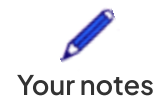




# HL IB Biology



## Photosynthesis

### Contents

- \* The Process of Photosynthesis
- \* Separating Photosynthetic Pigments: Skills
- \* Absorption Spectra
- \* Absorption & Action Spectra: Skills
- \* Limiting Factors of Photosynthesis: Skills
- \* Carbon Dioxide Enrichment Experiments
- \* Photosystems (HL)
- \* Light Dependent Reactions (HL)
- \* Photophosphorylation (HL)
- \* Light Independent Reactions (HL)
- \* Interdependence of Photosynthetic Reactions (HL)

## The Process of Photosynthesis



Your notes

### Transformation of Light Energy During Photosynthesis

- **Simple, inorganic compounds** are converted into complex organic ones by photosynthesis
  - The energy required is provided by **light**
- Photosynthesis occurs in autotrophic organisms such as **plants, algae** and **cyanobacteria**
- Photosynthesis is a form of **energy conversion**, from **light energy** to **chemical energy**, stored in biomass
- **Energy is stored** within the bonds of these organic compounds and provides most of the **chemical energy** needed for **life processes** in ecosystems

#### Examiner Tip

Remember, energy is never created or destroyed; it is only ever converted from one form to another!

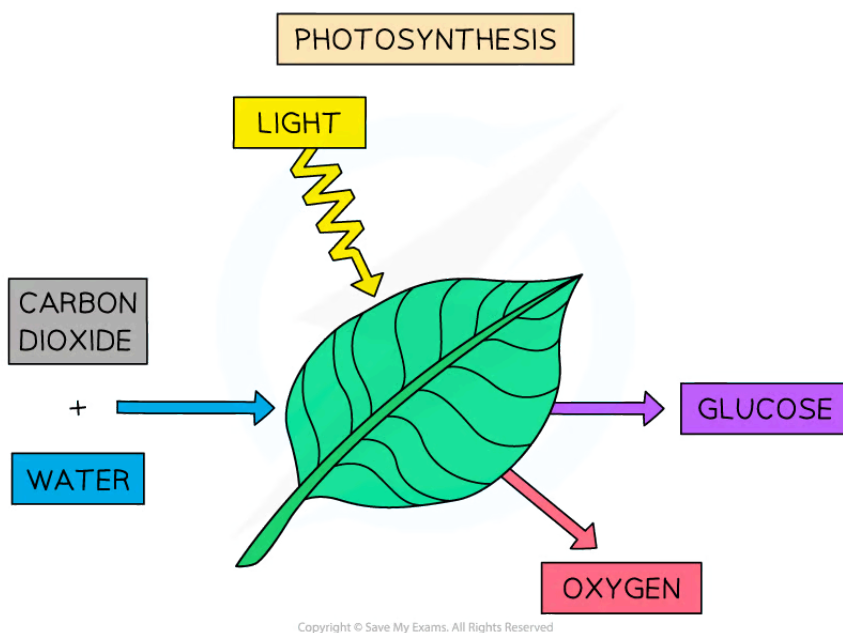


Your notes

## Conversion of Carbon Dioxide to Glucose

- During photosynthesis, **carbon dioxide** is converted to **glucose** using hydrogen released when a water molecule is split
  - Oxygen is released as a waste product
- The **reactants** of photosynthesis are **carbon dioxide** and **water**
- The **products** of photosynthesis are **glucose** and **oxygen**

### Reactants and products of photosynthesis diagram



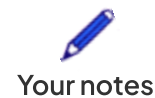
### Photosynthesis as it takes place in a leaf

- We can represent this chemical reaction in a word equation

### Photosynthesis word equation



### A word equation to represent photosynthesis



### 💡 Examiner Tip

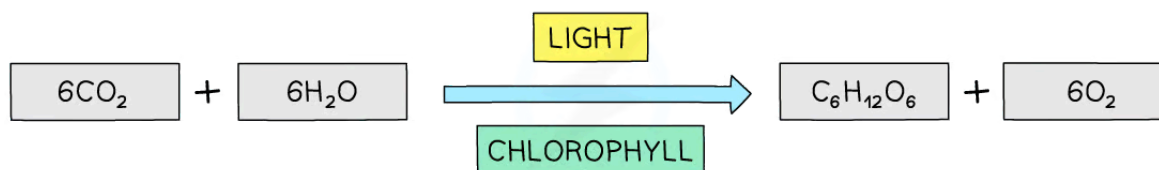
The glucose and oxygen formed during photosynthesis are the reactants of aerobic cell respiration while carbon dioxide and water released during respiration are used as the reactants of photosynthesis

- Respiration is the process by which **energy is released** from organic molecules in living cells

## Release of Oxygen

- Photosynthesis is carried out in **plants, algae and cyanobacteria**
- The **oxygen** that is released comes from the **water splitting** process which also provides **hydrogen** to allow the synthesis of **glucose**
- The paths of the oxygen and hydrogen can be seen more clearly when looking at the **chemical symbol equation** for photosynthesis

### Chemical symbol equation for photosynthesis



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### 💡 Examiner Tip

Note that you are only expected to know the word equation for photosynthesis for exam purposes



Your notes

## Separating Photosynthetic Pigments: Skills

### Separating Photosynthetic Pigments: Skills

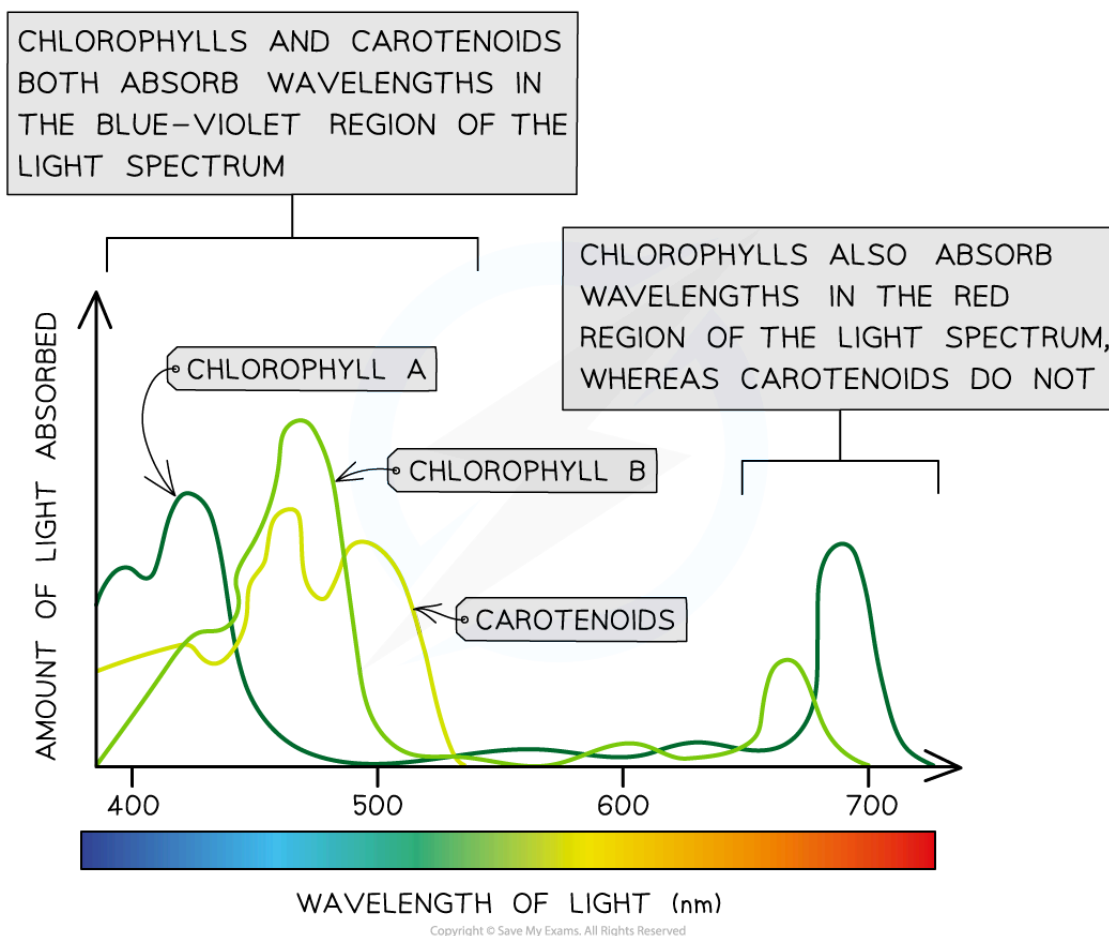
#### Separation of photosynthetic pigments by chromatography

- Plants contain several different **photosynthetic pigments**, which **absorb different wavelengths of light**
- There are **two groups** of pigments: **chlorophylls** and **carotenoids**
- Carotenoids surround the chlorophyll and absorb both similar and different wavelengths of light to chlorophyll
  - This **expands the range of wavelengths** that can be absorbed from light for use in photosynthesis

**Chloroplast Pigments Table**

Pigment group	Name of pigment	Colour of pigment
Chlorophylls	Chlorophyll a Chlorophyll b	Blue- green Yellow - green
Carotenoids	$\beta$ carotene Xanthophyll	Orange Yellow

- Chlorophylls** absorb wavelengths in the **blue-violet and red regions** of the light spectrum
  - They reflect green light, causing plants to appear green
- Carotenoids** absorb wavelengths of light mainly in the **blue-violet region** of the spectrum



**Chlorophyll and carotenoids absorb light across the visible light spectrum to use in the light-dependent reaction of photosynthesis**

## Chromatography

- Chromatography is an experimental technique that is used to **separate mixtures**
  - Different components** within the mixture travel through the material at **different speeds**
  - This causes the different components to **separate**
  - A retardation factor ( **$R_f$  value**) can be calculated for each component of the mixture
- Two of the most common techniques for separating these photosynthetic pigments are:
  - Paper chromatography** - the mixture of pigments is passed through paper (cellulose)
  - Thin-layer chromatography (TLC)** - the mixture of pigments is passed through a thin layer of adsorbent (eg. silica gel), through which the mixture travels faster and separates more distinctly
- Paper chromatography can be used to separate photosynthetic pigments although **TLC gives better results**

## Apparatus



Your notes

- Leaf sample
- Distilled water
- Pestle and mortar
- Filter paper
- Capillary tube
- Chromatography solvent
- Propanone
- Pencil
- Ruler

## Method

- Draw a straight line in pencil approximately 1cm above the bottom of the filter paper being used
  - **Do not use a pen** as the ink will separate into pigments within the experiment and obscure the results
- Cut a section of leaf and place it in a mortar
  - It is important to **choose a healthy leaf** that has been in direct sunlight so you can be sure it contains many active photosynthetic cells
- Add 20 drops of propanone and use the pestle to grind up the leaf sample and release the pigments
  - Propanone is an organic solvent and therefore fats, such as the lipid membrane, dissolve in it
  - The combination of propanone and mechanical pressure breaks down the cell and chloroplasts to **release the pigments**
- Extract some of the pigment using a capillary tube and spot it onto the centre of the pencil line you have drawn
- Suspend the paper in the chromatography solvent so that **the level of the solvent is below the pencil line** and leave the paper until the solvent has reached the top of the paper
  - The mixture is **dissolved** in the **solvent** (called the mobile phase) and the dissolved mixture then passes through a static material (called the stationary phase)
- Remove the paper from the solvent and draw a pencil line marking where the solvent moved up to
  - The pigment should have separated out and there should be different spots on the paper at different heights above the pencil line, these are the separate pigments
- Calculate the  $R_f$  value for each spot

$$R_f \text{ value} = \frac{\text{distance travelled by component (pigment)}}{\text{distance travelled by the solvent}}$$

