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Using Herbicides to Understand the Light-Dependent Reactions of Photosynthesis

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The electron transport chain (ETC) is a critical part of both photosynthesis and cellular respiration. However, it is frequently one of the topics least understood by our General Biology students. In this lab students first observe the production of oxygen in spinach leaves and the consumption of carbon dioxide by *Elodea* leaves during photosynthesis. Next, students perform a skit in which they act out the process of photosynthesis, focusing on the ETC and the light-dependent reactions. Finally, students design an experiment that will allow them to determine if a given herbicide blocks the ETC by stealing electrons or by blocking electron movement. This lab increases our students' basic understanding of photosynthesis and the role of the ETC in metabolism.

Keywords: electron transport chain, photosynthesis

Introduction

Metabolism is a significant component of an introductory biology course. We have observed that our students struggle with understanding how the electron transport chain operates. This two-week lab was designed to provide our students with hands-on experience with the electron transport chain and its role during the process of photosynthesis. During the first week they observe photosynthesis under normal conditions, then in the second week our students design an experiment that will allow them to determine if a given herbicide is stealing electrons from the electron transport chain or if it is blocking electron movement.

The first week of lab is focused on ensuring our students have a basic understanding of what is happening during photosynthesis. Prior to the first week students complete a prelab reading assignment and answer questions regarding the purpose and process of photosynthesis. In class, we first review the prelab material, ensuring that all students understand the basic purpose of the light-dependent and light-independent reactions of photosynthesis. We also discuss the reactants needed for each process and the products being created. Next, the students use spinach disks to observe oxygen production (spinach leaves will float) and then *Elodea* in a phenol red solution to observe the consumption of CO_2 (pH will become more basic) during photosynthesis (Wickliff and Chasson, 1964). We also use class time to practice proper microscope use while observing leaf structure and identifying the cells responsible for photosynthesis and gas exchange.

The second week of class is focused on ensuring our students understand the finer details of the light-dependent reactions. We have found that our students struggle in lecture with understanding how the electron transport chain operates. We begin week two by reviewing the previous week's experiments and results. Next, the students perform a skit acting out the light-dependent reactions. We have received very positive feedback from students indicating that after completing the skit they better understood how light energy is transferred first to electrons, then used to create a proton gradient, and then finally transferred to ATP and NADPH. While performing the skit our instructors (or students) take on the role of an herbicide by either stealing an electron from an electron carrier or "clogging" the ETC by preventing the electron from moving. The students observe what happens when the ETC stops working and correctly predict whether oxygen, ATP, NADPH, and sugars are produced when the ETC is inhibited.

After completing the skit, students design an experiment that will allow them to determine which of the two provided herbicides, DCMU or DCPIP, inhibits electron movement in the ETC and which steals electrons from the ETC. The two experiments performed during week one are essential to answering the proposed experiment question. The floating spinach disk assay allows students to look for oxygen production. Oxygen

will still be produced when the disks are placed in a solution containing the electron stealer, but will not when placed in a solution with the ETC clogger. The *Elodea* and phenol red experiment will allow students to confirm that the electron stealing herbicide is in fact inhibiting photosynthesis (and is therefore not simply non-functional or even a "fake"). This confirmation experiment is important because the positive control (no herbicide) and the electron-stealing herbicide will give the same results with the floating spinach disk assay because both result in the production of oxygen.

At the conclusion of this two-week lab our students submit a one-paragraph summary of their experiment, similar to an abstract for a scientific paper. This assignment forces them to articulate the role and operation of the ETC and allows the instructors to determine which students need additional help with the material.

Student Outline

Lab #: Photosynthesis

Learning Objectives

- To outline the role of the electron transport chain during photosynthesis
- To predict the effect of herbicides on the electron transport chain
- To use the scientific method to design an experiment
- To accurately summarize an experiment

Post-Lab Note: There will be a written assignment to be completed and turned in one week after completing the second week of this lab. The assignment will be "writing an abstract for a scientific paper." An abstract is a brief one-paragraph summary of your experiment. Detailed instructions on the format for this assignment will be given to you in lab.

Pre-Lab Reading (Week One)

In preparation for this week's lab you will need to read the following material and answer the questions embedded within the reading. This lab is a two-week lab. The first week you will be introduced to photosynthesis and methods we can use to observe photosynthesis. Your lab group will test the ability of spinach leaves to perform photosynthesis when exposed to light. You will also observe the effect of photosynthetic plants on the pH of an aqueous environment. After setting up the experiments, your lab group will discuss how to design your own experiment in order to investigate where two different herbicides act within the electron transport chain to disrupt photosynthesis.

Photosynthesis Overview

Photosynthesis is the process in which photoautotrophic organisms harvest energy from sunlight and use this energy to produce carbohydrates. For simplification, we will use glucose for the photosynthesis equation; however remember that plants also produce other sugars. These carbohydrates are used both for long-term energy storage as well as for building new plant tissue. The entire process takes place within the chloroplasts of plant leaves. The chloroplast is made up of two outer membranes and a series of stacked membranes called the thylakoid membranes (Figure 1).

Here is a url for a good image that can be printed for educational purposes:

http://www.nature.com/scitable/topicpage/plant-cells-chloroplasts-and-cell-walls-14053956

Figure 1. Image of a chloroplast and its structures.

Photosynthesis Equation

6 carbon dioxide + 12 water + light energy → glucose + 6 oxygen + 6 water

$$6CO_2 + 12H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$$

This entire process takes place in two steps. During the first step, called the light-dependent reactions, energy is harvested from light and stored in ATP and electrons. These high-energy electrons are carried by NADPH. During the second step, called the light-independent reactions (or the Calvin cycle), the energy in the ATP and electrons in the NADPH made in the light-dependent reactions are used to convert CO_2 into glucose.

Light-Dependent Reactions

The light-dependent reactions take place within the thylakoid membranes and are dependent on solar energy from white light (visible light). White light consists of multiple wavelengths of light: violet, blue, green, yellow, orange, and red. The chlorophyll pigments within the thylakoid membranes can only harvest energy from red and blue wavelengths of light.

Steps of the Light-Dependent Reactions

(1) When light enters a leaf and hits a chlorophyll pigment within photosystem II, an electron absorbs the energy.

