Problem Set 5 **(Due February 25th)**

1. Show how glucose can be converted to two equivalents of pyruvate. Include mechanisms for each of these reactions. See ~~attached page~~ lecture notes

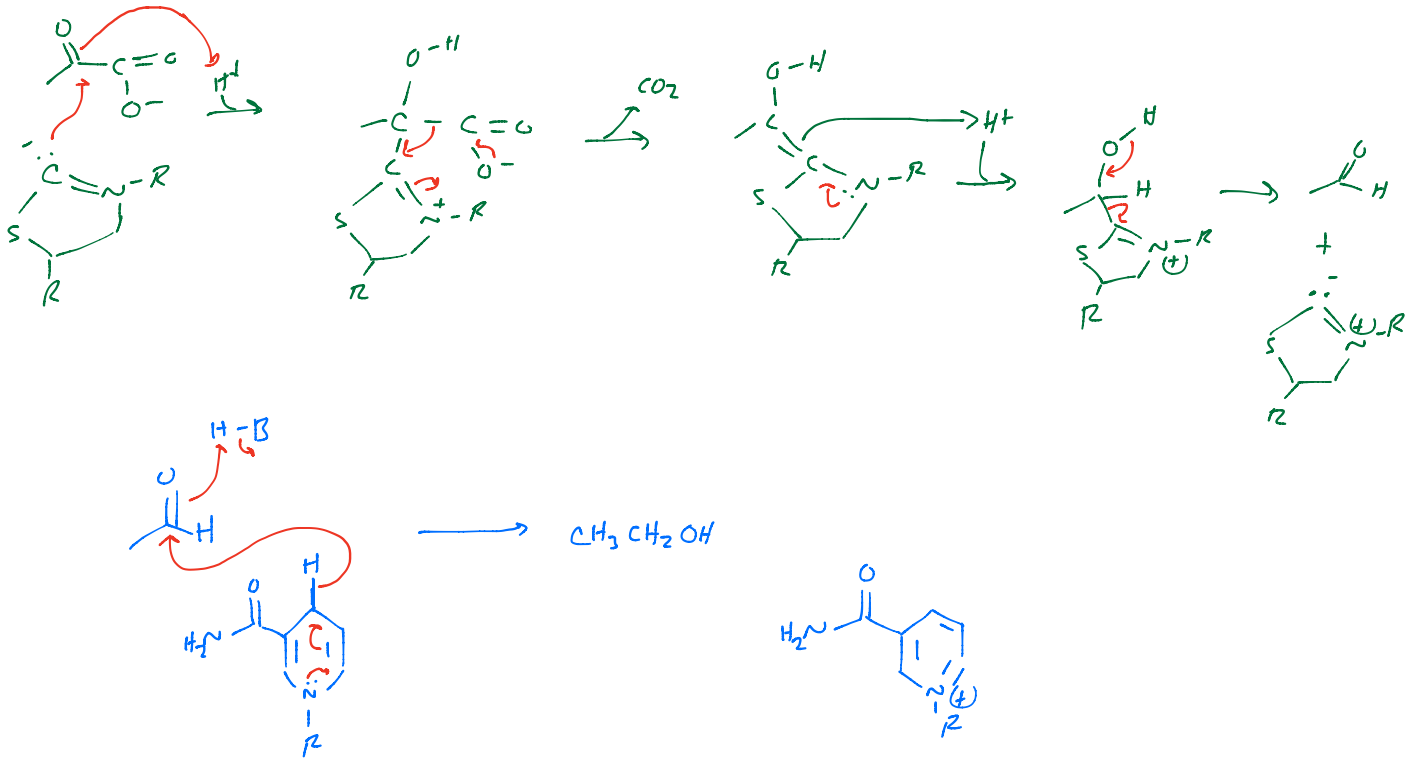
2. What role does the conversion of an aldose to a ketose play in the net glycolytic reaction scheme? This is necessary to prepare the hexose for the reverse aldol condensation.

3. Some organisms replenish the NAD^+ pool using alcoholic fermentation which yields ethanol and carbon dioxide. This is a 2 step process according to the following chemical scheme:

$$\text{Pyruvate} \rightleftharpoons \text{Acetaldehyde} + CO_2$$

$$\text{Acetaldehyde} + NADH + H^+ \rightleftharpoons \text{Acetaldehyde} + NAD^+$$

   a. How does this process "*complete*" glycolysis? It provides a way to regenerate NAD+ so that the NAD+ pool does not become depleted.
   b. What category of metabolic reactions do each of these fall into? C-C bond cleavage and oxidation-reduction
   c. Name the enzyme responsible for catalyzing each reaction (I suggest using KEGG for this). For each enzyme, name the enzyme class (e.g. hydrolase). Pyruvate decarboxylase does both steps. Lyase followed by oxidoreductase
   d. Draw a mechanism for each of these reactions. Make sure to include any important cofactors.



e. What role does the (+) charge on the TPP nitrogen play? It's an electron sink that stabilizes the carbanion.

