Lab Section: _____ **Name:** _____

Pre-lab Homework for Lab 5: Enzymes & Diffusion

After reading over the lab and the enzyme, diffusion and osmosis topics from your textbook, answer these questions to be turned in at the beginning of the lab!

1. In this lab we will make up mock cells using a material called dialysis tubing. Dialysis tubing is semi-permeable, allowing small molecules through while preventing large molecules to pass. Look over the molecules we will be testing and their molecular masses in the **Assay Table** on page 6 of this lab manual.

   A. Predict what molecule(s) you think cannot cross the dialysis tubing. (don't use potassium because we do not have a way to test for its ability to move)

   B. Why do you think this molecule(s) cannot cross? (your hypothesis)

2. For the above setups, we will be trying to determine which way water is moving (either into or out of the mock cell bags). What is the term used to describe the movement of water through a membrane from an area where the concentration of water is high to where the concentration of water is low?

3. When we discuss how your cells control chemical reactions, the following terms will be useful to know. Define them below. They can be found in this lab manual chapter, and in the energy/metabolism chapter of your textbook, or via the Google.

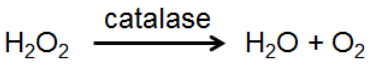
   - Catalyst

   - Enzyme: (and indicate which type of macromolecule)

   - Reaction Substrate:

   - Reaction Product:

4. Now label the following chemical reaction. (use circles and arrows to clarify where are the substrate/s, product/s and enzyme/s). see bottom of page 10 for help.



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5. What does the suffix "**-ase**" usually mean when you see at the end of a word in a biology class? (Hint: read the enzyme introduction to this lab!)

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Name: _____ **Date/Lab time:** _____

Lab 5: Enzymes & Diffusion

LAB SYNOPSIS:

We will cover two topics; osmosis/diffusion and enzymes.

- Osmosis/Diffusion; we will do an experiment to explore the relationship between concentration of a substance and its ability to move through a dialysis tube membrane.
- Enzymes; we will do two experiments studying how changing conditions effect the ability of an enzyme to catalyze a chemical reaction.
    - The effect of changes in enzyme concentration.
    - The effect of either changes in temperature or changes in pH.

Note: if these labs are being done on two separate days: do the enzyme lab first!

OBJECTIVES: After successfully completing this lab, a student will be able to:

- Understand and diagram how a concentration gradient drives diffusion.
- Understand and diagram how a water concentration gradient drives osmosis.
- Know what enzymes are and what enzymes do.
- Investigate the effect of enzyme concentration on the rate of reactions.
- Explain how changes in environmental factors can change enzyme-catalyzed reaction rates.

Introduction to Diffusion:

If you have ever been in a room with someone wearing too much perfume, or have ever put sugar in your tea or coffee, you are familiar with diffusion.

Diffusion- is the passive spreading out of a substance down its concentration gradient.

Concentration gradient- means that there is a difference in concentration of a substance in one area versus another area (fig. 1A).

Passive- means no added energy is used. This means that organisms and cells do not use their own energy for diffusion. So where does the energy of motion come from?

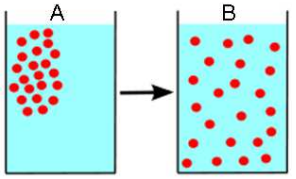


Figure 1. Diffusion to equilibrium

Energy of motion is due to intrinsic thermal energy of vibration.

The driving force for movement- is the concentration gradient (substance move from an area where their concentration is high to where their concentration is low). This movement continues until equilibrium is reached (fig. 1B). This means that the concentration will become equal throughout.

Diffusion can happen within a container or can occur into and out of cells, crossing the cell's plasma membrane.

How do substances diffuse into and out of cells? They have to move through the cell's plasma membrane.

Selectively permeable membranes- allow some substances to easily cross cell membranes, while other substances either cannot cross the membrane or they need assistance to cross the membrane.

