Photosynthesis

Objectives

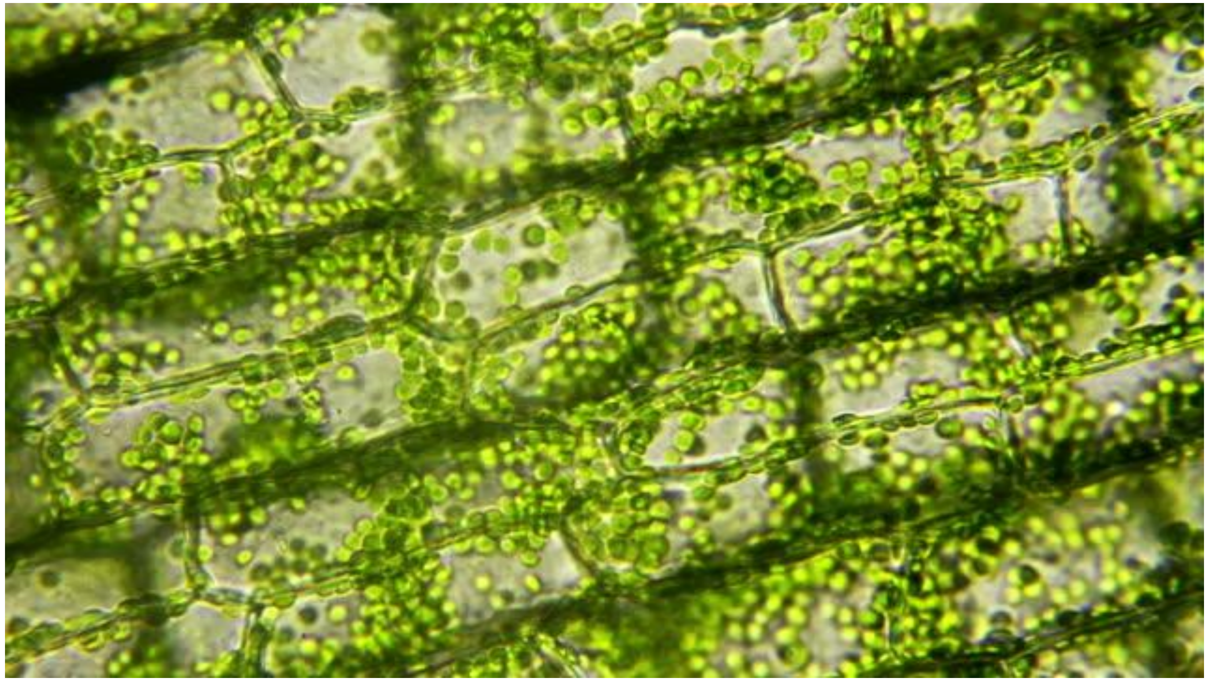
- Construct an action spectrum and describe the effect of light on photosynthetic organisms
- Describe the effect of metabolic poisons on photosynthesis
- Describe and explain the effect of metabolic poisons on the ability to convert light and chemical energy to kinetic energy

Safety

- **Gloves, goggles, closed-toe shoes required**
- Working in groups of 4
- Tips in tip discards ONLY
- Sodium azide - **toxic waste container**
- DCMU – **solvent waste container**
- Untreated Chlamydomonas – **“untreated Chlamydomonas waste”**

What is Photosynthesis? Where does it take place?

Fig: Microscopic view of plant cells



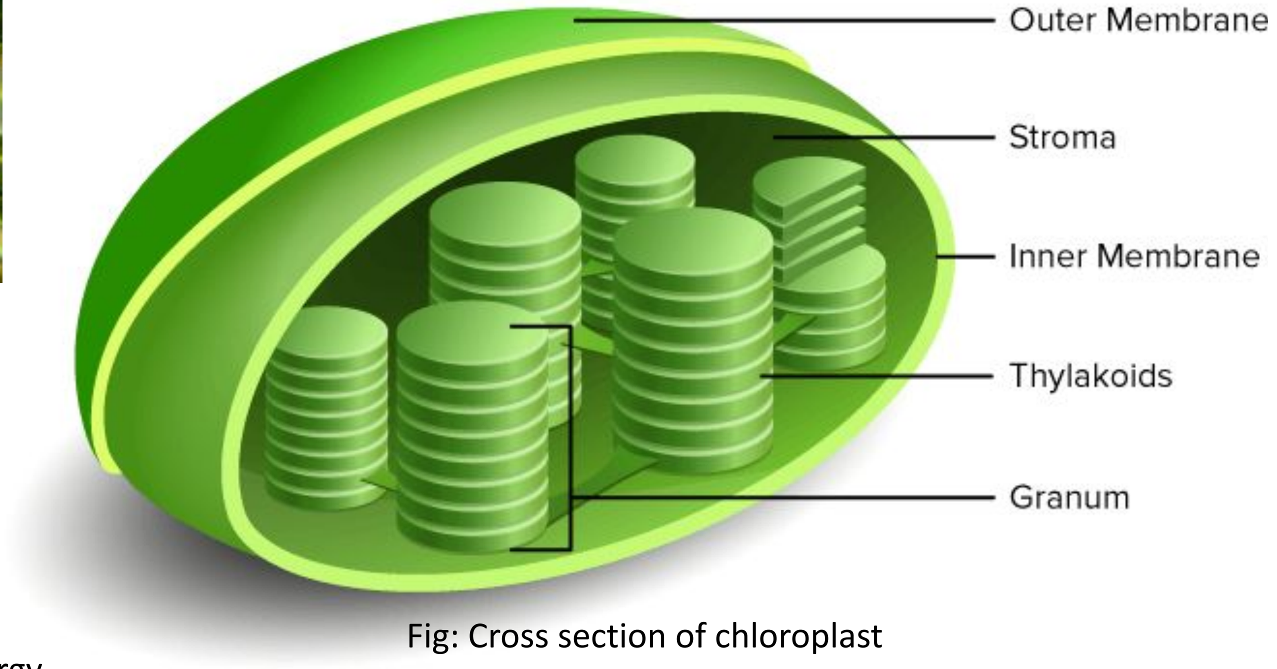


Fig: Cross section of chloroplast

Energy (visible light)

