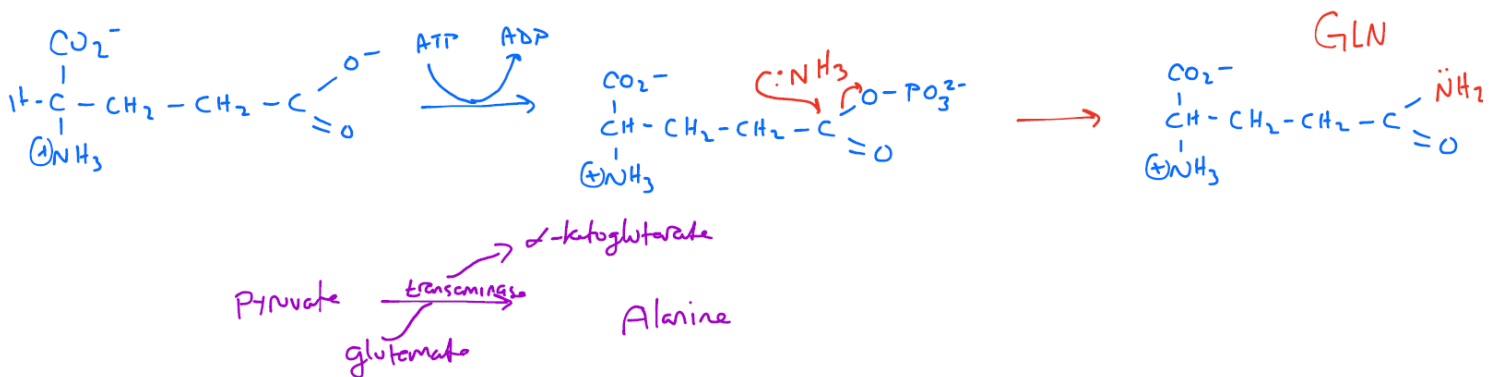


**Problem Set 9**

**(Due April 14<sup>th</sup>)**

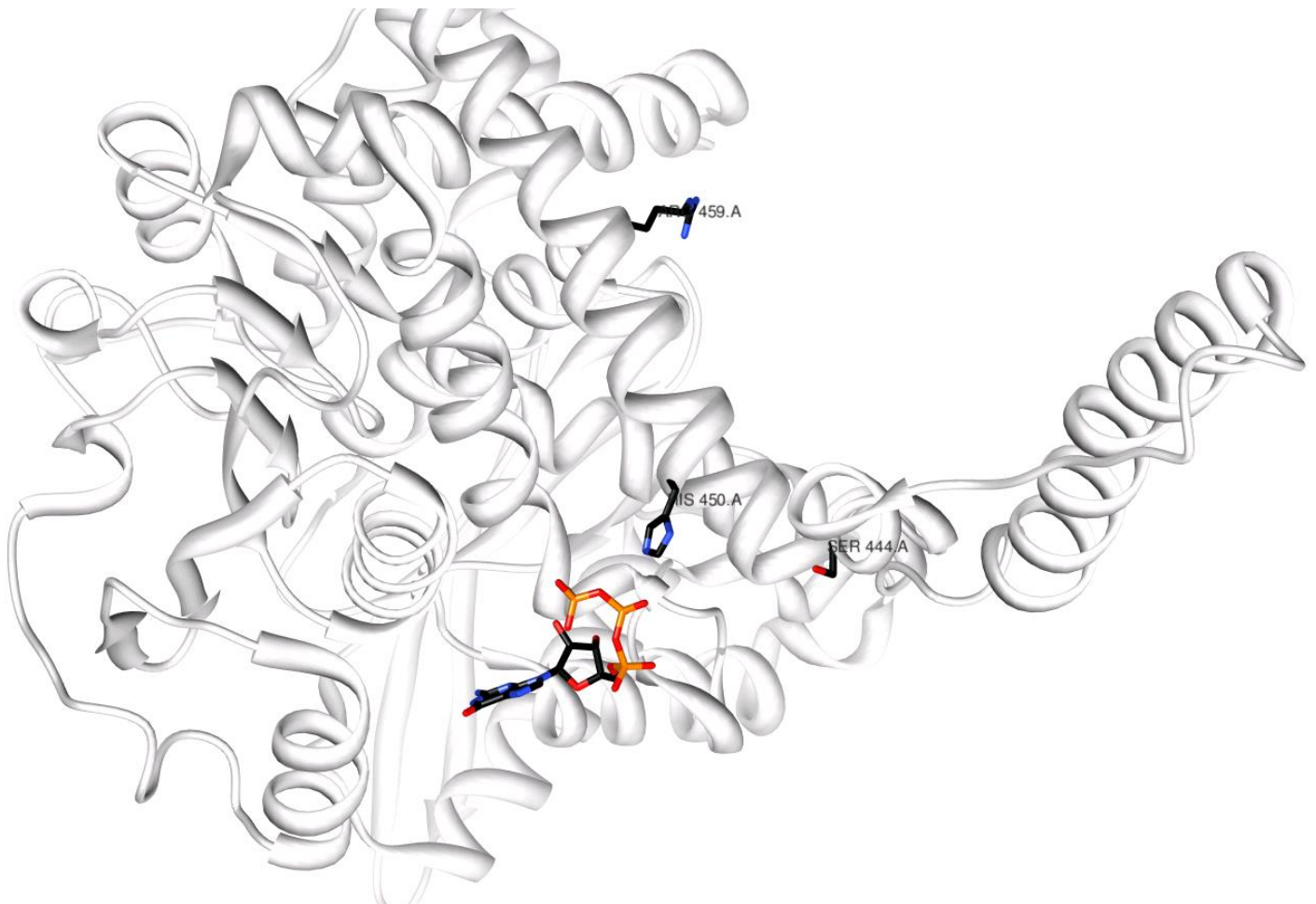
1. Describe the process of delivering amino acids to the liver from:
  - a. Dietary proteins **Gastrin Hormone is secreted by gastric mucosal cells which signals the release of HCl and Pepsinogen (pepsin zymogen) by gastric glands. The low pH triggers Secretin release, which stimulates pancreas to release bicarbonate to neutralize the small intestine. Amino acids in the duodenum trigger the release of cholecystokinin into blood which signals the release of pancreatic proteases (e.g. trypsin) into the intestine. Complete peptide hydrolysis is completed by these and other intestinal peptidases. Free amino acids are transported to the blood by intestinal mucosa cells where they move to the liver.**
  - b. Degraded cellular proteins: **Proteins are tagged for proteasomal degradation by polyubiquitination. This process involves three enzymes: E1, E2 and E3. E1 activates ubiquitin, E2 serves as an intermediate of ubiquitin transfer, and E3 transfers ubiquitin to a target protein. Once tagged, the protein is sent to the proteasome and degraded to individual amino acids.**
  
2. Ammonia is transported through the blood from extrahepatic cells to the liver as glutamine or alanine.
  - a. Why is this necessary? **Ammonia is quite toxic at elevated levels. It needs to be chaperoned for transport between different tissue.**
  - b. How are these two molecules generated in other tissues?



3. The first step in the degradation of most amino acids is removal of the amine group.
  - a. What is the class of molecules that are produced when amino acids are deaminated?  
 **$\alpha$ -ketoacids**
  - b. How is aspartic acid deaminated? Please show a mechanism describing this process. **See next page**
  - c. In addition to aspartic acid metabolism, Aspartate transaminase has a special role in nitrogen metabolism. What is this role? **Mitochondrial Aspartate transaminase catalyzes the transfer of  $\text{NH}_3$  from Glu to oxaloacetate and generates  $\alpha$ KG and aspartate. The Asp produced is shuttled out of the mitochondria and condenses with citrulline to form argino-succinate. This is a major step in the Aspartate-argino-succinate shunt**



