# Chem 135: Exam II

**30 March 2010**

*Please provide all answers in the space provided. Extra paper is available if needed. You may not use calculators for this exam. Including the title page, there should be 12 pages in this exam booklet.*

**! Good luck ¡**

Name: KEY

(1) _____ (5 points)
(2) _____ (20 points)
(3) _____ (25 points)
(4) _____ (15 points)
(5) _____ (20 points)
(6) _____ (15 points)

**TOTAL** _____ (100 points)

1. For **two** of the following 6 cofactors, draw the "business ends" for both forms listed. Also, write **one sentence** describing your chosen cofactor's general chemical function.

a. ADP / ATP

b. $NAD^+$ / NADH

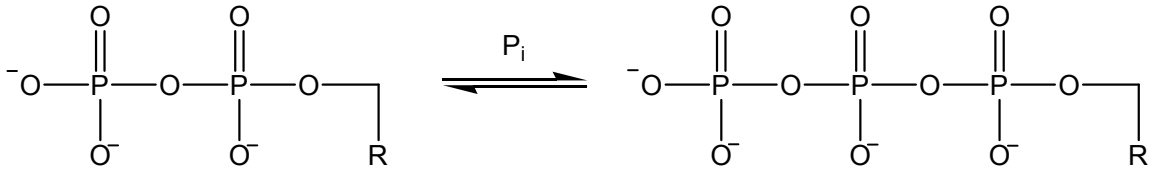
c. TPP / hydroxyethyl TPP

d. lipoic acid (oxidized) / lipoic acid (reduced)

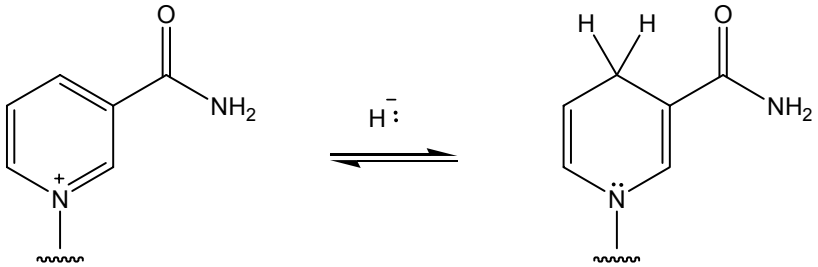
e. biotin / carboxybiotin

f. quinone / hydroquinone

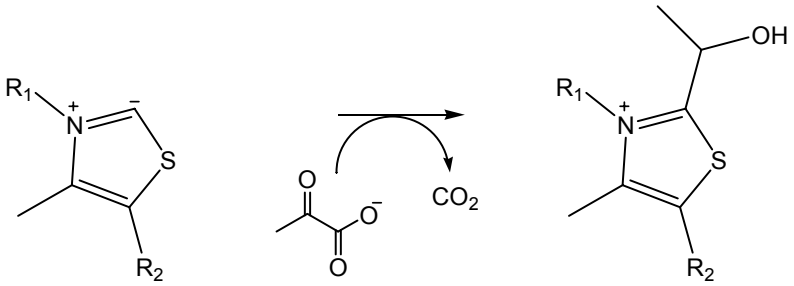
a. The cell's energy currency used to activate bonds



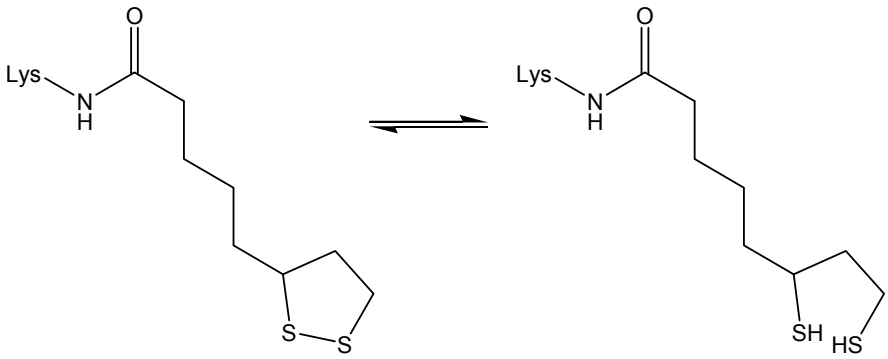

b. Hydride acceptor/donor used to transfer electrons in the cell for oxidation and reduction reactions



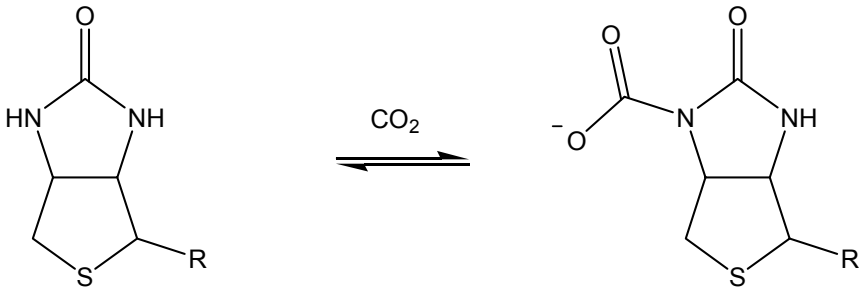

c. An electron sink (we saw it used for α-decarboxylation)