Photosynthesis

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

Respiration

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + Energy

Two parts of photosynthesis

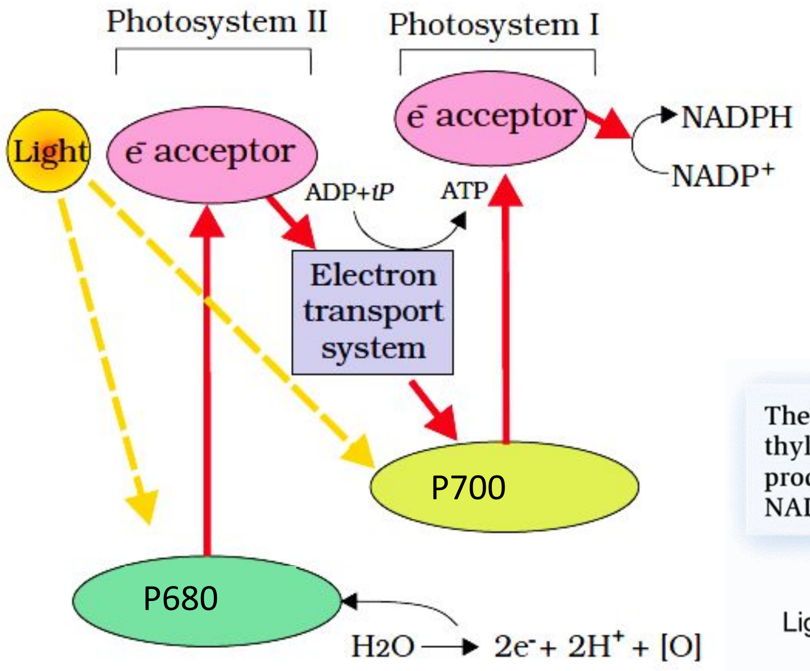
- In the light-dependent reactions, energy from sunlight is absorbed by pigments and that energy is converted into stored chemical energy.
  - **"Light reactions"**
    - **Light energy $\rightarrow$ chemical energy (ATP)/NADPH**

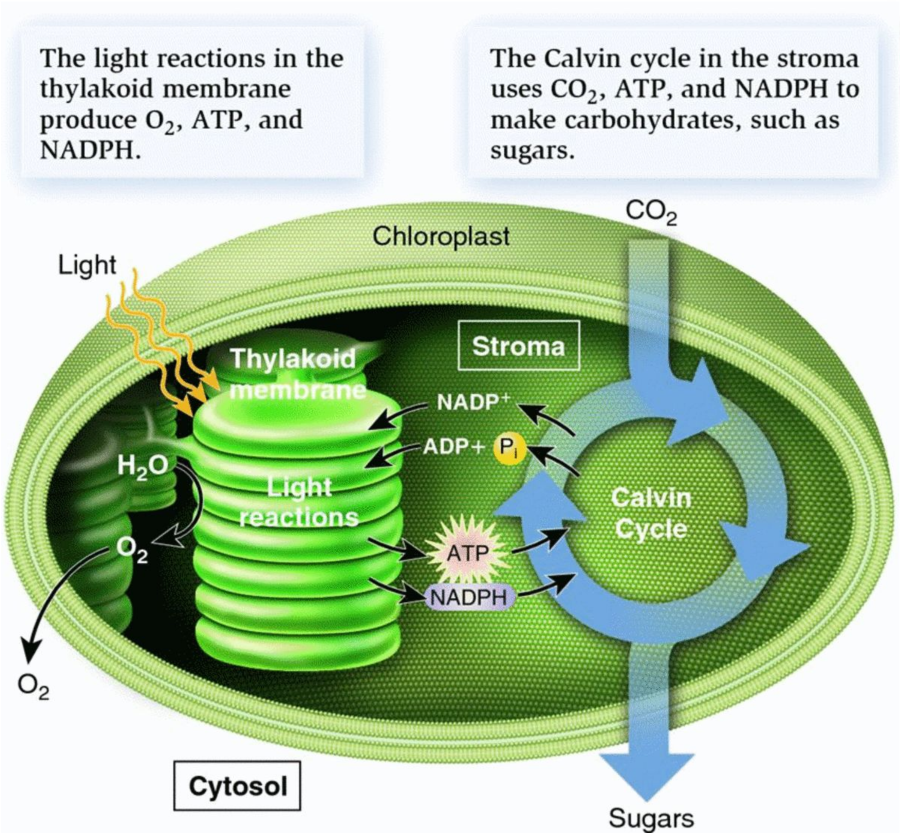
- In the light-independent reactions, the chemical energy harvested during the light-dependent reactions drives the assembly of sugar molecules from carbon dioxide.
  - **"Calvin Cycle"**
    - **$CO_2 \rightarrow$ glucose (sugars)**