(2) This high-energy electron is then handed off to the electron transport chain where the energy is harvested from the electron and used to pump protons across the thylakoid membrane.

(3) The electron that was removed from photosystem II is replaced by the splitting of water, resulting in the release of molecular oxygen.

(4) When the excited electron from photosystem II reaches the end of the electron transport chain it is now a low-energy electron and is passed to photosystem I where it is once again excited by solar energy.

(5) This high-energy electron is added to $NADP^+$ to create NADPH.

(6) The protons that were pumped across the membrane by the electron transport chain create a proton concentration gradient.

(7) The protons will then flow down their gradient through ATP synthase, allowing for the production of ATP. The ATP and NADPH created by the light-dependent reactions are used by the light-independent reactions.

Figure 2 represents the thylakoid membrane and the molecules found within the membrane. Label each portion of the figure with the numbers (above) representing each step of the light-dependent reactions.

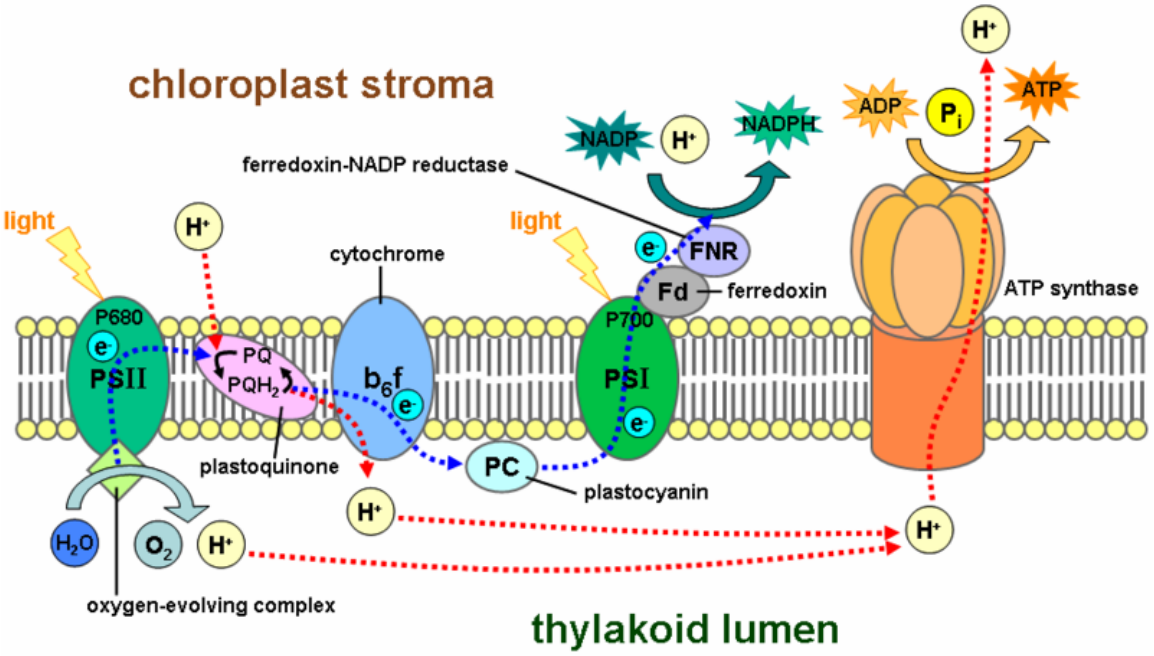

Figure 2. The light-dependent reactions of photosynthesis. Image source: https://commons.wikimedia.org/wiki/File:Thylakoid_membrane_3.svg

Light-Independent Reactions

The light-independent reactions take place within the stroma of the chloroplasts.

Steps of the Light-Independent Reactions

(1) During this process the Rubisco enzyme adds CO_2 to RuBP to create two three-carbon molecules (3PG).

(2) Energy from ATP and electrons from NADPH are used to produce three-carbon molecules (G3P) that can be used by the plant to produce glucose (and other carbohydrates). (Note: During this process ATP is converted back to ADP and NADPH is converted back to $NADP^+$. These products are then sent back to the light-dependent reactions and recycled to make new ATP and NADPH.)

(3) For every 2 G3P that are used to make glucose, 10 G3P are used to produce more RuBP in order to repeat the light-independent reactions.

Figure 3 represents the light-independent reactions within the stroma. Label each portion of the figure with the numbers (above) representing each step of the light-independent reactions.

