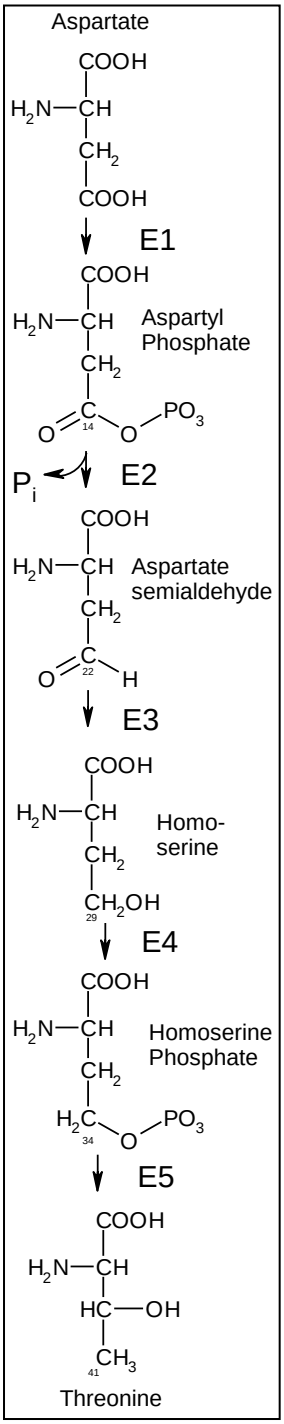
Due Tuesday, November 19 Estimated time: ~ 75 minutes

1. (10 pts, 15 min) The metabolic pathway for the *synthesis* of threonine is shown on the right. Note that not all reactants/products are shown for each reaction.



   i) The first step in this pathway is phosphorylation of a carboxylic acid. The phosphorylation of a carboxylic acid by inorganic phosphate is unfavorable, with a ΔG^{o} of approximately +30 kJ/mol yet the reaction proceeds spontaneously in the forward direction. How can the Gibbs energy, ΔG, become negative for this step in the pathway (2 pts).

Direct coupling using ATP as the phosphate donor. Energy is released by transferring the phosphate from ATP to aspartate, i.e. the energy of ATP is higher than Aspartyl phosphate

   ii) Provide a name for the enzyme (E1) that catalyzes the first step of this reaction, based on your answer to part i) (2 pts).

A phosphate group is added, most likely from ATP, so this is a kinase – Aspartate kinase.

   iii) How would you describe the chemical changes that occur between aspartyl phosphate and aspartate semialdehyde (catalyzed by enzyme E2)? What cofactor/co-substrate is likely involved in this step? Sample *incorrect* answer: This step is catalyzed by a phosphatase because a phosphate is released from the substrate). [Hint: a similar reaction occurs in glycolysis, but in reverse. You can consider the reaction to be a change between a protonated carboxylic acid and an aldehyde – i.e. pretend the phosphate is not there.] (2 pts)

The similar reaction in glycolysis is glyceraldehyde-3-phosphate dehydrogenase. In that case the aldehyde group on glyceraldehyde is converted to a phosphorylated carboxylic acid. In this reaction the reverse is occurring, a phosphorylated carboxylic acid is converted to an aldehyde. This is a reduction, the electrons are provided by NADH.

   iv) Show, by balancing the reaction (or counting electrons on the relevant atoms in the reactant and product), that the step catalyzed by E3 is a redox reaction. Is it an oxidation or a reduction? (2 pts)

This is a reduction of an aldehyde to an alcohol.

Method 1: Since there is no change in the number of oxygens, you need only use hydrogens and electrons to balance the reaction. (aldehyde + $2e^-$ + $2H^+$-> alcohol) Since the aldehyde gains electrons when converted to the alcohol, it's a reduction.

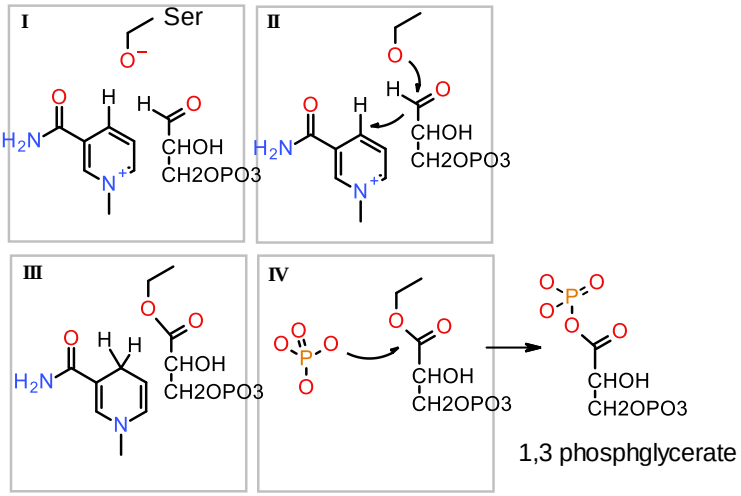
Method 2: By counting electrons you will count only three on the carbon in the aldehyde and five on the same carbon that is now reduced upon conversion to the alcohol. The carbon has gained electron = reduction.

   v) What *general* conclusion can you draw about synthetic pathways? Do they produce or consume energy? Do they contain oxidative or reductive steps? Briefly justify your answer with reference to the pathway for threonine biosynthesis (2 pts)

- Synthetic pathways require energy and produce reduced compounds, making more complex molecules from simpler ones.
- Degradative pathways produce energy and produce oxidized compounds. Typically taking more complex molecules and breaking them down to simpler ones.