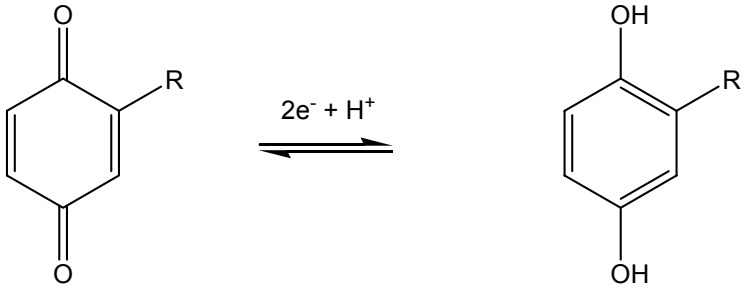

d. Redox cofactor (we saw it attached to Lys used in the PDH complex)




e. Used to find $CO_2$ for carboxylation reactions

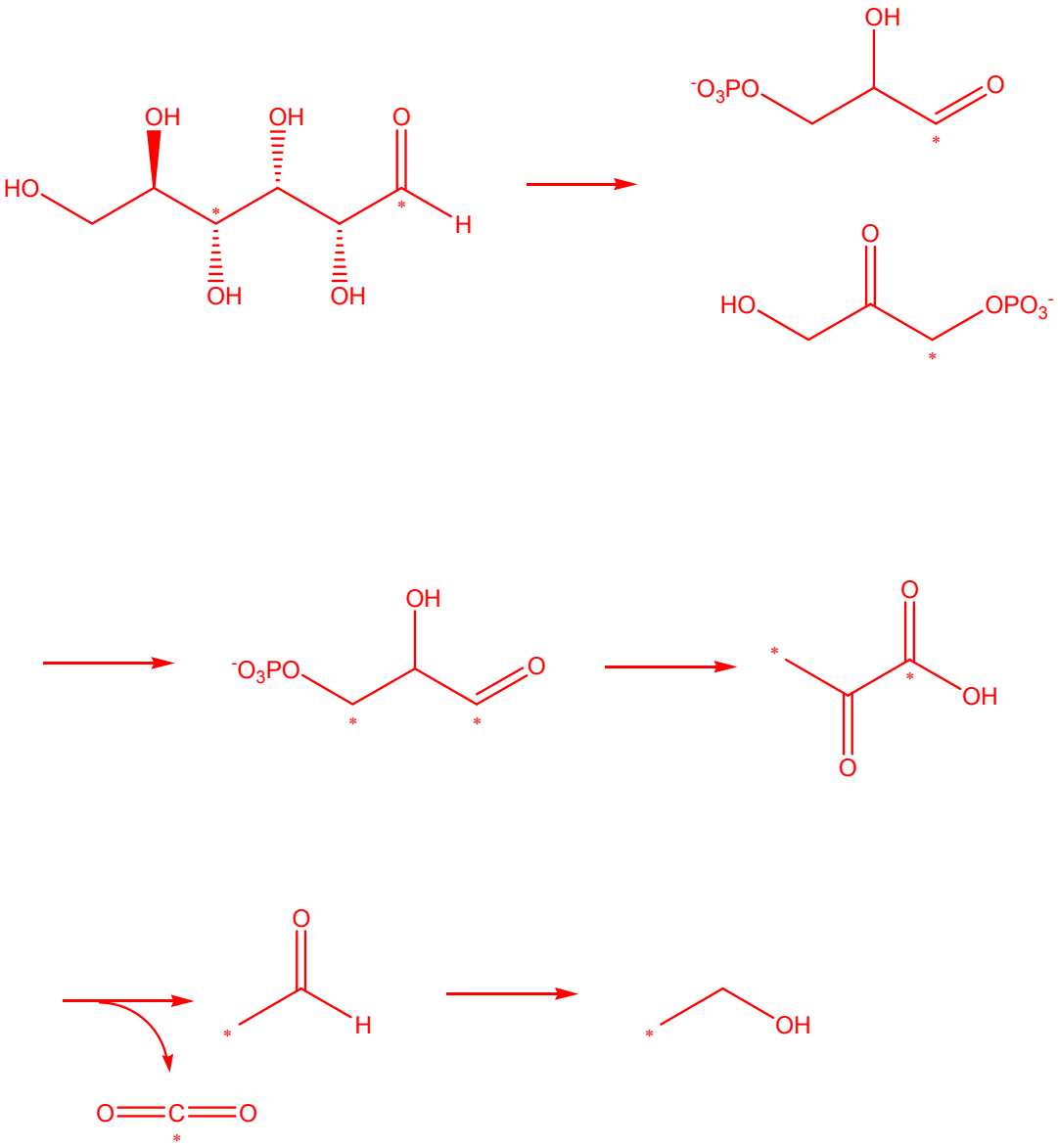



f. Used as a H acceptor/donor for electron **and** H+ transfer



2. *Zymomonas mobilis* is an ethanol-producing bacterium that yields an unusually high yield of ethanol from glucose (92-94%). In order to study glycolysis and fermentation in this organism, you decide to feed it labeled glucose to find out where the label ends up on ethanol and carbon dioxide.

a. When glucose is labeled at $C_1$ and $C_4$ (C1 is the aldehyde carbon of glucose), there is no labeled carbon in ethanol and it is found exclusively in the evolved carbon dioxide. Is this the labeling pattern that you expect based on what you learned in class about glycolysis and fermentation of ethanol? Please explain your answer using chemical structures.