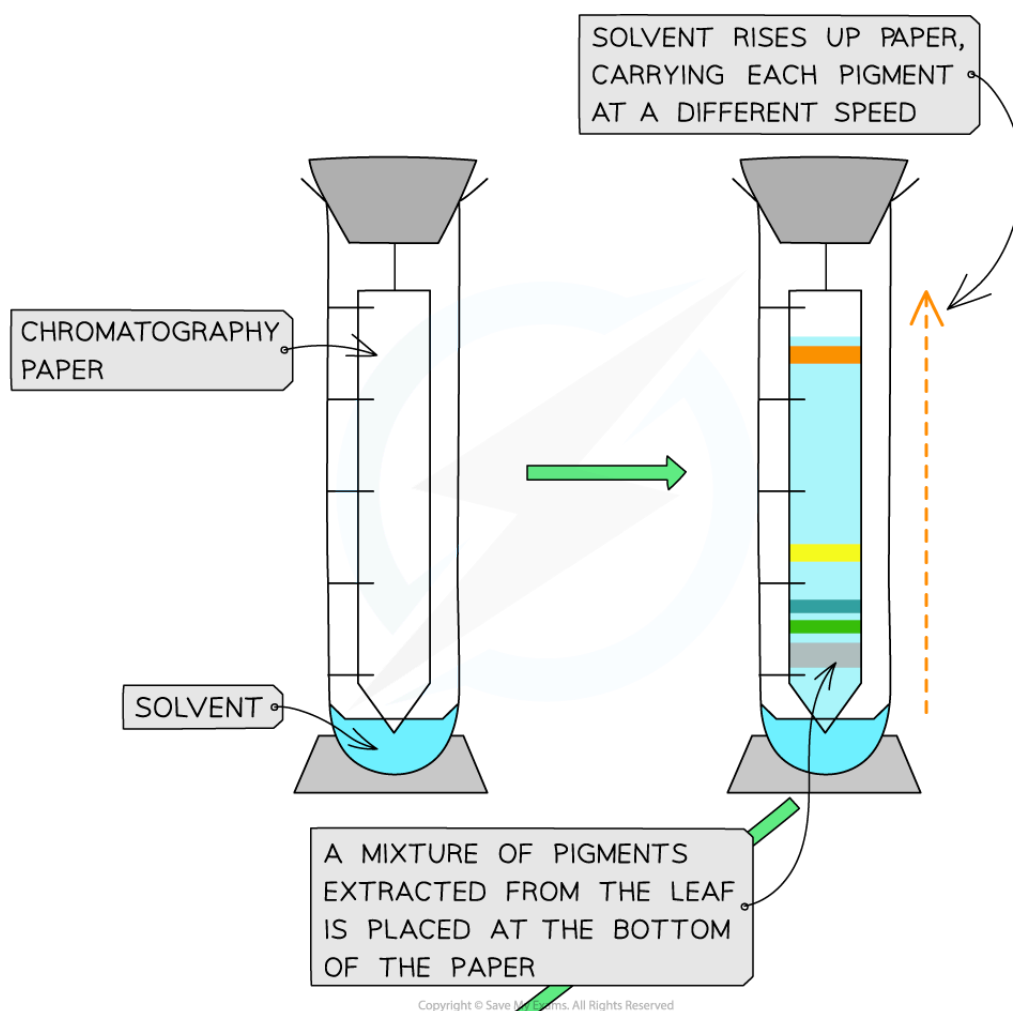
- Always measure to the centre of each spot

## Results

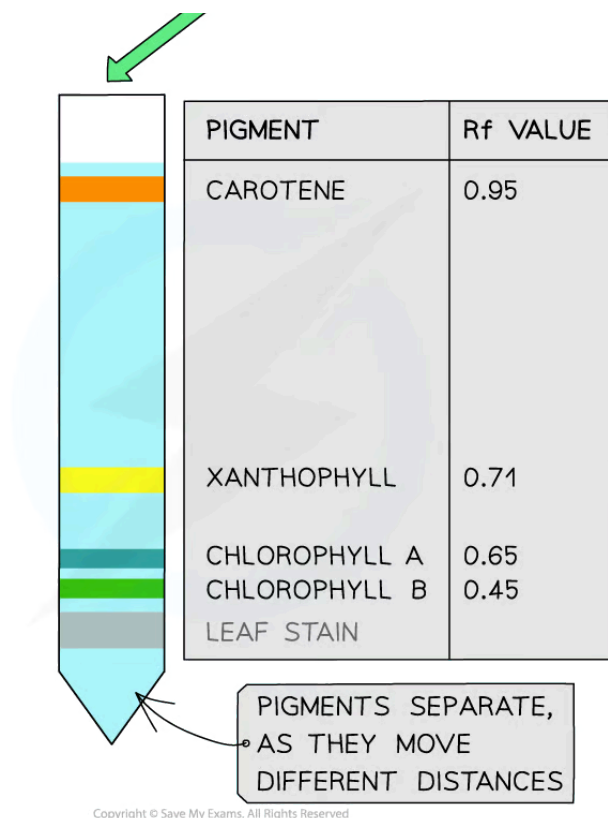


Your notes

- Chromatography can be used to **separate and identify chloroplast pigments** that have been extracted from a leaf as each pigment will have a unique  $R_f$  value
- The  $R_f$  value demonstrates how far a dissolved pigment travels through the stationary phase
  - Molecules with a higher affinity to the stationary phase, such as large molecules, will travel slower and therefore have a **smaller  $R_f$  value**
  - Molecules that are more soluble in the mobile phase will travel faster and therefore have a **larger  $R_f$  value**
- Although specific  $R_f$  values depend on the solvent that is being used, in general:
  - Carotenoids** have the **highest  $R_f$  values** (usually close to 1)
  - Chlorophyll b** has a **much lower  $R_f$  value**
  - Chlorophyll a** has an  $R_f$  value somewhere **between** those of carotenoids and chlorophyll b
  - Small  $R_f$  values** indicate the pigment is **less soluble** and/or **larger** in size







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**Paper chromatography is used to separate photosynthetic pigments. These pigments can be identified by their  $R_f$  values. In this example, a line of the mixture (rather than a spot) is added to the paper.**

### Limitations

- Paper chromatography is not as specific as other chromatography techniques
  - It is sufficient to separate and distinguish different pigments and to calculate their  $R_f$  value
- Chromatography does not give data on the amount of each pigment present or the wavelengths that they absorb
  - Colorimetry can be used to calculate these values

### Examiner Tip

Remember – the pigments themselves have colour (as described in the table). This is different from the colours of light that they *absorb*. You don't have to remember specific  $R_f$  values, just know that they differ between each type of pigment.

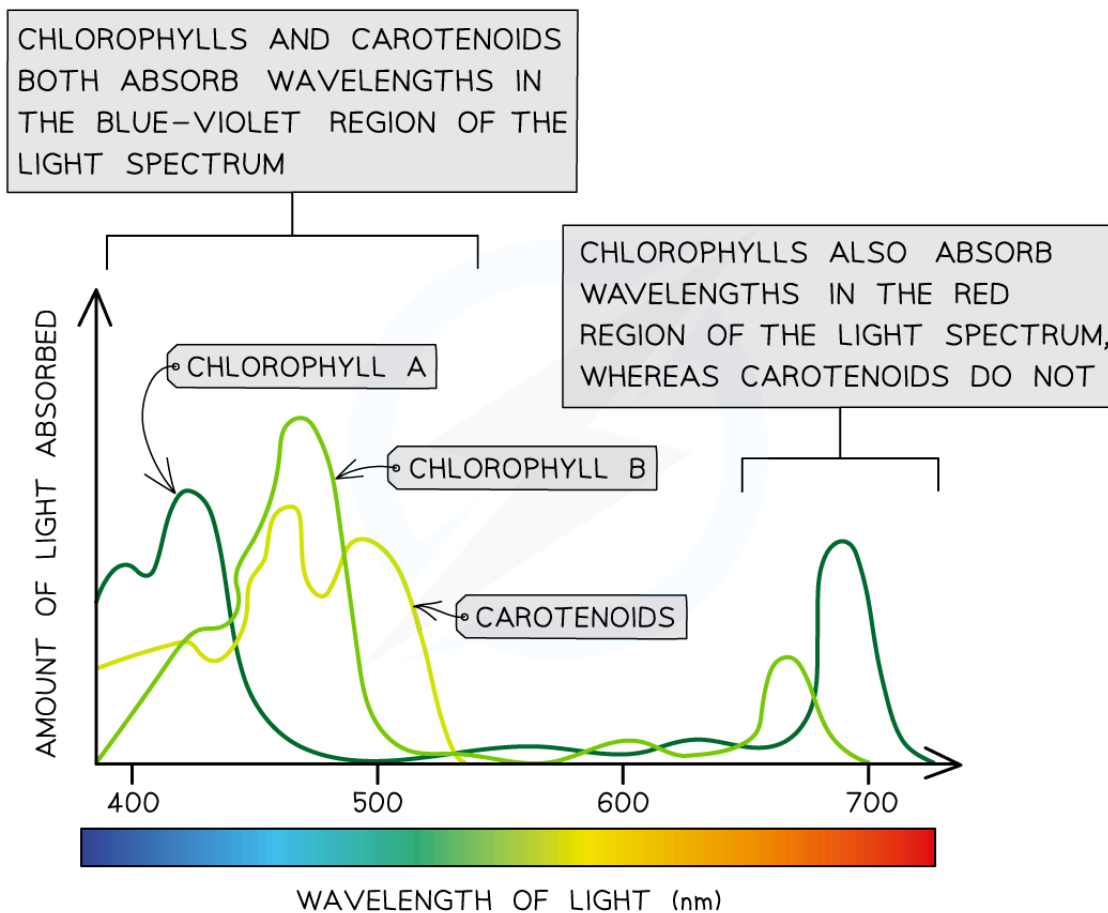


Your notes

## Absorption Spectra

### Absorption Spectra

- Light is made up of a mixture of all the **visible wavelengths** to include red, orange, yellow, green, blue, indigo and violet
- An **absorption spectrum** is a graph that shows the **absorbance** of different wavelengths of light by a particular pigment in the chlorophyll
- Within the chlorophyll, light energy results in the **excitation of electrons** which triggers transfer of electrons leading to a series of reactions which make up the process of **photosynthesis**
  - During photosynthesis, **light energy is transformed to chemical energy** when glucose is formed
- Chlorophylls** absorb wavelengths in the **blue-violet and red regions** of the light spectrum
- Carotenoids** absorb wavelengths of light mainly in the **blue-violet region** of the spectrum
- The chemical structure of these molecules determines the wavelengths of light that can be absorbed
- The green part of the spectrum is largely **reflected** from the leaf and this is why leaves usually appear green