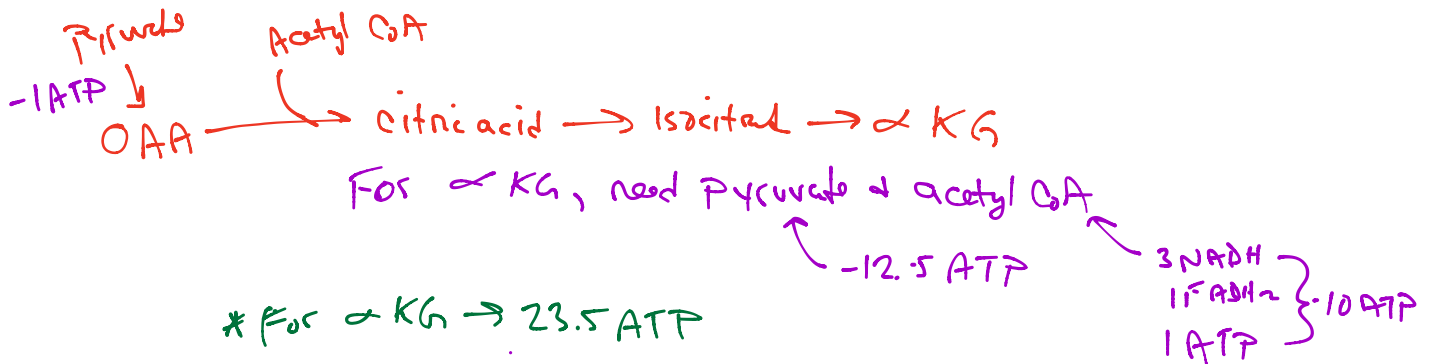
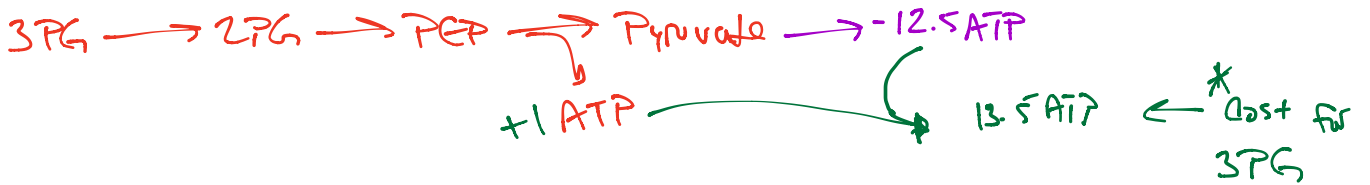
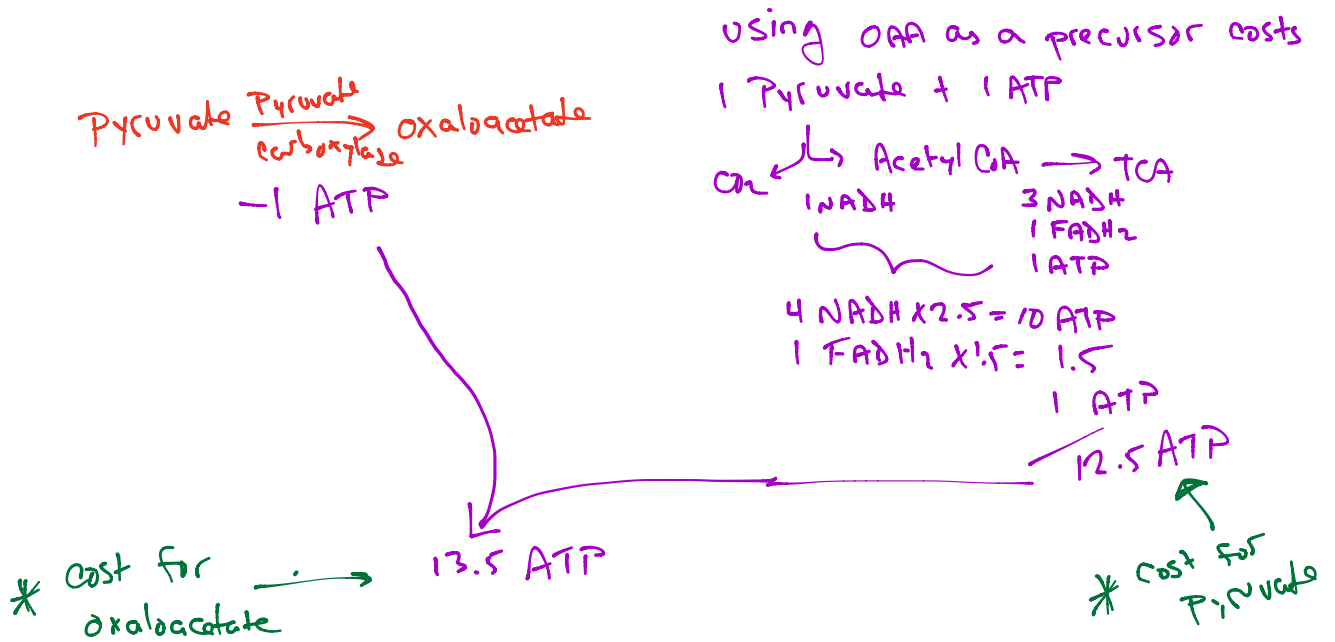
4. Glutamate Dehydrogenase is allosterically inhibited by GTP.
- How does this process occur? GDH can adopt two conformations – open (inactive) and closed (active). GTP binds to and stabilizes the closed conformation. By doing this, it dramatically increases the affinity of the enzyme for reactants AND products. Therefore, once the products are generated, they cannot be released and the enzyme is not able to turnover more cycles.
  - Using the crystal structure of GDH (pdbID 1HWX), discuss the inhibition graph found in your lecture notes (Lecture 18, slide 8) based on what you see in the structure. Note that the numbering of amino acids in the pdb is off by 4 relative to the book (H454 = H450, S448 = S444, and R463 = R459). **Please include an image of ONE subunit of this protein with GTP and these three residues shown as stick images.** His454 makes direct contact with the gamma phosphate of GTP, so mutation of this residue would be expected to have a profound impact on the ability of GTP to inhibit the enzyme. The other two mutations are spatially distinct from the FTP site, so their mutations should not hinder GTP binding as much. Serine→Proline is more potent than Arg→Ala; this should be expected since proline dramatically influences secondary structure.