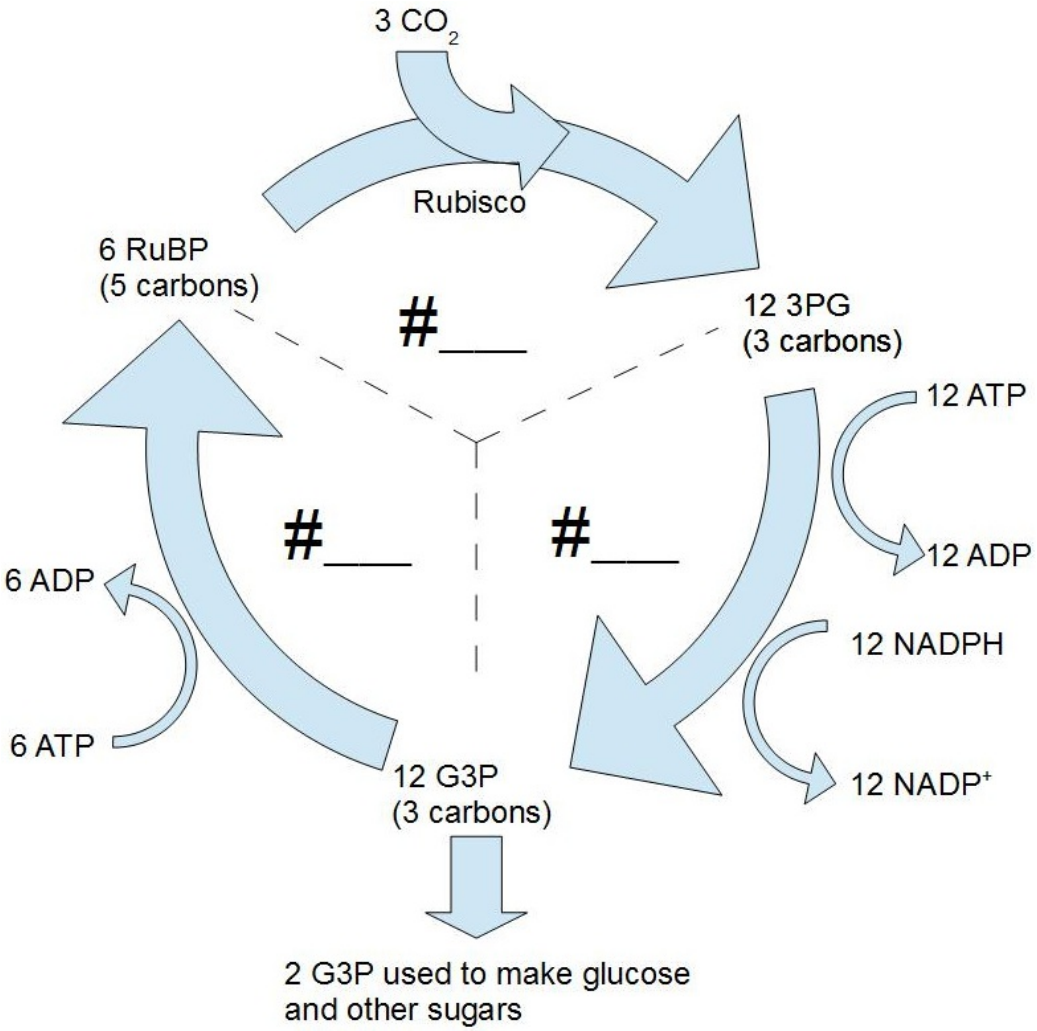

Figure 3. Light-independent reactions of photosynthesis. Image source: Dr. Nicholas Bongio

Pre-Lab Questions

Be sure you follow the instructions within your prelab reading above and label Figure 2 and Figure 3.

1. What is the purpose of photosynthesis?

2. What is the function of chlorophyll pigments during photosynthesis?

3. Where within a leaf does photosynthesis take place?

4. How are the light-independent reactions and light-dependent reactions dependent on each other?

5. What is the function of the electron transport chain during photosynthesis?

6. What gas is produced during the light-dependent reactions?

7. What gas is consumed during the light-independent reactions?

Lab – Week One

This week you will first practice proper microscope technique while viewing leaf cross-sections. You will identify mesophyll cells, where the majority of photosynthesis occurs, and the stomata and guard cells, which are responsible for allowing gas exchange in and out of the plant leaf. Next, you will practice two photosynthesis experimental methods. These experimental methods will help you design an experiment next week that will allow you to determine how the ETC is being affected by treatment with two different herbicides.

In the first experimental method you will visualize the production of oxygen gas (O_2) by photosynthesizing spinach leaves. For this study you will use the floating spinach disk model. Small disks of spinach, from which the air has been removed, will be sunk in two beakers of water, then one placed in the dark and one placed under a light. If photosynthesis occurs, oxygen will be produced, and the disks will float to the surface of the water. If photosynthesis does not occur, the disks will remain at the bottom of the beaker.

The second investigation will allow you to determine the effect of photosynthesis in an aquatic environment. For this study you will place the aquatic plant *Elodea* into a test tube containing diluted phenol red. Phenol red is a pH indicator that changes color based on the pH of the solution. Phenol red is orange when neutral, yellow when acidic, and pink when basic. CO_2 levels within water will affect the pH of the solution. If the CO_2 concentration decreases the solution will become more basic (decreased H^+ concentration). If the CO_2 concentration increases, the solution will become more acidic (increased H^+ concentration). By using phenol red, you will be able to observe any changes to the pH of the water as *Elodea* carries out photosynthesis.

What You Will Do in Lab This Week

- (class) Review the basic process of photosynthesis
- (individual) practice proper microscope usage
- (individual) observe a leaf cross-section and identify structures within the leaf
- (individual) practice making spinach disks and observe them under light
- (group) set up phenol red experiment, discuss expected results, return tomorrow to observe results

Photosynthesis Experimental Methods

Today you will practice two experimental methods that may be useful for next week's experimental design.

Method One

Below is a basic protocol for the spinach disk investigation. Your instructor will also demonstrate how to prepare the spinach disks. Once you read through the protocol, each student should practice creating spinach disks, then disks from each student will be collected into a single Petri dish. Next, divide equal numbers of disks into two beakers. Place one inside of a dark cabinet and leave the other one under a bright light and wait 10 minutes. While you are waiting, discuss what you expect to occur in each beaker and write your prediction below.

1. Prepare a solution of 0.2% $NaHCO_3$. This solution will act as a pH buffer environment to keep your leaf disks healthy throughout the experiment.

2. Use a hole puncher to cut out 10–20 disks (more if needed) from the provided spinach leaves. These should be cut over a 50mL beaker half filled with the buffer solution. Be sure to cut out more disks than you will ultimately need.

3. Transfer the disks and buffer into a 10mL syringe. Add buffer until the syringe is nearly full. Insert the plunger.

4. Point the syringe upwards and push out all of the air, being sure not to douse everyone in your vicinity.

5. Put your finger over the syringe tip and pull back on the plunger to create a vacuum within the syringe. Try pulling back 1–2cm to start with; be gentle, you don't want to damage the leaf disks. You should see bubbles forming around the edges of the leaf disks. Tap the syringe gently so that the majority of the leaf disks are surrounded by buffer.

6. While still under vacuum, remove your finger from the syringe tip. As the vacuum is released, the leaf disks will refill with buffer.

7. Tap the syringe several times to see if the leaf disks sink or float.

8. Repeat steps 4–7 until all or most of your disks sink. You may need to adjust the amount of vacuum you are creating if your disks don't sink after 3–4 trials. Your goal is to get the disks to the point where they JUST sink. Applying too much vacuum could result in damaging the disks.

9. Pour the disks into a Petri dish containing buffer.

10. Transfer the disks that sink into two 50mL beakers of buffer. Use the edge of a pair of forceps to slide under and grab the disks and sink them. Be careful not to crush them. Put an equal number of disks into each beaker.