*Absorption spectra of chlorophyll A, chlorophyll B and carotenoid pigments*



Your notes



Your notes

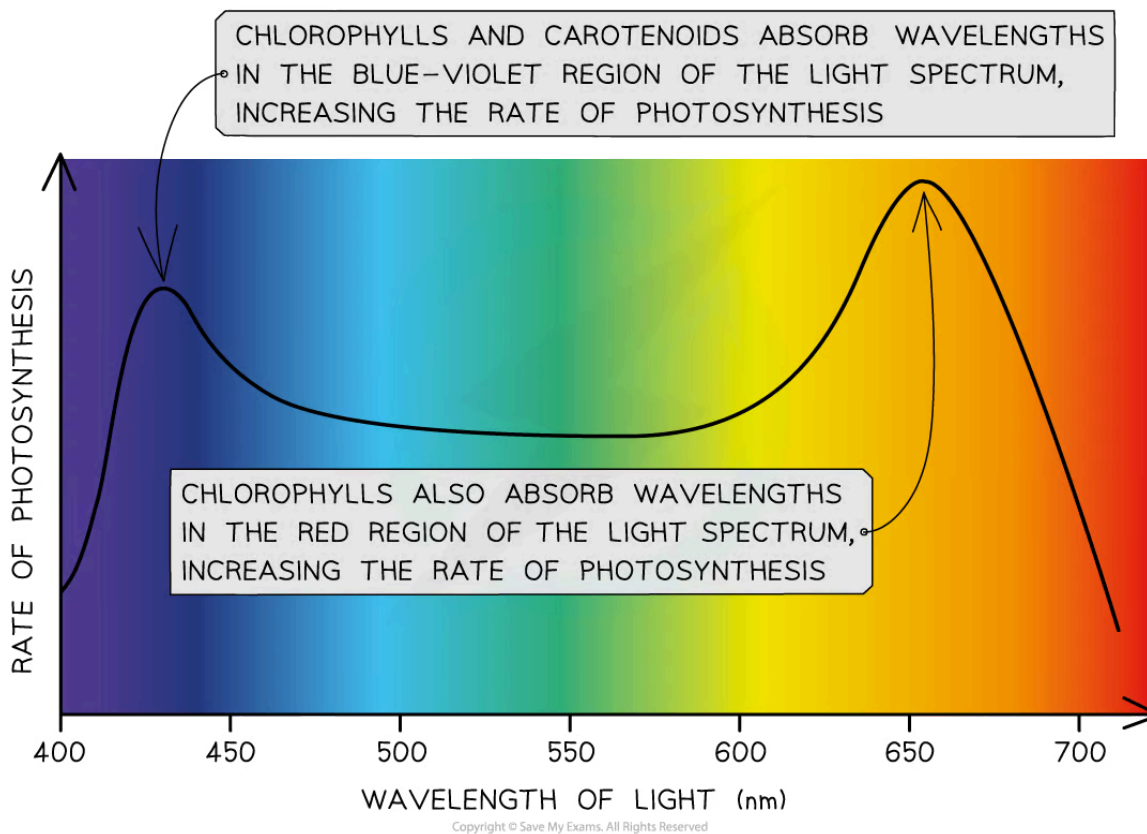
## Absorption & Action Spectra: Skills

### Comparing Absorption & Action Spectra

#### What is an action spectrum?

- An **action spectrum** is a graph that shows the **rate of photosynthesis** at different wavelengths of light
- The rate of photosynthesis is **highest** at the **blue-violet** and **red** regions of the light spectrum, as these are the wavelengths of light that plants can **absorb** (i.e. the wavelengths of light that chlorophylls and carotenoids can absorb)

#### Diagram to show the action spectrum of chlorophyll pigments



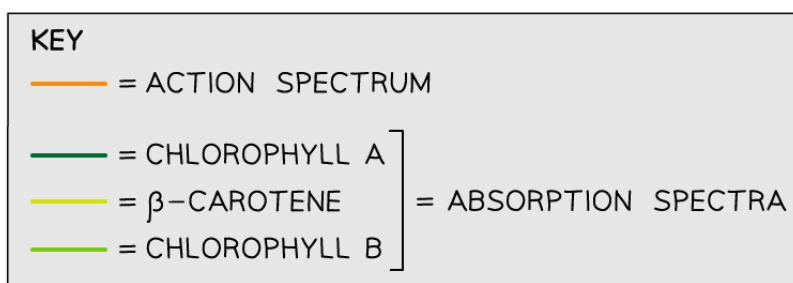
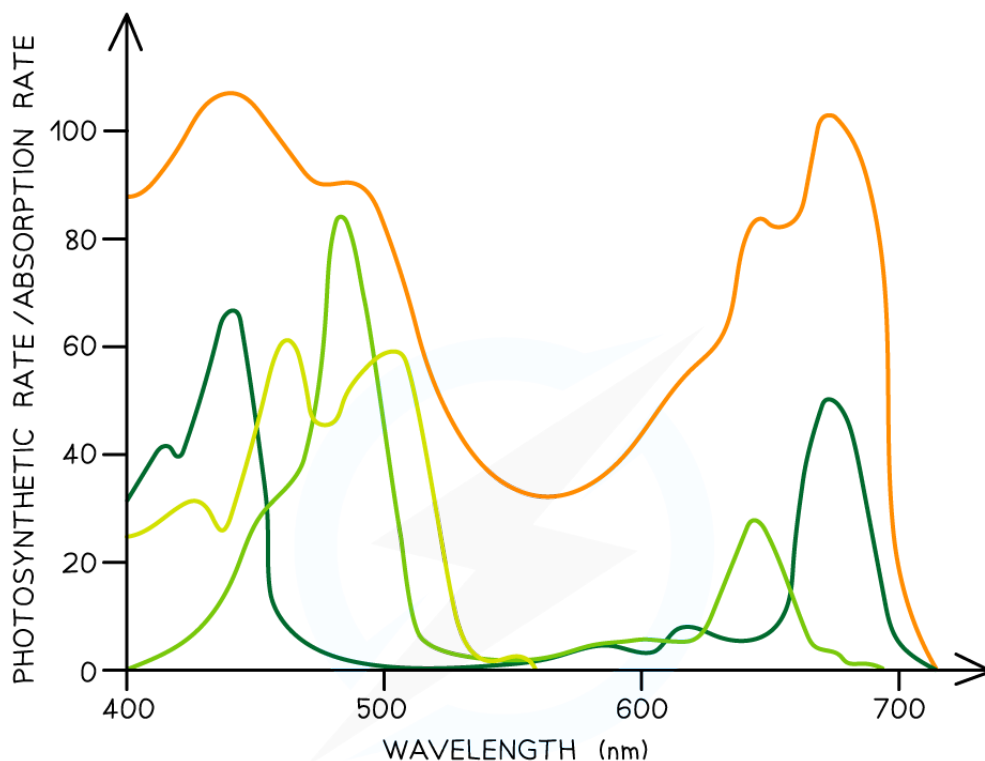
The photosynthetic action spectrum shows the rate of photosynthesis at different wavelengths of light

#### Comparing action and absorption spectra

- There is a strong **correlation** between the cumulative absorption spectra of all pigments and the action spectrum:

- Both graphs have **two main peaks** – at the **blue-violet** region and the **red** region of the light spectrum which supports the idea that the **most light energy** is absorbed at these wavelengths leading to the fastest rate of photosynthesis
- Both graphs have a **trough** in the **green-yellow** region of the light spectrum which supports the idea that the **least light energy** is absorbed at these wavelengths leading to the slowest rate of photosynthesis

**Diagram to show the correlation between action and absorption spectra**



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*An overlay of the photosynthetic absorption and action spectra shows there is a strong cumulative correlation*

**Determining the rate of photosynthesis**



Your notes

- The rate of photosynthesis can be determined by measuring the **volume of oxygen produced** or the **carbon dioxide consumption** at different wavelengths of light
- An experiment can be set up similar to the one [investigating the effect of light intensity](#) on photosynthesis
  - Remember that the lamp should be **kept the same distance** from the pondweed as we are investigating the effect of different wavelengths of light only
  - Place different colour filters (covering the full light spectrum) in front of the lamp to change the colour of light the pondweed is exposed to
  - Measure the volume of oxygen produced or the number of bubbles released from the pondweed per minute for each colour
    - Include an experiment with no filter in front of the lamp to investigate the effect of white light on the rate of photosynthesis
  - Repeat the experiment several times to obtain reliable results

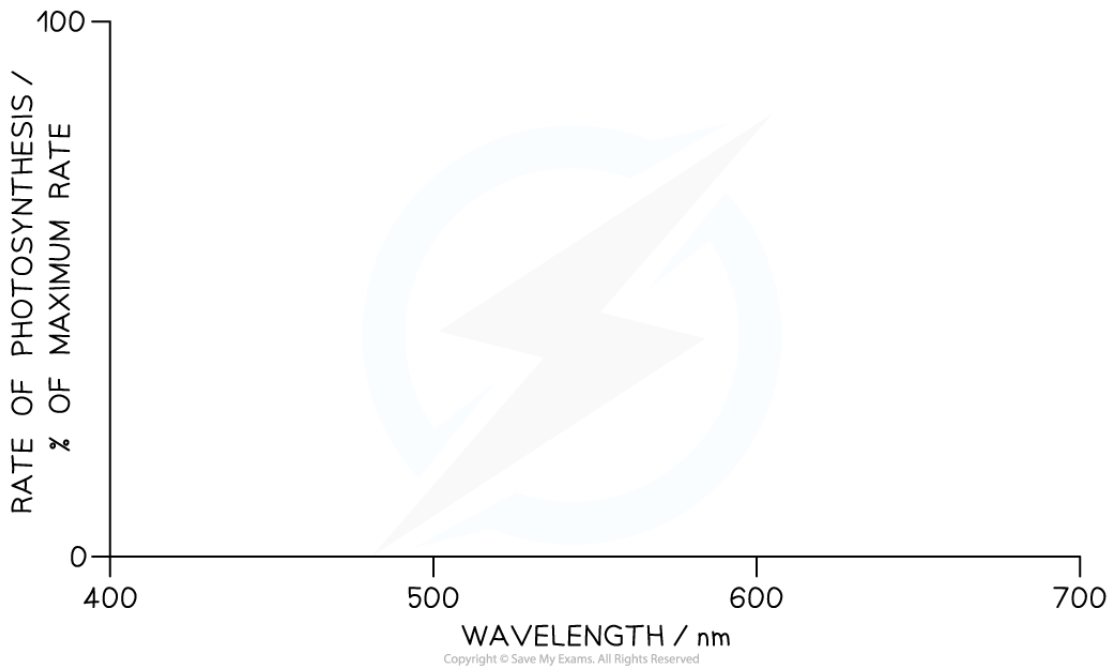
## Drawing an action spectrum for photosynthesis