4. Glycolysis relies on 4 different phosphorylated variations of glycerate. What are these variations and what role do they play?
   1,3 BPG → 1st high energy phosphate compound
   3PG→Product of 1st ATP production step
   2PG → preparation for PEP
   PEP → 2nd high energy phosphate compound

5. Determine how many equivalents of ATP are synthesized from one molecule of Glucose.

	NADH	ATP	$FADH_2$
Glucose → pyruvate	1x2	2	
Pyruvate → Acetyl CoA	1x2		
TCA Cycle	3x2	1x2	1x2

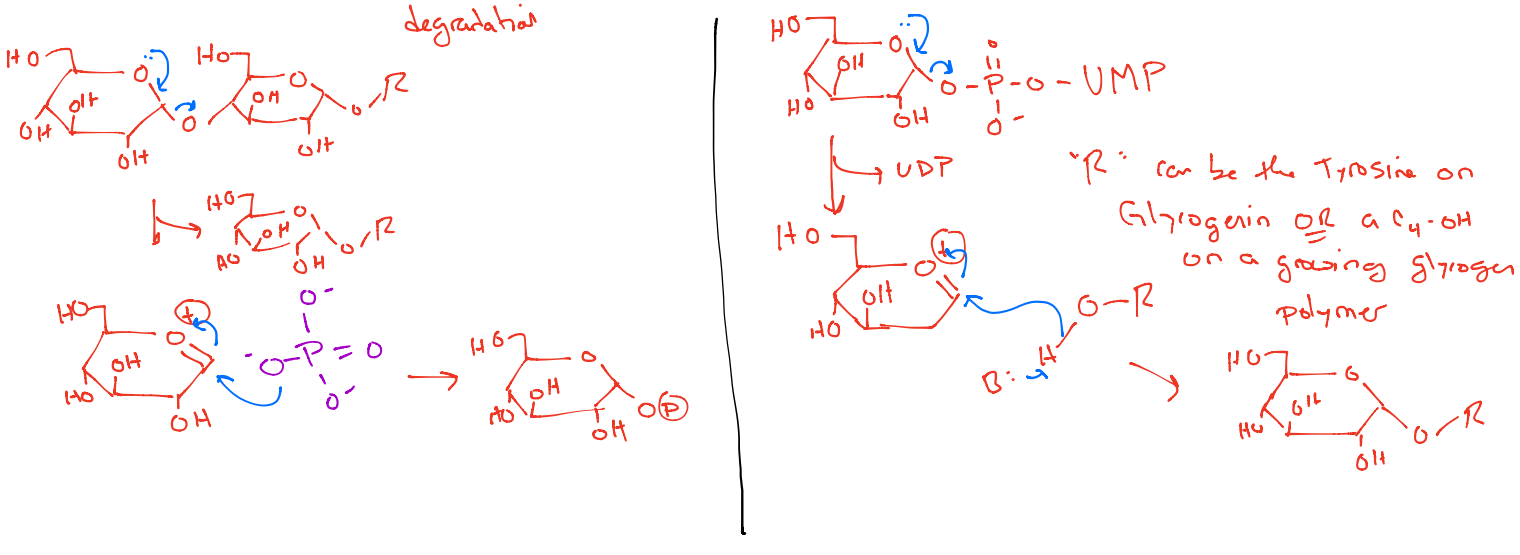
   Assuming a P/O ration of 2.5 for NADH and 1.5 for $FADH_2$
   10x2.5 + 2x1.5+ 4 = 32

6. Determine how many equivalents of ATP are produced when four glucose units are removed from a glycogen polymer. Glycogen degradation does not require energy and it results in G6P, so the 1st ATP consumption step of glycolysis is avoided. So, for every round of glycolysis, a net 3 ATP is generated instead of 2. Each G6P results in 33 ATP