12. Place one beaker under a light source and one beaker in a dark cabinet.

11. Wait 10 minutes and observe how many of your disks float during the experimental period.

Predicted results:

Observed results:

Method Two

Below is a basic protocol for an *Elodea* investigation that will allow you to indirectly observe the use of carbon dioxide during photosynthesis. Once you read through the protocol, work as a group to set up two test tubes. One will be placed in a dark cabinet and the other under a light. You will need to return tomorrow (as a group) to check your results. Be sure to discuss your predicted results in the space below prior to leaving.

1. Create a solution of phenol red by adding 5 drops of concentrated phenol red to 40mL of water (NOTE: Do NOT use buffer for this experiment). The phenol red may change color as a result of adding water, depending on how acidic your tap water is.

2. If your solution is not orange, use a straw to gently blow air into the solution until the solution reaches a neutral pH.

3. Transfer your solution evenly into two test tubes and label each tube either light or dark.

4. Place a cut 3cm piece of *Elodea* stem (including leaves) into each test tube.

5. Cover your test tubes with Parafilm to minimize reactions with the ambient air.

6. Place your test tubes in the appropriate treatment areas.

7. You will need to return tomorrow to record your results.

Predicted results:

Observed results:

Post-Lab Questions

1. Why do sunken spinach leaves float when placed under white light? (Do not merely say, "Because they undergo photosynthesis.")

2. Did the pH of the water surrounding your *Elodea* increase or decrease? Explain why the pH changed. A complete answer will discuss both the *Elodea* kept in the dark and the *Elodea* kept in the light.

Pre-Lab (Week Two) – Questions

This week you will first perform a skit designed to demonstrate the role of the electron transport chain (ETC) during photosynthesis. Each student will act out the role of one component required for the light-dependent reactions. During the skit you will discuss and observe how the ETC would be affected by an herbicide that steals an electron and an herbicide that prevents electron movement within the ETC.

After completing the skit you will design an experiment that will allow your lab group to determine how the provided herbicides disrupt the electron transport chain. The two herbicides are called DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) and DCPIP (dichlorophenolindophenol). Both prevent proper function of the electron transport chain (ETC) and thus disrupt photosynthesis. One acts by stealing electrons from the ETC and the other acts by binding to one of the components of the ETC and preventing electrons from being moved through the ETC (i.e. clogs up the ETC).

Review the ETC function from week one and in your textbook. 1. Below is a list of components of the ETC. Write one sentence, in your own words, describing the role of each component during photosynthesis.

Light

Photosystem II (PSII)

H_2O

Plastoquinone (PQH_2)

Cytochrome

Plastocyanin (PC)

Photosystem I (PS1)

Ferredoxin

ATP synthase

Ferredoxin reductase (FNR)

NADPH

Lab (Week Two)

What You Will Do In Lab This Week

- (class) Act out a skit demonstrating how the light-dependent reactions function during photosynthesis.
- (class and groups) Discuss how photosynthesis would be affected if the ETC were inhibited.
- (group) design an experiment to determine how two different herbicides inhibit the ETC during photosynthesis

Skit Instructions

Each student will be assigned a role to perform during the light-dependent reactions of photosynthesis. Prior to beginning the skit, use your prelab assignment to discuss the role of each of the items below during the light-dependent reactions of photosynthesis. You will then act out the conversion of light energy into chemical energy (stored within ATP and NADPH molecules). Your instructor will provide guidance the first time you perform the skit.

The student roles for the skit are:

Light	Photosystem II (PSII)	H_2O
Plastoquinone (PQH_2)	Cytochrome	Plastocyanin (PC)
Photosystem I (PS1)	Ferredoxin	ATP synthase
Ferredoxin reductase (FNR)	$NADP^+$/NADPH	

Lab Experimental Design

Today you need to design and execute an experiment to determine whether DCPIP and DCMU stop photosynthesis by stealing electrons from the ETC or by clogging up the ETC. You also need to think about how you can confirm that your herbicide really is inhibiting photosynthesis if O_2 is still being produced.

Complete the following information PRIOR to setting up your experiment. You may want to write your answers in pencil in case you decide to restructure parts of your experiment after discussing your ideas with your instructor. You will need to incorporate your knowledge from lab 6 week 1 in order to design your experiment. Think about which experiment would allow you to test for oxygen production and which experiment would allow you to test for carbon dioxide consumption.

During this lab you will be provided stock solutions of 0.2mM DCPIP and 2.0mM DCMU. You will need to make a 0.04mM DCPIP solution and a 0.3mM DCMU solution.

1. Outline your basic experimental design. Which protocol(s) do you plan to use and why?

2. What is your hypothesis?

3. What are your independent variables?

4. What are your dependent variables?

5. What are your standardized variables?

6. What are your experimental groups?

7. What are your control groups?

8. What is your sample size?

9. How much DCPIP solution will you make? How much DCMU solution will you make? Show your calculations for making each in the space below.

10. Will you make solutions for the spinach disk assays with buffer or water? Why?

11. Will you make the solutions for the phenol red experiment assays with buffer or water? Why?

12. Outline the steps of your experiment. Bullet points are okay.

Results

Create a table (or two if needed) below to record your results.

Post-Lab (Week Two) - Questions

Answer the following questions regarding your lab. You will also be expected to complete a written assignment.

1. Briefly summarize your results below. Write your answer in complete sentences.

2. Based on your results, do you accept or reject your hypothesis? Explain your response.

3. Would O_2 be produced if the ETC was clogged and unable to receive electrons?

4. Would O_2 be produced if the ETC was receiving electrons, but the electrons were then being stolen prior to passing through the entire ETC?

5. Would the CO_2 levels around a plant change if the ETC was clogged and unable to receive electrons? Why or why not?

6. Would the CO_2 levels around a plant change if the ETC was receiving electrons, but the electrons were then being stolen prior to passing through the entire ETC? Why or why not?

7. For the photosynthesis lab you will need to complete the assignment titled "writing an abstract for a scientific paper." An abstract is a brief one-paragraph summary of your experiment. Detailed instructions on the format for this assignment will be given to you in lab and posted online. The assignment will be due at the beginning of lab next week.