### Step 1: Draw and label the axes

- Draw an x-axis
- Label the axis **wavelength**
- Add the units / **nm**
- Make 400 the smallest value and 700 the largest value
  - Label 500 and 600 nm on the x-axis
- Draw a y-axis
- Label it **Rate of photosynthesis / % of maximum rate**
- Make 0 the lowest value and 100 the highest value
  - No units are required because the y-axis is showing a percentage scale



Your notes



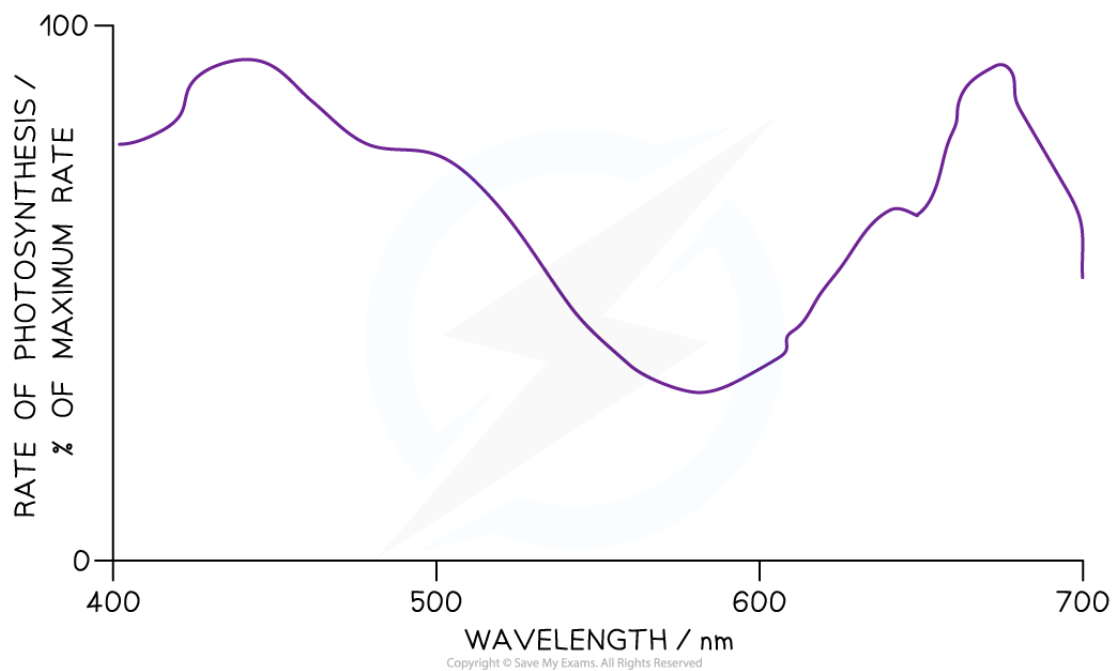
**Step 1: Draw and label the axes**

**Step 2: Draw the plot**

- There should be **two peaks of rate of photosynthesis**
  - One peak at either end, in the blue and red areas of the spectrum
  - And a **trough** in the middle, which represents green light
  - As below, with a smooth curve



Your notes



**Step 2: Sketch the Curve. An action spectrum for photosynthesis (colour range labels are not required)**

### Examiner Tip

Remember - the pigments themselves have a distinctive colour. This is different from the colours of light that they *absorb*. Key points to remember:

1. Label 400 - 700nm on the x-axis, in 100nm increments
2. Use a % scale on the y-axis
3. Smooth curve
4. Peaks at either end
5. Trough in the middle for green light





Your notes

## Limiting Factors of Photosynthesis: Skills

### Limiting Factors

- An **aquatic plant** such as *Elodea* or *Cabomba* is a good choice for investigating photosynthesis in plants, because the rate of photosynthesis can be measured by **counting oxygen bubbles** that come off a cutting of this plant
  - Oxygen output from terrestrial plants (that grow on land) would not be observable

### NOS: Hypotheses are provisional explanations that require repeated testing

- A hypothesis is a proposed explanation for an idea which may be true or false
- In an investigation the hypothesis can be tested through observations or experiments to provide either support or opposition to the proposed hypothesis
- The following investigation looks at the effect of limiting factors on the rate of photosynthesis
- A suggested **hypothesis** for an investigation into the effect of light intensity on photosynthesis could be:
  - **Light intensity will have an effect on the rate of photosynthesis**

### Identifying the variables in an investigation

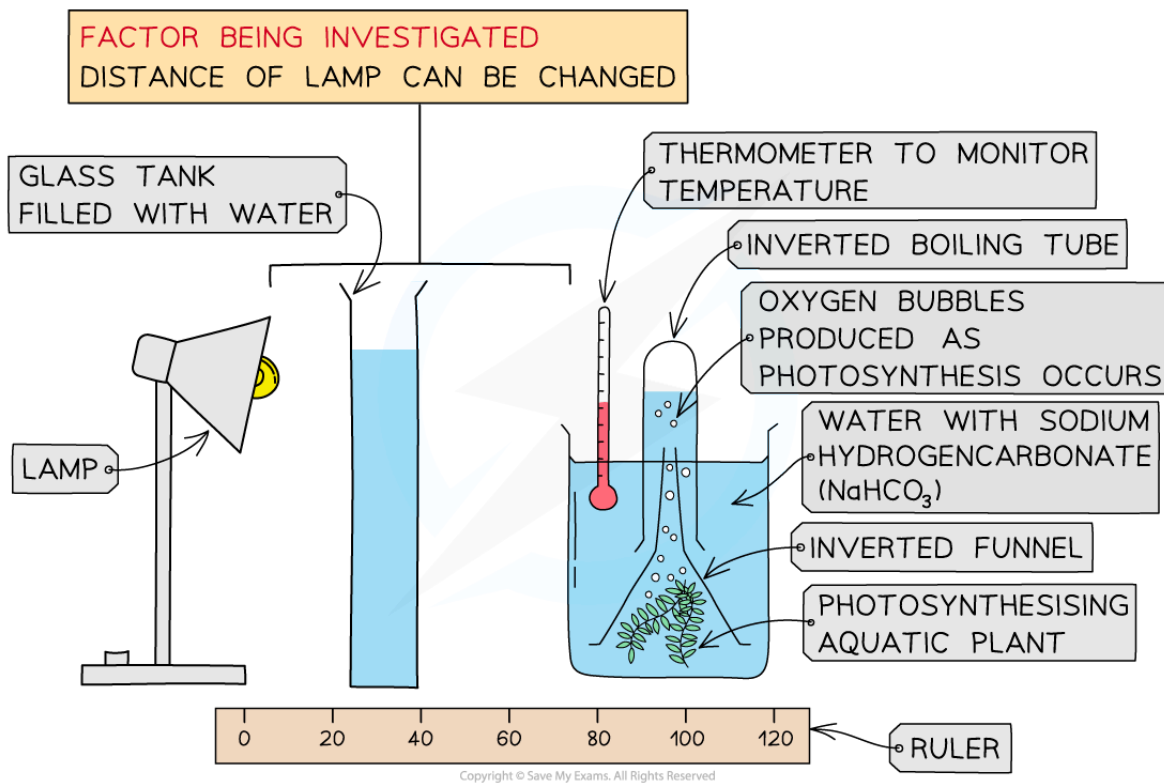
- When designing an experiment it is crucial that all variables (apart from the independent and dependent variables being investigated) are controlled
  - The independent variable is the factor that is **deliberately manipulated** between a specific range throughout the experiment
  - The dependent variable is the factor that is **measured** during the experiment (to see if it is affected by the changes to the independent variable)
  - Other variables must be **controlled** so that it can be said the independent variable is the only factor affecting the dependent variable during the experiment
- **Changes in light intensity, carbon dioxide concentration and temperature** are all limiting factors that affect the rate of photosynthesis and **can be altered experimentally** to measure the effect on the rate of photosynthesis
  - Any of these limiting factors could be selected as the **independent variable** in the investigation

### Effect of light intensity – experimental design

- Basic Experimental Setup
  - Aquatic plant cutting in water
  - Powdered sodium hydrogencarbonate ( $\text{NaHCO}_3$ )
  - Glass funnel
  - Boiling tube
  - Lamp for illumination
  - Glass tank filled with water



Your notes



### Measuring the effect of light intensity on the rate of photosynthesis in pondweed

#### Research Question

Does the rate of photosynthesis (number of bubbles released per min) of *Elodea* increase as the light intensity increases?

#### Method

- Place a piece of aquatic plant (*Elodea* or *Cabomba* are often used), into a beaker of water
- Place a lamp a set distance from the plant
- Record the number of bubbles observed in three minutes
- Repeat these steps for different distances between the lamp and plant

#### Improvements

- Use a **gas syringe** to collect and measure the volume of gas produced
- For **reliability** of data, **repeat** the experiment at least twice for each distance and calculate the mean number of bubbles
- Use of a **data logger** to measure results continuously

#### Variables to Be Controlled

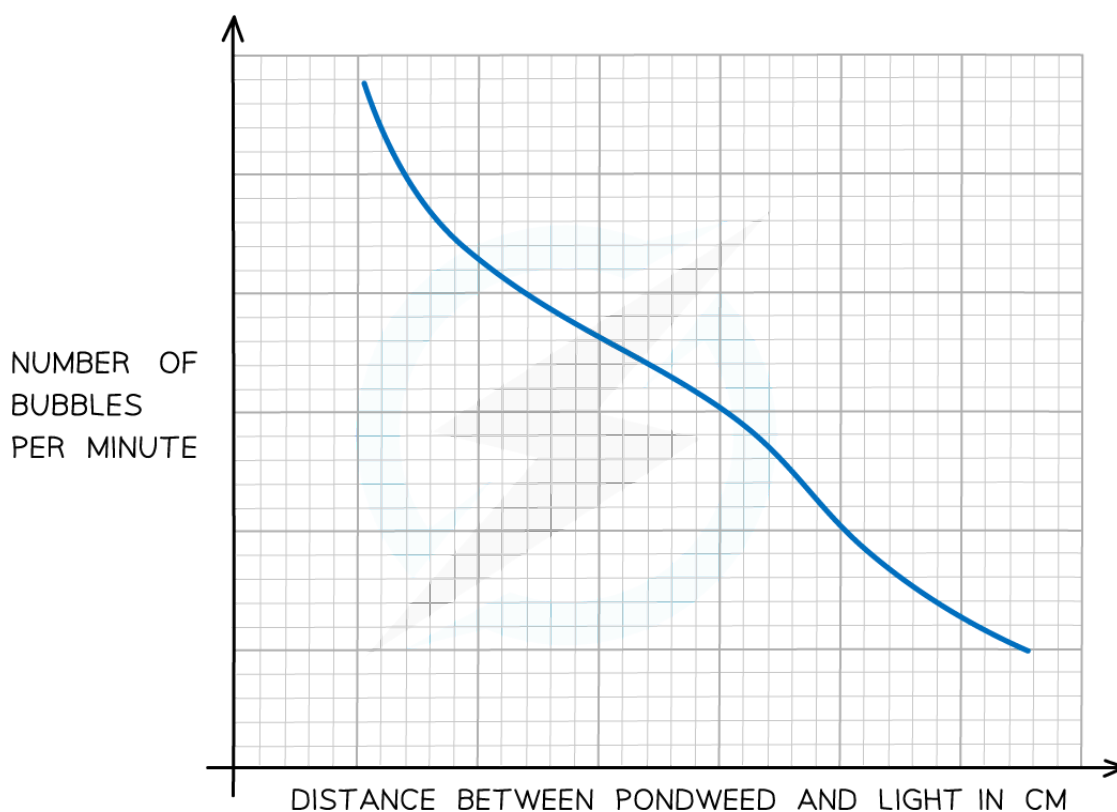


Your notes

- **Temperature**
  - The glass tank filled with water absorbs any heat that is emitted from the lamp
  - Modern LED bulbs can be used as they give off less heat than filament bulbs
- **CO<sub>2</sub> concentration**
  - The water used around the plant is first **boiled and re-cooled** to remove any dissolved carbon dioxide
  - A **set mass of sodium hydrogencarbonate** is added to the water that surrounds the plant to make the concentration approx.  $0.1 \text{ mol dm}^{-3}$ 
    - This will ensure that the carbon dioxide concentration is not limiting the rate of photosynthesis

