Laboratory 4: Role of Membranes in Controlling Entry and Exit of Molecules to the Cell

General Purpose: The ability of cells to perform the complex functions of living organisms requires the capacity for **homeostasis**, or regulation of the internal environment. In particular, the cell must regulate its internal chemistry in the face of a changing external environment. One way to achieve this is to regulate the movement of different molecules into or out of the cell. Using a few simple experiments, we will observe this phenomenon and enhance our understanding of the concepts of osmosis and diffusion.

Background: Transport of molecules can occur in several ways, the most common way being diffusion. **Diffusion** is the movement of molecules from a high concentration to a low concentration. This process continues until the concentration of molecules becomes evenly spaced, or an equilibrium is reached. The rate of diffusion is dependent on the difference between the concentrations of the molecules in one place compared to another. If the two concentrations are very different, the highly concentrated molecules will rapidly move towards the area of low concentration. If the difference is slight, diffusion will occur more slowly. Diffusion may not necessarily involve the presence of a membrane. It can occur in air, like smelling perfume from across a room, or in liquid, like food coloring in a bowl of water. We will observe diffusion of potassium permanganate crystals in distilled water in this lab.

2diff
↑ cons.

A special type of diffusion is called **osmosis**, which is the diffusion of molecules across a selectively permeable membrane, like the plasma membrane. This membrane allows some molecules to move freely across, while others pass slowly or not all. In general, small molecules will move freely across a membrane, while large or highly charged molecules will not. In this lab we will observe osmosis by evaluating the ability of iodine (a small molecule) to cross a selectively permeable membrane and bind with starch (a large polymer of sugars which does not cross the membrane). We will also look at the movement of water across the selectively permeable membrane of a chicken egg by measuring the weight lost or gained by eggs placed in different sucrose solutions.

Procedure:

- **Wear gloves throughout the experiments—potassium permanganate and iodine can stain hands and clothes, the eggs have been soaking in acetic acid that can burn.**
- **Do not throw the eggs directly in the trash, but place in the plastic bag indicated by the TAs**

X	Y
tim	diameter
0 min	4 mm
2min	2.9cm
4min	4.2cm
6min	5.9cm

time	diameter
8min	6.4 cm
10 min	7.0 cm

Diffusion of Potassium Permanganate ($KMnO_4$)

$KMnO_4$ is a purple crystal compound which is soluble in water, acetone and methanol. It is a powerful oxidizing reagent that can be used as a disinfectant.

1. Obtain a small petri dish. Use a marker to draw a small circle on the bottom center of the dish to guide the placement of the crystals.
2. Place the dish on a level surface, like a countertop. Your bench may not be level due to the bench paper.
3. Cover the bottom of the dish with distilled water and let it sit until the surface is even.
4. Using a small metal spatula, carefully place a very small amount of potassium permanganate inside the circle you marked. Immediately use a mm ruler to measure the diameter of the dye circle.
5. Continue to measure the diameter of the expanding dye circle every 2 minutes, or until the dye has reached the edge of the dish. If your circle is expanding in only one direction, that's still okay—just measure the diameter the best you can.
6. Graph the movement of the dye over time (diameter vs. minutes). Your graph should have a title and each axis should be labeled with its unit of measurement. You should also use a ruler to draw a best fit line and determine the rate of diffusion (slope of your graph). You may use a computer program to make your graph if you wish.

rate = Slope

$$\text{Slope} = \frac{Y_2 - Y_1}{X_2 - X_1} \left(\frac{\text{rise}}{\text{run}}\right)$$

Sucrose

Travel of Iodine through an artificial semi-permeable membrane

Color → cloudy Clear (white)

1. Obtain a piece of dialysis tubing from the beaker at the front bench. It should be closed at one end with a clip.

Color turns blue

2. Open the top end of the dialysis tubing by rubbing it between your fingers. You may need to use a Pasteur pipette to help you keep it open.
3. Use a 10 ml pipette to add 10 ml of amylose to the dialysis tubing.
4. Fold over the top of the tubing and close it with another clip. Note the color of the amylose solution in the tubing.

5. Add your filled tubing to the beaker of iodine solution on your stir plate. Note the color of the solution in the beaker.

→ dark red

6. At the end of lab today, observe the solution in the beaker and the tubing again. **What happened?**

mosis in De-shelled Chicken Eggs

1. Each group should have 3 de-shelled chicken eggs. Use a marker to carefully label each egg with a group identifier and the number 1, 2, or 3.
2. Place a weigh boat onto the balance. Press TARE to zero the balance, and carefully transfer one of your eggs to the weigh boat. Record the egg's weight, and repeat for the other 2 eggs. It is very important that you keep track of which egg is being placed in which solution.

3. Place one pre-weighed egg into each of the solutions—0% sucrose (distilled water), 20% sucrose, and 60% sucrose.

4. About 30 minutes before the end of lab, carefully remove your eggs from the solutions. Gently but thoroughly dry each egg, and then weigh it as before.

5. Record the new weight of each egg, as well as the difference in weight. **Did it gain or lose weight? Why?**

Results

Eggs

egg	#1	#2	#3
weight	53.64g	61.73g	61.55g
final weight	53.97g	63.09	62.22g
Sucrose	0%	20%	60%
difference	0.27g	1.36g	0.67g