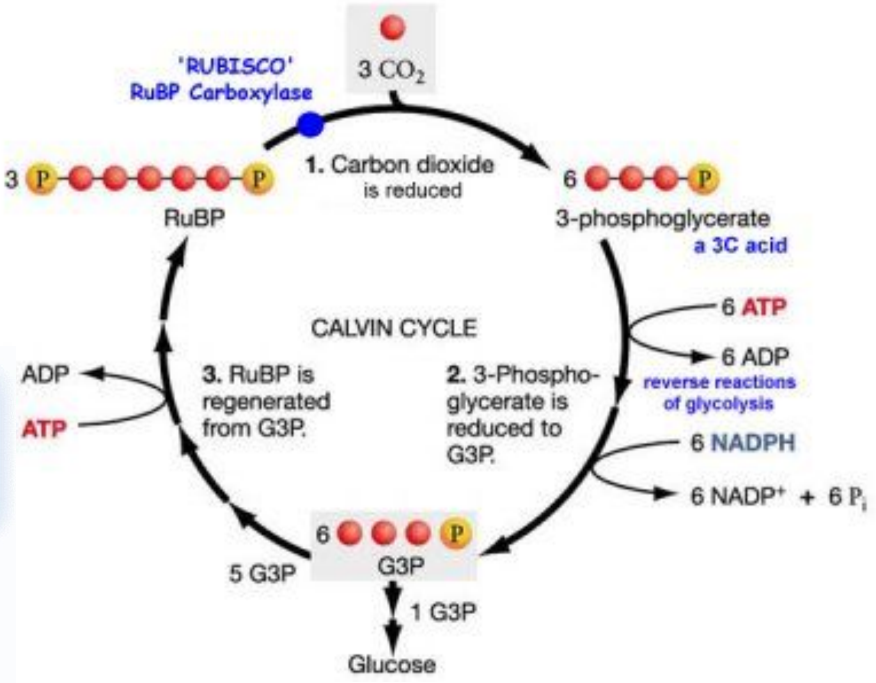
What happens in photosynthesis

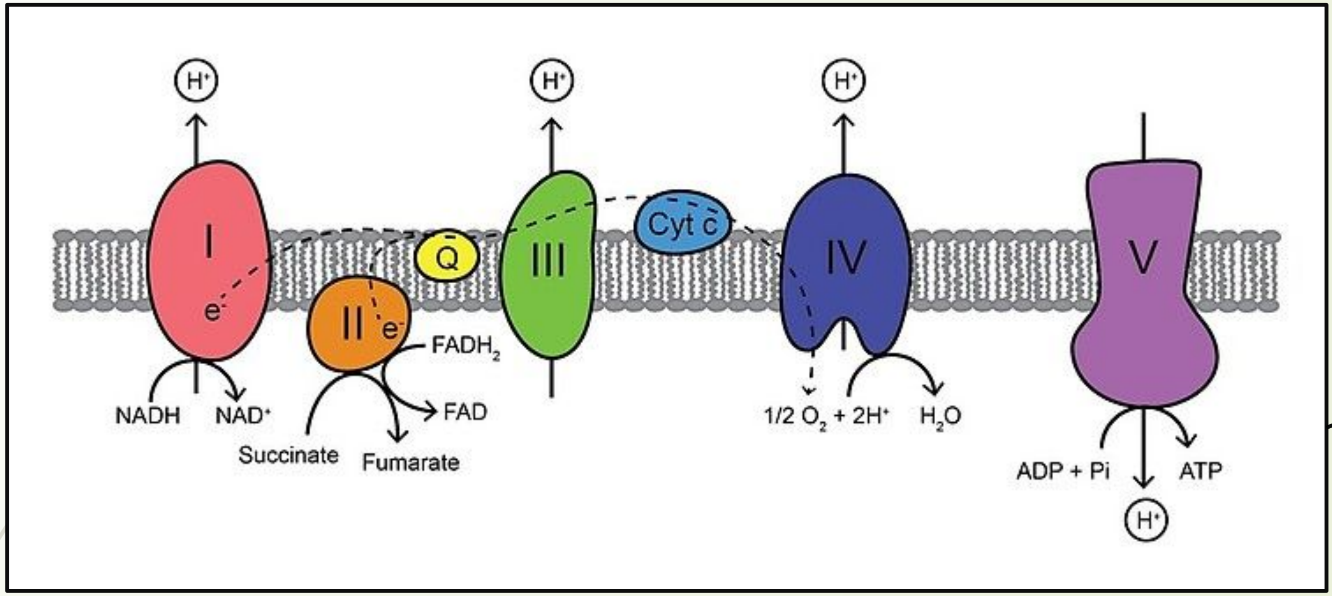
Photochemical reaction

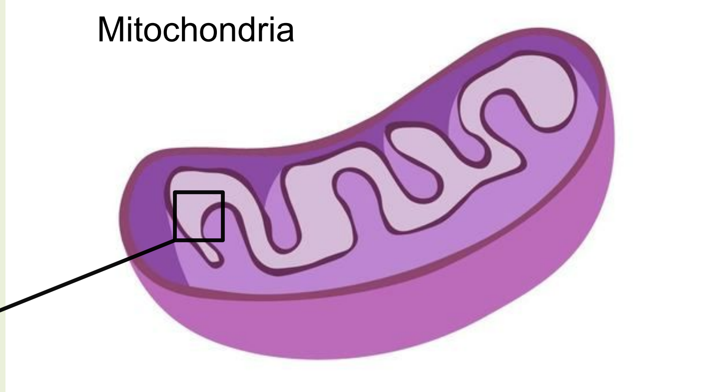
Biochemical reaction

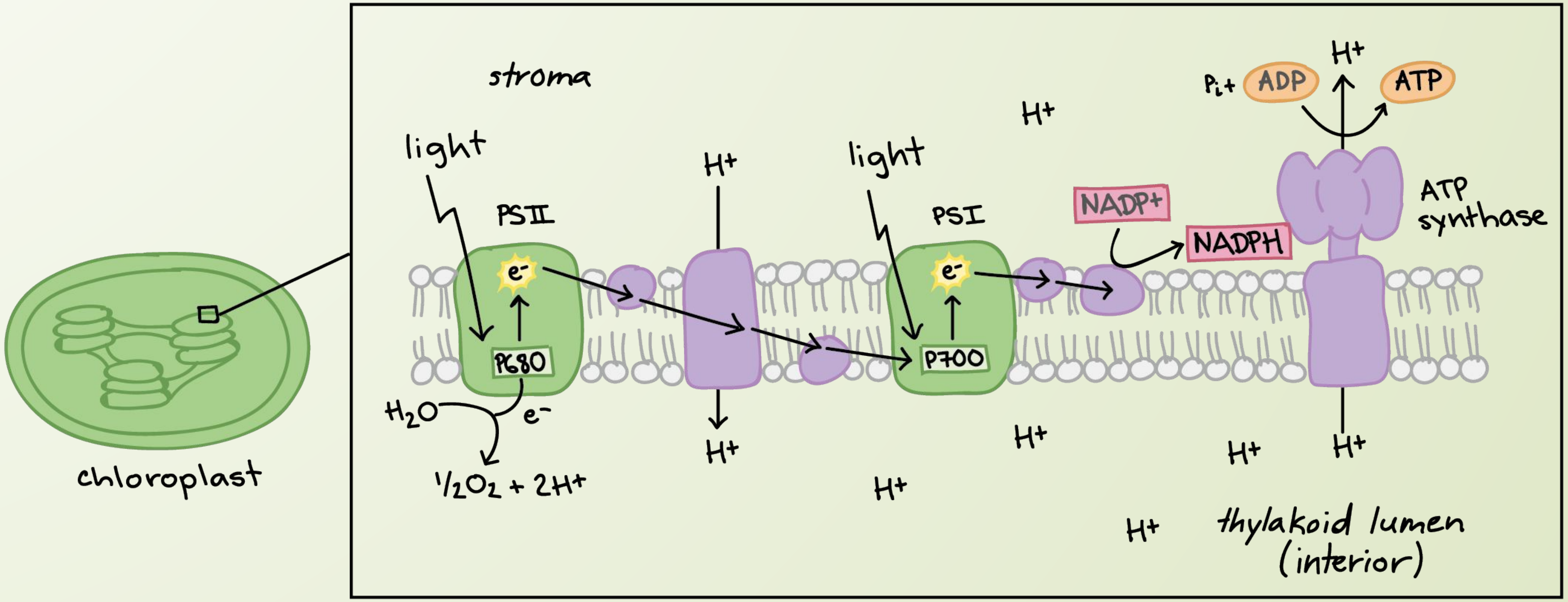






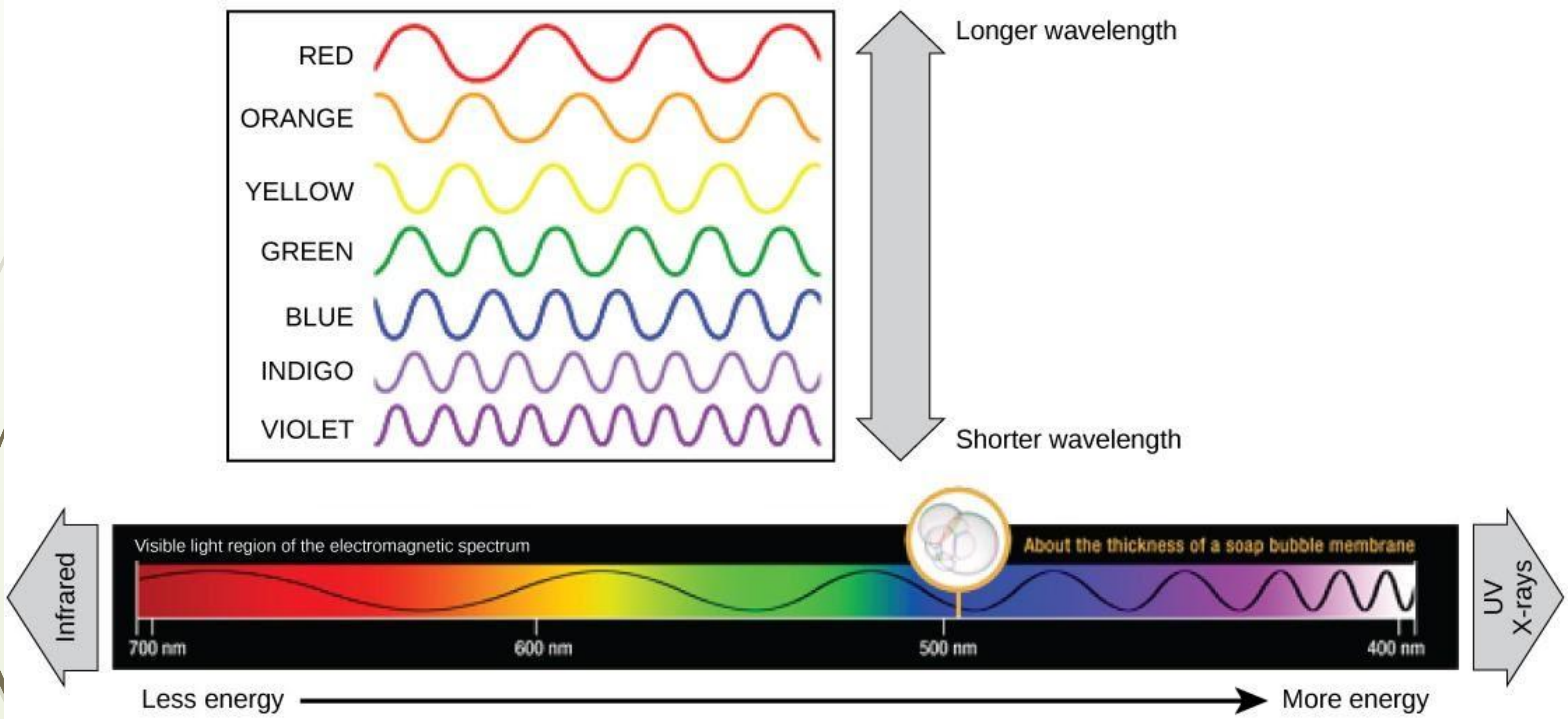

H+
I
e-
NADH
NAD+
II
e-
FADH2
FAD
Succinate
Fumarate
Q
III
Cyt c
IV
1/2 O2 + 2H+
H2O
V
ADP + Pi
ATP


Mitochondria


chloroplast
stroma
light
PSII
e-
P680
H2O