Materials

Lab groups are assumed to contain 4 students.

Weeks One and Two – Experiments

$NaHCO_3$
- several liters will be needed to soak the spinach leaves and each lab group will need about 200mL of buffer

Analytical Scales
Spinach (5 leaves per lab group for each week)
Hole Punches (two per lab group)
50mL beakers (4 per group)
10mL syringes (two per lab group)
Petri dish
Spatula or forceps for picking up spinach disks
100mL beakers (4 per group)
Elodea
Phenol red (Wards Cat# 8508001)
- each lab group needs an eye dropper with phenol red and will likely use 5-10 drops

Straws
Test tubes
Test tube racks
Lab tape
Parafilm

Week One – Microscopy

Leaf cross-section slides (one per student)
Light microscope (one per student)

Week Two – Experiments

DCMU – Sigma D-2425 (also called Diuron)
DCPIP – Sigma D-1878

Week Two – Skit

20 empty soda cans
20 Ping-Pong balls
15 Nametags
36 single space Megabloks or Legos
2 flashlights
2 green T-shirts (large men's)

DCMU and DCPIP Stock Preparation:

0.2 mM DCPIP solution (this solution is the "5X" which students further dilute).
1. Add 0.29 g DCPIP powder into 1000 mL dH_20 to get stock solution 1.0 mM DCPIP
2. Add 200 mL 1.0 mM DCPIP stock solution to 800 mL d H20
3. Store in 1L amber bottle, refrigerated at 4°C up to one month

2.0 mM DCMU (Use Caution)

1. Add 0.466 g DCMU powder into 1000 mL of 50% acetone
2. Dissolve by stirring (takes about 20 minutes).

Notes for the Instructor

Week 1

The purpose of week 1 is to introduce our students to the process of photosynthesis and for them to practice setting up two protocols for observing photosynthesis.

We start week one by reviewing the basic steps of photosynthesis and the relationship between photosynthesis and cellular respiration. The level of detail provided will depend on whether photosynthesis has already been covered in lecture. It is most important that the students realize that oxygen is being produced when electrons are removed from water and that carbon dioxide is used to make sugars. We usually draw a diagram on the board depicting the relationship between the light-dependent and light-independent reactions as well as a diagram depicting the relationship between photosynthesis and cellular respiration.

Before we begin the experiment portion of the lab we first discuss the structure of a leaf. The students are shown a diagram and a model of a leaf cross section. We then ask the students to identify the mesophyll, stomata, and guard cells within a leaf cross section. We have a variety of cross section slides for the students to use while practicing proper microscope technique.

Once the students have observed the leaf structures, we discuss the two protocols they are to perform. The first protocol is the floating spinach disk experiment. In this process the students use a hole punch to create spinach disks and replace the air within the spinach leaf with buffer by creating a vacuum with a syringe. The instructor will need to demonstrate how to properly put the spinach disks in the syringe and remove the air from the disks. There are often times when all but one or two spinach disks will sink, and these last one or two remain floating. Instruct your students to simply toss the stubborn floaters. The sunken disks can become damaged while you are trying to get the last couple disks to sink. Once placed in the buffer the spinach disks in the light should start floating within ten minutes. The spinach disks should not float when placed in a dark cabinet. The

rare occasions when disks do float, it is always significantly fewer than when placed in the light. There are several YouTube videos online demonstrating how to sink spinach disks.

For the second experiment the students will place a 3-6cm piece of *Elodea* in a test tube with a phenol red solution. One test tube is placed in a dark cabinet and the second test tube is placed under intense light overnight. The students should return 12-24hrs after set up to record the color change. The solution left in the light should become more basic (magenta in color) because the process of photosynthesis removed CO_2 from the surrounding solution. The solution in the tubes left in the dark cabinet will become more acidic (yellow/orange in color) because CO_2 is being added to the surrounding solution. When in the dark the *Elodea* is unable to perform photosynthesis, but it will still be performing respiration (as is the tube in the light). You can also ask your students to make a tube without *Elodea* as a "no color change" reference.

Week 1 can be accomplished within a 2hr time period.

Week 2

The purpose of week 2 is to improve our students' understanding of the photosystems and the electron transport chain. We start the lab by reviewing the process of photosynthesis and the results of the experiments from the previous week. We then assign each student a role within the light-dependent reactions and then act out the entire process. This skit allows our students to observe the movement of electrons through the light-dependent reactions and the transfer of energy from the light-dependent reactions to the Calvin cycle. After practicing the skit, students are asked to use the experiments from the previous week to design an experiment that allows them to determine if two provided herbicides steal electrons from the ETC or clog the ETC during the light-dependent reactions. As a part of this process we ask the students to predict whether O_2, NADPH, ATP, and glucose would be produced if the ETC is clogged or an electron is being stolen during the process.

Performing the Skit

Establish the locations for the thylakoid membrane (an aisle works well), the thylakoid space (one side of the aisle) and the stroma (the other side of the aisle). Label these so students can refer to them when they forget the names. (I hang signs on differently colored paper.) When setting up the skit, be sure you do not start with a gradient of protons for ATP synthase to use; this way the students have to wait for a gradient to form before ATP synthase can start making ATP.

Show the props that will be used. We use ping-pong balls from Target for the electrons (store in a 1 gallon zip-loc bag), empty soda cans for protons, Megabloks or Duplo Lego blocks for phosphate groups in adenosine nucleotides, and one or two flashlights.

Then start assigning roles. We have prepared name tags that can be used from one year to the next. This not only allows the student to remember what their role was, but also allows other students to remember what their role was.