   33 x 4 = 132

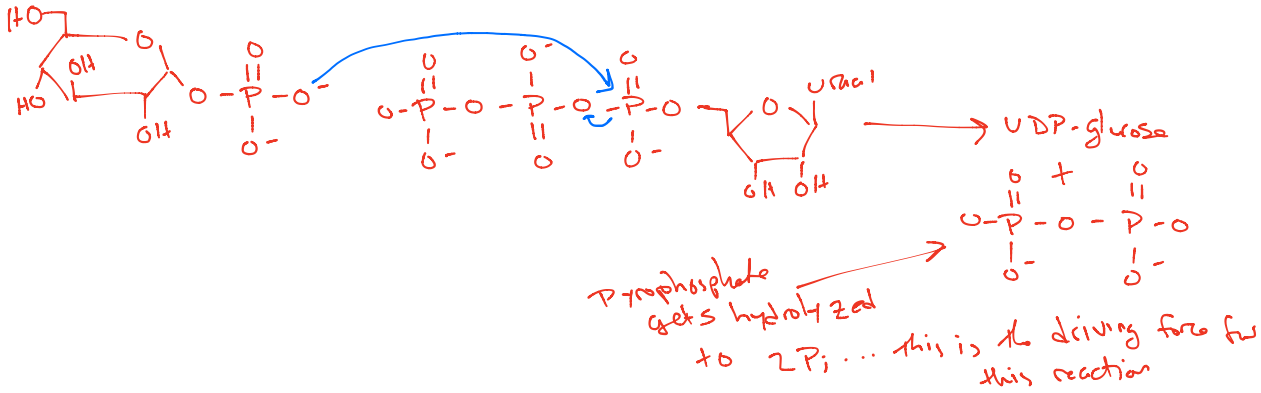
7. Phosphate transfer from PEP to ADP to yield ATP and enolpyruvate is not an exergonic process ($\Delta G^{o'}$ = 14.6 kJ mol^{-1}). With this in mind, please propose a reason that PEP is a high energy phosphate compound that can transfer its phosphate to ADP in a net exergonic process under standard conditions. PEP is a high energy phosphate compound because the unfavorable phosphate transfer is coupled to a very favorable enol tautomerization that produces pyruvate.

8. The formation of oxonium ions are a staple in glycogen metabolism.
   a. What is the purpose of the oxoinum ion? Be specific – this was stressed in lecture. The oxonium ion is the result of cleavage of the alpha C1-Oxygen bond. This, in turn, lets the incoming nucleophile to attack from the alpha side so that stereochemistry is retained.
   b. Show how the oxonium ion is used in the synthesis and degradation of glycogen.



9. Fat is much more prevalent and has a larger energy density (more ATP can be generated per volume) than glycogen. Why then is glycogen so important?
   - Energy production from fat is much slower than the energy generated by oxidizing glucose.
   - Fat metabolism requires aerobic conditions – this is not always possible, particularly in active muscles.
   - Fat cannot be converted to glucose and can therefore not maintain blood glucose levels

10. Glycogen Phosphorylase uses inorganic phosphate to generate glucose-1-phosphate (G1P) from glycogen
    a. How does G1P enter glycolysis? It's converted to G6P by Phosphoglucomutase.
    b. Why is it significant that this enzyme removes a glucose from the non-reducing end of glycogen? There are a lot more non-reducing ends in glycogen than reducing ends. This makes G6P production much more rapid.
11. Glycogen synthase uses UDP-glucose as a substrate for glycogen synthesis.
    a. What is the role of the UDP? The UDP-glucose bond is high energy, so it can readily be displaced during glycogen synthesis and will provide energy for the endergonic formation of the glycosidic bond.
    b. Show how UDP-glucose is formed.