1/2O2 + 2H+
H+
PSI
P700
NADP+
NADPH
Pi+
ADP
ATP
ATP synthase
thylakoid lumen (interior)

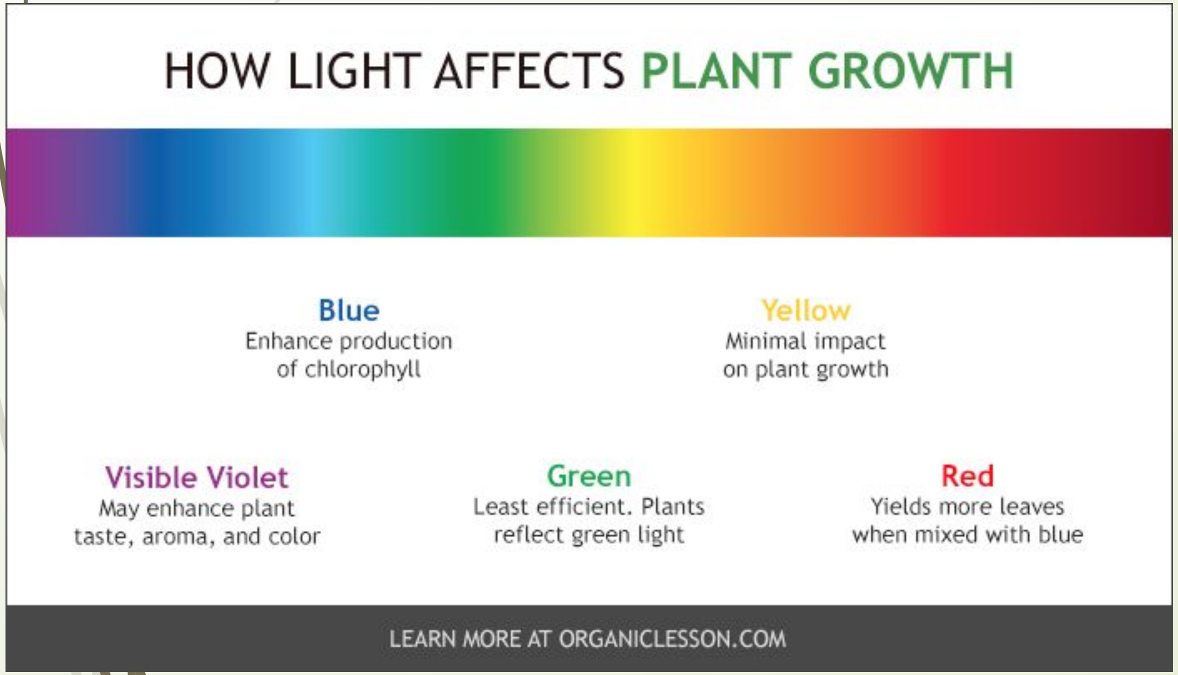
Action Spectrum

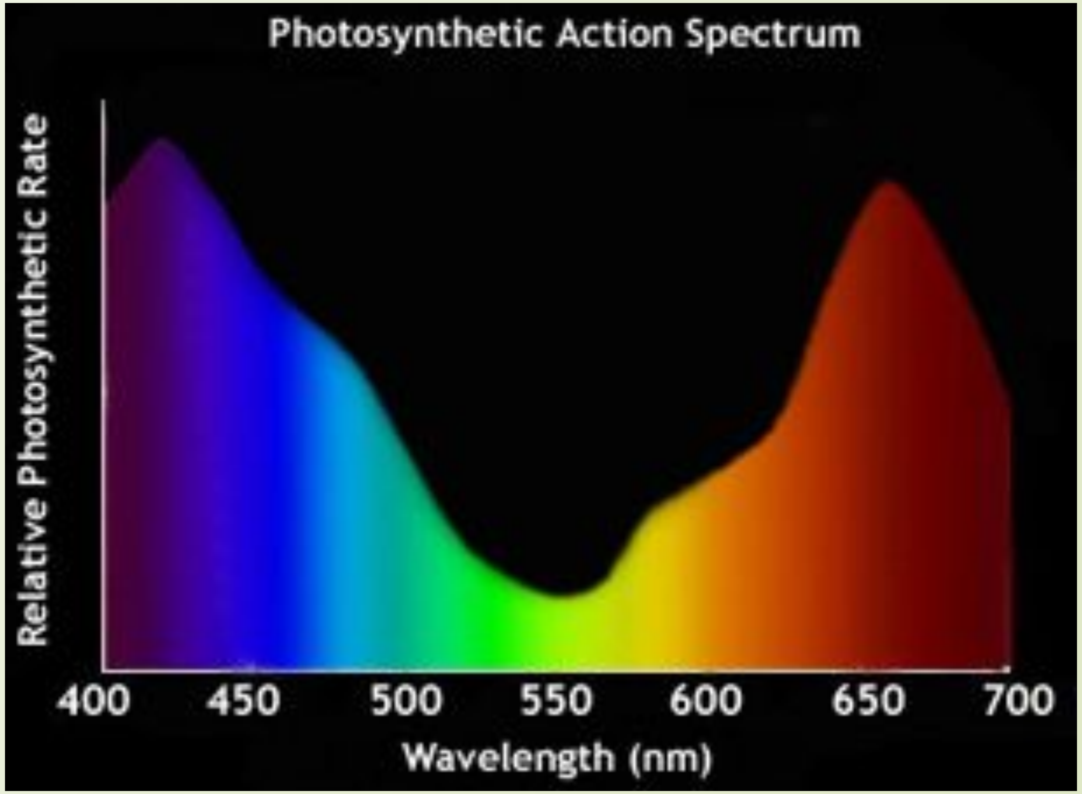
- Energy levels lower than those represented by red light are insufficient to raise an orbital electron to a excited (**quantum**) state. Energy levels higher than those in blue light will physically tear the molecules apart, in a process called **bleaching**.



