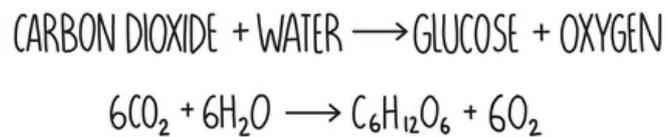


Topic 5: Energy Transfers in and between Organisms

Photosynthesis

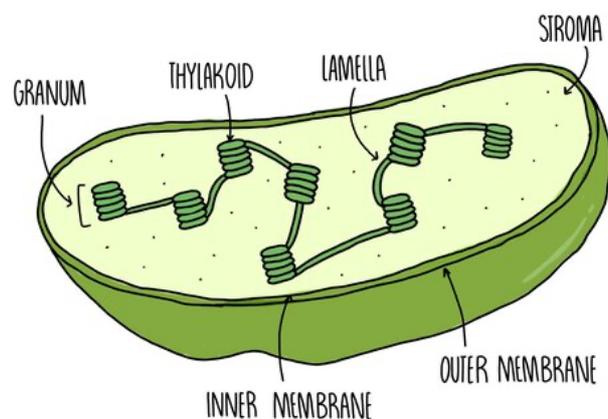
Photosynthesis reaction

Photosynthesis combines **carbon dioxide** with **water** to form **glucose** and **oxygen**. The glucose is used in respiration to produce energy while the oxygen is released from the plant through the stomata. The energy released can be used for active transport of mineral ions, DNA replication and protein synthesis.



Chloroplast structure

Photosynthesis takes in the **chloroplasts** of plant cells. Chloroplasts contain fluid-filled sacs called **thylakoids**. Thylakoids are stacked up like pancakes to form structures which we call **grana**. Each granum is connected together by pieces of thylakoid membrane called **lamellae**. The gel-like substance which surrounds the thylakoids is called the **stroma**.



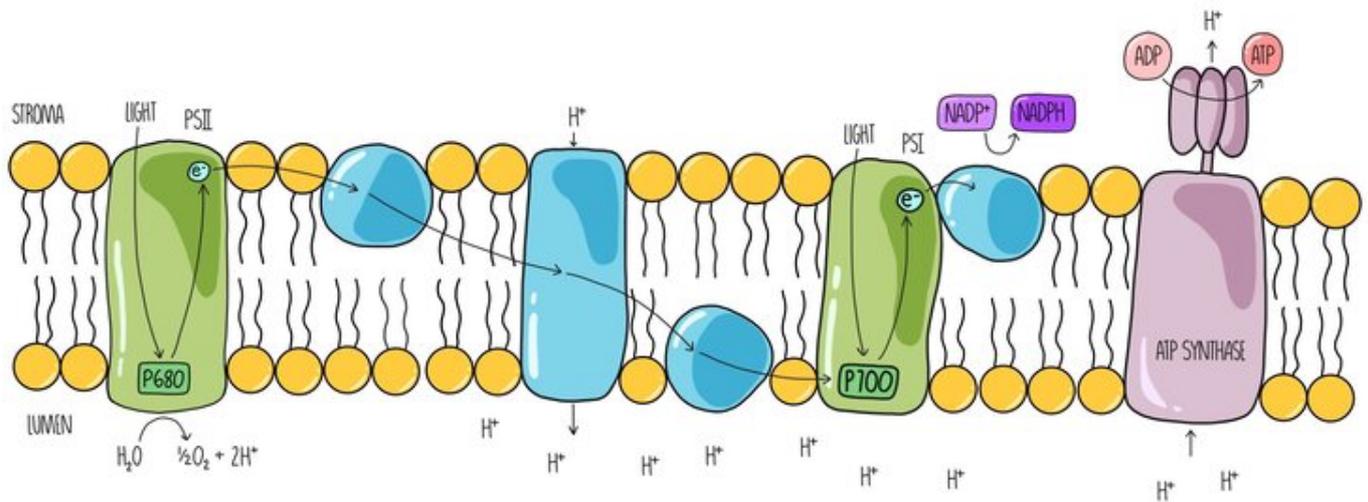
The thylakoids within the chloroplast provide a **large surface area** to allow as much light to be absorbed as possible. Within the thylakoid membrane are **photosystems** which consist of a pigment molecules attached to proteins. The pigment is what gives plants their colour and includes **chlorophyll a**, **chlorophyll b** and **carotene**. Plants contain two photosystems, called **photosystem I** and **photosystem II**. PSI absorbs light at a wavelength of 700 nm while PSII absorbs light at a wavelength of 680 nm.

The overall process of photosynthesis can be split into two stages: the first one stage is known as the **light-dependent reaction** and (unsurprisingly) requires light to get going. The second is called the **light-independent reaction** which doesn't need light but does need the products that were generated in the first stage.