1. Reaction center for photosystem II. (If possible pick a student wearing green, to make the point that this is where the chlorophyll pigments are that are absorbing light. Or give them a green T-shirt to wear.) Give this student an electron. It gets energized by
2. Light: Give a student a flashlight and make sure they shine it on the pigment to energize their electron. Suggest that the pigment do some sort of silly dance or motion to indicate receipt of the energy. Properly energized, the electron is now passed to (technically the electron is taken by)
3. Plastiquinone (PQ), which passes the electron (technically PQ takes two at a time before it passes any) to
4. Cytochrome *bf*, which is a multi-subunit transmembrane protein. Have them figure out where the name cytochrome comes from (*cyto*=cell, *chrome*=color; cytochrome *c* is bright red!) Cytochrome *bf* "sucks" some of the energy out of the electron and uses that energy to move a proton from the stroma to the thylakoid space. Make sure they realize that now there is an imbalance in the concentration of protons on the two sides of the membrane, establishing a proton concentration gradient. Cytochrome *bf* passes the electron to
5. Plastocyanin (PC), which passes the electron to photosystem I.
6. Reaction center of photosystem I, similar to photosystem II, a collection of pigments absorbing light. Since the electron has lost energy by this point (both from cytochrome *bf* sucking out energy and simple Second Law of thermodynamics energy loss with each transfer), it needs this boost of energy from the light to continue. (Perhaps refer them to the "Z scheme" diagram that shows this energy drop (Figure 4).) So you again need light.

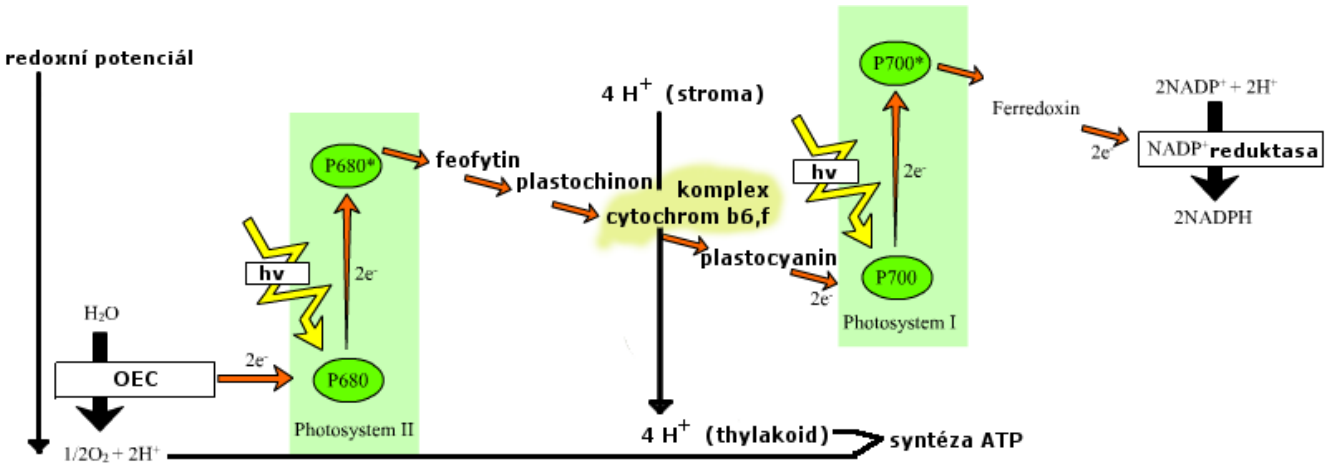

Figure 4. Z-scheme depiction of energy flow during photosynthesis. Source: public domain Wikipedia.com

7. Light (Same person as before or a new person depending on class size), uses a flashlight to shine on the pigment to energize their electron. Again, suggest the pigment do some sort of silly dance or motion to indicate receipt of the energy. Properly energized, the electron is now passed to
8. Ferredoxin (Fd), which passes the electron to
9. $NADP^+$ reductase, which reduces $NADP^+$ to NADPH with the electron (technically two) and a proton from the stroma (which adds to the imbalance of protons on the two sides of the thylakoid membrane). Perhaps point out that the electron is still fairly high in energy (easily seen on the Z scheme).
10. Students playing the role of $NADP^+$ will flip their name over to read NADPH after they receive the electron (technically two) and a proton from the stroma.

    Now that the reaction center of photosystem II has passed on its electron, it needs a replacement. It is so desperate for this that it strips the electrons out of
11. Water. If you want to make this point, be sure that you instruct the reaction center to *take* the electrons from water, rather than having the water simply hand over the electrons. Water has two electrons to donate; the leftover protons (2) are dumped into the thylakoid space (contributing to the proton imbalance). The leftover oxygen atoms from two water molecules bond to make molecular oxygen (O_2). (Make sure they understand the difference between oxygen atoms and oxygen molecules.)

    So let them do this awhile, with water taking electrons out of a bag and NADPH putting the electrons into a bag. Stop them once there is a significant proton concentration gradient and point out that this is a form of energy: ask where did energy come from to make it and what form of energy it is (kinetic or potential). Make sure they understand that the energy is in the gradient, not the protons themselves (unlike the electrons). You can also review the three contributors to the proton concentration gradient:
    1. the protons from water being dumped into the thylakoid space after electrons are stripped
    2. the moving of a proton from the stroma to the thylakoid space by the cytochrome *bf*
    3. the removal of a proton from the stroma to make NADPH.

    Point out that the potential energy can be released if the protons go back across the membrane down their concentration gradient, but can they? Can protons cross a phospholipid bilayer? Why not? So they will need a transport protein to get them across. And this transport protein is able to use the energy in the gradient to synthesize ATP, which cannot happen without energy.
12. So assign someone to be ATP synthase, which is a multi-subunit transmembrane protein. Have them carry three protons at a time back across the thylakoid membrane then stack a Lego block (representing a phosphate group) onto two that are already stacked (representing ADP) to make ATP.

Have students continue electron transport and ATP synthesis until most of the ADP is converted to ATP. Ask them what will be done with this ATP (and NADPH). You can assign a student to be the Calvin cycle to use up the ATP and NADPH.

Once the students have completed the skit under normal conditions, repeat the skit in the presence of an herbicide that steals electrons from the ETC and an herbicide that blocks electron movement. During each version of the skit, be sure the students are observing which products are not made when the ETC is affected by the herbicide. It does not matter where you steal or block

the electrons within the ETC chain. The results, not being able to make enough products for the Calvin cycle, will be the same. However, if you want to recreate the exact mechanism of these particular chemicals, DCPIP takes electrons from ferredoxin and DCMU blocks the ability of plastoquinone to bind to PSII.