Substances that can cross without assistance are small, non-polar and uncharged. These include; oxygen, carbon dioxide, nicotine, alcohol, sterol hormones, non-polar amino acids, etc.

Substances that cannot easily cross need assistance from membrane transport proteins. These substances are large, polar or charged. This includes **all ions** (H^+, Na^+, K^+, Cl^-, etc.), **sugars**, **the polar amino acids**, etc.

Two major components of cells membranes allow them to "select" what enters and what leaves the cell (a phospholipid bilayer, and membrane proteins)

- **Phospholipid bilayer**- is composed of two oppositely oriented layers, with hydrophilic heads to the outside and interaction hydrophobic tails towards the inside (fig. 2). The bilayer is a barrier to many hydrophilic substances (large molecules, polar molecules and charged ions). So how do those types of materials get into or out of cells? They must pass via membrane transport proteins.
- **Membrane transport proteins**- aid in the movement of substances through the membrane (fig. 2)

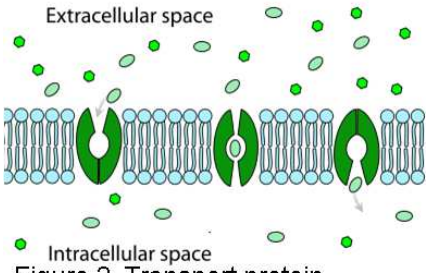


Figure 2. Transport protein

Osmosis- the diffusion of water through a membrane.

How does water get into and out of cells? Water has to cross the cells selectively permeable membrane.

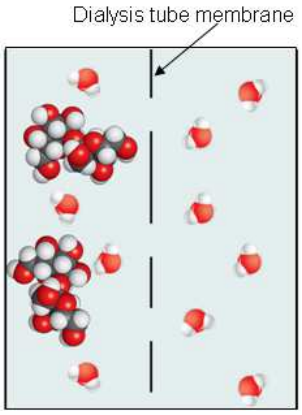
Water is a small polar molecule. Scientists are not sure how polar water is able to cross the cell's hydrophobic membrane. Some cells have aquaporins, membrane transport proteins for water. However, water can still move into and out of cells that lack aquaporins.

Osmosis is another case of diffusion. Water moves into and out of cells down its concentration gradient (from high → low water concentration). Osmosis is the special name given to water's diffusion into and out cells.

Exercise 1: Osmosis and Diffusion in a Mock Cell

OBSERVATION: water and other materials can move into and out of cells.

QUESTION: What determines the direction a substance moves across a dialysis tube membrane?



HYPOTHESIS: Molecules will move through a semi-permeable membrane from an area where their concentration is high to where their concentration is low.

We will test the ability of water, glucose, I_3^-, K^+ and starch to move through a selectively permeable membrane. To do this, we will make an artificial (mock) cell using dialysis tubing and put it into a beaker solution (see below for beaker and mock cell solutions). A true cell membrane is selectively permeable for small, non-polar molecules. Dialysis tubing is only selective for size, allowing smaller molecules to pass freely while larger molecules are blocked. Which direction will substances move? You will do an experiment to find out!

Experimental Procedure for the Osmosis/Diffusion Experiment:

I. Beaker set-up

1. Get one 400 mL beaker and set it up as follows:

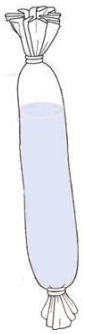
2. Add ~300 mL of de-ionized water

3. Add ~30 drops of Lugol's iodine to the water. (if water not noticeably yellow, add more drops)
Note: Lugol's Iodine (I_2KI) dissolves in water to form I_3^- + K^+ ions.