Light-Dependent Reaction (LDR)

The LDR takes place in the **thylakoid membranes** of the chloroplasts. It takes place in the following stages:

1. Light energy is absorbed by **PSII** (even though PSII is involved before PSI, it was discovered afterwards - hence the confusing naming system). Light **excites electrons** within PSII and causes them to move into a **higher energy state**. The electrons are passed onto a series of electron carriers within the **electron transport chain** to **PSI**.
2. The electrons which have been lost from PSII need to be replaced. This happens through the **photolysis of water** - light energy causes a water molecule to split apart and release hydrogen ions, electrons and oxygen. The electrons from water replace the electrons lost from PSII.
3. As the electrons move along the electron transport chain, they **move from high to low energy**. The energy lost by the electrons is used to **pump hydrogen ions** from the stroma into the thylakoids. This generates a **proton gradient** across the thylakoid membrane.
4. Protons flow down their concentration gradient through **ATP synthase**. The energy from the movement of protons is used to **phosphorylate ADP to ATP (photophosphorylation)** in a process called **chemiosmosis**.
5. Light is absorbed by **PSI** causing another electron to become excited and be passed along the rest of the electron transport chain.
6. The **electron is passed onto NADP** to form **reduced NADP (NADPH)**. NADPH is an **electron carrier** which transfers electrons from one molecule to another.
7. The **ATP** and **reduced NADP** move into the **stroma** for the next stage of photosynthesis, the light independent reaction.



This process is known as **non-cyclic photophosphorylation**. There is another process called **cyclic photophosphorylation**, in which electrons repeatedly cycle through PSI. Electrons leave PSI but instead of being accepted by NADP they flow back down the chain to the first electron acceptor. This means that ATP is produced but no NADPH and may happen when NADPH is in plentiful supply. Cyclic photophosphorylation is more common in plants with especially high ATP needs and may prevent excess light damaging photosynthetic proteins.

The Light-Independent Reactions (aka the Calvin Cycle)

The Calvin Cycle takes place in the **stroma** of the chloroplast and uses the products of the LDR (ATP and reduced NADP) to form glucose. The reactions which take place can be divided into three main stages: **carbon fixation**, **reduction** and **regeneration**.

Carbon fixation

- Carbon dioxide is 'fixed' by adding it to a 5-carbon molecule called **ribulose biphosphate (RuBP)**, forming a 6-carbon molecule. This reaction is catalysed by an enzyme called **Rubisco**.
- The 6C molecule is unstable and immediately breaks down to form two 3-carbon compounds called **glycerate-3-phosphate (GP)**.