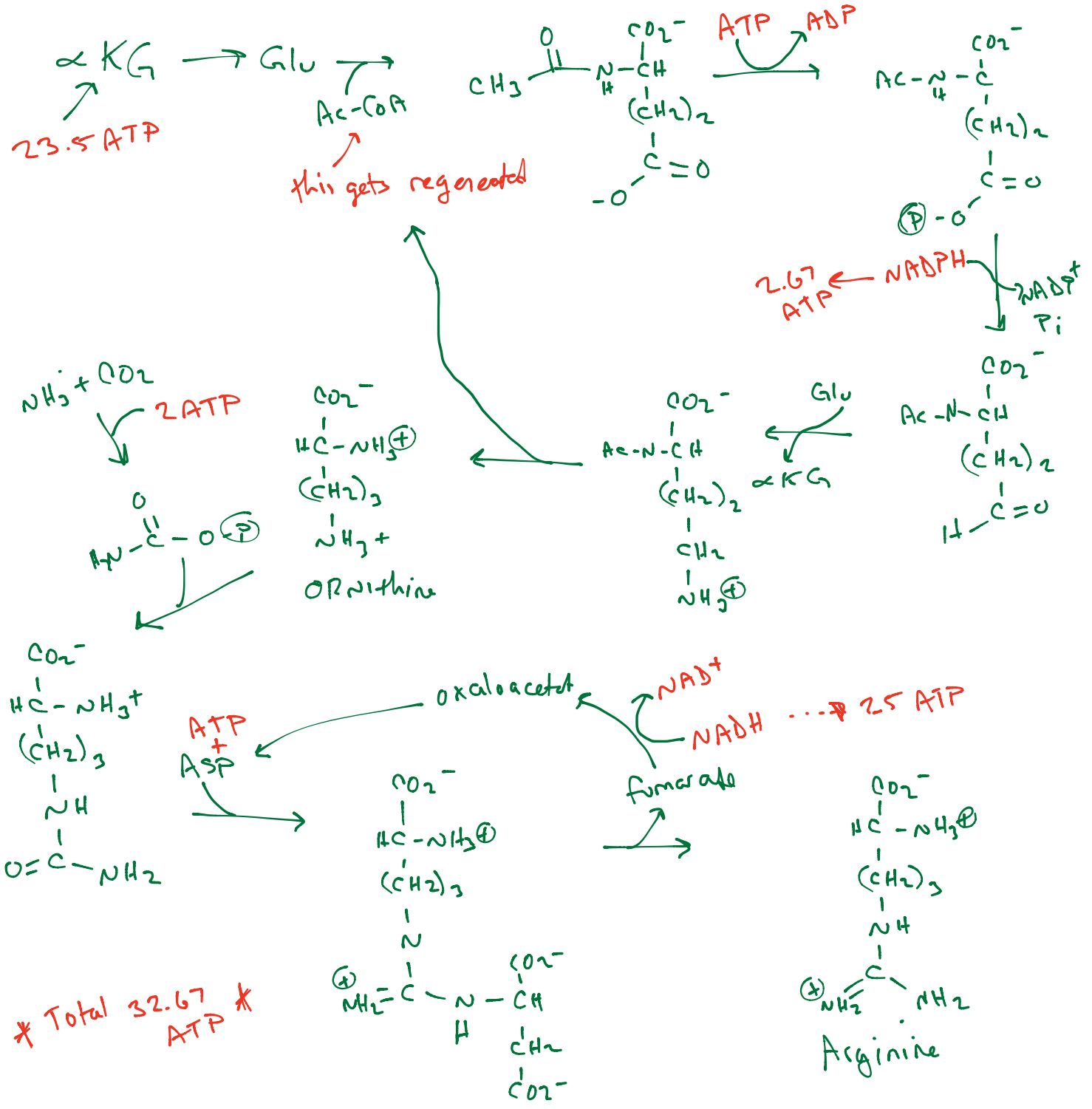
5. Although mammals can synthesize Arginine, it is often considered an essential amino acid. Why is this? Arginine is an intermediate of the Urea Cycle; as such, most of it is hydrolyzed to form urea and ornithine.