Not all the reaction above are shown, but you can follow it through in more detail from the class notes. The marked C1 is what becomes the phosphorylated carbon in DHAP, while the marked C4 is what becomes the aldehyde on G3P.

b. From the experiment described in 2a, do you believe that *Z. mobilis* to utilizes the same reactions for glycolysis that we learned in class?

No, I do not believe that is utilizes the same reactions for glycolysis because if it did then there would be two different carbons labeled in pyruvate. When the pyruvate is converted to ethanol and $CO_2$ the label could not be situated only in the evolved gas.

c. In order to produce ethanol as the main fermentation product, do you expect *Z. mobilis* to use pyruvate decarboxylase or pyruvate dehydrogenase? Please briefly explain your answer.

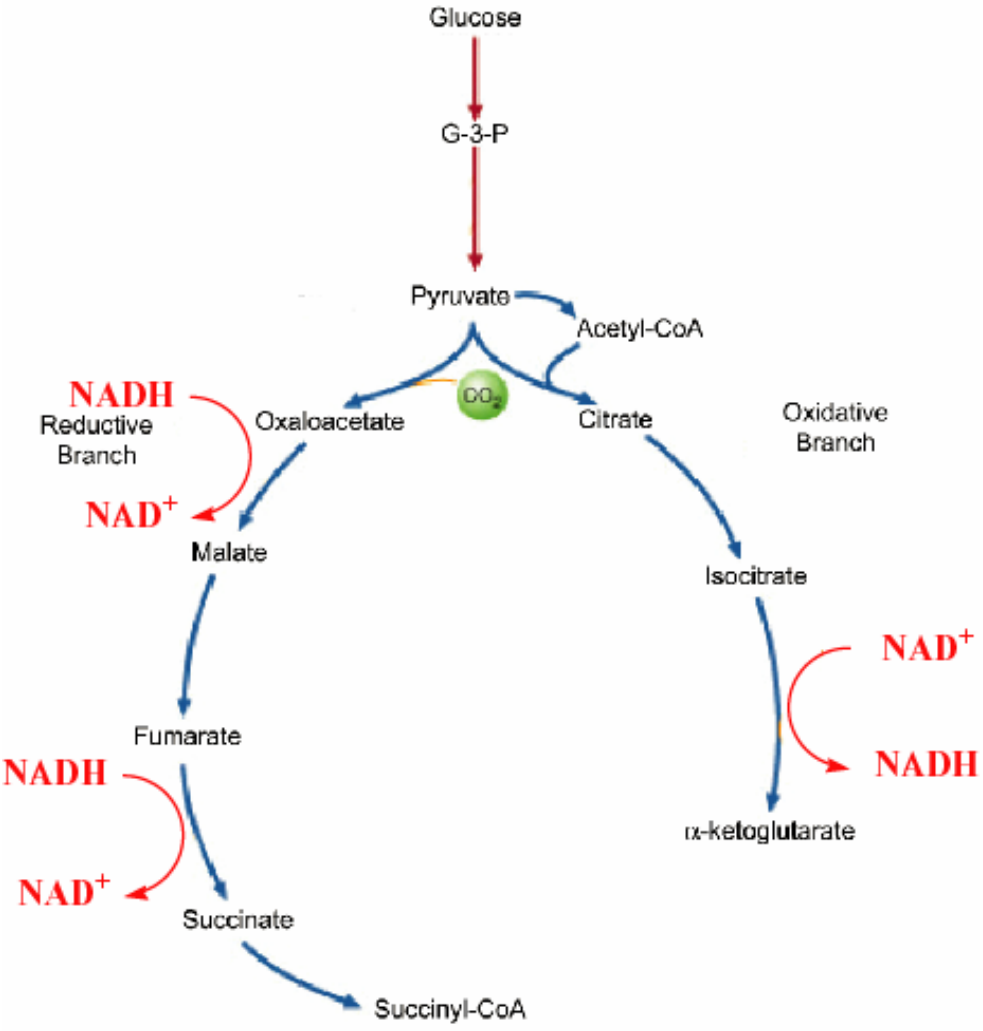
I expect *Z. mobilis* to use pyruvate decarboxylase.

To convert one glucose to 2 pyruvate the cell must use 2 NAD+ to make 2 NADH. When the two acetaldehyde are reduced to 2 ethanol, the two NADH can be oxidized back to 2 NAD+.

If pyruvate dehydrogenase was used then the 2 pyruvate would be converted to acetyl-CoA which must be reduced twice to form ethanol. This would require 4 NADH to be oxidized back to 4 NAD+ per glucose, which is no longer balanced with glycolysis.