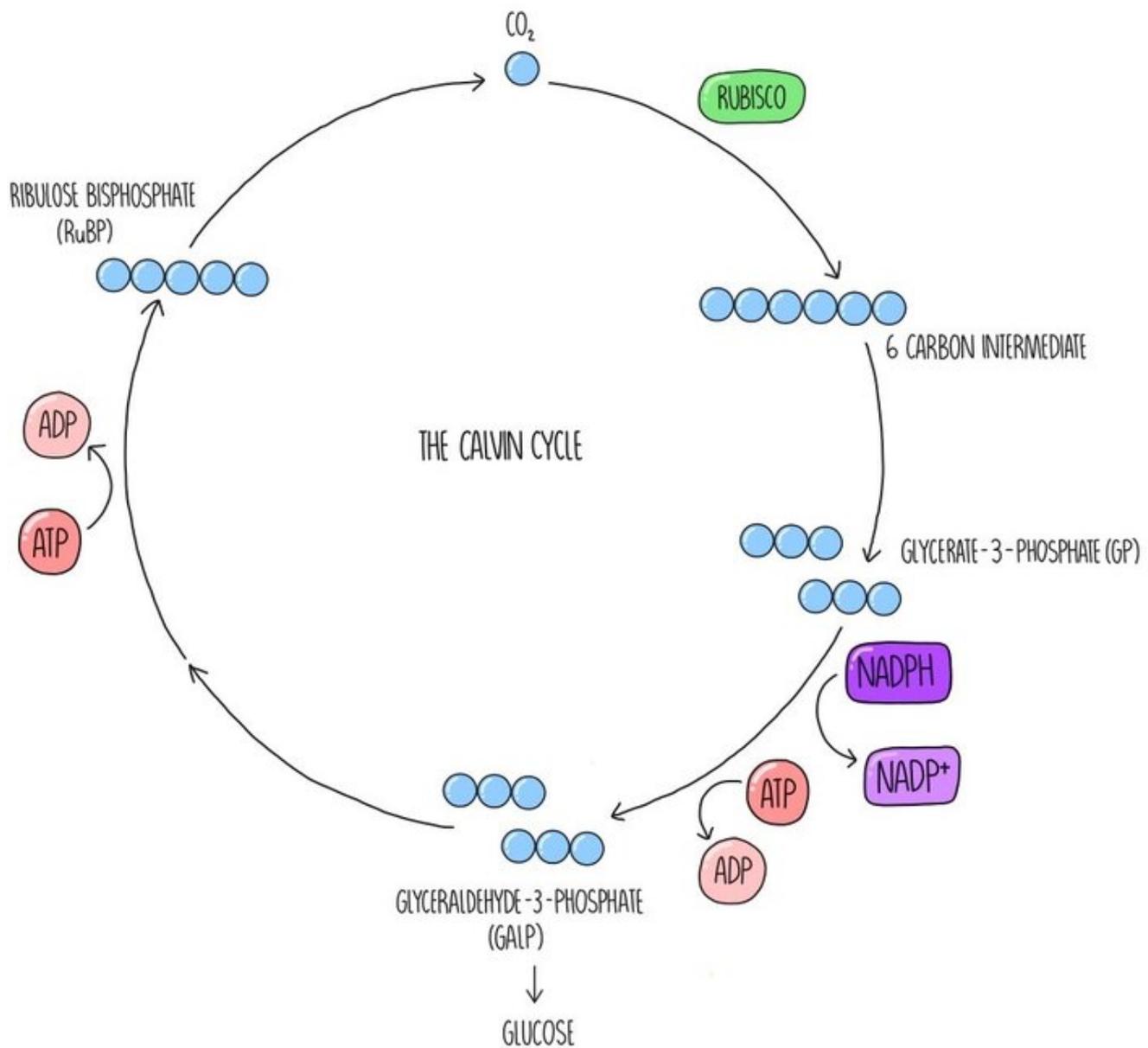
Reduction

- An isomerisation reaction occurs which converts GP into a different 3-carbon compound called **glyceraldehyde-3-phosphate (GALP)**. GALP is also known as **triose phosphate (TP)**. This reaction requires energy so **ATP** (from the light-dependent reaction) is hydrolysed into ADP.
- This reaction also requires electrons from the electron carrier reduced NADP (also from the LDR). **Reduced NADP transfers electrons** to GP, reducing it to GALP.

- Some GALP is **converted into organic molecules**, such as glucose, but some will be used to **regenerate RuBP**. For every 6 molecules of GALP, 1 is used to produce organic molecules whereas 5 will be used for RuBP regeneration.

Regeneration

- GALP is **converted back into RuBP** - this process requires energy which is generated by **ATP hydrolysis**.
- The cycle is completed and another round of carbon fixation can take place.



Synthesis of organic substances

GP and GALP (aka TP) are used to make all of the biological molecules that a plant needs to grow:

- **Glucose** is made by joining **two GALP molecules** together. The glucose can then be used to build polysaccharides like starch and cellulose.
- **Amino acids** are made from **GP**.
- **Glycerol** is made from **GALP** and **fatty acids** are made from **GP**. Glycerol and fatty acids are joined by ester bonds to form triglycerides.

Optimum conditions for photosynthesis

Light

- **High light intensity** means that the light-dependent reaction can work faster. So the more light, the more photosynthesis.
- But it needs to be the **right wavelength** – in the red or blue part of the spectrum (chlorophyll reflects any light in the green part of the visible spectrum).
- Gardeners grow plants in **transparent greenhouses or polytunnels** which let in light. They may also use **lamps** to provide light at night.

Temperature

- Temperatures around 25°C allow **photosynthetic enzymes** to work quickly. At lower temperatures, enzymes become inactive and at higher temperature they can denature.
- At high temperatures, the **stomata** will also **close** to conserve water. This stops gas exchange and reduces the rate of photosynthesis.
- Greenhouses **trap heat energy** from sunlight. Fancier greenhouses will have **heaters, cooling systems and air circulation systems** to ensure an optimum temperature is maintained year-round.