6. Oxaloacetate,  $\alpha$ -Ketoglutarate, pyruvate and 3-phosphoglycerate are all metabolic precursors for amino acid biosynthesis. Please determine the energetic cost (e.g. how much ATP is sacrificed) of using one of each of these molecules. For the two TCA cycle intermediates, recall that the product of the pyruvate carboxylase catalyzed reaction is in equilibrium with some, but not all TCA intermediates.

3PG  $\rightarrow$  ~~14.5~~ <sup>13.5</sup> ATP      Pyruvate  $\rightarrow$  12.5 ATP      Oxaloacetate  $\rightarrow$  13.5 ATP       $\alpha$ -Ketoglutarate = 23.5 ATP



7. How much energy is required to biosynthesize arginine? **32.67 ATP**



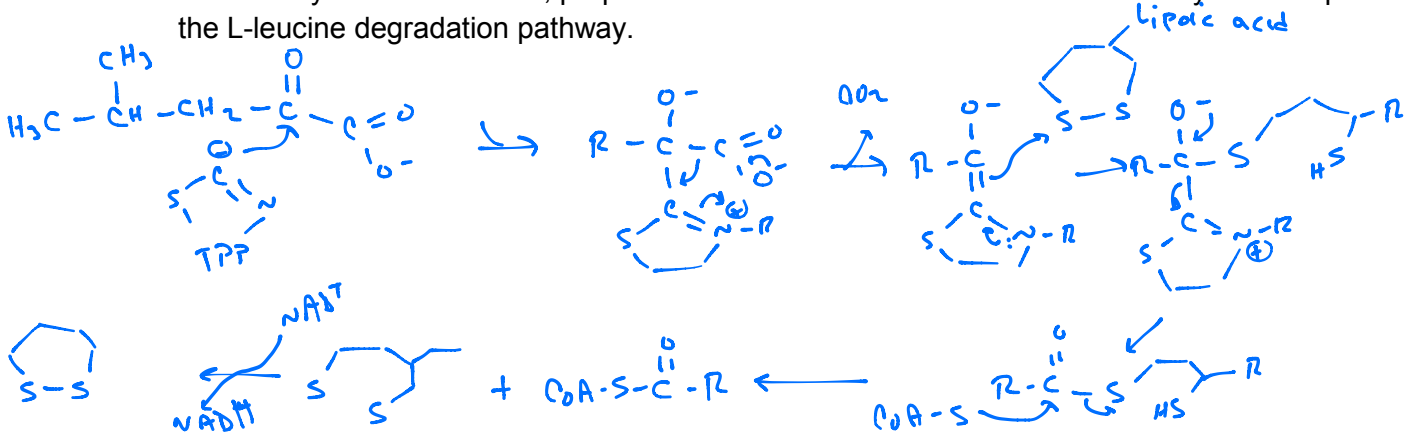
8. Please compare the energetic cost vs. yield of proline anabolism and catabolism. → P. 893      → P. 721