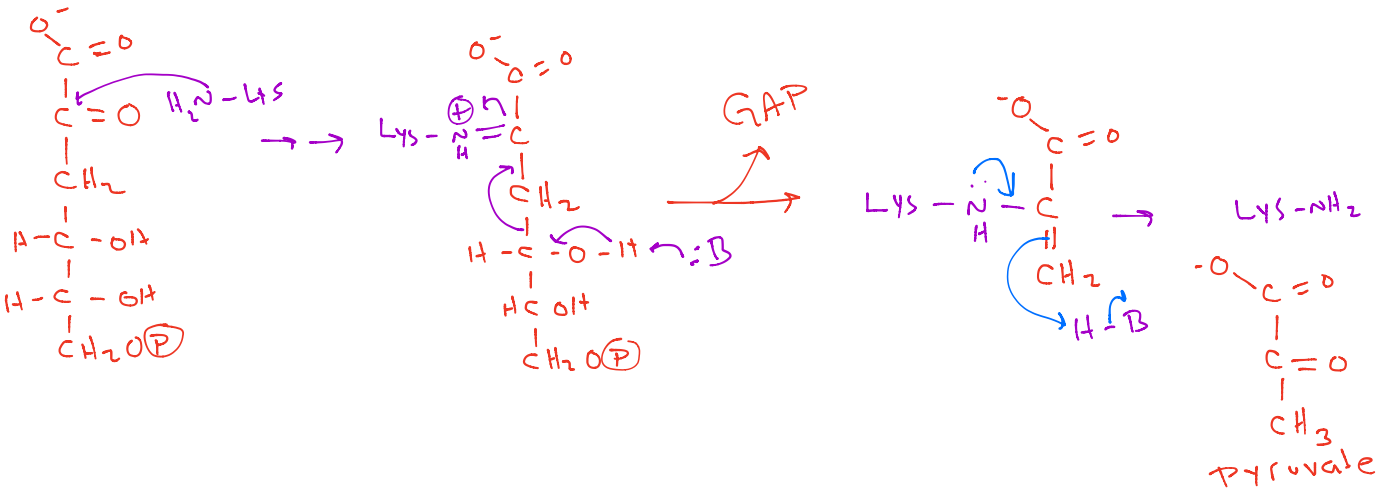


    c. What is glycogenin and why is it important in glycogen synthesis? Glycogenin is a protein that exists at the center of glycogen. The initial primer (~6 glucose units) is built on a tyrosine residue of glycogenin.
    d. How is UTP regenerated and why is this significant when thinking about the energy landscape of this pathway? A phosphate is transferred from ATP.

12. Familiarize yourself with the JBC article below and answer the following questions:
    a. The *Thermoproteus tenax* genome contains two genes that code for a GAPDH. What is the difference in the chemical reaction catalyzed by these two proteins? One catalyzes the oxidation phosphorylation of GAP while the other catalyzes the oxidation reaction, but NOT a phosphorylation. What is the difference in cofactor dependence? The phosphorylating GAPDH requires NADP+ while the phosphate independent enzyme uses NAD+.
    b. The introduction to this article mentions two metabolic pathways that are able to convert glucose to pyruvate, the Embden-Meyerhof-Parnas and the Entner-Doudoroff pathways. Investigate these pathways.
       i. For the pathway that is different than what we've discussed (Embden-Meyerhof-Parnas is also called glycolysis), name and draw the structure of the 6 carbon intermediate that is cleaved into two 3 carbon units. 2-dehydro-3-deoxy-6-phospho-D-gluconate

ii. What are the two products and how are they related to glycolysis? Pyruvate and Glyceraldehyde-3-phospate are both glycolytic intermediates.

iii. The enzyme that catalyzes this reaction is in the aldolase class of enzymes. Predict a mechanism.



c. What does Figure 2 tell us about the enzyme of interest? As described in the text, the enzyme contains the catalytically relevant Glu and Cys residues. Additionally, NAD+ binding residues are present.

d. How was enzyme activity monitored? Monitoring the reduction of NAD+ to NADH spectrophotometrically – I noticed that the experimental section refers to another paper, so I apologize if this gave you a headache.

e. Figure 5 has a lot of important information.

   i. What does this figure tell us about Glucose-1-phosphate and AMP – are they activators or inhibitors? They are activators of the enzyme. Does this make sense based on the role of this enzyme? Yes, a lot of AMP suggests that more energy is needed – this enzyme is involved in an energy producing pathway. An abundance of G1P indicates that glycogen is rapidly being phosphorylzed, suggesting the cellular conditions are appropriate for energy generation.

   ii. The shape of the curve in the presence of $NADP^+$ is very different than the others. What does this tell us? NADP+ is an inhibitor of the enzyme.

f. Comment on the meaning of Hill coefficients determined in this paper. Recall that a Hill coefficient of 1 means that there is no cooperativity in the interactions of the substrate and the effector molecule. A Hill coefficient > 1 means positive cooperativity and less than one is negative cooperativity. This protein is a tetramer, so it's very possible that there are multiple active sites. Taken together, the Hill coefficients in this paper suggest that the inhibitors are bound in a way that has the effect of activating other subunits upon NAD+ binding – this is evident by the positive cooperativity in all cases. This could be taking place at an allosteric site or it could be NAD+ displacement of the inhibitor at the active site. The fact that presence of NADP+ and NADH induce the most positive cooperativity suggests that this is a competitive inhibition.