Thus final the beaker's solution contains ~0.03% of both I_3^- + K^+ ions. What is the concentration of water in the beaker? 0.03% + 0.03% = _____, subtract that from 100% to determine the concentration of water in the beaker. _____ Record this water concentration of beaker in Table 1

4. Record the color of the beaker solution in Table 2 on page 7 ("Color of beaker solution" "Initial color")

II. Bag set-up



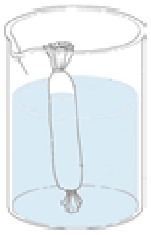
1. Make a bag from a section of pre-wet dialysis tubing:
   Open one end of the tube (like opening a grocery store bag).
   Run tap water through the tube to open it all the way through.
   Tie a knot near the bottom of the tube (tight, but not too tight).

2. Using the labeled pipettes, add the following solutions to the bag:
These solutions have already been made up, you just need to add them to the bag.
   Add 5 mL of 30% glucose into the bag.
   Add 2.5 mL of 1% starch into the bag. } Combined the final water concentration in the tube is 80%

3. Loosely fold the end of the bag and squeeze it gently to push out trapped air. Twist the open end of the bag and tie it as you did the other end. The bag should be limp after it is tied; there needs to be room inside in case water enters the "cell".

4. Squeeze your bag gently to check for leaks. Leaks will cause you to have odd results. Rinse the bag with tap water and gently blot the bag dry with paper towel.

5. Add the following information to Table 2 on page 7
   Initial color of the bag & beaker solutions.
   Initial weight of the bag to the nearest 0.1 g.



III. Put the dialysis bag into the beaker

Record the initial time here: _____. The bag must remain in the beaker for at least 45 minutes. (You can work on the other lab exercises during this time.)

IV. Obtain results

1. After ~45 minutes (or more) remove the bag and gently blot the outside with a Kimwipe or paper towel to remove any excess solution.

2. Weigh the bag to the nearest 0.1 g. Record the final weight of the bag and also the change in weight in Table 2. Indicate a gain in weight with a plus (+) and a loss in weight with a minus (-).

3. Record the final color of the beaker solution.

4. Record the final color of the bag solution.

5. Test for glucose in the beaker solution. Dip the test strip into the beaker solution for 2 seconds. Remove. Wait 3 minutes to read results. If the test pad changes from yellow to any shade of green, glucose is present.

V. Clean Up!

Pour beaker contents down the sink. Rinse and replace beaker in its drawer. Cut open bag and empty contents into the sink. Dialysis tube & glucose test strip go into trash.

Recall the question & hypothesis:

QUESTION: What determines the direction a substance moves across a dialysis tube membrane?

HYPOTHESIS: Molecules move through a semi-permeable membrane from an area where their concentration is high to where their concentration is low.

Assay Table: You will use the following assays to determine whether water, glucose, I_3^-, K^+ and/or starch are able to cross the dialysis bag membrane.

Substance &Their Molecular size		**Assay**
Water (H_2O)	18	Weight change
Glucose ($C_6H_{12}O_6$)	180	Glucose dip stick
Iodine (I_3^-)	378	Iodine stains starch black
Potassium (K^+)	39	We do not have a test for potassium
Starch $(C_6H_{12}O_5)_n$	100,000's	Iodine stains starch black

---

PREDICTIONS: Complete Table 1 below with the details of the bag and beaker contents at the start of the experiment. Then predict which substances will be able to move across the dialysis tubing membrane and which direction (into or out of the bag), after the 45 minutes.

Table 1. Initial contents of bags & beakers and your predictions. See I. Beaker set-up and II. Bag set-up. (hint: note which area has the higher concentration in the beaker and bag setup)

Substance	**Concentration in the bag**	**Concentration in the beaker**	**Hypothesis/Prediction:** Based on its concentration inside and outside the bag; Which direction should it move?
Water	~80%		
Glucose	~30%		
I_3^-		~0.03%	
K^+		~0.03%	
Starch	~1%		

Review the procedure set-up and determine the experimental variables.
Hint: see your predictions in Table 1

What is the **independent variable** in this experiment?

Hint: How do the substances/situations differ between the bag and the beaker?