Organic pigments found in the thylakoid membrane.

- Light energy initiates the process of photosynthesis when pigments absorb specific wavelengths of visible light.
- Organic pigments, have a narrow range of energy levels that they can absorb.





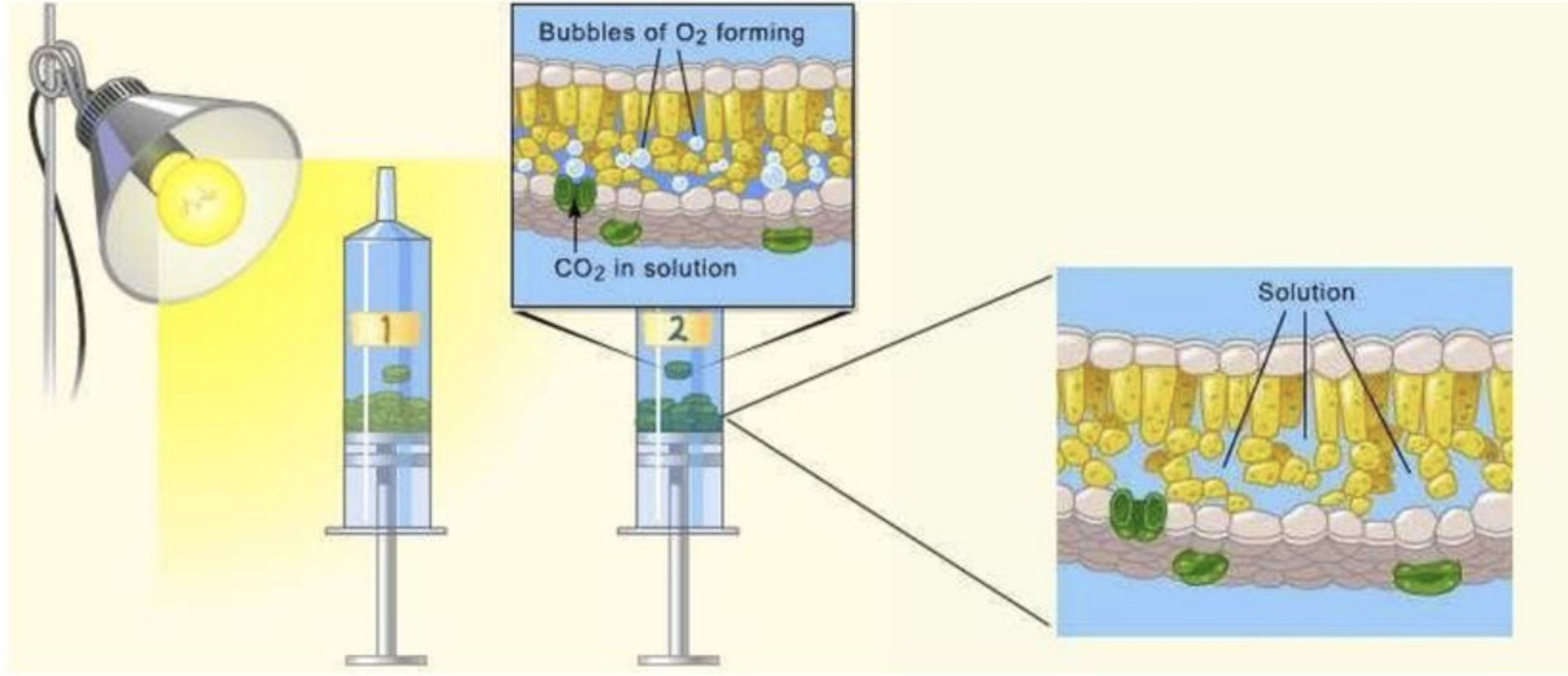
Lab activity 1

- Label small beakers with light color + group name
- Cut spinach leaf disks and place them in syringe
- Sodium bicarb in beaker, fill syringe, “vacuum” until all disks are no longer floating – keep disks in dark until transfer to beakers
- Add 40mL sodium bicarb to each small beaker, add 10 disks to each
- Immediately place in appropriate light box, **start timer counting up**, turn on light (note time)
- Every 5 min, count the number of floating disks. **Record in table 1**

