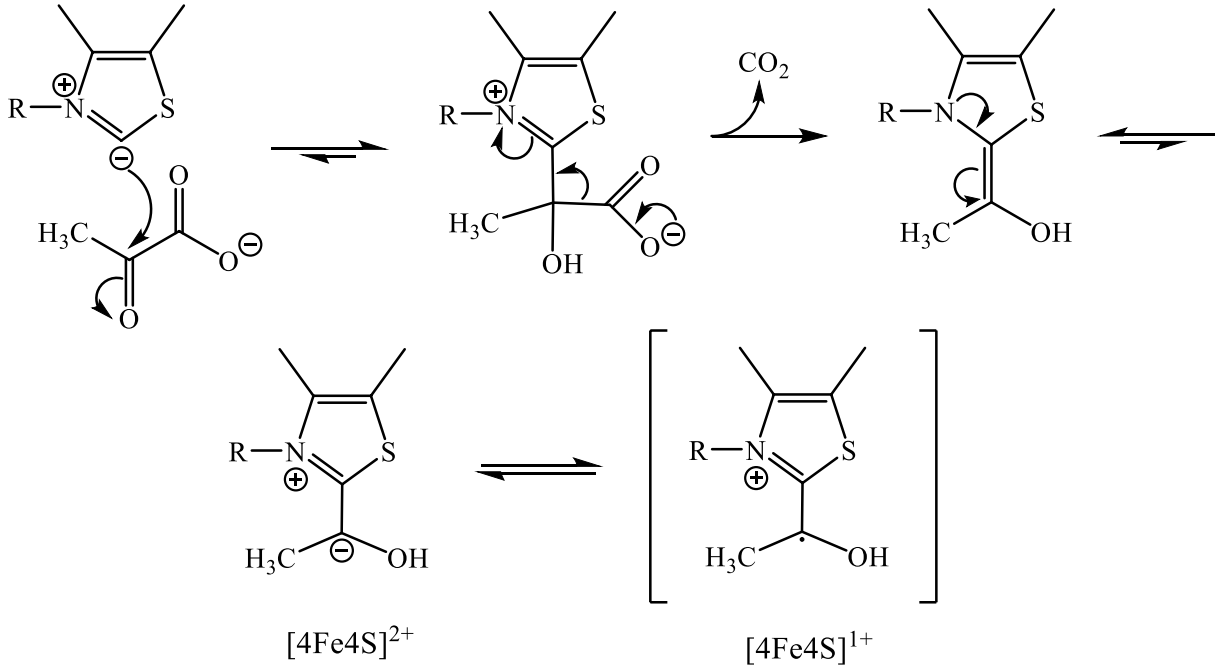
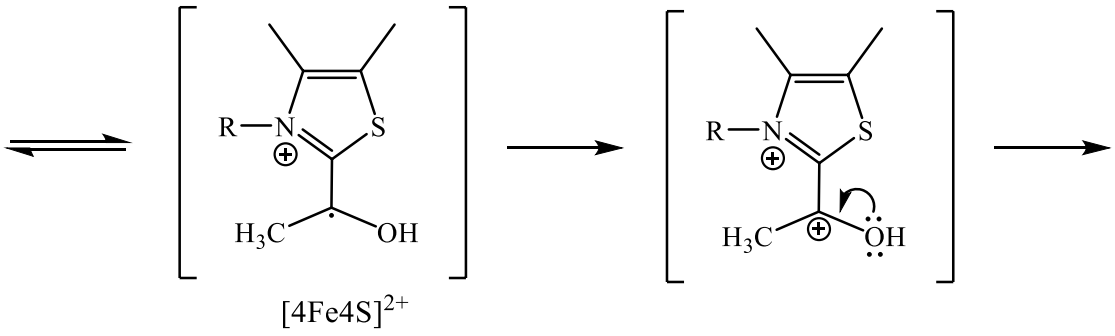
ANSWERS Problem Set 6