## Results

- A graph of the **number of bubbles produced per minute** against the **distance between the lamp and the plant** used can be drawn to see the pattern or trend
  - Distance between the lamp and the plant is linked to the light intensity



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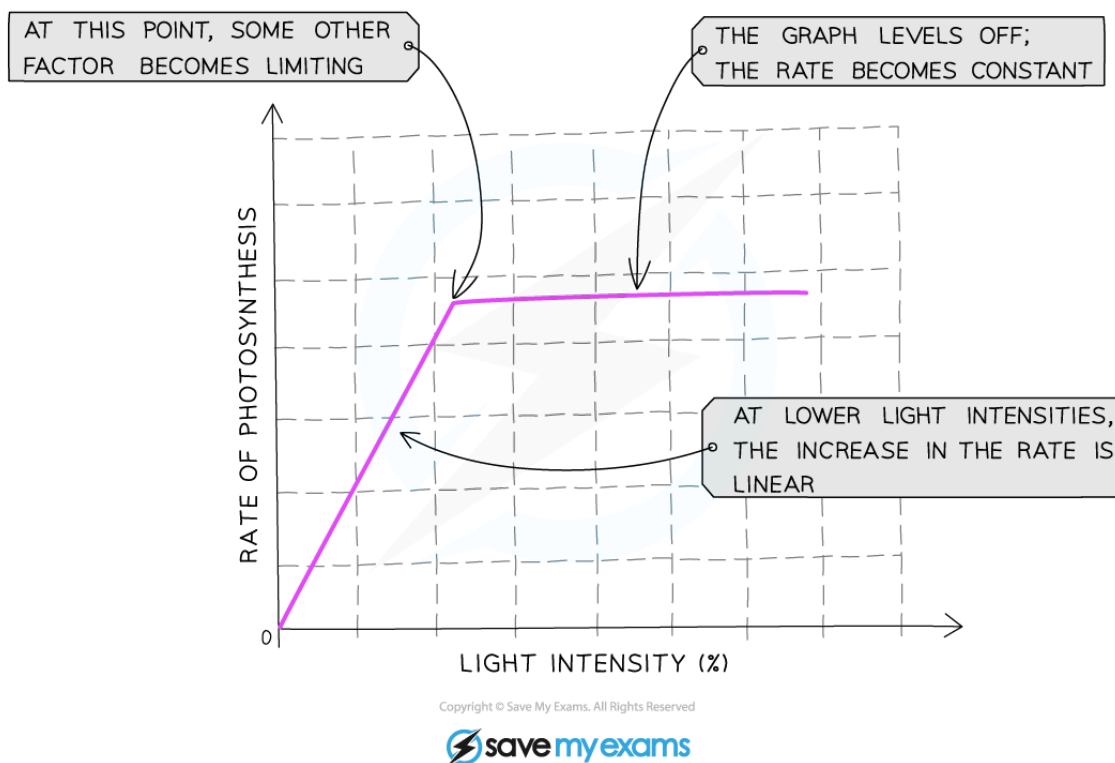


*A graph of distance from the lamp against number of bubbles per minute*



Your notes

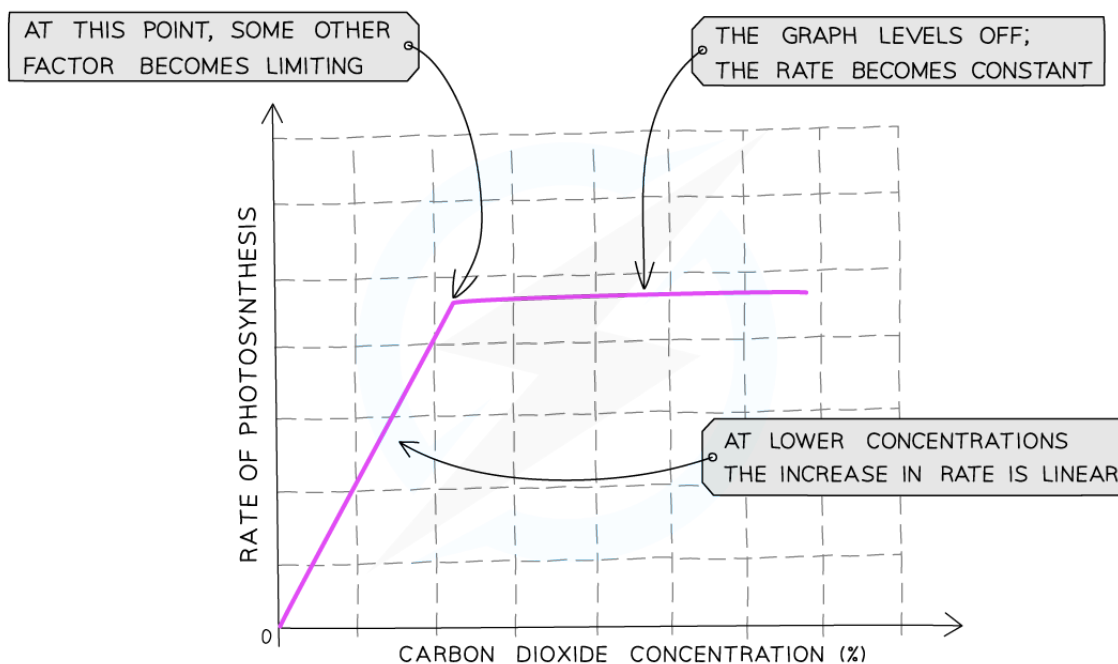
- A graph can also be drawn showing the effect of different light intensities on the rate of photosynthesis
- It can be seen that:
  - As light intensity increases so too does the rate of photosynthesis (**positive correlation**)
    - At this stage light intensity is the limiting factor
  - At some point, there will be no further increase in the rate of photosynthesis if the light intensity is increased
    - Now temperature or carbon dioxide concentration may be limiting factors



*The effect of light intensity on the rate of photosynthesis*

## Carbon dioxide concentration

- The **same basic experimental setup** can be used, but with varying use of the following variables
  - Start with boiled and re-cooled water as before
  - Add **successive masses of sodium hydrogencarbonate** to increase the concentration in increments of  $0.01 \text{ mol dm}^{-3}$ , and record the rate of photosynthesis in bubbles  $\text{minute}^{-1}$
  - **Keep the temperature constant** at  $25^\circ\text{C}$  using a water bath, monitoring with a thermometer in the water surrounding the aquatic plant
  - **Keep the light intensity constant** by keeping the lamp a fixed distance from the plant
- A graph of the effect of carbon dioxide concentration against the rate of photosynthesis shows a similar trend to what was observed with light intensity



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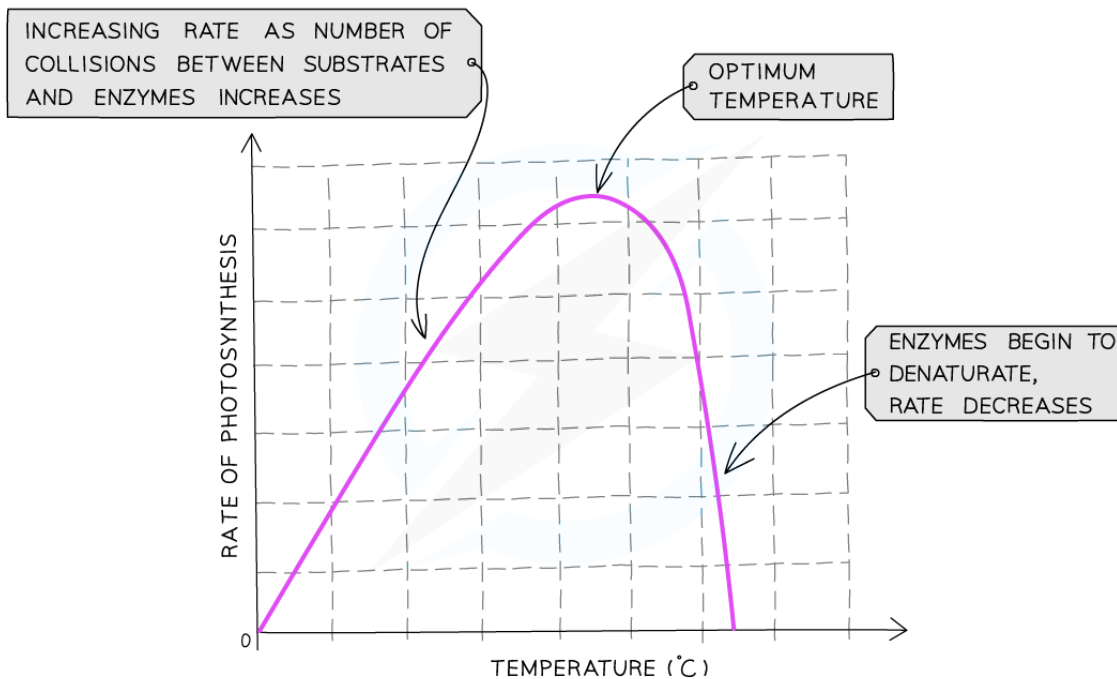
### The effect of carbon dioxide concentration on the rate of photosynthesis

## Temperature

- The **same basic experimental setup** can be used, but with varying use of the following variables
- Start with boiled and re-cooled water as before, with **sodium hydrogencarbonate at a fixed concentration** of  $0.1 \text{ mol dm}^{-3}$ , and record the rate of photosynthesis in bubbles  $\text{minute}^{-1}$
- **Vary the temperature from  $5^\circ\text{C}$  to  $50^\circ\text{C}$**  using water baths, monitoring with a thermometer in the water surrounding the plant
- **Keep the light intensity constant** by keeping the lamp a fixed distance from the plant
  
- It can be seen from a graph of the effect of temperature on the rate of photosynthesis that:
  - An increase in temperature will result in an increased rate of photosynthesis
    - This is due to the increase in **kinetic energy** of enzyme and substrate molecules which results in more collisions and formation of more enzyme-substrate complexes
  - This increase will continue until the **optimum temperature** for the enzyme is reached
  - Any further increase in temperature will see a **decrease** in the rate of photosynthesis
    - As enzymes begin to denature, they cannot form enzyme-substrate complexes anymore and therefore cannot catalyse the reaction



Your notes



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### The effect of temperature on the rate of photosynthesis

#### 💡 Examiner Tip

The key to this part of the spec is to appreciate how an experimental investigation can be controlled so that any effects we observe **are directly due to the one variable** that we are deliberately changing.