3. Prior to the "Great Oxidation", primitive prokaryotes developed a precursor to the modern TCA cycle that we learned in class. These organisms still exist today and are missing the α-ketoglutarate dehydrogenase complex. Instead of a full cycle, these organisms carry out two branched pathways from pyruvate, labeled in the figure in 3a as the oxidative and reductive branch.

a. The partial TCA cycle below only uses $NAD^+$/NADH and not FAD/$FADH_2$. Fill in the redox cofactors used for the individual reactions of the partial TCA cycle. What is the net yield of NADH per glucose if half the pyruvate goes towards the reductive branch and half goes towards the oxidative branch? Compare to the standard TCA cycle that you learned in class.




For each glucose molecule, partial TCA cycle yields:

1NADH (oxidative branch) + 2 NAD+ (reductive branch) = **1NAD+ net yield compared to 6 NADH of standard TCA cycle (for 2 pyruvate from glc)**

b. These organisms grow slowly and prefer to preserve pyruvate for biosynthetic reactions rather than using it for fermentation to ethanol or lactate. Instead, the partial TCA cycle can be used to balance glycolysis and also preserve carbon flux through the TCA cycle. Which branch would you predict would be used for fermentation? Is it balanced with glycolysis? Please briefly explain.

The reductive branch would be used for fermentation since it produces NAD+ to recycle the NADH produced during glycolysis. It is, however, not balanced, since through glycolysis of one glucose, 2 NADH are produced, and through the reductive branch for 1 glucose (since during fermentation both pyruvates will go through the reductive branch) 4 NAD+ will be produced.

c. Although these organisms do not produce oxygen during respiration, NADH is produced during the incomplete TCA cycle that can be used to generate ATP. It is believed that elemental sulfur ($S^0$) could be used as the terminal electron acceptor in these organisms, producing $H_2S$ as the product. Write a balanced reaction for this reaction and calculate the $\Delta E°'$ for the reaction using the following standard reduction potentials. Based on this calculation, would you expect the efficiency of respiration on sulfur to be higher or lower than that of aerobic respiration? Please briefly explain.

$NAD^+ + 2\ e^- + H^+ \rightarrow NADH$ $\quad E°' = -0.3$ V

$S^0 + 2\ e^- + 2H^+ \rightarrow H_2S$ $\quad E°' = -0.23$ V

$½\ O_2 + 2\ H^+ + 2\ e^- \rightarrow H_2O$ $\quad E°' = 0.8$ V

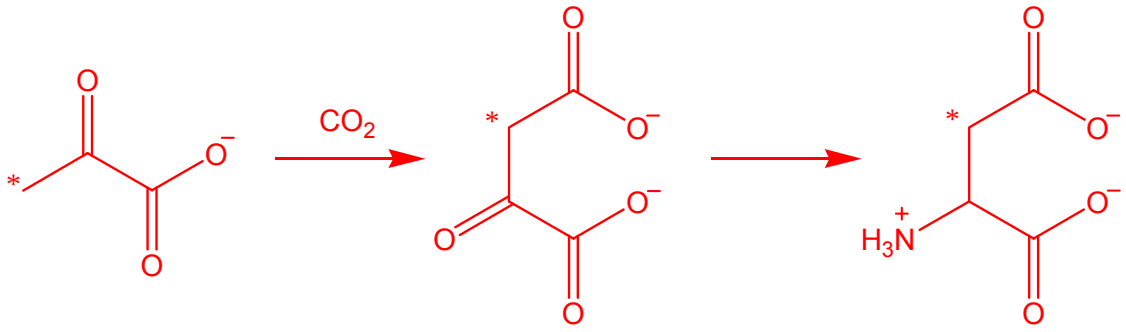
$S^0 + NADH + H^+ \rightarrow NAD^+ + H_2S$ $\quad \Delta E°' = 0.3 + (-0.23) = 0.07$ V

For aerobic respiration, the $\Delta E°'$ is significantly larger (1.1 V).

Since $\Delta G°' = -nF\Delta E°'$, the greater + the $\Delta E°'$, the more favorable (negative) the Gibbs Free Energy. Thus, S respiration is much less efficient

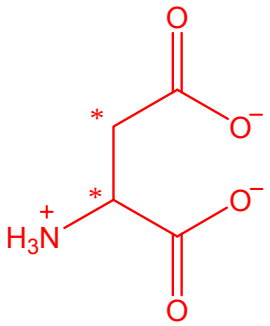
d. The major purpose of the TCA cycle in these organisms is the production of biosynthetic intermediates for other metabolic pathways. If pyruvate were labeled at the $C_3$ position (methyl group), where would you expect the label to appear in the aspartate? Would this be different than what you observe for organisms with complete TCA cycles? Please briefly explain using chemical structures.