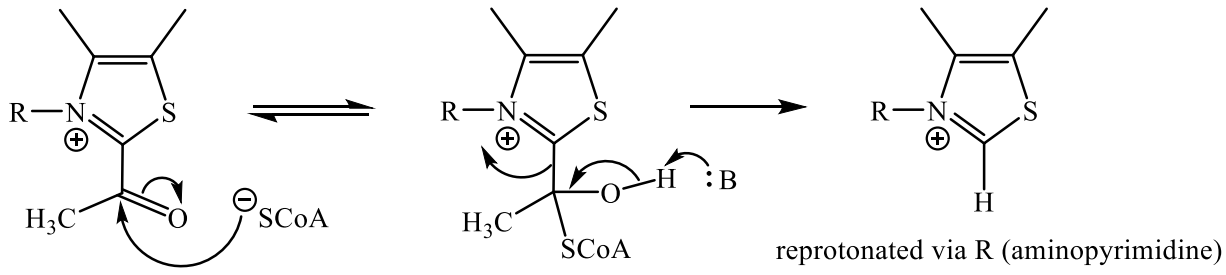
Problem 1

1. The ylid is generated via amino-pyrimidine (R) – see textbook and PP

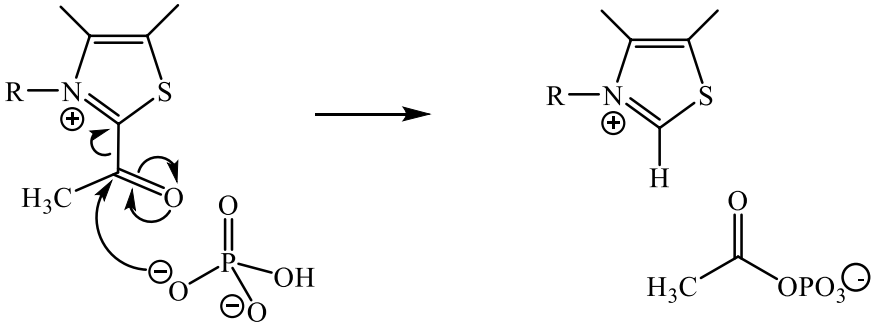


The $[4Fe4S]^{1+}$ must be reoxidized by the next cluster in the pathway so that it can accept a second e^-

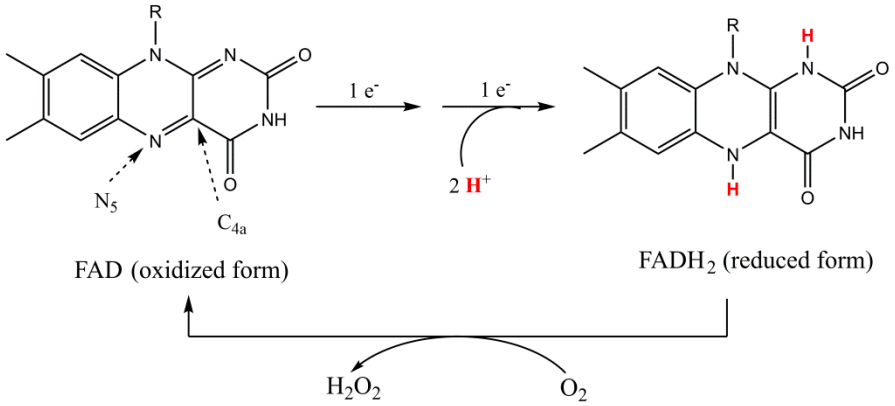




2. POX also requires a redox cofactor. Since it is not given in the Eq 2, it must be a flavin, which always remains bound to its protein (covalently or tightly). A number of mechanisms are possible, it is likely two 1e$^-$ transfers to generate the same intermediate as above