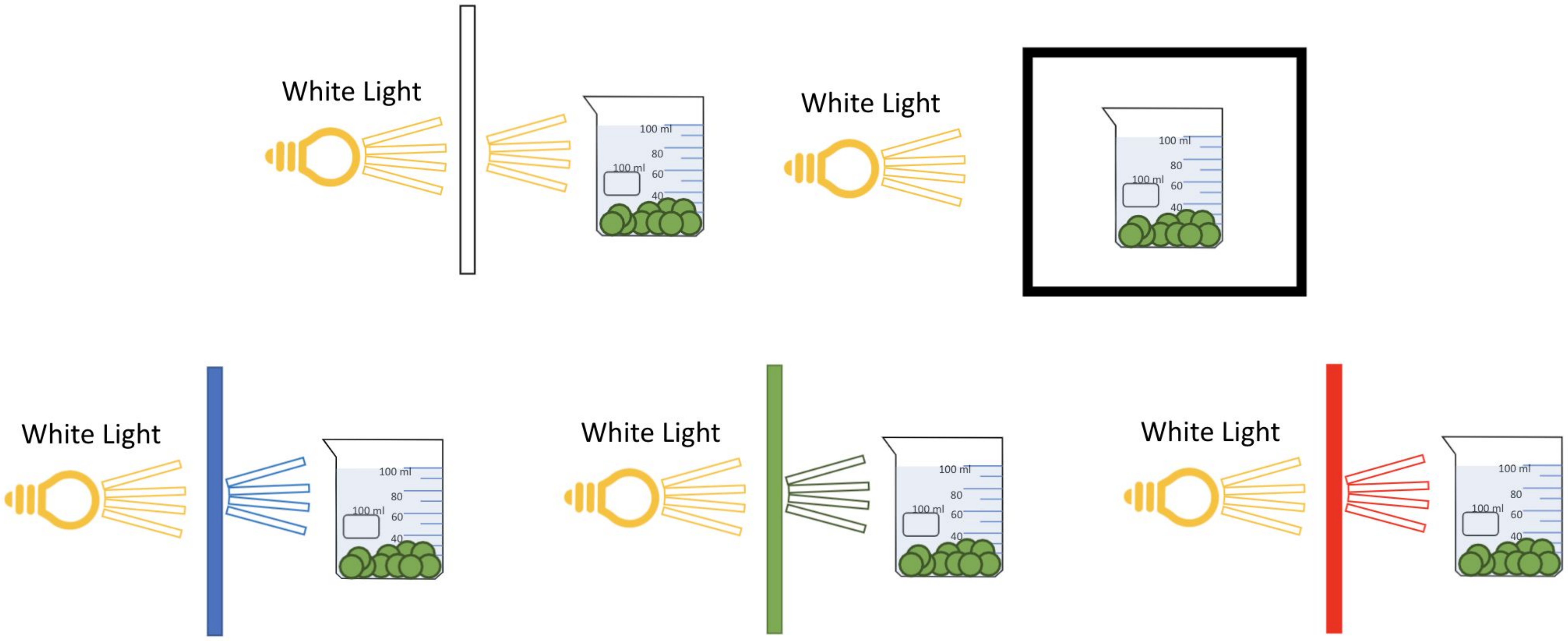
Lab Activity 1: Table 1

Time	0 min. # disks floating	5 min. # disks floating	10 min. # disks floating	15 min. # disks floating	20 min. # disks floating	25 min. # disks floating	30 min. # disks floating
White light							
Red Light ??							
Green Light							
Blue Light ??							
Dark							

Visual Protocol

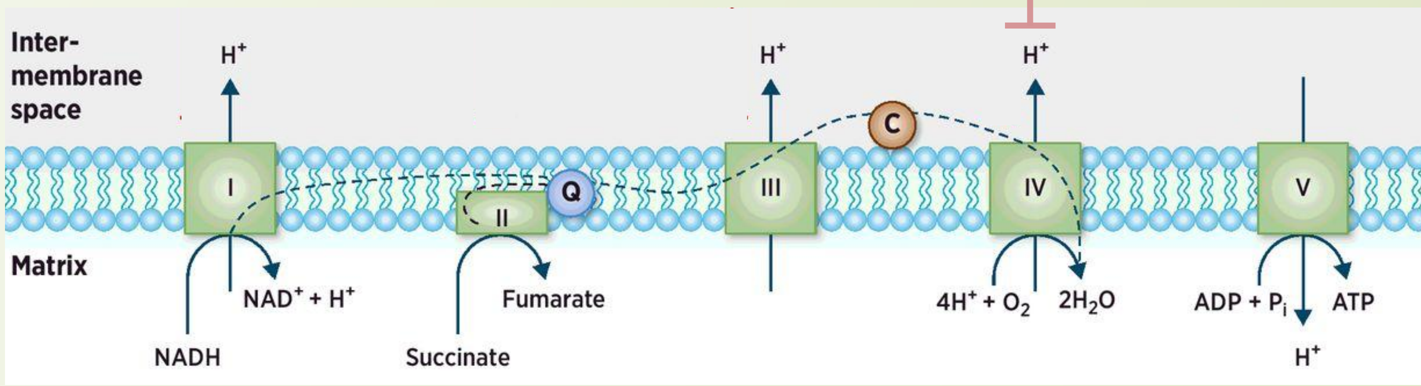


Visual Protocol

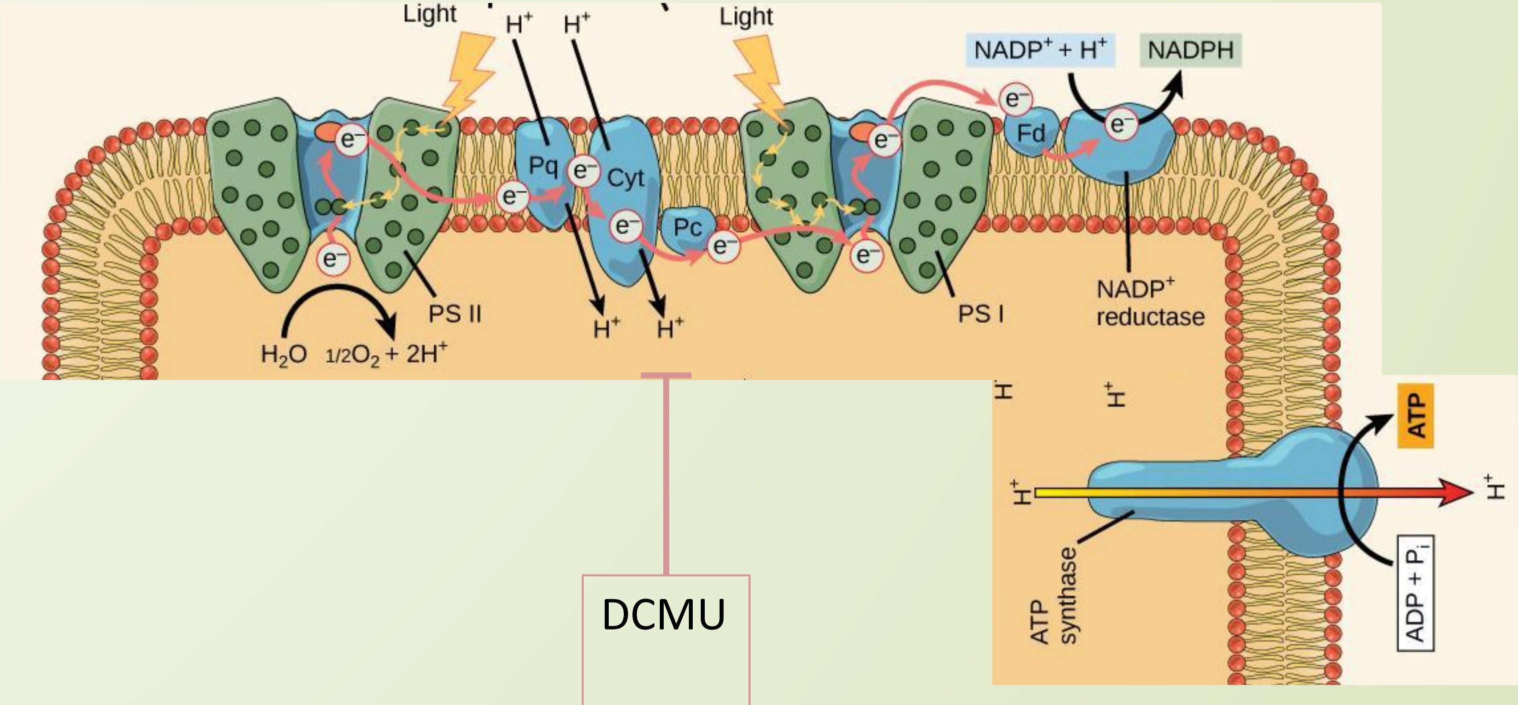


Metabolic Poisons

- Sodium azide inhibits the ETC of the mitochondrion and therefore, respiration.



- DCMU specifically inhibits the flow of electrons from PSII, thereby disrupting photosynthesis in the chloroplast.