_____

What is the **dependent variable** in this experiment? **What should change in response to the independent variables?**

What are some things you are keeping constant in the experiment? (**standardized variables**)

RESULTS (DATA COLLECTION):

Table 2: Changes over time

Test	Initial	Final
Color of beaker solution		
Color of bag solution		
Weight of bag		
Glucose in beaker (result of dipstick test)	Should be none!	

Record any additional observations made before, during or after the experiment (e.g. did the bag change shape/color/texture/etc.?)

CONCLUSIONS:

Based on your results record your conclusions as to whether or not the substance can move, and what directions it moved. Some things to think about as you make your conclusions:
What does it mean if the yellow iodine in the beaker turns black?
What does it mean if the cloudy starch inside the bag turns black?

Substance	Dialysis membrane crossing? Can / Can't / don't know	Direction? Into / Out of Bag
Water		
Glucose		
I_3^-		
K^+		
Starch		

Did you find any substance that **did not** move down its concentration gradient? If so, which? _____

Why did this substance not cross the dialysis tube membrane?

Can this substance cross a living cell membrane? You might ask your instructor for help with this one.

Questions:

1. Recall starch from the topic of macromolecules: how does the monosaccharide glucose relate to starch?

2. In this experiment, we expected the weight of the bag to change. Based on your results is this change due the osmosis of water? To the diffusion of glucose? Both? Explain your answer.

2. Review your original hypothesis. Did your results support or refute your hypothesis? Why? Why not? Remember, error analysis is an important part of the scientific method.

3. Experiments often lead to further questions. For example, what do you think would have happened if you had done this experiment for a longer time? Or if you had used sucrose instead of glucose? Recall sucrose is a disaccharide made up of two monosaccharides, a molecule of glucose covalently bound to a molecule of fructose. Note: the molecular size of glucose and fructose are both 180. Consult with your group: Record some questions that could be explored.

4. Recall living cells are not surrounded by dialysis tubes, they are surrounded by a selectively permeable membrane. What part of the membrane allows for living cells to be selective?

Introduction to Enzymes:

Catalyst- A substance that speeds up a chemical reaction. Are not used up in the process.
Enzyme- A biological catalyst. Enzymes speed up the rate of a chemical reaction in living systems.
Active site- The part of an enzyme where the chemical reaction occurs.

Without enzymes chemical reactions would be too slow to allow for life. Enzymes are very specific, speeding up (catalyzing) only a single type of reaction. Since enzymes are not used up they can catalyze the same reaction many many times. How do enzymes catalyze chemical reactions? Research shows that enzymes have a specially shaped region, called an **active site**. The active site binds to the chemicals taking part in a reaction (these chemicals are referred to as the **substrate(s)**). The binding forms what is called an "enzyme-substrate complex". This complex holds the substrate(s) and helps make the transition from one form to another (fig. 3).

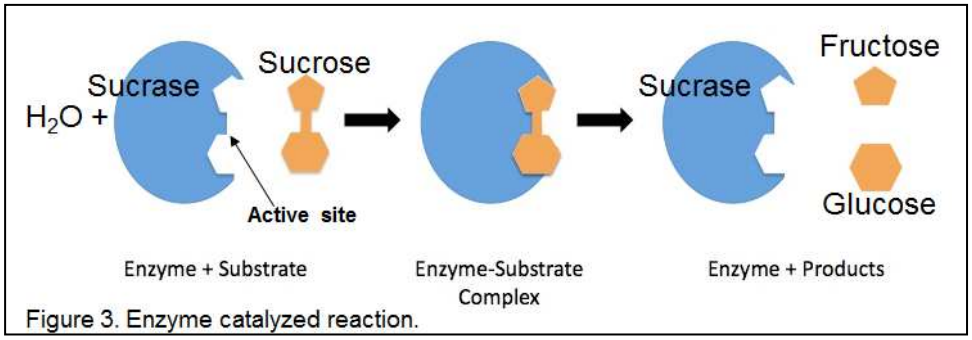


Figure 3. Enzyme catalyzed reaction.