Transamination will occur from oxaloacetate, so labeling $C_3$ will label aspartate at 1 location




oxaloacetate aspartate

For organisms with complete TCA cycles, the molecule will go through the symmetric molecule, succinate, so the resulting products may be labeled at 1 of 2 locations



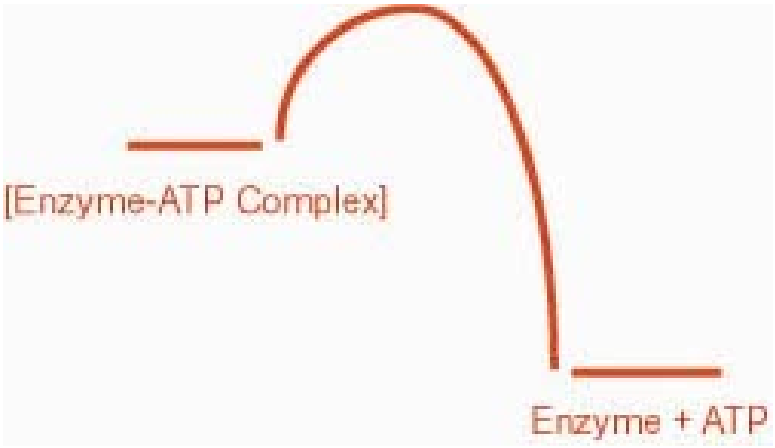

aspartate

4. ATP synthase is a highly efficient machine that efficiently couples chemical and mechanical energy. You are fascinated by its ability to synthesize ATP and continually check YouTube for new video uploads. Since you haven't found anything new lately, you've decided to go into the lab and do some experiments while thinking about everything you've learned in class.

a. Please briefly describe the different roles of the two functional domains, $F_0$ and $F_1$, in the ATP synthase reaction.

$F_0$ is a proton channel that is in the membrane and allows protons to go from the P-side of the membrane toward the N side. The protons are then channeled into $F_1$ causes it to turn catalyzing the synthesis of ATP. Without its association with $F_0$, $F_1$ hydrolyzes ATP.

b. As we learned in class, ATP synthase uses a rotary catalysis mechanism for ATP synthesis. Draw an energy diagram for one of the three active sites for the low-affinity ATP state with respect to enzyme and ATP.




In the low-affinity ATP state, the [Enzyme-ATP Complex] is higher energy than the Enzyme and ATP free in solution. This is what facilitates the release of ATP following the reaction.

c. You have developed a method in the lab to prepare vesicles formed from the inner mitochondrial membranes that retain the ability to make ATP. When you treat the vesicles with detergent, which removes membrane-associated proteins but leaves integral membrane proteins intact, your vesicles lose the ability to make ATP and the pH of the solution increases. Please briefly explain what is happening in your experiment.

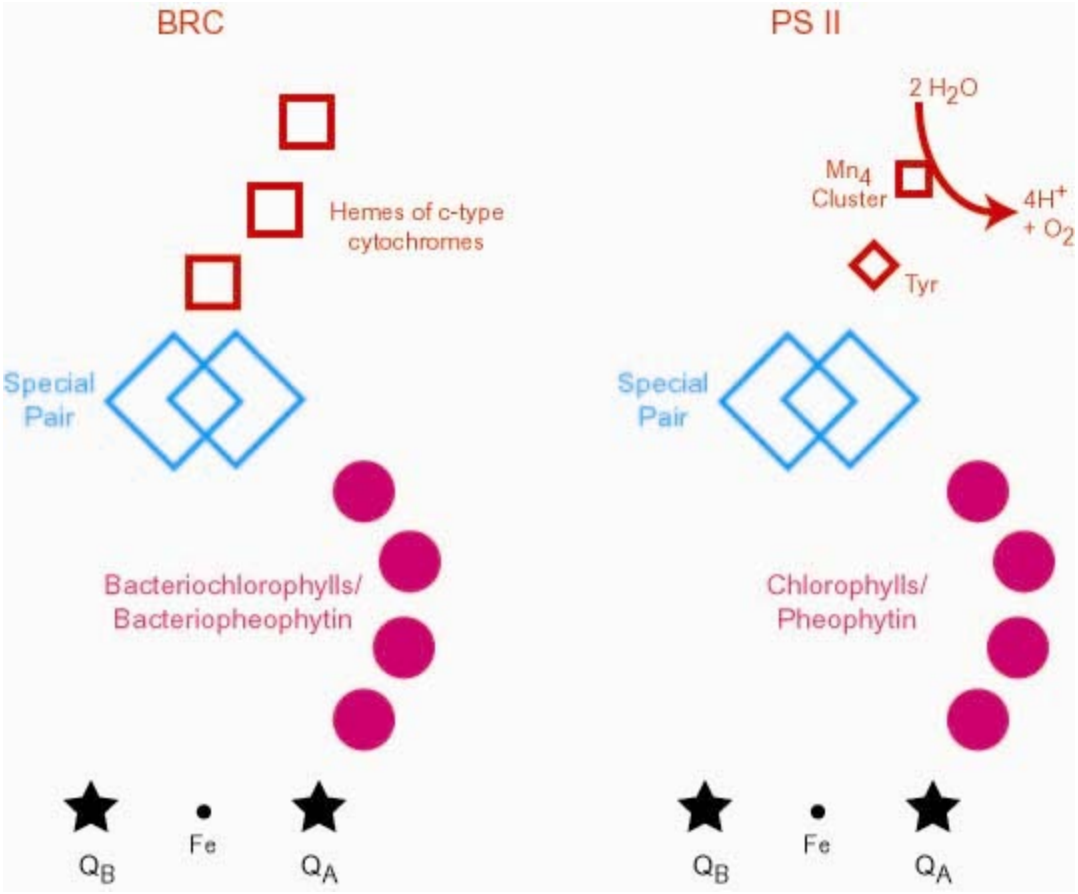
When the detergent is added, removing membrane associated proteins, the F1 module disassociates. This leaves the F0 proton channel in place, but it is no longer coupled with ATP production. The increase of the solution's pH indicates the protons are flowing into the vesicles (decreasing the concentration of protons in the solution) and removing proton gradient that had been powering the synthesis of ATP.