Your notes

## Carbon Dioxide Enrichment Experiments

### Carbon Dioxide Enrichment Experiments

- **Future rates** of photosynthesis and plant growth can be predicted using experiments such as
  - enclosed greenhouse experiments
  - free air carbon dioxide enrichment experiments (FACE)
- Due to the impact of global warming already documented and rising levels of **greenhouse gases**, including **carbon dioxide**, it is fundamental that studies are carried out to establish the effect of **carbon dioxide on plant growth and photosynthesis** to develop a clearer idea of the potential future risks that we may encounter

#### Enclosed greenhouse experiments

- Monitoring photosynthesis and growth can be done using an **enclosed greenhouse** or polytunnel set up
- This allows variables to be **manipulated** or **controlled** in order to establish the impact of different factors
- Only **small species** that can be contained in a greenhouse can be studied using this method
- **Variables** that would be manipulated might include
  - light
  - carbon dioxide
  - temperature
  - wavelengths of light
- Other variables should be controlled so as to ensure that the effect of only **one variable** is being considered at any one time

#### Free air carbon dioxide enrichment experiments (FACE)

- These experiments are carried out in natural ecosystems where carbon dioxide is pumped into the area to increase the localised carbon dioxide concentrations
- This set up allows larger plants and trees to be studied
- Other variables cannot be controlled in these scenarios but they can be monitored to establish any relationships that may become apparent in the data

#### NOS: Finding methods for careful control of variables is part of experimental design

- In an experiment, a variable is any factor that could change or be changed
  - The **independent variable**: the only variable that should be changed throughout an experiment
  - The **controlled/confounding variables**: any other variables that may affect the results of the experiment that need to be controlled or monitored
  - The **dependent variable**: the variable that is measured to determine the outcome of an experiment (the results)
- It is essential that any variable that may affect the outcome of an experiment is controlled in order for the results to be **valid**

- **Preliminary research** and preliminary studies can be used to **identify variables** within an experiment and to determine ways of controlling these variables effectively
- The science surrounding the issue/problem being investigated is likely to contain information about different factors or variables that may exist



Your notes





Your notes

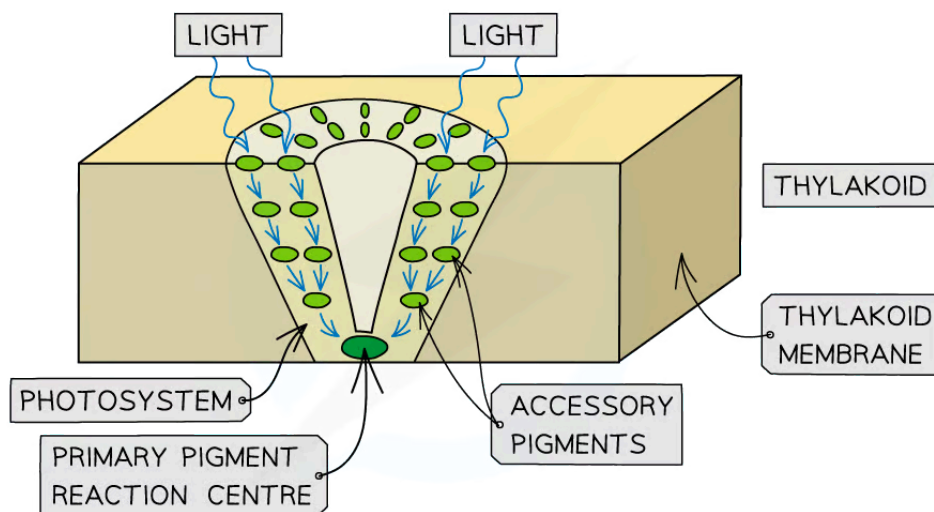
## Photosystems (HL)

### Photosystems

#### What are photosystems?

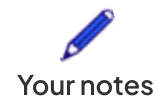
- Chloroplasts contains the pigment **chlorophyll**, plus other accessory pigments
- These are grouped together as structures called **photosystems** which are located in the **thylakoid membranes** in **cyanobacteria** and photosynthetic **eukaryotes**
- Photosystems contain many chlorophyll molecules and accessory pigments (carotene and xanthophylls) as well as a **reaction centre**
- Two types of photosystems exist:
  - Photosystem I** - contains the reaction centre P700 (as it is activated by a wavelength of light of 700nm)
  - Photosystem II** - contains the reaction centre P680 (as it is activated by a wavelength of light of 680nm)
- Chlorophyll molecules and accessory pigments within Photosystem II absorb light energy, in the form of photons, and pass it to a chlorophyll molecule in **reaction centre P680**
- Electrons** within the reaction centre of Photosystem II are then excited to a **higher energy level** by the photons of light
- The chlorophylls within the reaction centre are said to be **photoactivated**
- Excited electrons are able to be **donated** to an **electron acceptor** in a **reduction reaction**

#### Diagram to show excitation of electrons in a photosystem



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**A photosystem used in the light-dependent reaction to excite electrons**



### Examiner Tip

Rather confusingly, the first photosystem to be activated in the light-dependent reaction is Photosystem II. Later in the reaction, Photosystem I is involved. This is because Photosystem I was the first to be discovered and therefore was named first.

## Advantages of Photosystems

### Why are there multiple pigments in a photosystem?

- In each photosystem, the presence of many different types of pigment, each with a specific role, allows the photosystem to efficiently absorb light of different wavelengths
  - The structured arrangement of these pigments and accessory pigments allows for electrons to be excited in a controlled manner
  - These can then be directed along the [electron transport chain](#)
- All the pigments in photosystem I and II are required in order for photosynthesis to occur
  - A single pigment molecule would not be able to perform any part of photosynthesis

**Table to show the pigments involved in light harvesting in the light dependent stage of photosynthesis**

Pigment	Role
Chlorophyll (a, b)	Absorb wavelengths of light in the blue to violet and red regions of the spectrum
Carotenoid accessory pigments (xanthophyll, carotene)	Absorb wavelengths of light in the blue to violet region of the spectrum
Light harvesting complex proteins: enzymes	To catalyse: <ul style="list-style-type: none"> <li>▪ formation of ATP from ADP + P<sub>i</sub></li> <li>▪ reduction of NADP<sup>+</sup> to NADPH + H<sup>+</sup></li> </ul>
Light harvesting complex proteins: electron carrier molecules	Pass electrons down an electron transport chain



Your notes

## Light Dependent Reactions (HL)

### Location of the Light Dependent Reactions

- Photosynthesis takes place in two distinct stages:
  - The **light-dependent reaction**, which relies on light directly
  - The **light-independent reaction**, which does not use light directly

### Where do the light dependent reactions take place?

- Both stages of photosynthesis take place within the **chloroplast**
- The **light-dependent reaction** takes place in the **thylakoid intermembrane space** and across the **thylakoid membrane**
  - Thylakoids** are disc like structures which make up the grana in stacks of up to 100. They contain the photosynthesis pigment chlorophyll. Some may have tubular extensions (intergranal lamellae) which join up with thylakoids in adjacent grana
  - The **thylakoid membrane** contains a transfer chain where electrons are passed along a number of electron carriers in a series of oxidation-reduction reactions

### What happens in the light-dependent reaction?

Three key processes which occur during the light-dependent reaction in the thylakoid membrane include

- Photolysis**: The splitting of a water molecule using light energy
  - This occurs in photosystem II
- Chemiosmosis**: The synthesis of ATP using an electrochemical gradient produced by  $H^+$  protons
  - The proton gradient forms across the thylakoid membrane when protons are pumped from the chloroplast matrix into the thylakoid spaces
- Reduction of NADP**:  $NADP^+$  accepts electrons (from photophosphorylation) and  $H^+$  protons to become NADPH
  - This occurs in photosystem I

### Products of the light-dependent reaction

- During the light-dependent reaction light energy is converted into chemical energy in the form of **ATP** and **reduced NADP**
- Oxygen** is given off as a **waste product** of the light-dependent reaction
- The useful products of the light-dependent reaction are transferred to the light-independent reaction within the chloroplast

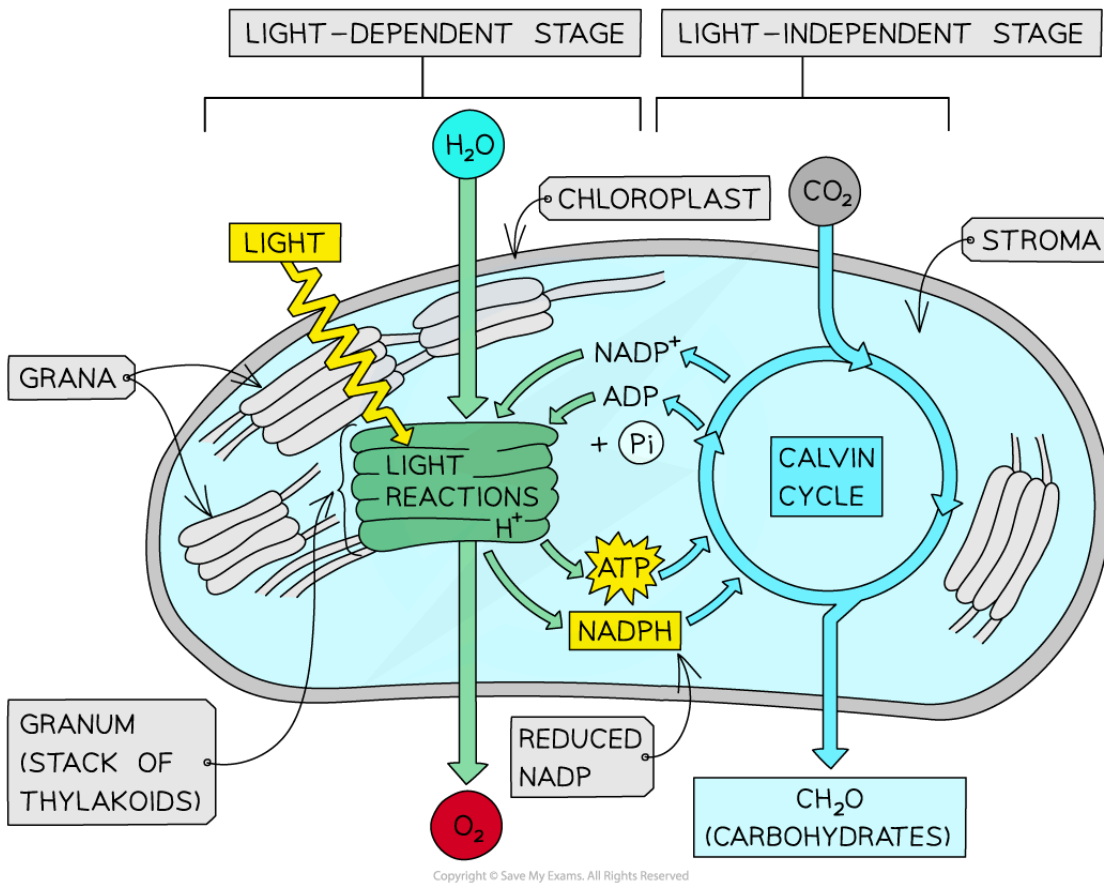
#### Examiner Tip

The thylakoid intermembrane space is also referred to as the thylakoid lumen.