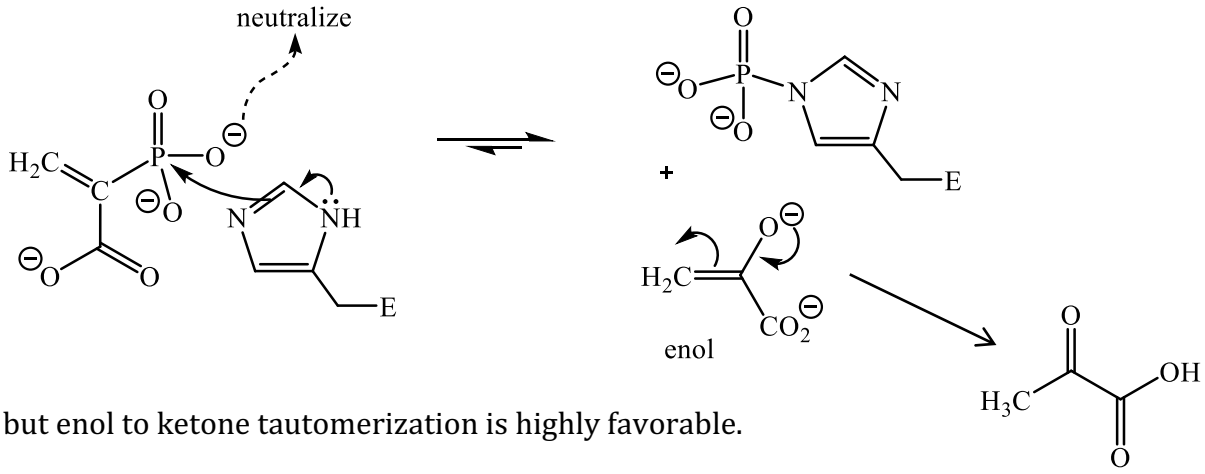
3. Since



We did not discuss the detailed chemistry, but this reoxidation involves a $C_{4a}OOH$ (C_{4a} hydroperoxide) intermediate and involves radical (1e$^-$) chemistry.

Problem 2

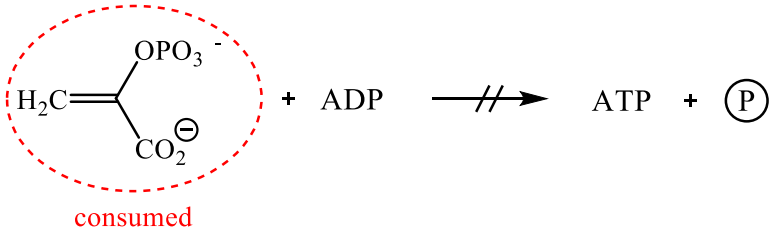
1. PEP is a "high energy" phosphate bond (see next page)



but enol to ketone tautomerization is highly favorable.

The energetics are excellent for phosphoryl transfer. Furthermore, PEP looks like 2-PGA and could "easily" bind in the active site. Thus this is chemically and enzymatically feasible.

2. Now PEP is consumed by PGlym reaction and is NO longer available to produce ATP by the pyruvate kinase reaction.



Thus NO net ATP is produced by glycolysis.

Phosphate Compound	**Hydrolysis products**	**$\Delta G^{0'}$ (kJ/mol)**
Phosphoenolpyruvate (PEP)	Pyruvate	-61.9
Adenosine triphosphate (ATP)	Adenosine diphosphate (ADP)	-30.5

Problem 3

1. ATP-Mg^{2+} is a substrate. The effect at low concentrations of ATP is binding at the active site to carry out the phosphorylation. At "high" concentrations of ATP, it binds to a second active site, an allosteric site, which then converts the R state (active state) to T (an inactive state) and shuts down formation of FBP. The activity (see dotted line) is dropped down >80%. This makes sense as ATP is one of the key end products of glycolysis. When ATP levels are high, you don't need to generate more.