Note that the enzyme (sucrase) is the same at the beginning and the ending of the reaction, but the substrate (sucrose) has now become the products (fructose and glucose). This is a **chemical reaction**! This reaction can occur only if the shape of the enzyme active site holds the substrate in just the right way as to allow for a chemical reaction. The shape of the enzyme is the key to its activity!

The above reaction is the hydrolysis of sucrose → fructose + glucose. And is catalyzed by the enzyme sucrase. This reaction occurs in your small intestine. The fructose and glucose diffuses through membrane transport proteins into your intestine cells, ultimately entering your blood.

Note: Almost all enzymes end in the suffix -**ase**, so if you see a word ending in **ase** you are probably looking at an enzyme.

So enzymes:

- Are proteins
- Catalyze chemical reactions (speed up reaction rates)
- Have specific structures that allow for their function
    - Have an active site- region on enzyme that binds to substrates
- Are not used up during the reaction
- End in the suffix –ase (for example; the enzyme catalase)

Catalase

Catalase- An antioxidant enzyme that catalyzes the reduction of the substrate (hydrogen peroxide) forming two products (water + oxygen). Bubbles of oxygen are formed via this reaction if you have ever used hydrogen peroxide on a cut.

Catalase is an enzyme found in many organisms, plants, fungi and even you. This enzyme speeds up the reaction of hydrogen peroxide (H_2O_2) into water and oxygen gas. You are familiar with this reaction if you have ever put hydrogen peroxide on a cut and seen the product, oxygen bubbles. Peroxisomes, an organelle in your cells, contain catalase to deal with toxic H_2O_2 (a common byproduct of metabolism).

The reaction is usually written as a chemical equation, like this:

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + O_2$$

Note: Since enzymes are not part of the substrates or products of reactions, they are usually written above or below the reaction arrow.

We will be testing how fast this reaction occurs when we change the concentration of the enzyme, then we will see how the reaction changes when we change the pH or the temperature.

Exercise 2A: The Effect of Enzyme Concentration on Reaction Rate

All groups do this experiment!

To set up our experiment, we will go through the first steps of the scientific method:

Observation → Question → Hypothesis → Test → Analyze Results

Since we are setting up much of this experiment for you, we will give you much of the information you will need for this process.

OBSERVATION: Many people have observed that changing conditions (ex. temperature, amount of enzyme, pH, etc.) can change reaction rates for enzyme catalyzed reactions (AKA change how fast they work)

QUESTION: How will the reaction rate change, if we change one of these conditions?

HYPOTHESIS: For this first experiment, each group will test the same hypothesis:
Changing the amount of the catalase enzyme will cause changes in the rate of a reaction.

In Exercise 3, you will get to choose another condition to test.

EXPERIMENT: The first experiment is to test for rate of the reaction at different amounts of the enzyme, catalase (i.e. its concentration). Remember, enzymes are proteins. Proteins are large complex, macromolecules that can only be made in living cells. We will not be using your blood cells, we will be using potato cells. The more potato cells, the more enzyme. For this experiment the important variables are:

- **Independent variable**: the amount of enzyme added. In this case, we will vary the amount of potato added and so change the amount of enzyme.
- **Dependent variable**: enzyme activity -we will measure this by looking at how fast the O_2 bubbles come off the potato.
- **Standardized variables**: What are some other things that may affect enzyme activity? These are variables you want to make sure are the same from one experiment to the next. After looking over the procedure, we will ask you about these again (see "Conclusions", below).