Lab activity 2

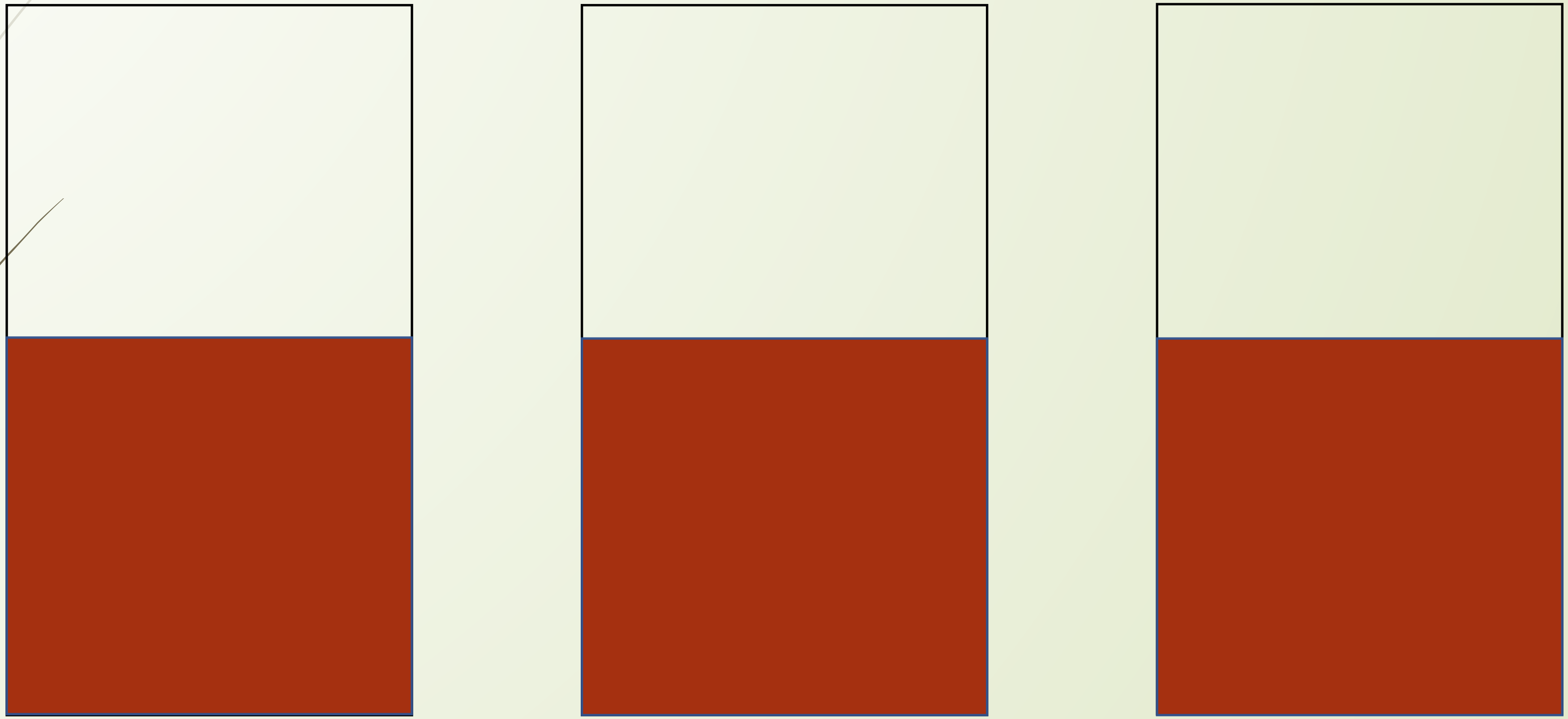
- Label 3 – 15mL tubes: “untreated”, “DCMU”, “Azide”
- Transfer 10mL Chlamydomonas (unicellular and flagellated algae) into each tube
- Add 100 uL of 10mM DCMU to the “DCMU” tube (discard tip!)
- Add 100 uL of 1M Sodium azide to the “azide” tube (discard tip!)
- Add 100 uL of sterile water to the “untreated” tube
  - Cap and mix each tube
- Pour each tube into a **labeled** locomotion chamber
- Arrange foil tent over ½ of the locomotion chambers
- Place light source over chamber. Observe distribution.
- Turn on light. Start timer. Observe after 10 minutes, then again at 20 minutes. **Record observations in Table 2.**

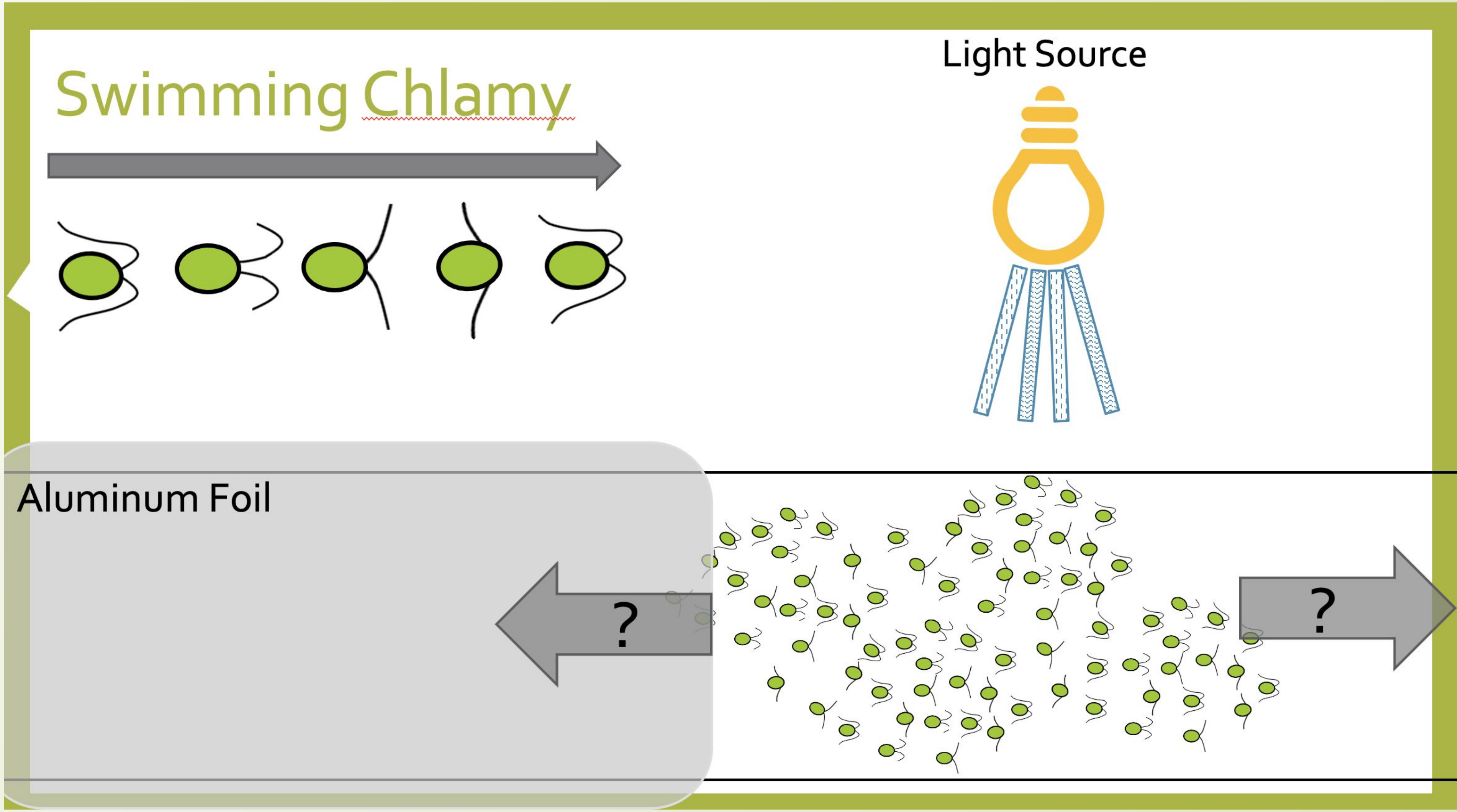
POUR FROM TUBES:

Untreated:
- 10mL Chlamy
- 100uL sterile water

DCMU:
- 10mL Chlamy
- 100uL 10mM DCMU

Azide:
- 10mL Chlamy
- 100uL 1M Sodium azide





Swimming Chlamy
Light Source
Aluminum Foil
?
?