**Diagram to show the location of the light dependent and light independent stages of photosynthesis**



Your notes



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**The two stages of photosynthesis**

**Examiner Tip**

NADP is an electron carrier that is important in photosynthesis. When it takes up protons the NADP becomes reduced and can be written as NADPH.

When writing about this electron carrier, you should use consistent notation from the following two options:

- NADP which is converted to reduced NADP
- OR
- NADP<sup>+</sup> which is converted to NADPH



Your notes

## Photolysis

### Photolysis and the light-dependent reaction

- Photolysis occurs in Photosystem II during the **light-dependent reaction** of photosynthesis
  - The reaction centre acts as an **oxidising agent** and causes water molecules (that have been moved into the leaf by transport up the xylem vessels) to split during photolysis
- **Water splits into protons, electrons and oxygen**
  - The oxygen is released as a **waste product**, it diffuses out of the leaf through stomata
  - The electrons are passed into the **electron transport chain**
  - The protons are picked up by the carrier molecules NADP forming reduced NADP
- The reaction can be summarised as  $2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$
- The photolysis of water generates the electrons needed for:
  - **Replacement** of the electrons lost from the reaction centre in Photosystem II
  - **Subsequent reactions** of the light-dependent reaction

### The effect of oxygen

- Changes to the Earth's atmosphere, oceans and rock deposition occur due to photosynthesis, and more specifically **photolysis**
- The **first life forms** emerged around 4 billion years ago
  - At the time, there was **no oxygen in the atmosphere**
- About 3.5 billion years ago photosynthetic prokaryotes became the first organisms to carry out photosynthesis
  - **This began the release of oxygen** into the atmosphere
- Millions of years later algae and plants evolved and also carried out photosynthesis
- Around 2.2 billion years ago, the oxygen concentration in the atmosphere reached 2%
  - This is known as the Great Oxidation Event
- Other changes to the Earth occurred due to photosynthesis
  - **Minerals** in the oceans were **oxidised**
  - Photosynthetic bacteria released oxygen into the ocean
  - When dissolved iron was oxidised it formed iron oxide which is a red precipitate that lies on the sea bed
  - Over time a distinctive rock formation was produced - the banded iron formation. Layers of red iron oxide alternate with other mineral oxides
  - Banded iron formations are the most important source of iron **ores** (and consequently our supply of steel)
  - Methane and  $\text{CO}_2$  levels in the air fell, which resulted in an **Ice Age**
    - This is because methane and  $\text{CO}_2$  are important greenhouse gases
- By 600 million years ago, life had evolved into **large multicellular organisms**, many of which were photosynthetic (plants)
  - This pushed the oxygen concentration of the air up to 20%, **peaking at 35%** 300 million years ago
  - This contributed to the **large size of the animals** that roamed the Earth at that time
- The current atmospheric oxygen level is around 21%, due to **increased human activity**, e.g. burning of fossil fuels, deforestation which remove oxygen from the atmosphere



Your notes

## Photophosphorylation (HL)

### Chemiosmosis in Photosynthesis

#### Types of photophosphorylation

- The photophosphorylation of ADP to ATP can be **cyclic** or **non-cyclic**, depending on the pattern of electron flow in photosystem I or photosystem II or both
  - In **cyclic** photophosphorylation, **only photosystem I** is involved
  - In **non-cyclic** photophosphorylation, **both photosystem I and photosystem II** are involved

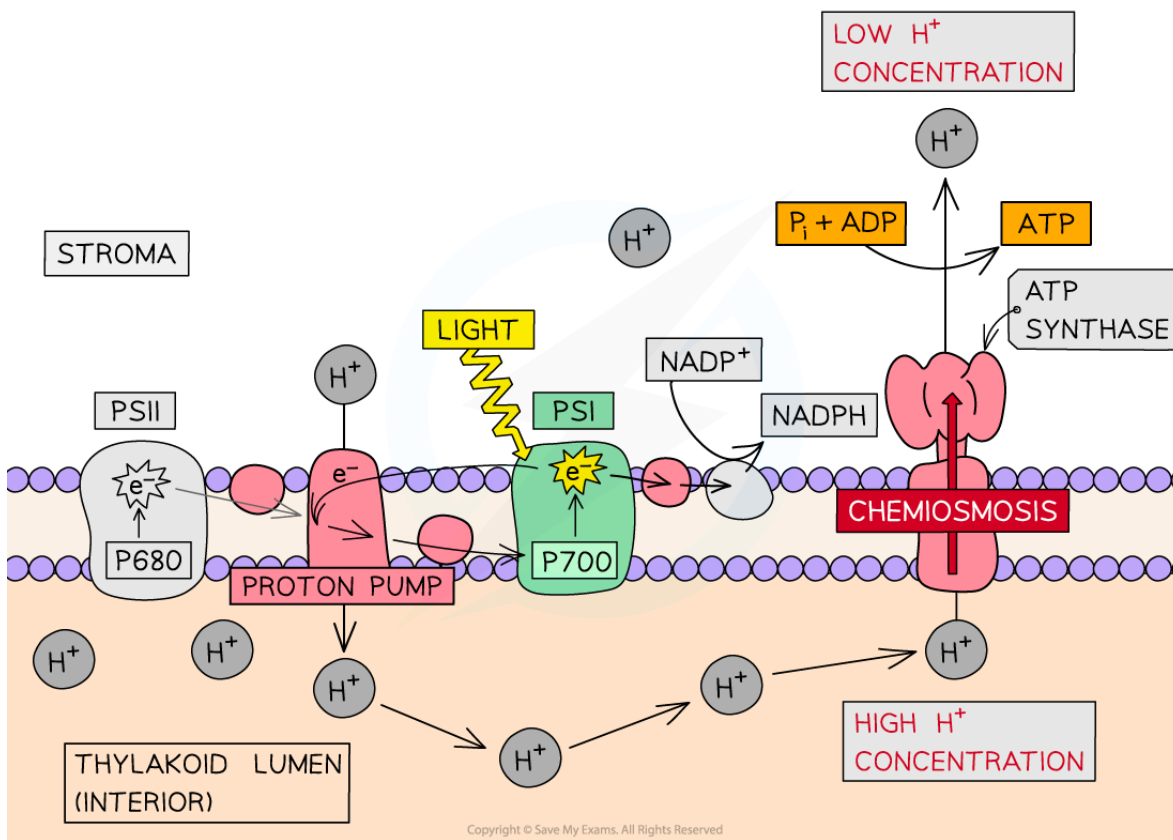
#### Cyclic photophosphorylation

- Cyclic photophosphorylation involves **photosystem I (PSI) only**
- **Light is absorbed** by photosystem I (located in the thylakoid membrane) and passed to the photosystem I **primary pigment (P700)**
- An **electron** in the primary pigment molecule (i.e. the chlorophyll molecule) is **excited** to a **higher energy level** and is emitted from the chlorophyll molecule in a process known as **photoactivation**
- This excited electron is captured by an electron acceptor, transported via a **chain of electron carriers** known as an **electron transport chain** before being passed back to the chlorophyll molecule in photosystem I (hence: cyclic)
- As electrons pass through the electron transport chain they provide energy to transport protons ( $H^+$ ) from the stroma to the thylakoid lumen via a **proton pump**
- A build-up of protons in the thylakoid lumen can then be used to drive the **synthesis of ATP** from ADP and an inorganic phosphate group ( $P_i$ ) by the process of **chemiosmosis**
- **Chemiosmosis** is the movement of chemicals (protons) down their concentration gradient, the energy released from this can be used by ATP synthase to synthesise ATP
- The ATP then passes to the **light-independent reactions**

#### Cyclic photophosphorylation diagram



Your notes



*Cyclic photophosphorylation in photosynthesis involves only photosystem I*

 **Examiner Tip**

Remember a redox reaction is one where reduction reactions (gain of electrons or hydrogen, loss of oxygen) and oxidation reactions (loss of electrons or hydrogen, gain of oxygen) happen alternately. This happens along the series of electron carriers in the thylakoid membrane as part of the electron transport chain.

**Non-cyclic photophosphorylation**

- Photophosphorylation is the term for the overall process of using **light energy** and the **electron transport chain** to **generate ATP** from ADP
- During photophosphorylation excited electrons (from Photosystem II) are passed down a series of **electron carriers** that form the **electron transport chain**
- The electron transport chain occurs on the **thylakoid membranes** within the chloroplast
- Thylakoid membranes contain the following structures:
  - Photosystem II
  - ATP synthase
  - A series of electron carriers

- Photosystem I
- An electron acceptor carries a pair of **excited electrons** from Photosystem II to the start of a chain of electron carriers
- The electron carriers undergo a series of **redox reactions** as electrons are gained and lost from each carrier
- Excited electrons gradually release their energy as they pass through the electron carriers which is used to generate a **proton gradient**
- The **excitation** of the electrons **falls** and they are eventually picked up by the reaction centre in **Photosystem I**
- Finally the pair of electrons are used to **reduce NADP** (along with protons from the photolysis of water) which is then passed into the light-independent reaction
- The pathway of electrons is linear, photophosphorylation is referred to as **non-cyclic photophosphorylation**
- **ATP** and **reduced NADP** are the **main products** of photophosphorylation and are immediately passed to the light-independent reaction

### Non-cyclic photophosphorylation diagram



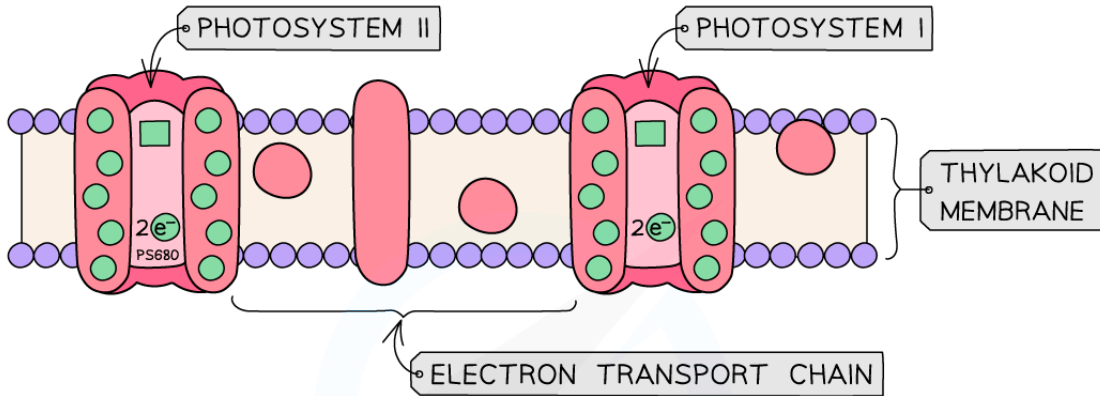
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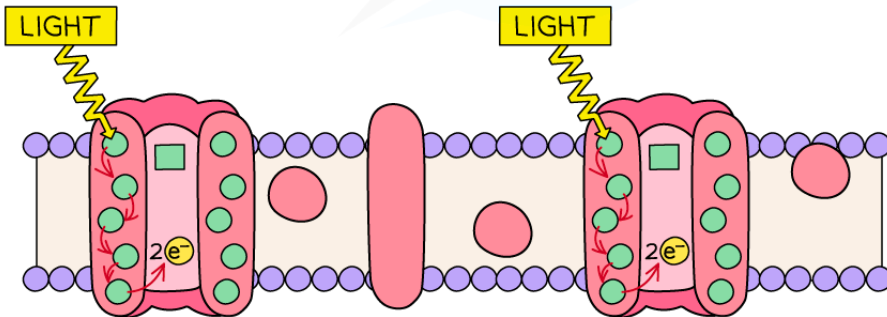