d. If you add back purified $F_0$ and $F_1$, you still cannot recover ATP synthase activity in your solution. Please briefly explain this observation.

It is possible that the ATP synthase complex is unable to reform even after adding back the purified protein. However, even if the ATP synthase does reform in the active form, there will be no proton gradient to give the energy needed for ATP synthesis.

5. The light reactions of photosynthesis of plants/cyanobacteria share many important features with those from photosynthetic bacteria. In this question, we will discuss the similarities and differences between the two systems.

a. Draw a schematic of the bacterial reaction center (BRC) and compare it to Photosystem II (PS II) from cyanobacteria. Outline the similarities and also indicate the differences.




In general, the two systems are very similar. In both cases light activates a special pair made up of two chlorophylls (or bacteriochlorophylls) an electron is then transports through (bacterio)chlrophylls and (bacterio)pheophytin to a quinone ($Q_A$) and then through an iron to a second quinone that can diffuse out ($Q_B$). The main difference is the terminal electron donor. In the BRC the electron comes from the hemes of cytochromes. In PS II The electron comes from the splitting of water which transfers an electron to a $Mn_4$ cluster and then through a Tyr to the special pair.

b. Absorption of a photon or exciton from a light-harvesting complex in PS II initiates a chain of events that leads to formation of $QH_2$. Given the

REDUCTION potentials below, calculate the potential for the reduction of Q by the special pair when this occurs. Please briefly explain your answer.

$(Chl)_2^* \rightarrow \bullet(Chl)_2^+ + e^-$ $E°' = -0.8$ V

$(Chl)_2 \rightarrow \bullet(Chl)_2^+ + e^-$ $E°' = 1.1$ V

$Q + 2\ H^+ + 2\ e^- \rightarrow QH_2$ $E°' = -.05$ V

Q is reduced by the electrons taken from an activated special, so only the $(Chl)_2 \rightarrow \bullet(Chl)_2^+ + e^-$ reduction potential is NOT used. In addition, since the special pair is oxidized, we must use the negative of the reduction potential for that half reaction.

$2\ (Chl)_2^* \rightarrow 2\ \bullet(Chl)_2^+ + 2\ e^-$ $E°' = -0.8$ V

$Q + 2\ H^+ + 2\ e^- \rightarrow QH_2$ $E°' = -.05$ V

$2\ (Chl)_2^* + Q + 2\ H^+ \rightarrow 2\ \bullet(Chl)_2^+ + QH_2$ $E°' = 0.8\text{ V} - .05\text{ V} = 0.75$ V

While the balanced reaction uses two activated special pairs, you should remember that it is actually the same special pair that is regenerated after the first electron is transferred.

c. PS II utilizes water as its terminal electron donor, while a cytochrome serves an equivalent role in the BRC. Given the reduction potentials below, briefly explain why the BRC does not utilize water splitting.

$(Chl)_2^* \rightarrow \bullet(Chl)_2^+ + e^-$ $E°' = -0.8$ V (PS II) and $-1.0$ V (BRC)

$(Chl)_2 \rightarrow \bullet(Chl)_2^+ + e^-$ $E°' = 1.1$ V (PS II) and 0.5 V (BRC)

$NADP^+ + 2\ e^- + H^+ \rightarrow NADPH$ $E°' = -0.3$ V

$\frac{1}{2}\ O_2 + 2\ H^+ + 2\ e^- \rightarrow H_2O$ $E°' = 0.8$ V

Refilling the hole in the special pair, following the donation of an electron to the quinine, will regenerate the special pair in its unactivated form so we'll use the $(Chl)_2 \rightarrow \bullet(Chl)_2^+ + e^-$ reduction potentials. In this reaction the special pair is being reduced, but in order to donate an electron the water must be oxidized so we will use the negative of its reduction potential.