2. (5 pts, 5 min) Glyceraldehyde-3-phosphate dehydrogenase catalyzes the oxidation of glyceraldehyde to a phosphorylated carboxylic acid in glycolysis. Briefly discuss how changing the cysteine residue to serine would affect the mechanism.

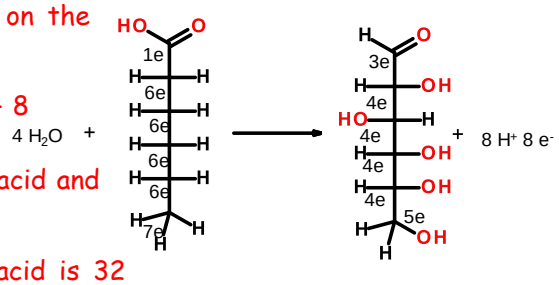
The overall reaction mechanism would remain the same, however the oxygen on the serine sidechain would react with the aldehyde, forming an ester instead of a thioester. This would be broken by a phosphate, generating the phosphorylated carboxylate group. The kinetics would be altered.



3. (5 pts, 5 min) Is the hypothetical conversion of hexanoic acid (a 6 carbon fatty acid) to glucose an oxidation or a reduction? Show by either balancing or electron counting. Which compound is higher in energy?

By balancing, 4 water molecules need to be added to the left side to give the same number of oxygens on the right.

To balance the hydrogens, a total of 8 hydrogen atoms (8 H+ + 8 e-) need to be added to the right. Since there are excess electrons on the right, they were removed from the hexanoic acid and this is a 8-electron oxidation.

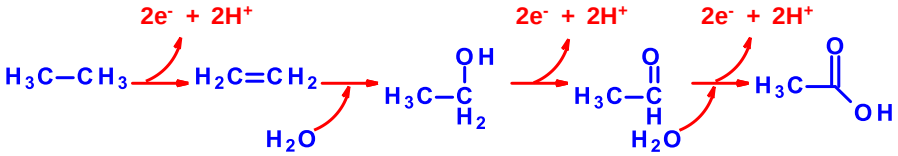


By electron counting, the sum of the electrons on hexanoic acid is 32 while the number of electrons associated with the carbons on glucose are 24, i.e. an 8 electron oxidation.

The least oxidized compound will be higher in energy, so hexanoic acid is higher in energy than glucose. The heat of combustion for glucose is -2800 kJ/mol and hexanoic acid is -3500 kJ/mol.

4. (6 pts, 10 min) Fill in the steps in the conversion of an alkane (e.g. ethane) to a carboxylic acid (e.g. acetic acid) using a series of two electron oxidations, plus any additional reactions that might be required. Give the generic name (e.g. type of reaction catalyzed) by each enzyme in each step. A skeleton outline is given below:

Alkane → alkene	ethane dehydrogenase (redox)
Alkene → alcohol	ethane hydratase (addition of water)
Alcohol → aldehyde	ethanol dehydrogenase (redox)
Aldehyde → carboxylate	ethaldehyde dehydrogenase (redox)

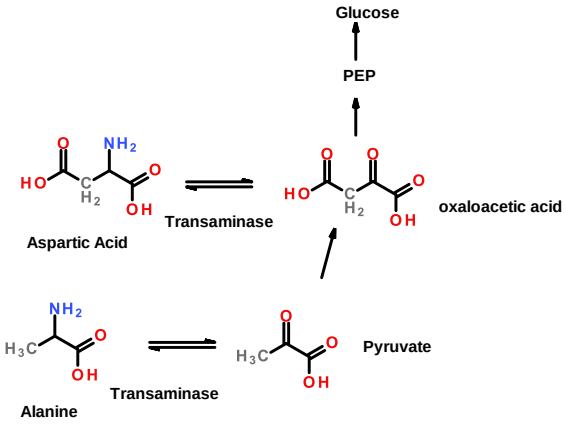


5. (8 pts, 10 min) In recitation we investigated how PFK was controlled by ATP and ADP.
   i) Does the regulation make physiological sense? Briefly justify your answer (4 pts).
   ii) Propose a mechanism by which this occurs, based on the ***structure*** of PFK (4 pts)

i) Yes, ADP is produced from the hydrolysis of ATP. So as the cell uses ATP up, it generates ADP. The ADP will turn on PFK-1 in glycolysis, regenerating the ATP by oxidation of glucose.

