Problem Set 8 (**Due October 30th**)

1. What factors influence flux for non-mediated diffusion.
   - Energy of Dehydration ($\Delta G_{dehydration}$) → All solutes must be dehydrated to enter the lipid bilayer - this is the major contributor to the activation energy
   - Permeability Coefficient → This tracks very well with the oil/water partition coefficient. Basically, molecules that are not soluble in oil cannot pass across the membrane through non-mediated diffusion.
   - Concentration gradient → If the solute concentration is at equilibrium, there is not driving force for transport therefore flux will be zero.

2. Describe how aquaporin is selective for H_2O. the protein adopts an hourglass configuration. The bottleneck pore diameter is pretty much exactly the VDW diameter of H_2O so bigger things cannot get through. Cations are excluded because of the electrostatic repulsion from an Arg just above the bottleneck. H^+ are excluded because H_2O does not H-bond with itself within the pore; instead, all H-bonds are tied up in with side chains. Further, there is a dipole reorientation that must occur for transport to happen; this ensures that H_2O has the appropriate H-bonds.

3. Sodium has a much smaller ionic radius than potassium; this suggests that it should be able to fit through the pore formed by potassium channels. What are two reasons why sodium is not transported by these proteins? The dehydration energy for Na^+ is much greater than K^+, so there is a much bigger activation energy. Also, there selectivity filter (four δ- oxygen atoms) is situated perfectly so that ideal contacts are made with K^+; Na^+ is too small and won't be able to strongly interact with the filter.

4. What role do the P, N, and A domains of P-type ATPases play in ion transport. ATP binds at the interface of the N and P domains; the N domain interacts with the sugar and base while the gamma phosphate is oriented into the P domain. When hydrolysis occurs, the gamma phosphate is transferred to an Asp in the P domain. Formation of this unstable phosphate adduct is what causes the Actuator domain to reorient and open the pore for the metal ion to pass through the membrane.

5. Chemical potential (ΔG):
   a. If the sodium ion concentration inside the cell is 50mM and outside it is 560 mM, determine the minimum membrane potential that would be needed to drive sodium transport out of the cell. Remember that body temperature is 37 °C.

$$\Delta G = 0 = RT ln \frac{[Na]_{out}}{[Na]_{in}} + ZF\Delta E \qquad -1(96485)\Delta E = 8.314(310.15) ln \frac{560}{50} \qquad \Delta E = -64.6\ \text{mV}$$

   b. Imagine an antiport system uses a pH gradient across the membrane to transport sodium against its chemical gradient. If the $[Na^+]_{out}$ = 100 mM and $[Na^+]_{in}$ = 300 mM, calculate the pH gradient that would be necessary to overcome the unfavorable transport of Na^+ AND provide an additional 1 kJ mol^{-1} of energy.

$$\Delta G = RT ln \frac{[Na]_{out}}{[Na]_{in}} = 8.314(310.15) ln \frac{300}{100} = 2832.9\ J\ mol^{-1}$$

So 2832.9 J mol^{-1} is necessary for DG to drive transport. For a net -1000 kJ mol-1, we need 3832.9 J mol^{-1}

$$-3832.9 = 8.314(310.15) ln \frac{[H]_{in}}{[H]_{out}} \qquad \frac{[H]_{in}}{[H]_{out}} = 0.226 \qquad [H]_{in} = 0.226\,[H]_{out}$$

$$-\log[H]_{in} = -\log 0.226 + -\log[H]_{out} \qquad pH_{in} = pH_{out} + 0.646$$

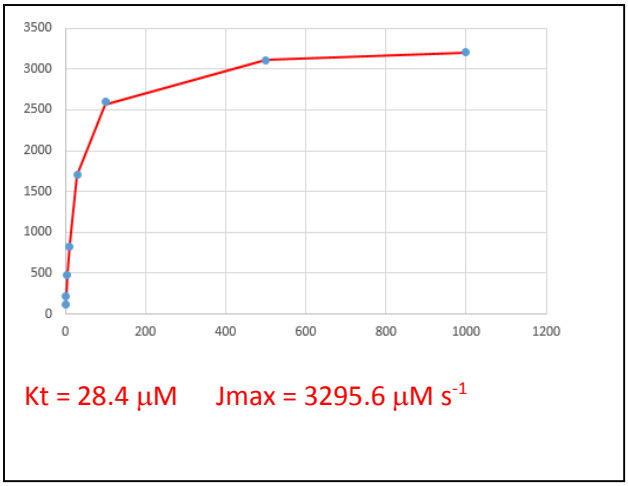
c. What other transport mechanism might be able to pump sodium against its chemical gradient?It can be coupled to an ATPase