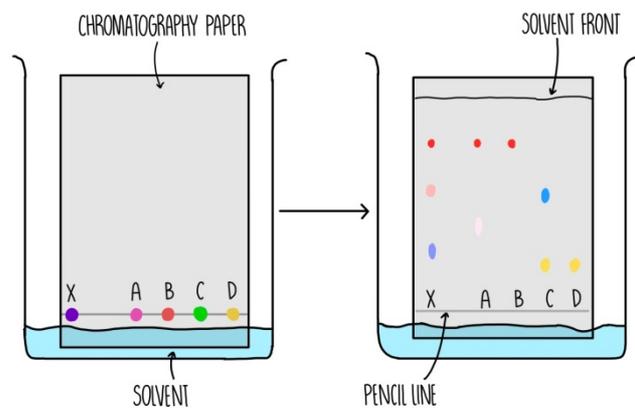
Carbon dioxide concentration

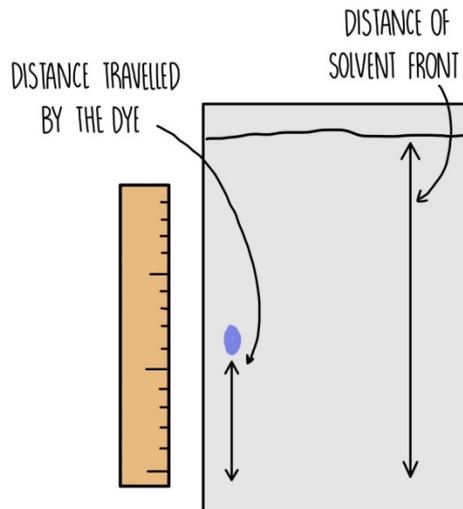
- Atmospheric carbon dioxide concentration is around 0.04%. Concentrations ten times higher (**0.4%**) can maximise photosynthesis.
- Concentrations above 0.4% can cause **stomatal closure** and a reduction in photosynthesis.
- Gardeners can add carbon dioxide to the greenhouse by **burning propane**.

Investigating leaf pigments using chromatography

Leaves of a plant may contain several types of photosynthetic pigment, each of which absorbs light at a specific wavelength. Other, non-photosynthetic pigments may be present and perform functions such as protecting the plant from UV radiation. You can investigate which pigments are present using thin-layer chromatography:

- Extract pigment from the leaves of a plant – **grind** up the leaves with **anhydrous sodium sulfate** then add a few drops of **propanone**.
- Transfer to a test tube and shake with **petroleum ether**. You should get two separate layers – the top layer contains the pigments. Transfer this top layer into a test tube containing anhydrous sodium sulfate.
- Place drops of the extract on a pencil line drawn along the bottom of the **TLC plate**. This is the **stationary phase**, made of glass containing a thin layer of silica gel.
- The TLC plate is placed in a tank containing a **solvent** (the **mobile phase**). You can use a mixture of propanone, cyclohexane and petroleum ether as the solvent.
- Place a lid on the tank.
- As the solvent moves upwards through the gel, the pigments dissolve in the solvent and are carried up with it.
- The **more soluble** the pigment, the **further it will travel** up the stationary phase. Since different pigments have different solubilities, they separate out. Draw a line at the point on the TLC plate where the solvent has reached – this is the **solvent front**.
- You can identify each pigment by calculating the **R_f value** and looking it up in a database.





$$R_f \text{ VALUE} = \frac{\text{DISTANCE TRAVELLED BY THE DYE}}{\text{DISTANCE TRAVELLED BY THE SOLVENT}}$$

This experimental set-up can be used to compare the pigments found in shady vs sunny plants. You'd expect to find that plants adapted to grow in the shade have a larger range of pigments so they can absorb as many wavelengths of the limited light available to it.

Investigating photosynthesis using chloroplast extract

This practical measures the rate of the **light-dependent reaction** by investigating how fast an electron is transferred from the electron transport chain to NADP. The reduction of NADP is catalysed by the **dehydrogenase enzyme**. It uses **DCPIP**, a redox indicator dye, which changes colour (from blue to colourless) when it accepts an electron.

Method:

- Remove 2-3 leaves from a plant and add cold isolation solution. Grind the leaves into the solution using a pestle and mortar.
- Filter to remove the leafy pieces and centrifuge the filtrate at high speed for 10 minutes.
- Pour off the liquid so that you just have the solid pellet. Re-suspend in cold isolation solution to form the chloroplast extract. Store on ice until ready to use.
- Zero a colorimeter using a cuvette just containing chloroplast extract and distilled water.
- Add a certain volume of chloroplast extract to a series of test tubes. Place a lamp at a certain distance from the tubes.
- Add a certain volume of DCPIP to each test tube and mix.
- Straight away, take a sample from the mixture and transfer into a new cuvette. Record its absorbance using the colorimeter. Repeat every 2 minutes for the next 10 minutes.
- Repeat two more times and calculate a mean absorbance for each time interval.
- **Control tubes:**
 - DCPIP + isolation solution – shows that the chloroplasts are responsible for the colour change
 - DCPIP + chloroplast extract with tube wrapped in tin foil – shows that light needs to be present to cause the colour change
- **Results:** DCPIP loses colour as the reaction progresses. This experiment can be adapted to determine the effect of light intensity. The closer the lamp to the test tubes, the quicker DCPIP loses colour.
- **Control variables:** concentration and volume of DCPIP, temperature, volume of chloroplast extract