Representative Membrane Transport HW Problems

1. Oocytes are particularly difficult to cryopreserve because they have a low membrane water permeability, which slows removal of cellular water and leads to intracellular freezing. We would like to increase the water permeability by adding aquaporins to the oocyte membrane (e.g., by microinjecting aquaporin mRNA). Water transport in unmodified oocytes (no aquaporins) at 300 K can be described using the following equation:

   $$J_w A = \text{moles water/time} = P_w A(C_s^i - C_s^e)$$

   where $P_w A$ = 2x10^{-13} m^3/s, C_s^i is the intracellular solute concentration and C_s^e is the extracellular solute concentration. The molar flux (moles area^{-1} time^{-1}) of water through a single cylindrical aquaporin channel can be described by:

   $$J_w = \left(\frac{r^2 RT}{8\eta v_w \delta} + \frac{D_w}{\delta}\right)(C_s^i - C_s^e)$$

   where r is the pore radius (0.5 nm), R is the ideal gas constant (8.314 J mol^{-1} K^{-1}), η is the viscosity of water (10^{-5} Pa s), v_w is the molar volume of water (1.8x10^{-5} m^3/mole), δ is the thickness of the cell membrane (4 nm), and D_w is the diffusivity of water (10^{-9} m^2/s).

   a. Compare water transport via the solubility-diffusion mechanism and via aquaporins. Include sketches of the driving forces (concentration, pressure, chemical potential) for water transport. You may assume that solute molecules are excluded from the aquaporin channels.

   b. Calculate the rate of water transport (moles water/second) for modified oocytes that contain 1000 aquaporins/cell, relative to unmodified oocytes. Assume that the area of the phospholipid membrane is unchanged in the modified oocytes.

   c. Sketch a plot of the relative water transport rate against the number of aquaporin channels/cell.

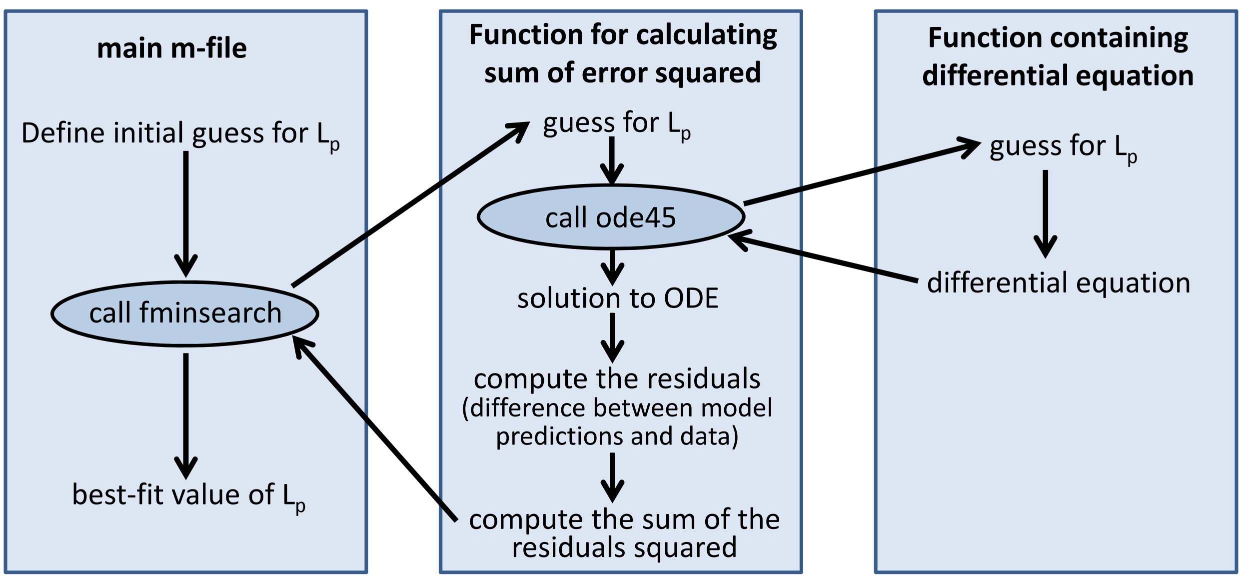
2. Use the osmotic transport virtual laboratory software to answer the following questions. To launch the software, save the files OsmoticTransportGUI_3.p and OsmoticTransportGUI_3.fig to your computer, open MATLAB and set the current folder to the folder containing the files, and then type OsmoticTransportGUI_3 into the MATLAB prompt. The virtual laboratory software allows you to do experiments on a cell type with unknown osmotic properties.

   a. Expose the cell to the following solutions at 37°C and create a plot of the results. Explain the trends.
      i. Ethylene glycol at a concentration of 300% of isotonic
      ii. Sucrose at a concentration of 300% of isotonic
      iii. Saline at a concentration of 300% of isotonic

b. Expose the cell to isotonic saline solution at 37°C in at least 3 replicate experiments. What is the isotonic cell volume? Report your answer as the mean ± standard error of the mean.

c. Expose the cell to saline solutions ranging from 50% of isotonic to 300% of isotonic at 37°C and determine the steady-state cell volume. Plot the steady state cell volume against the relative saline concentration $C_{s,0}/C_s$, where $C_{s,0}$ is the isotonic concentration. This is known as a Boyle-van't Hoff plot. Fit a line to the data and use it to estimate the osmotically inactive volume V_b.

d. Expose the cell to saline solution with a concentration of 300% of isotonic at temperatures of 0°C, 22°C and 37°C. Fit the resulting data with a membrane water transport model to determine the best-fit hydraulic permeability L_p at each temperature. You can use the MATLAB code provided on Blackboard. It uses 2 built-in MATLAB functions, fminsearch and ode45, as illustrated in the figure below. The function fminsearch automatically iterates the value of L_p to minimize the sum of the error squared between the model predictions and the "experimental" data.



e. The temperature dependence of the water permeability can be described using an Arrhenius model:

$$L_p = L_{p,\infty} \exp\left(-\frac{E_a}{RT}\right)$$

where $L_{p,\infty}$ is the water permeability at infinite temperature, E_a is the activation energy for water transport, R is the ideal gas constant and T is the temperature in Kelvin. Use your data from part c above to estimate the activation energy for water transport.