Then have each student tell the rest of the class what their role was, where they are located (good to have them say thylakoid membrane over and over), and exactly what they did. It can get repetitive, but it's worth it. If you have a lab quiz after lab, ask them to describe their role.

Designing an Experiment

For the second part of the lab, I usually tell the students that, between DCPIP and DCMU, one will steal electrons from the ETC in the light-dependent reactions and one will block the movement of electrons or one of them could be a fake and have no effect on the ETC. The students are tasked with designing an experiment that allows them to distinguish between these possibilities. At this point I will turn the students loose and let them work on their experiment design without help for five to ten minutes. Once they start to panic I will circulate around the room and help each group individually. The final experiment will include both the spinach disk experiment and the *Elodea* experiment. You may opt to first let the students complete the spinach disk experiment, and then point out the need for the *Elodea* experiment.

If you think your students will struggle with developing a hypothesis, you could place post-lab questions 3, 4, 5, and 6 within the pre-lab or in the lab right after the skit in order to help students see the difference in the outcome when a plant is treated with each herbicide.

The floating disk experiment will allow students to determine which herbicide is stealing electrons and which is clogging the ETC. If the ETC is clogged, the leaves will no longer produce oxygen because electrons will no longer be stripped from water. If the electron is being removed from the ETC by the herbicide, then the spinach will float. Even though the process of photosynthesis is inhibited, oxygen is still being produced when electrons are being removed from the ETC because electrons are still being stripped from water and moved through PSII.

Once the students have determined that DCPIP steals electrons and DCMU clogs the ETC, they will next need to confirm that DCPIP is actually inhibiting photosynthesis. This experiment is necessary because spinach disks without an herbicide and spinach disks with DCPIP will both float. Students can confirm that DCPIP is working properly by setting up the *Elodea* experiment that was performed in week 1. DCPIP turns blue in solution. This color will make it more difficult for students to interpret the color change they do see. To overcome this challenge students will need to set up four test tubes: two tubes with phenol red solution and *Elodea* and two tubes with phenol red solution, DCPIP, and *Elodea*. One of each tube will be placed in light and one will be placed in dark. The two tubes without DCPIP are control tubes in order to ensure that the *Elodea* is functioning properly. The two tubes with DCPIP are the experimental tubes. The tube in the dark will represent the expected color change if photosynthesis is not functioning. Students compare the color of the DCPIP tube placed in the light to the tube placed in the dark. If the color change is the same, then photosynthesis did not occur in the tube that was placed in the light, confirming that DCPIP inhibits photosynthesis. (The problem created by the blue color of the DCPIP can also be avoided by in fact giving them a "fake", no herbicide at all. In this case, the light and dark tubes will be different colors, the same as the no herbicide control.)

We conclude this lab by requiring our students to write a one-paragraph summary of the experiment they designed and performed during the second week. A copy of this assignment is provided at the end of this submission.

Chemical Safety and Waste Disposal

Make sure students are wearing gloves and goggles at all time when working with chemicals. Do not pour DCPIP, DCMU, or phenol red down the sink. They can all be collected in the same bottle for pick up by your chemical waste disposal company.

Using a Fake Herbicide

Having one of your herbicides in fact be a "fake" (not really an herbicide) provides an interesting twist that will force your students to have to think a little harder. So you can tell the students that either solution could be an electron stealer, an electron blocker, or a fake. Since the fake would give the same results on the spinach disk experiment as a stealer (disks float in the light), they have to do the phenol red experiment to see if CO_2 is being consumed to make sugar by photosynthesis (if it is a fake) or is being produced by aerobic respiration, observable because no photosynthesis occurring (if it is a real herbicide). If they do in-the-light controls (positive control: no herbicide added and negative control: the blocker herbicide, which they will already know from the spinach disk experiment) for the phenol red experiment, then by comparison, then they can easily match up their unknown with one of them to conclude whether they have been given a stealer or a fake.

The use of a fake avoids the problem of DCPIP's blue color, which complicates the color changes in the phenol red experiment. Also, if a course is taught in

consecutive semesters, this allows the experiment to be changed slightly, forcing new thinking from the repeating students. (And then their roommates can't tell them the results either.) Warn the students in the second semester that different herbicides have been used compared to the first semester; Home Depot has many, many herbicides on their shelves!

Students often try to use clues to guess whether you are using a fake. Since the DCMU must be made in an organic solvent, students smell this and then deduce that the other herbicide is a fake if it also does not smell. This can be easily avoided by adding the same solvent to the fake solution.

Adapting the Skit for Aerobic Respiration

It is easy to adapt the photosynthesis skit for aerobic respiration due to the many similarities in the processes; in fact, having students do both skits will reinforce this. The same materials and location can be used for both skits.

The thylakoid membrane is now the inner mitochondrial membrane and is the location of the electron transport chain. Electrons start in NADH and are passed through a sequence of carriers, several of which are pumping protons from the matrix to the intermembrane space:

Complex I (pumps protons)
Ubiquinone (CoQ_{10})
Complex III (pumps protons)
Cytochrome *c*
Complex IV (pumps protons)

The final electron acceptor at the end of the chain is O_2, making H_2O. With three proton pumps, the proton concentration gradient builds up quickly, so you may need to have more than one student in the role of ATP synthase. If you have additional students, you can have $FADH_2$ feeding electrons into Complex II, which would then pass electrons to Ubiquinone. (So both Complex I and Complex II would be passing electrons to Ubiquinone.)

To add a twist similar to the herbicides in the photosynthesis skit, have one student be the poison *cyanide*. They will block electrons from being passed from complex IV to O_2, similar to the electron blocker herbicide. Students will see that everyone gets "stuck" with their electrons, so no proton concentration gradient can be formed, and therefore no ATP will be made. Students can then consider that anaerobic respiration can still be used to make ATP, but it is not enough to survive.