Proline is biosynthesized from Glu in 3 enzymatic steps which consumes  
 $1 \text{ ATP} + 2 \text{ NADPH} \rightarrow 6 \text{ ATP}$

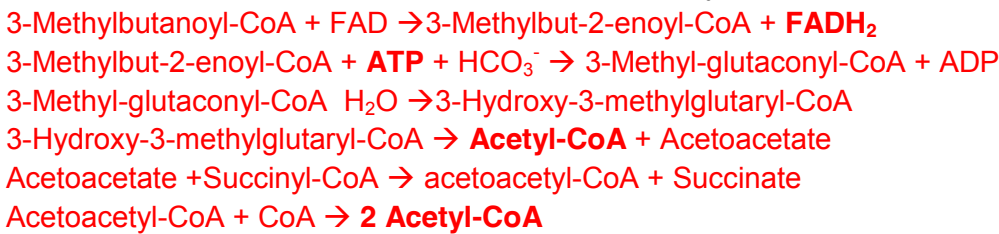
Proline degrades to Glu in a process that yields 0.6 NADH  
 $\rightarrow 2.5 \text{ ATP}$

So it takes more energy to produce histidine than what is yielded by its degradation

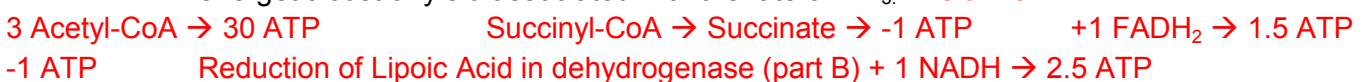
9. Using KEGG Pathways, navigate to the page that describes the degradation of branched chain amino acids.
- As you can see, the first steps in each case are the same. Once transamination occurs, the next step (or 3, depending on how you look at it) should look very familiar to you. What reaction does this resemble? Hint: looking closely at the cofactors should be very helpful here. **Pyruvate Dehydrogenase**
  - Based on your answer to 9a, propose a mechanism for the oxidative decarboxylation steps in the L-leucine degradation pathway.



- Show a reaction scheme for the rest of the degradation of L-Leucine. Make sure to include the steps that convert acetoacetate to Acetyl-CoA.



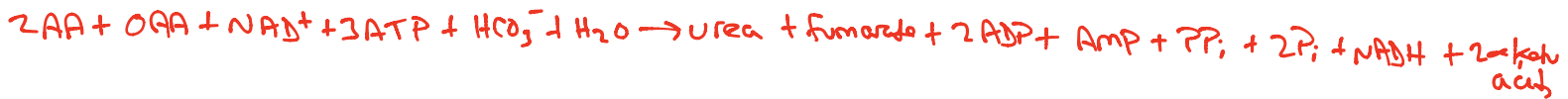
- Determine the energy yield for the complete oxidation of L-Leucine. You may ignore any energetic cost or yield associated with the fate of  $\text{NH}_3$ . **Yield = 32 ATP**



10. Determine the net reaction for the Urea Cycle.



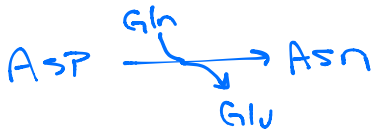
Overall:



11. Please show a mechanism for the enzyme-catalyzed steps of the Urea Cycle. See next page

12. How is flux through the Urea Cycle regulated? Major regulatory point is carbamoyl phosphate synthase. This enzyme is allosterically activated by N-acetyl glutamate, a product of enzyme mediated acylation that is proportional to the [glutamate] in the cell. So, as [Glu] increases (an indicator of protein degradation), the urea cycle is activated.

13. How are the biosynthesis of arginine and glutamine linked? Please show a reaction scheme to reinforce your answer.



production of Asn is dependent on Gln ... Gln is synthesized from  $\text{NH}_4^+ + \text{Glu}$

14. Please draw the fully reduced form of folic acid (tetrahydrofolate) and discuss how this essential cofactor plays a role in glycine and serine metabolism.  $N_5,N_{10}$ -Methylene-THF can readily accept or donate a methyl group; this facilitates the interconversion of glycine and serine. Serine is biosynthesized from 3PG and degrades to pyruvate; biosynthesis and degradation of glycine typically proceeds through serine as an intermediate in the THF-dependent fashion described above.