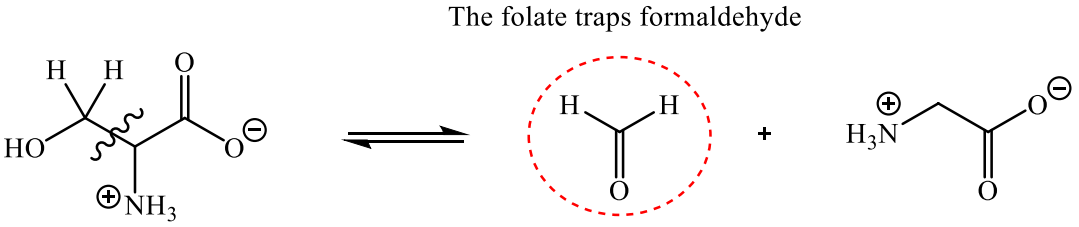
PFK1 is a kinase with a complex quaternary structure (see PP for R, T states – active site, allosteric site locations). You will see in studying gluconeogenesis additional allosteric regulation of this "irreversible", flux controlling step.

2. AMP can "buffer" the shutdown of PFK-1 and one can calculate how a change in [ATP] affects the concentrations of ADP and AMP, assuming K_{equil} remains the same, and that adenylate kinase is the key enzyme

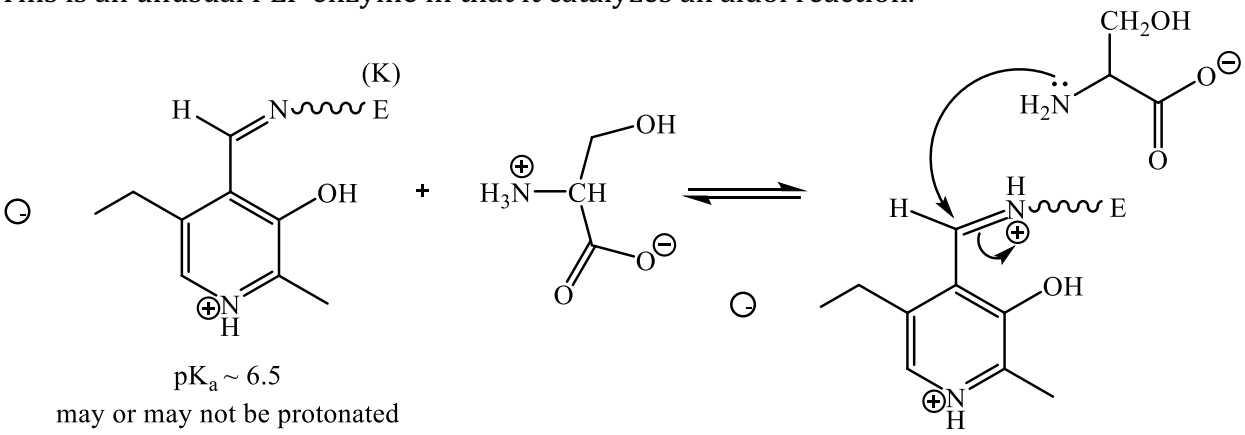
$$ATP + AMP \rightleftharpoons 2\,ADP \qquad \overset{\approx 1}{\underset{\downarrow}{X}} = K = \frac{[ATP][AMP]}{[ADP]^2}$$

If you know the equilibrium for this reaction, you can calculate how changes in ATP affect changes in AMP. Then AMP/ATP compete for some allosteric binding site on PFK-1, and their binding affinity must be tuned to make the regulation work.

