Problem Set 1

1. Using the KEGG PATHWAY website:

   a. Sugar Metabolism:
      i. What metabolite can either continue through glycolysis or enter the Pentose phosphate pathway? **Glucose-6-phosphate**
      ii. The conversion of Glucose-6-phosphate (G6P) to Gluconate-6-phosphate happens in two steps.
         1. Draw and name the intermediate. **D-Glucono-1,5-lactone 6-phosphate**

        ![Diagram of Glucono-1,5-lactone 6-phosphate]

         2. If cofactors are required in this process, draw the cofactor and explain its role.
            NADP+ → the first step in the reaction is the oxidation of Glucose-6-Phosphate. The cofactor is the hydride acceptor.

        ![Diagram of NADP+]

   3. In humans, there are two possible paths from G6P to Gluconate-6-phosphate. Describe the difference in these two related paths. One path requires 2 enzymes while the other is facilitated by a single enzyme.

   4. Consider the one step pathway. What is the name of the enzyme and the Gene ID in humans (HSA)? **Hexose-6-phosphate dehydrogenase (H6PD)**. Gene ID 9563

   5. Is this enzyme important in any other pathways? **Yes – Glutathione metabolism, Biosynthesis of secondary metabolites, Microbial metabolism in diverse environments, carbon metabolism.**

   6. What disease is linked to this enzyme in humans? **Cortisone Reductase deficiency**
      a. A direct role in this disease seems unlikely, but the link is made in KEGG. Please describe how this enzyme is related to this disease. The Disease page notes that mutations in H6PD is important because it play a role in supplying important cofactor for cortisone reductase.
      b. If you navigate to the Steroid hormone biosynthesis page directly from the Disease page, the relevant enzyme is highlighted in red. What is the name of this enzyme and what cofactor does it rely on? How might this be related to the oxidation of G6P? The enzyme is hydroxysteroid (11-beta) dehydrogenase. The relevant cofactor is not stated on the KEGG page, but a quick Google search of the enzyme name shows that it is an NADPH-dependent enzyme (this should make sense since it’s an oxidoreductase). NADPH is a major product of the pentose phosphate pathway.
b. In Nitrogen Metabolism:
   i. What is the name of the enzyme that facilitates nitrogen fixation? Nitrogenase
   ii. Click on the Taxonomy button. What are three species that can fix Nitrogen? (red highlights = genomes with this gene). There are a bunch. A few examples are Pectobacterium, Enterobacter, Klebsiella, Dickeya, etc.
   iii. What is the net chemical reaction?
   \[
   \text{N}_2 + 16 \text{ATP} + 8 \text{Ferridoxin}_{\text{reduced}} + 8 \text{H}^+ + 16 \text{H}_2\text{O} \rightleftharpoons 2 \text{NH}_3 + 16 \text{H}_3\text{PO}_4 + \text{H}_2 + 8 \text{Ferridoxin}_{\text{oxidized}}
   \]
   iv. This enzyme is composed of two independent proteins. Name these proteins and the cofactors are involved with each? Dinitrogen Reductase (requires a 4Fe-4S Cluster) and Dinitrogenase (Molybdenum and Iron)

2. What cofactors are important for energy storage? Draw each and explain why they are important.

![ATP and NAD(P)H/NAD(P)⁺]

Flavin containing cofactors (FADH₂ and FMN)

3. Most transmembrane regions of transporters are composed of α-helices. Describe how multiple α-helices can combine to form a pore for aqueous molecules. Be specific. Need amphipathic helices – one face needs to be non-polar to interact with the interior of the bilayer and the polar face needs to create a pore. Your book discusses this in Figure 11-30.
4. Chemical potential (ΔG):

   a. If the sodium ion concentration inside the cell is 50 mM and outside it is 560 mM, determine the chemical potential difference across the membrane if the membrane potential is 100 mV (inside negative). Inside is negative, so the electronic potential should be favorably additive with concentration gradient

   \[
   \Delta G = 2RT \ln \left( \frac{560}{50} \right) + 1(96485)(0.1) \]

   \[
   \Delta G = 15,978 \text{ KJ/mol}
   \]

   b. Calculate the maximum ratio \([\text{glucose}]_{\text{in}}/\[\text{glucose}]_{\text{out}}\) that can be transported with this potential. Make sure to consider the Na/glucose stoichiometry for this symporter.

   \[
   \Delta G = 2RT \ln \left( \frac{[\text{glucose}]_{\text{in}}}{[\text{glucose}]_{\text{out}}} \right)
   \]

   \[
   \frac{3176 \text{ KJ/mol}}{8.314 \text{ J/mol} \cdot \text{K}} = \ln x
   \]

   \[
   x = \text{concentration ratio} = \frac{224495}{1000} \mu \text{M} = 1
   \]

5. Clearly describe the affect that a sodium ionophore would have on the system described in 4. A sodium ionophore would bind to the ion and passively diffuse it across the membrane until the potential across the membrane is nullified.

6. You isolate a new strain of bacteria that has evolved to rely heavily on leucine and ethylene glycol for energy. Of course, these molecules need to get inside the cell to be useful. One of these molecules enter in a mediated fashion and the other through passive diffusion.

   a. Based on the experimental data below, determine which is which a passive diffuser and which is mediated.

   b. For the passive diffuser, determine the permeability coefficient. Assume that \([A]_{\text{in}}\) is equal for all trials.

   c. For the mediated diffuser, determine \(K_t\) and \(J_{\text{max}}\).

<table>
<thead>
<tr>
<th>Leucine (mediated – subject to saturation)</th>
<th>Ethylene glycol (non-mediated – no saturation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (µM)</td>
<td>Initial Uptake Rate (µM s⁻¹)</td>
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<tr>
<td>---------------------</td>
<td>-----------------------------</td>
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<tr>
<td>1</td>
<td>110</td>
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<td>2600</td>
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<tr>
<td>500</td>
<td>3100</td>
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You could use a Lineweaver-Burk analysis to determine $K_t$ and $J_{\text{max}}$, but using the solver function of Excel is much more reliable.

7. Familiarize yourself with the attached journal article (Wakisaka, M, et. al. Biochim. Biophys. Acta 1362, 87-96.)
   a. How did the authors monitor glucose uptake? They used heavy isotope labeled glucose
   b. Carefully inspect Figure 2. Based on this data, what type of glucose transporter (GLUT or sodium symporter) is present in each cell type? Retinal pericytes (top image) have a sodium dependent glucose uptake mechanism. Retinal endothelial cells do not.
   c. Phlorizin is an inhibitor of sodium-coupled glucose transporters. Look up the structure of this molecule and propose a reason for this observation. The molecule contains a glucoside functional group that competes with D-glucose for binding at the sodium dependent transporter.
   d. Which figure confirms that phlorizin effectively inhibits sodium-dependent glucose transport in retinal pericytes? Figure 3 – addition of phlorizin significantly dampens the uptake of the heavy isotope labeled glucose.
   e. Why was choline chloride chosen as a control? Choline chloride is a good control be
   f. Figure 7a shows us that phlorizin is not able to completely prevent glucose uptake. Propose a reason for this observation. One of two possibilities is possible: phlorizin is not 100% efficient at these concentrations – some glucose can still sneak through the transporter. The other possibility is that retinal pericytes have a sodium independent glucose transporter (this is very likely).