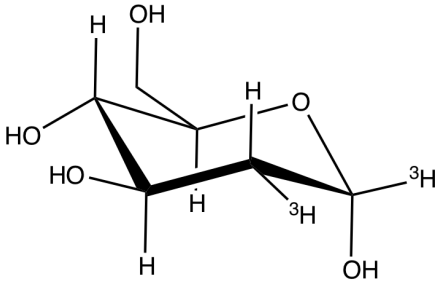
NAME:_____ Exam 1

The following questions are based on Fontaine, K.A.; Sanchez, E.L.; Camarda, R.; and Lagunoff, M. (2015). Dengue Virus Induces and Requires Glycolysis for Optimal Replication. **J. Virol. 89**: 2358-2366.

1. All the culture media used by the authors contained 2 mM glutamine. Imagine that you prepared a 100 mM stock solution of glutamine that you dilute while preparing the culture media. If you plan to prepare 1 L of culture, how much of the 100 mM stock solution of glutamine will you need to add to the culture flask? What fold dilution does this represent (e.g., 1:X)?

2. To monitor glucose uptake, the authors fed cells 2-deoxy-D-glucose radioactively labeled with tritium (^{3}H) at two positions.



   a. What are the possible metabolic fates of glucose within the cytoplasm of the cell (neglect efflux out of the cell)?

   b. 2-deoxy-D-glucose cannot be metabolized through the complete process of glycolysis. Try to run 2-deoxy-D-glucose through glycolysis (write this out including chemical structures, enzyme names, and any co-reactants or co-products). Which step is not possible?

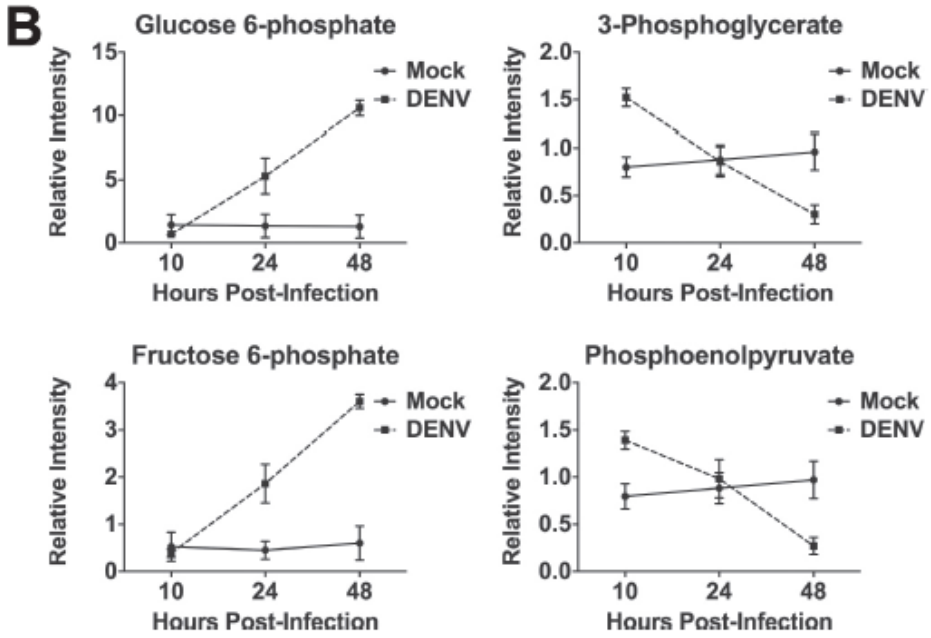
c. Could 2-deoxy-D-glucose be incorporated into glycogen? Write out the process for glycogenesis to provide evidence for your answer. Include chemical structures, enzyme names, and any co-reactants or co-products. Would the resulting glycogen be radioactive?

d. Could 2-deoxy-D-glucose be metabolized through the pentose phosphate pathway? Write out the process for the pentose phosphate pathway to provide evidence for your answer. Include chemical structures and any co-reactants or co-products. Would the resulting ribose-5-phosphate be radioactive?
   i. Draw glucose.
   ii. React glucose with hexokinase.
   iii. Convert C1 to a carbonyl (i.e. ester). This is named 6-phosphoglucolactone.
   iv. Linearize 6-phosphoglucolactone and convert C1 to a carboxylate. The enzyme that catalyzes this reaction is 6-phosphoglucolactonase, while the product is 6-phosphogluconate.
   v. C1 is released as CO_2. This enzyme is named 6-phosphogluconate dehydrogenase. The final product is named ribulose-5 phosphate.
      1. A base abstracts the proton from the C3 hydroxyl group.
      2. One lone pair from the resulting oxyanion move to form double bond with C3, while the hydride attached to C3 is transferred to $NADP^+$. C3 should be a keto group.
      3. A lone pair from one of the C1 carboxylate oxyanions moves to form a double bond with C1. The electrons forming the C1 to C2 bond are withdrawn towards the ketone. These electrons form a double bond between C2 and C3. The carbonyl oxygen on C3 accepts an additional lone pair to form an oxyanion. C1 leaves as CO_2.
      4. One lone pair of the oxyanion of C2 (note number change after CO_2 release) moves to reform the carbonyl, moving the pi-electrons towards C1, which picks up a proton.
   vi. Ribulose-5 phosphate undergoes a keto-enol tautomerization to the aldopentose to ribose-5-phosphate.

NAME:_____ Exam 1

e. Does the level of radioactivity accurately reflect all the glucose taken up by a cell (neglecting efflux out of the cell)? Explain.

3. The authors do no consider the actual activity of phosphofructokinase-1 after Dengue virus infection.
   a. Based on the following panels of results, do expect phosphofructokinase-1 to be more active or less active after Dengue virus infection? "Mock" is the negative infection control and DENV is for infected cells.



   b. Do you expect [fructose-2,6-bisphosphate] to be increaseing or decreasing after Dengue virus infection?

   c. Do you expect phosphofructokinase-2 to be more active or less active after Dengue virus infection?

   d. Do you expect fructose-2,6-bisphosphatase to be more active or less active after Dengue virus infection?

   e. If the regulation of phosphofructokinase-2 followed that of the liver (hepatocytes), would you expect the level of phosphorylation of phosphofructokinase-2 to be increasing or decreasing after Dengue virus infection?