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Appendix A
Research Summary Instructions and Rubric

How to Write a Research Summary

A research summary (frequently called an abstract) is used in the academic community to provide a brief description of your project. You frequently use a summary when applying for a conference or sharing information with potential research collaborators. Being able to write a concise summary is also a good exercise for determining the most important aspects of your project. It is not uncommon to find yourself in a position where you need to verbally explain your research project to another scientist or student. If you have already practiced writing a summary, then it is much easier to verbally explain your project in a short period of time. There are five main components of an abstract:

1. **Background information:** This is usually one or two sentences that provide the reader with enough information to understand your project.
2. **Your problem or question:** This is a single sentence summarizing the exact problem or question you are studying.
3. **Methods and procedures:** This is one or two sentences providing basic information about your research methods. You want your audience to know how you completed your project, but you do not need to provide enough detail here for them to repeat the experiment themselves.
4. **Results**: You usually only need one sentence to summarize your research findings. If you have a particularly large project you may need an additional sentence.
5. **Conclusion and implications:** Finish your paragraph with a sentence or two interpreting your results and the implications for future research projects.

When writing your abstract you should maintain the introduction/body/conclusion format that you use for large papers, but instead you are writing a single paragraph. A good abstract is unified, coherent, concise and can stand alone without further information from the author. In order to be unified and coherent you have to remain on topic throughout your paragraph and provide transitions from one sentence to the next.

When you start writing your abstract you should first create an outline of the information you intend to include. First, list each of the five summary components above. Next, write down the basic information needed in your paper for each component. DO NOT submit your outline with your assignment; instead use it as a tool for organizing your thoughts prior to writing.

Below is an example of a possible research summary for the osmosis lab. *(Remember you are writing a research summary for the second week of the PHOTOSYNTHESIS lab)*.

> **Finding the sugar concentration of sweet potatoes and russet potatoes.**
> Due to the process of osmosis, when a cell is placed in a hypertonic solution it will lose water and shrink. Conversely, when a cell is placed in a hypotonic solution it will gain water and swell. We estimated the solute concentration of sweet potatoes and russet potatoes by soaking pieces of each potato in known sugar concentrations ranging from 0M to 1M. If the potato piece gained mass when placed in a sugar solution, then the solution was hypotonic and contained less solute than the potato. If the potato piece instead lost weight, then the solution was hypertonic and contained more solute than the potato. Based on our results we concluded that the sweet potato contains a solute concentration between 0.6M and 0.7M and the russet potato contains a solute concentration between 0.2M and 0.4M. More sugar concentrations within this range need to be tested in order to determine the exact concentration of sugar in each potato type.

Basic Instructions for Your Assignment

Your paragraph should be no more than 250 words. This word limit will be no more than one page double-spaced. You need to type your assignment using 12-point font and double-spaced. Once completed, place your name and lab section on the top of your page and submit the assignment online. You MUST submit your first draft prior to your lab period next week.

Table 1. Research summary rubric.

	2pts	**1pt**	**0pts**
Format	Use 12pt font, double space, name is on document, no more than 250 words	One formatting item is incorrect	More than one formatting error is incorrect
Grammar (use of language and sentence structure)	All sentences structured correctly and no more than one minor grammar error	Entire paragraph contains no more than 3 errors	More than 3 grammar errors
Mechanics (punctuation, spelling, capitalization, etc.)	No more than one mechanical error	No more than 3 mechanical errors	More than 3 mechanical errors
Paragraph development	Paragraph is developed in a logical manner and all required components are included	One required component is missing or paragraph is not written in a logical order	More than one required component is missing
Coherence	Sentences are written logically and clearly articulate a point	1-3 sentences lack coherence.	Most sentences lack coherence
Concision	No wordiness or unnecessary information	Limited wordiness or unnecessary information	Significant wordiness or unnecessary information
	4pts	**2pts**	**0pts**
Scientific Accuracy	All science discussed in the assignment is accurate	Most of the science discussed in the assignment is accurate	None, or very little, of the science in the assignment is accurate
Lab Accuracy	The lab description, purpose, and results are reported correctly	One lab component is reported incorrectly	More than one lab component is reported incorrectly
Interpretation	Results are interpreted correctly and the suggested further research is logical	The results are not interpreted correctly or the suggested further research is not logical	The results are not interpreted correctly and the suggested further research is not logical

Appendix B
Answers to Lab Questions

Week 1 Pre-Lab Questions

1. To use energy from sunlight to make sugars and other macromolecules from CO_2 and water.

2. Chlorophyll pigments absorb light energy and transfer it to electrons.

3. Photosynthesis takes place in chloroplasts within the mesophyll cells.

4. The light-dependent reactions produce ATP and NADPH that are required for the light-independent reactions. The light-independent reactions produce ADP and $NADP^+$ that are required for the light-dependent reactions.

5. The purpose of the ETC is to use energy in electrons to create a proton gradient.

6. O_2 is produced during the light-dependent reactions.

7. CO_2 is consumed during the light-independent reactions.

Week 1 Post-Lab Questions

1. The sunken spinach leaves float because they are producing O_2, filling the space within the leaf and causing it to float.

2. In the light, the pH of the water surrounding the *Elodea* leaf will become basic as the CO_2 is being removed. In the dark, the pH of the water surrounding the *Elodea* leaf will become acidic because, even thought photosynthesis is blocked, respiration is still occurring and producing CO_2.

Week 2 Pre-Lab Questions

The function of each component listed in the prelab can be found in the instructor notes for the photosynthesis skit.

Week 2 in Lab Questions

1-9 and 12. Answers will vary.

10. Students should dilute the spinach disk assays in buffer. The spinach leaves tend to work more consistently when they are in buffer.

11. Students should dilute the phenol red in water because the buffer will prevent sufficient pH/color change (buffers buffer).

Week 2 Post-Lab Questions

1 and 2. Answers will vary.

3. No, O_2 would not be produced if the ETC was clogged because electrons would be unable to move through the ETC. Thus, electrons will not be removed from water and O_2 will not be produced.

4. Yes, O_2 would still be produced because electrons would still be moving out of PSII. As long as electrons are moving out of PSII, electrons will be removed from water and oxygen gas will be produced.

5. The CO_2 levels would increase around a plant if the ETC was clogged because photosynthesis would be inhibited, but respiration would still be occurring (at least temporarily) and producing CO_2.

6. The CO_2 levels would increase around a plant if electrons were removed from the ETC because photosynthesis would be inhibited, but respiration would still be occurring (at least temporarily) and producing CO_2.

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