6. Regarding nerve impulses
   a. How do ligand gated and voltage gated ion channels work together to facilitate nerve impulses? In your answer, think critically about what depolarization and hyperpolarization mean and why each occur. In neurons, there are two types of gated ion channels; ligand and voltage. The ligand gated sodium channel responds to an extracellular signal and initiates a nerve impulse. The voltage gated ion channels then respond to the change in membrane potential due to the influx of sodium; the voltage gated sodium channels bring more sodium into the nerve while the voltage gated potassium channels move potassium out of the cell. The rapid increase in intracellular sodium is what depolarizes the cell (the membrane potential become much less negative, in fact, it becomes quite positive) while the export of potassium, a much slower process, causes the hyperpolarization (the inside of the cell becomes much more negative and makes the membrane potential more negative).
   b. Clearly describe what affect valinomycin, a K^+ ionophore, would have on nerve impulses. Ionophores facilitate transport of ions across a membrane down the concentration gradient in a transport-protein independent manner. The K^+ gradient would be destroyed if valinomycin came into contact with neurons and, as such, the net driving force for ion transport would be 0.

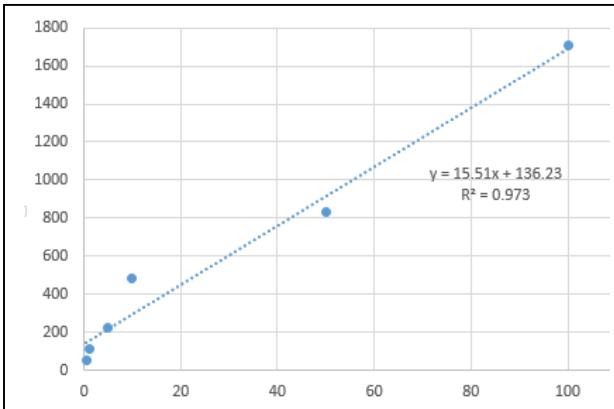
7. You isolate a new strain of bacteria that has evolved to rely heavily on leucine and ethylene glycol for energy. Of course, these molecules need to get inside the cell to be useful. One of these molecules enter in a mediated fashion and the other through passive diffusion.
   a. Based on the experimental data below, determine which is which.
   b. For the passive diffuser, determine the permeability coefficient. Assume that $[A]_{in}$ is equal for all trials.
   c. For the mediated diffuser, determine K_t and J_{max}.

Leucine - Mediated	
Concentration (μM)	Initial Uptake Rate (μM s^{-1})
1	110
2	220
5	480
10	830
30	1700
100	2600
500	3100
1000	3200

Ethylene glycol – Non-mediated	
Concentration (mM)	Initial Uptake Rate (mM s^{-1})
0.5	50
1	110
5	220
10	480
50	830
100	1709.8



Kt = 28.4 μM Jmax = 3295.6 μM s^{-1}



Slope = permeability coeff = 15.51 s^{-1}

8. GPCR:
    a. Clearly describe how GPCRs work. In your description, make sure to comment on the different types of alpha subunits (i.e. G_s, G_i, G_q) and any structural changes that are important for function. An extracellular signal is received by the GPCR. This triggers the movement of an intracellular helix that displaces the heterotrimeric G Protein, which is GDP bound at this time. GTP comes in and replaces the GDP triggering the dissociation of the α subunit (the actual G protein) from the β/γ. GTP-bound Gα interacts with Adenylate Cyclase. If this is a Gα,s, then the AC is stimulated and cAMP is produces. If this is a Gα,i, then cAMP production is inhibited. GTP hydrolysis (a slow process) results in three 'switch' regions to flip out and triggers Gα to dissociate from the AC. In the case of the Gα,q, this protein interacts with and activates phospholipase C (PLC). PLC hydrolyses PIP_3 to produce IP_3.

    b. Discuss the parallel role of PLC and AC in GPCR nucleated signal transduction cascades. PLC and AC are both activated in response to interacting with Gα and both produce secondary messengers (cAMP and IP3, respectively). cAMP activated PKA and IP_3 ends up activating PKC; these are both kinases that 'mass phosphorylate' enzymes in a regulatory manner.

9. Calcium as a secondary messenger:
    a. Calcium is released by an IP3 gated Calcium Channel in the ER. Clearly explain what this means and why it is relevant. IP3 is only present when PLC is activated. IP3 binds to the IP3-gated ion channel and releases Calcium. Calcium serves as a secondary messenger that activates other kinases, including PKC.

10. How is the insulin signal propagated to the nucleus? Please clearly articulate how the insulin receptor works and how the signal is passed on to IRS-1. I don't expect you to know the names of other proteins involved, but be familiar with how the signal is propagated. Insulin binding triggers autophosphorylation of the PTK (protein tyrosine kinase) domain. Three Tyr get phosphorylated which causes a loop to flip out of the substrate binding groove and allows IRS-1 to bind. IRS-1 get phosphorylated which, in turn, activates the G Protein RAS (through Grb2-SOS). RAS then begins the kinase cascade which terminates with a phosphorylation-dependent transcription factor.