For PS II: $E°' = 1.1\text{ V} - 0.8\text{ V} = 0.3$ V

For BRC: $E°' = 0.5\text{ V} - 0.8\text{ V} = -0.3$ V

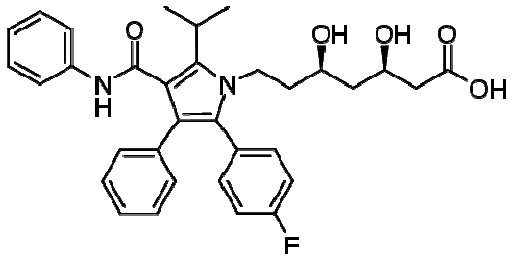
So the reaction is spontaneous for the PS II the reduction potential is high enough to drive the splitting of water spontaneously. The reduction potential for the BRC special pair is not as high, so if it were coupled with a water splitting reaction it would not occur spontaneously.

d. Bacterial light-harvesting complexes (LHCs) have higher excitonic coupling between the chlorophyll molecules, leading to absorption at

longer wavelength ($\lambda$ = 850 nm) compared to those from plants ($\lambda$ = 670 nm). Do you think that the bacterial LHCs would work with PS II from plants ($\lambda$ = 680 nm)? Please briefly explain your answer.

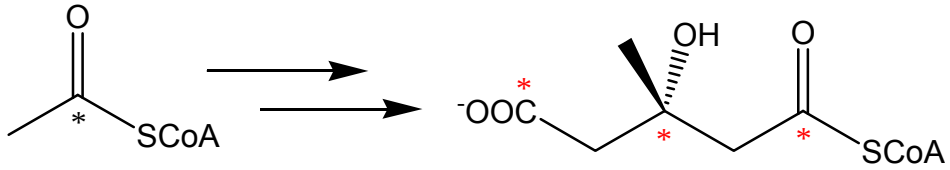
No, the bacterial LHC would not work with PS II from plants. Light with longer wavelengths have less energy. So when the plant LHC aborbs a photon of 670 nm it has enough energy to activate PS II from plants (which requires slightly less energy than that photon has). In contrast, the bacterial LHC absorbs light that is lower in energy than the PS II needs, so it couldn't be used to activate the plant photosystem.

6. Lipitor (Atorvastatin) is the top-selling drug in the world with sales in the US alone of over $12 billion per year. Lipitor belongs to statin family of drugs that is used to lower blood cholesterol and is a competitive inhibitor of HMG-CoA reductase, which is a key enzyme in the cholesterol biosynthetic pathway. Inhibition of this step decreases cholesterol biosynthesis, eventually lowering the level of LDL-cholesterol in the blood.

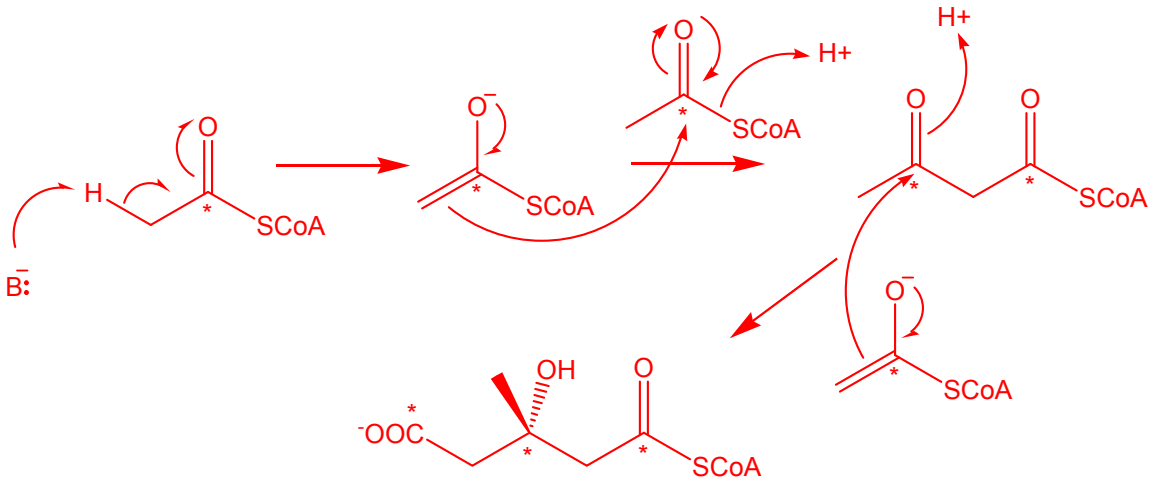



*Lipitor*

a. HMG-CoA reductase is an enzyme in the mevalonate pathway, which is the eukaryotic pathway to make the precursors to steroids like cholesterol and estrogen. Like fatty acids, steroids are made from acetyl-CoA units that are condensed together. The substrate of HMG-CoA reductase is hydroxymethylglutaryl-CoA (HMG-CoA) as shown below. If you fed cells a acetyl-CoA labeled at C1, where would you expect the label to exist on HMG-CoA? Please indicate the labeled carbons using stars.




b. Draw an arrow-pushing mechanism for the formation of HMG-CoA from acetyl-CoA and label the nucleophiles and electrophiles.