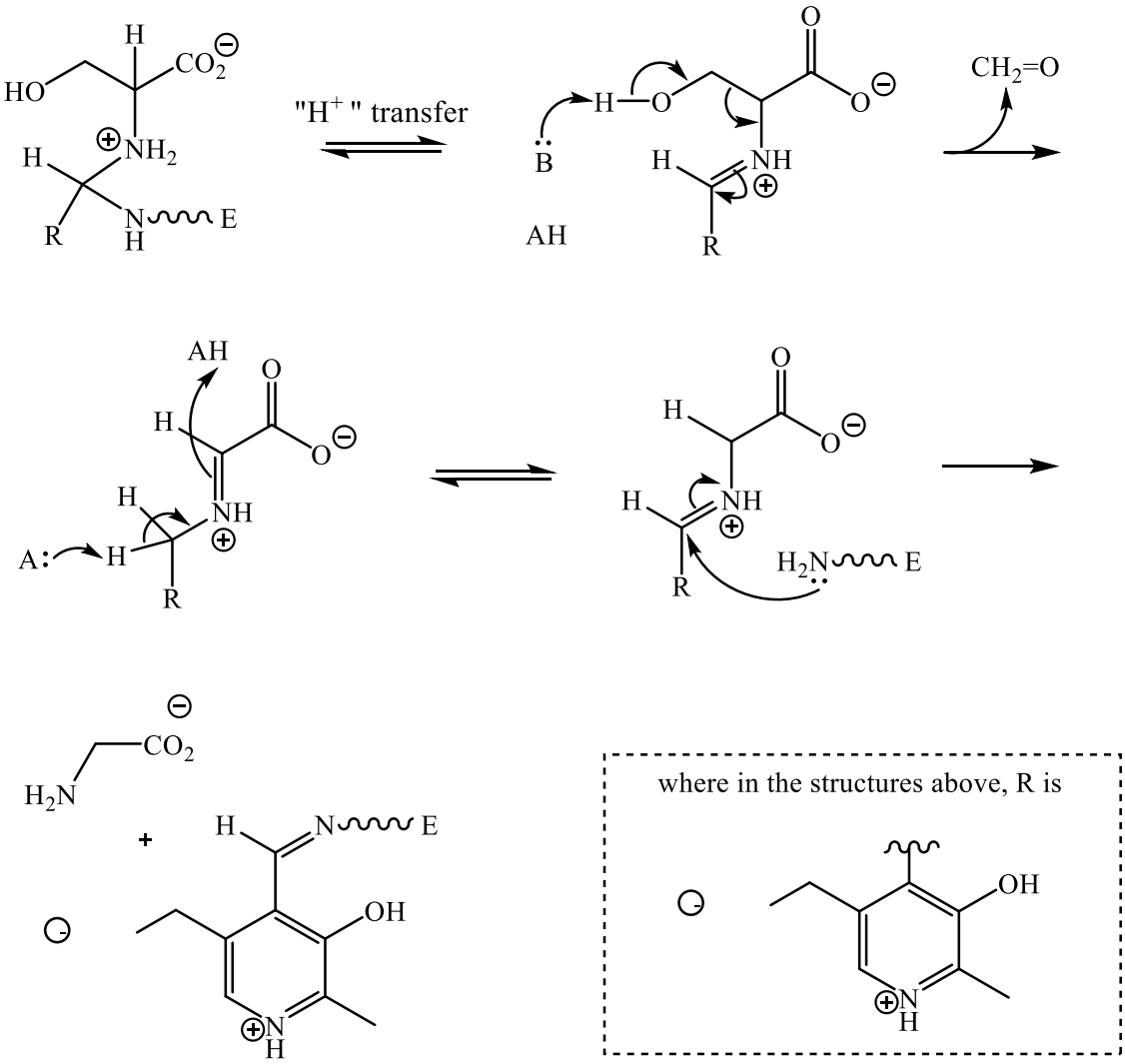
Problem 4



This is an unusual PLP enzyme in that it catalyzes an aldol reaction.



Proton transfer activates the amino group of serine and activates imine to iminium.



(directly reforms the aldimine with the enzyme)

Note this PLP-dependent reaction, along with decarboxylation, does not require removal of the α-hydrogen.

5.07SC Biological Chemistry I
Fall 2013