Procedure:

1. Collect 5 test tubes, a grease pencil, a test tube rack, a small ruler, and a 10ml graduated cylinder.

2. Cut the potato so that you have 4 cylinders ranging in size from 0.5 cm to 2 cm long (i.e. each should be ~0.5cm longer than the previous one). **Please be careful with the very sharp razors and scalpels.**

3. Label and set up 5 test-tubes as follows:

Tube#	Deionized water (ml)	Diluted Detergent	Size of potato cube	H_2O_2 WAIT!
1	3	1 drop	No potato!	see step 5
2	3	1 drop	Smallest potato cube	see step 5
3	3	1 drop	Next smallest potato cube	see step 5
4	3	1 drop	3rd smallest potato cube	see step 5
5	3	1 drop	Biggest potato cube	see step 5

HYPOTHESIS: based on what is in each tube, what do you think will happen after you add hydrogen peroxide? Record this prediction into table 3 below.

4. You will estimate the amount of enzyme activity by observing the amount of bubbling coming off the potatoes. Use the follow scale for your observations:

   - no bubbles
   + a few bubbles
   ++ moderate bubbles
   +++ lots of bubbles
   ++++ oh my gosh, the bubbles!

5. You are now ready to start your experiment. After waiting about 3 minutes, add 4ml of the substrate hydrogen peroxide to each of the test tubes, gently mix contents and observe the bubbles. Record your results into table 3.

RESULTS:

Table 3: Predictions and Results of Catalase Experiment

Tube #	Size of Potato Cube	Predictions	Observed Reaction
1	No potato!		
2	Smallest potato cube		
3	Next smallest potato cube		
4	3rd smallest potato cube		
5	Biggest potato cube		

CONCLUSIONS:

1. Do your results support your predictions? i.e. is the hypothesis supported or not supported? Why? Why not?

2. What does this tell you about the effect of enzyme concentration on enzyme activity? Explain.

3. What is the purpose of test tube 1? Explain your answer!

4. What are some additional standardized variables that could change your experimental outcomes?

5. What else could be done to improve this experiment? (trouble-shoot and error analysis)

Exercise 2B: The Effect of Changing Environment on Reaction Rate

In this exercise, we will investigate either the effects of pH or temperature on our enzyme-catalyzed reaction. Some groups will do the pH experiment, some groups will do the temperature experiment. Your instructor will help set this up.

OBSERVATIONS: Enzymes are proteins. The structure and function of proteins is maintained by their environment. Changing conditions (pH, temperature, solute concentration, etc.) can change the shape of enzymes and thus affect their function.

QUESTIONS:

- What is the effect of different pH on catalase enzyme activity?
- What is the effect of different temperature on catalase enzyme activity?

Your instructor will assign your group one of these questions to explore.

HYPOTHESIS: Develop a hypothesis as you think about an experiment to address your question.

1. For your experiment, define the following: *(if you are having one group member act as secretary to record information, inform your instructor and acknowledge who is recording info here _____)

Independent variable: (assigned by your instructor)

Dependent variable:

Standardized variables: remember each tube needs exactly the some conditions as the first experiment (volume, detergent, tube size, etc.). All your tubes need one single size of potato. Which of those sizes are you going to use?

2. Develop a hypothesis as to what you think will happen (your prediction)

and why this will happen (your hypothesis).

TEST:

Write out your procedure below. Recall this should be clear enough such that someone else could read, understand and repeat your experiment. Recall your experiment is designed to test your hypothesis to see if your prediction is valid. (write out each step!)

Step 1:

Step 2:

Step 3: (add more steps as needed…)

Once you have completed your procedure, have your instructor check it. Be sure that you can explain to your instructor what you are doing and why!

***INSTRUCTOR INITIALS**: _____

RESULTS:

1. Build a data table in the space below to help you collect your results.

2. Perform your experiment and record the results in your table.

3. Do your results match your prediction?

4. If they do, is your hypothesis supported? If they don't, is your hypothesis disproved? Explain. i.e. error analysis and trouble shoot.

How can your experiment be improved?

5. Talk to one of the groups that experimented on the other independent variable (pH or temperature). What was their hypothesis and what did their results show?

6. Briefly summarize what these experiments on pH and on temperature show about how enzymes work. Be sure to think about what happened and why it happened!