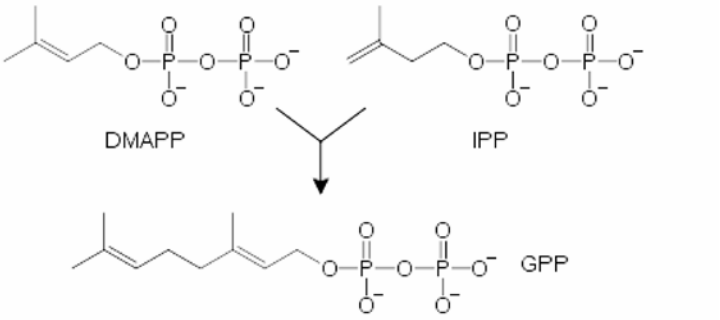


c. If you were to use HMG-CoA for energy production rather than steroid biosynthesis, how many net ATP could you make if all the products were used in the TCA cycle and oxidative phosphorylation?

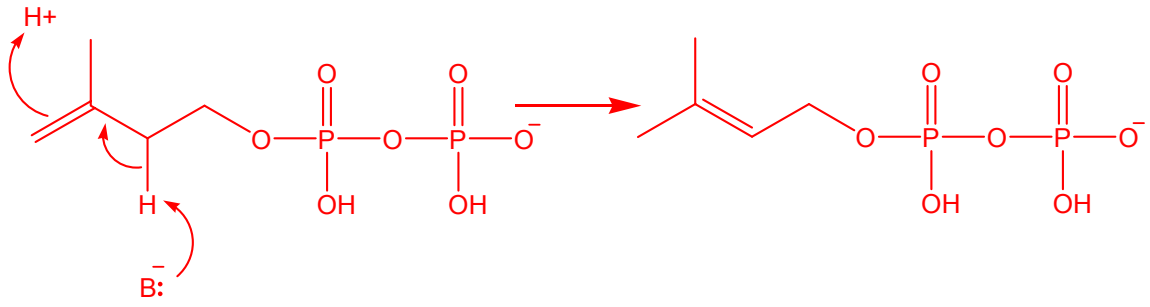
Breakdown of HMG-CoA will yield 3 Acetyl-CoA, which if taken through the TCA cycle will each yield 10 ATP; however, to get the full 3 Acetyl-CoA, we must activate one molecule with ATP to add the CoA-SH, so:

30 ATP – 1 ATP = **29 ATP**

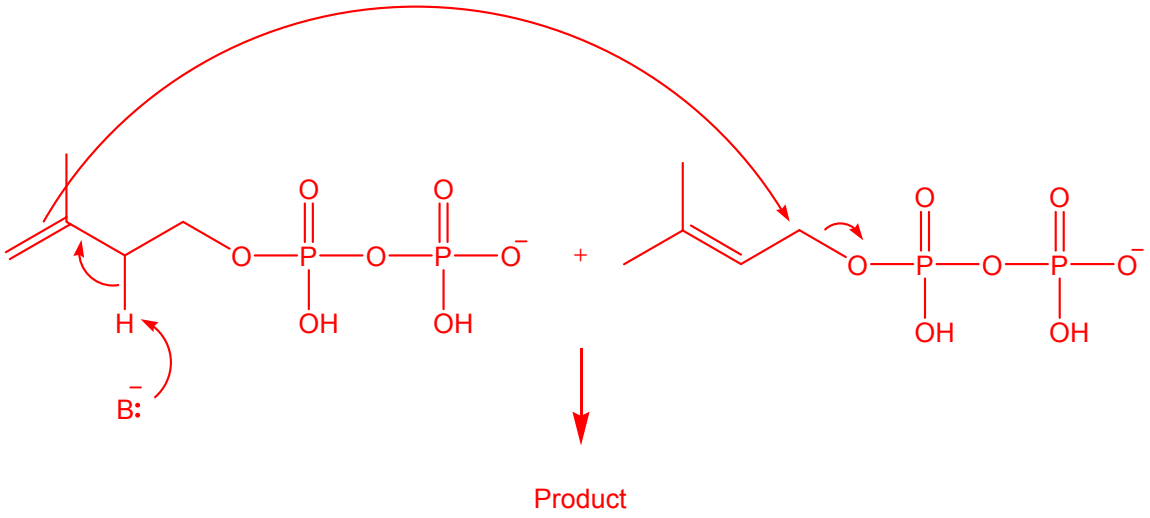
d. The end product of the mevalonate pathway is isopentenyl pyrophosphate (IPP). IPP is a five-carbon building block that is used to make steroids and other complex molecules. In order to initiate polymerization, IPP needs to be isomerized to dimethylallyl pyrophosphate (DMAPP). Elongation with additional IPP units produces a linear chain that is then cyclized to make the types of structures observed in part d. Show an arrow-pushing mechanism for the isomerization of IPP to DMAPP and the condensation of one DMAPP and IPP to form geranyl pyrophosphate (GPP).



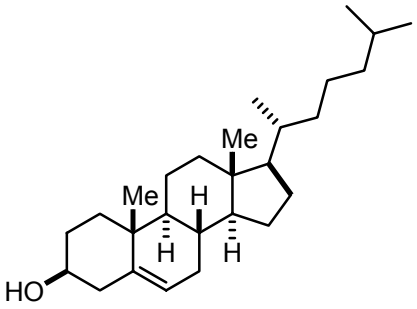

Isomerization




Reaction




Extra credit (1 pt): What do you say if someone asks if you have you ever seen this molecule?




Yes! It's a sterol (this one happens to be cholesterol).