Table 2 – each treatment

Sodium Azide	Time (minutes)	Observation
	0 min.	
	10 min.	
	20 min.	
	30 min.	

DCMU	Time (minutes)	Observation
	0 min.	
	10 min.	
	20 min.	
	30 min.	

Untreated	Time (minutes)	Observation
	0 min.	
	10 min.	
	20 min.	
	30 min.	

0min

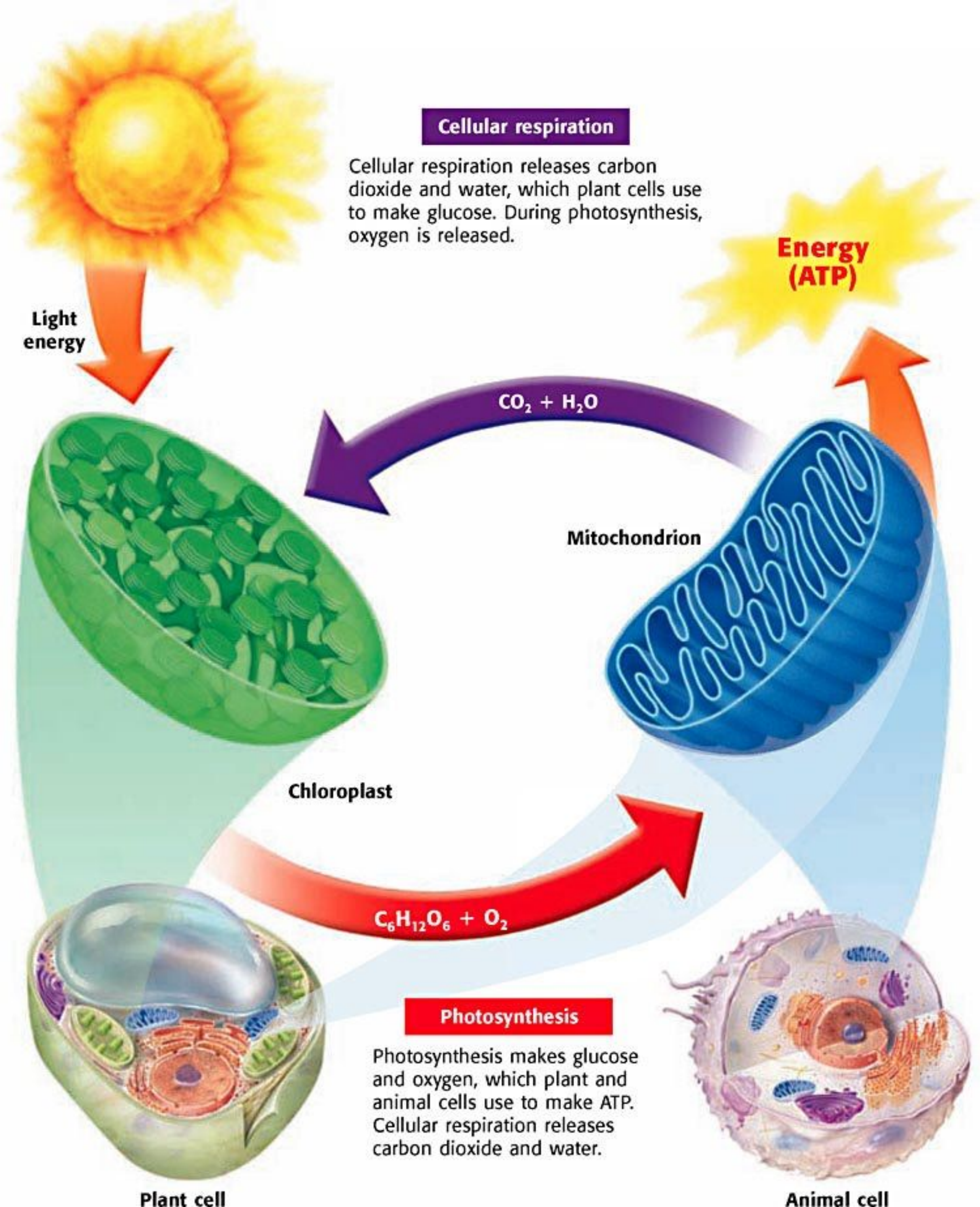
10min

20min

30min

Expectations from this experiment

- DCMU specifically inhibits the flow of electrons from PSII, thereby disrupting photosynthesis in the chloroplast.
- Sodium azide does not inhibit photosynthesis. Inhibits the ETC of the mitochondrion and therefore, respiration.
- Plants have BOTH mitochondria and chloroplasts, which is why we are using both metabolic toxins.
- The chlamy can't swim in the presence of azide because they can't produce enough ATP from sugar via the mitochondria (and respiration) to keep swimming.
- They can swim in the presence of DCMU because it's inhibiting the production of sugar, but not the utilization of that sugar. Eventually, the chlamy will run out of sugar and die.


Cellular respiration
Cellular respiration releases carbon dioxide and water, which plant cells use to make glucose. During photosynthesis, oxygen is released.
Light energy
Energy (ATP)
$CO_2 + H_2O$
Mitochondrion
Chloroplast
$C_6H_{12}O_6 + O_2$
Photosynthesis
Photosynthesis makes glucose and oxygen, which plant and animal cells use to make ATP. Cellular respiration releases carbon dioxide and water.
Plant cell
Animal cell

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Summary of the <u>two photosynthesis experiments</u>

- 1-2 Sentence introduction/purpose of the experiments
- 2-3 Sentence hypothesis:
  - Hypothesis for activity 1 (which wavelength of light would you expect to allow the spinach to start photosynthesis)
  - Hypotheses for activity 2 regarding each of the metabolic poisons and *Chlamydomonas* motility
- 1-2 Sentence Method summary
- 3+ Results – What happened in the experiments. What data did you get? What overall trends can you assume are happening.
- 3+Discussion – Does your data support or refute your hypothesis? (Your hypothesis is a guess and it's okay to be wrong). Why do you think your results came out they way they did? <u>What **biological processes** are being affected? How did altering those processes affect your dataset?</u> What possible errors could also be affecting your data?

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