Name: _____ Exam #3

The following questions are based on: Zhen Yang, Eric L. Matteson, Joerg J. Goronzy, and Cornelia M. Weyand. (2015). T-cell Metabolism in Autoimmune Disease. **Arthritis Research Therapy 17:** 29-39.

> "Among the amino acids, glutamine appears to be particularly important. T-cell activation induces a substantial increase in the import of glutamine, but not glutamate. T cells consume glutamine at rates comparable with or even higher than glucose. During glutaminolysis, the amino acid is diverted into metabolic intermediates, such as pyruvate and glutamate. Scientists have long known about the absolute requirement for glutamine in proliferating T cells and have supplemented tissue culture media for T-cell cultures with glutamine."

1. **Draw glutamine.**

2. **T cells are often grown in culture media that contains 2 mM glutamine. How many grams of solid glutamine would need to be added to each liter of media? Show all of your work.**
   H = 1 g/mole; C = 12 g/mole; N = 14 g/mole; and O = 16 g/mole

Glutaminolysis

3. **Glutaminase catalyzes the S_N2-like reaction between H_2O and glutamine to produce glutamate.**
   a. Draw glutamate.

   b. Identify the nucleophile. Be very specific.
   c. Identify the electrophile. Be very specific.
   d. Identify the leaving group. Be very specific.

4. **Glutamate is converted to α-ketoglutarate.**
    a. Draw α-ketoglutarate.

    b. If this reaction is catalyzed by a transaminase, what cofactor(s) are required?

    c. If this reaction is catalyzed by a transaminase, where does the nitrogen atom go?

    d. If this reaction is catalyzed by glutamate dehydrogenase, what cofactor(s) are required?

    e. If this reaction is catalyzed by glutamate dehydrogenase, where does the nitrogen atom go?

5. **Diagram the pathway for the conversion of α-ketoglutarate to pyruvate. Include the names of intermediates and/or the names of enzymes in your diagram. Chemical structures are not necessary.**

"In contrast to healthy CD4 T cells, rheumatoid arthritis T cells fail to upregulate glycolytic activity due to the insufficient induction of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 [PFKFB3; a PFK-2/F-2,6-BPase variant that contains the activity of both PFK-2 and F-2,6-BPase]"

"The study employed a gene expression screen for 29-glycolysis-related makers, and PFKFB3 was the only marker that was significantly suppressed in rheumatoid arthritis T cells"

6. **Besides expression levels, the phosphorylation state of PFKFB3 may be important. In separate cancer studies [Bando *et al.* (2005). Phosphorylation of the 6-phosphofructo-2-kinase/Fructose-2,6-bisphosphatase/PFKFB3 Family of Glycolytic Regulators in Human Cancer. Clin. Cancer Res. 11:5784-92.], it was found that phosphorylation up-regulates the kinase activity of PFKFB3.**

    a. In <u>healthy</u> CD4 T cells, do you expect PFKFB3 to be phosphorylated?

    b. Is this regulation similar to hepatocytes, lipocytes, cardiac myocytes, and/or skeletal myocytes?

    c. Explain your reasoning.