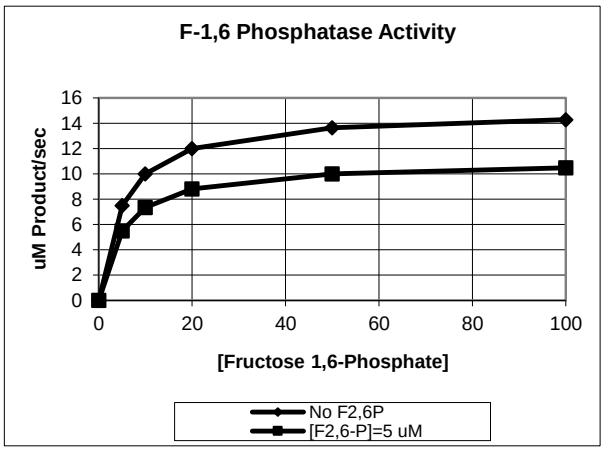
ii) The terminal phosphate of ATP is directed towards the core of the enzyme, forcing it into that region must distort the nearby active site to affect (reduce) catalysis. When ADP (or AMP) bind, then this phosphate is absent, so the enzyme assumes its active form.

6. (5 pts, 10 min) Transaminases are enzymes that reversibly convert α-keto acids to α-amino acids by the replacement of a ketone group by an amide group, thus providing a way to both synthesize and degrade amino acids. Discuss how the amino acids alanine and aspartic acid could be used to synthesize glucose.



Alanine is converted to pyruvate by the transaminase.
Aspartic acid is converted to oxaloacetate by a transaminase
Both of these can be converted to PEP by the 1st and 2nd steps in gluconeogenesis.

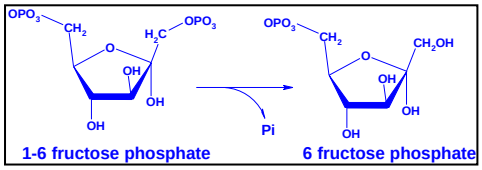
7. (10 pts, 20 min) The curves to the right show the effect of fructose-2,6-bisphosphate (5μM) on the activity of fructose-1,6-bisphosphatase. The data that was used to generate this plot is given in the table below:



[F-1,6-Phosphate] (μM)	uM Product/sec	
		+F-2,6P
0	0	0
5	7.5	5.5
10	10	7.3
20	12	8.8
50	13.6	10.0
100	14.3	10.5

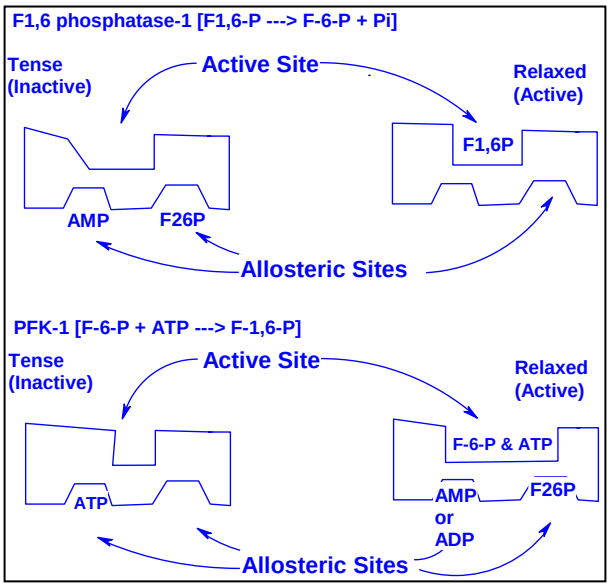
Please answer the following questions (most of these are straightforward and are designed to draw your attention to differences in F16P and F26P).

i) Draw the substrate and product of the reaction catalyzed by fructose-1,6-bisphosphatase. Include any cofactors/co-substrates that may be involved in the reaction (e.g. ATP, NADH, etc.) (1 pt)



ii) In which metabolic pathway does fructose-1,6 bisphosphatase operate (1 pt)? Gluconeogenesis

iii) What is the structural difference between F-1,6-Phosphate and F-2,6-Phosphate (1 pt)? The position of one of the phosphates, in F1,6 the phosphate is on C1, in F2,6 the phosphate is on C2.



iv) Would you characterize F-2,6-P as which of the following:(1 pt)

- Competitive inhibitor of F-1,6-bisphosphatase
- Allosteric activator of F-1,6-bisphosphatase
- Allosteric inhibitor of F-1,6-bisphosphatase

Briefly justify your answer on the basis of the experimental data (3 pts).

Allosteric *inhibitor*: The inhibition is non-competitive, since V_{max} is affected. F2,6 phosphate reduces the activity of the enzyme

v) Based on your answer to part iv, draw a *simple*, cartoon-like diagram of fructose-1,6-bisphosphatase. In your diagram should indicate the binding sites for F-1,6-P and F-2,6-P and indicate the active site region on the enzyme (3 pts).

- See the diagram to the right. Note that both F2,6-P and AMP bind, and stabilize, the tense or inactive state of fructose bisphosphatase.

- For purposes of comparison, also included is a diagram for PFK, the enzyme that performs the same reaction (in reverse) in glycolysis. ATP is shown binding in the allosteric site, in this case inhibiting PFK-1.
- Note that the location of the binding site of F2,6-P on PFK-1 is not known, allosteric sites for AXP and F26P are shown as separate sites, but they may overlap.