Your notes

1 NON-CYCLIC PHOTOPHOSPHORYLATION INVOLVES BOTH PHOTOSYSTEM I AND PHOTOSYSTEM II



2 LIGHT IS ABSORBED BY BOTH PHOTOSYSTEMS. THE LIGHT IS PASSED TO THE PRIMARY PIGMENT OF EACH PHOTOSYSTEM

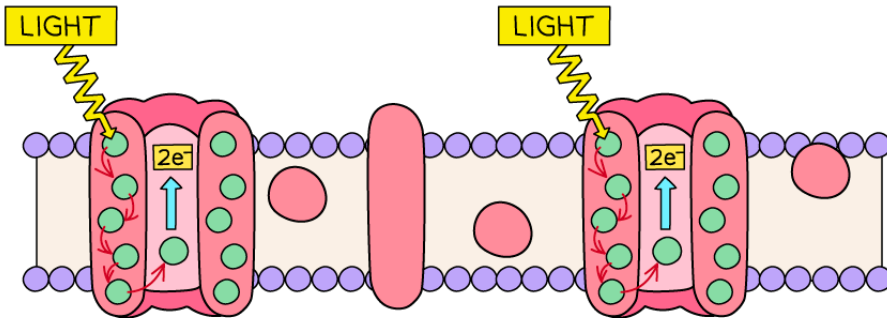


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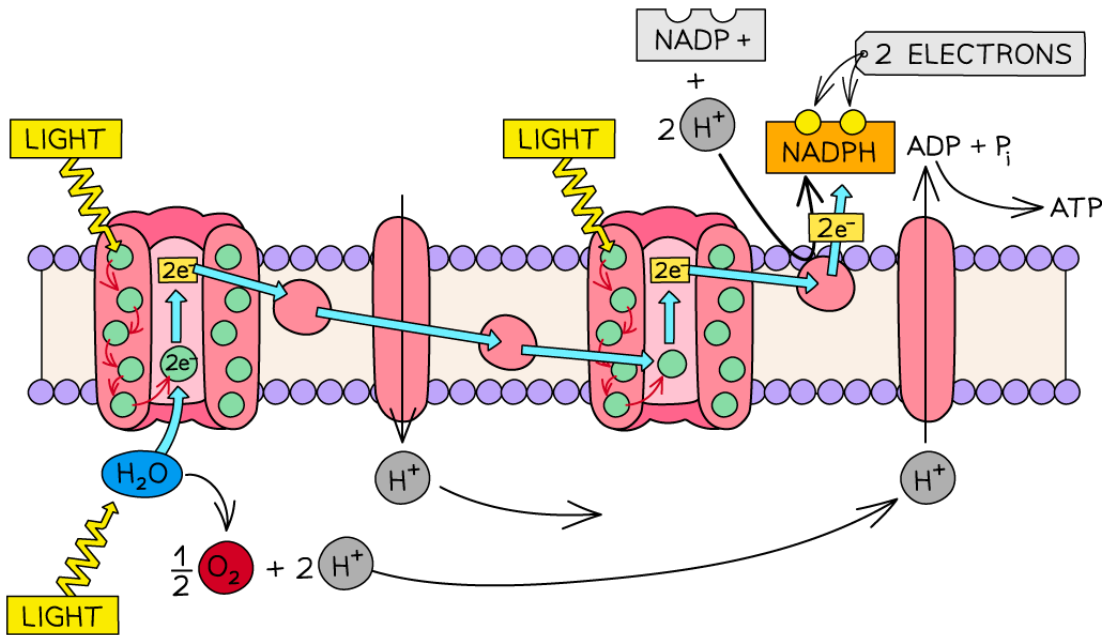


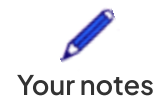
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3 PHOTOACTIVATION – ELECTRONS IN THE PRIMARY PIGMENT MOLECULE OF EACH PHOTOSYSTEM ARE EXCITED TO A HIGHER ENERGY LEVEL



4 EXCITED ELECTRONS FROM PHOTOSYSTEM II ARE PASSED TO PHOTOSYSTEM I VIA AN ELECTRON TRANSPORT CHAIN, RELEASING SUFFICIENT ENERGY TO SYNTHESISE ATP





5 ELECTRONS FROM THE PHOTOLYSIS OF WATER REPLACE THOSE LOST FROM PHOTOSYSTEM II

6 EXCITED ELECTRONS FROM PHOTOSYSTEM I AND HYDROGEN IONS FROM THE PHOTOLYSIS OF WATER BOTH COMBINE WITH NADP TO FORM REDUCED NADP

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### ***Non cyclic phosphorylation involving the electron transport chain and the production of ATP and reduced NADP***

#### **Examiner Tip**

Make sure you know the difference between the two forms of photophosphorylation! Cyclic photophosphorylation differs from non-cyclic photophosphorylation in two key ways:

- Cyclic photophosphorylation **only** involves photosystem I (whereas non-cyclic photophosphorylation involves photosystems I and II)
- Cyclic photophosphorylation does **not** produce reduced NADP (whereas non-cyclic photophosphorylation does)

## **Chemiosmosis**

- During the light dependent stages of photosynthesis, ATP is synthesized from ADP + P<sub>i</sub> using energy released from the movement of H<sup>+</sup> protons down an electrochemical gradient

### **Forming a proton gradient**

- Electrons are passed from carrier to carrier in the electron transport chain
- As they do so they **release energy** which is used to **pump protons** from the stroma across the thylakoid membrane and into the intermembrane space (also known as the the thylakoid lumen)
- The protons move via a proton pump
- A **high concentration** of protons builds inside the intermembrane space creating a concentration gradient
- **Photolysis of water** contributes to the proton gradient

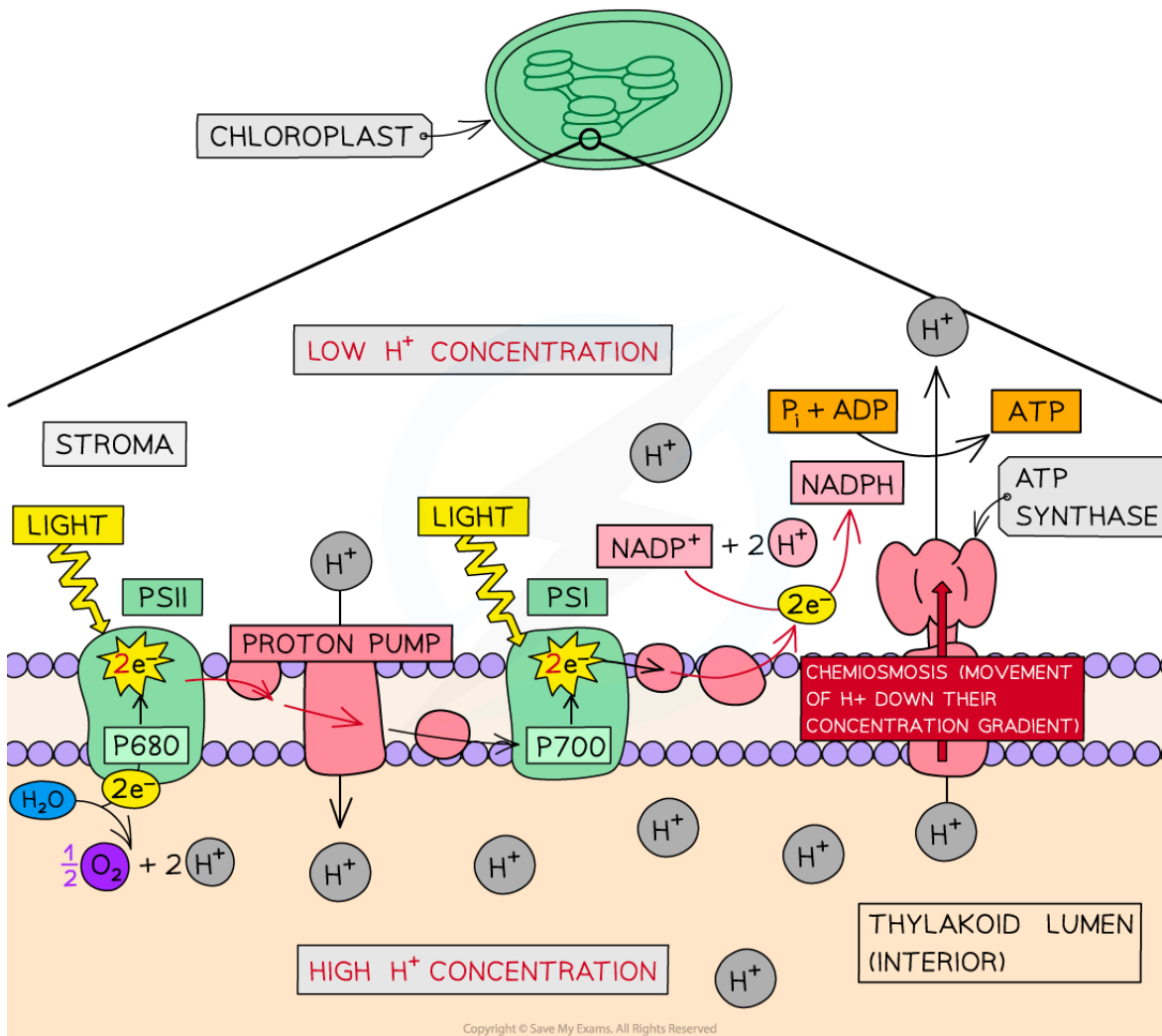
### **Synthesis of ATP**

- The proton gradient within the intermembrane space of the thylakoid powers the **synthesis of ATP**
  - The protons travel down their concentration gradient through the membrane protein **ATP synthase**
  - Energy is released by the movement of protons and is used to make ATP from the phosphorylation of ADP
- This process is called **chemiosmosis**
- The ATP produced is used in the light-independent reaction

#### **Chemiosmosis diagram**



Your notes



**Photophosphorylation and chemiosmosis in photosynthesis**

**Examiner Tip**

Remember - the oxygen produced during the photolysis of water is a waste product of this process. The hydrogen ions and electrons produced during the photolysis of water are useful products. The electrons replace those that have been lost from the primary pigment molecule of photosystem II (as photosystem II passes its electrons on to photosystem I). The hydrogen ions combine with the electrons from photosystem I to form reduced NADP (NADPH).

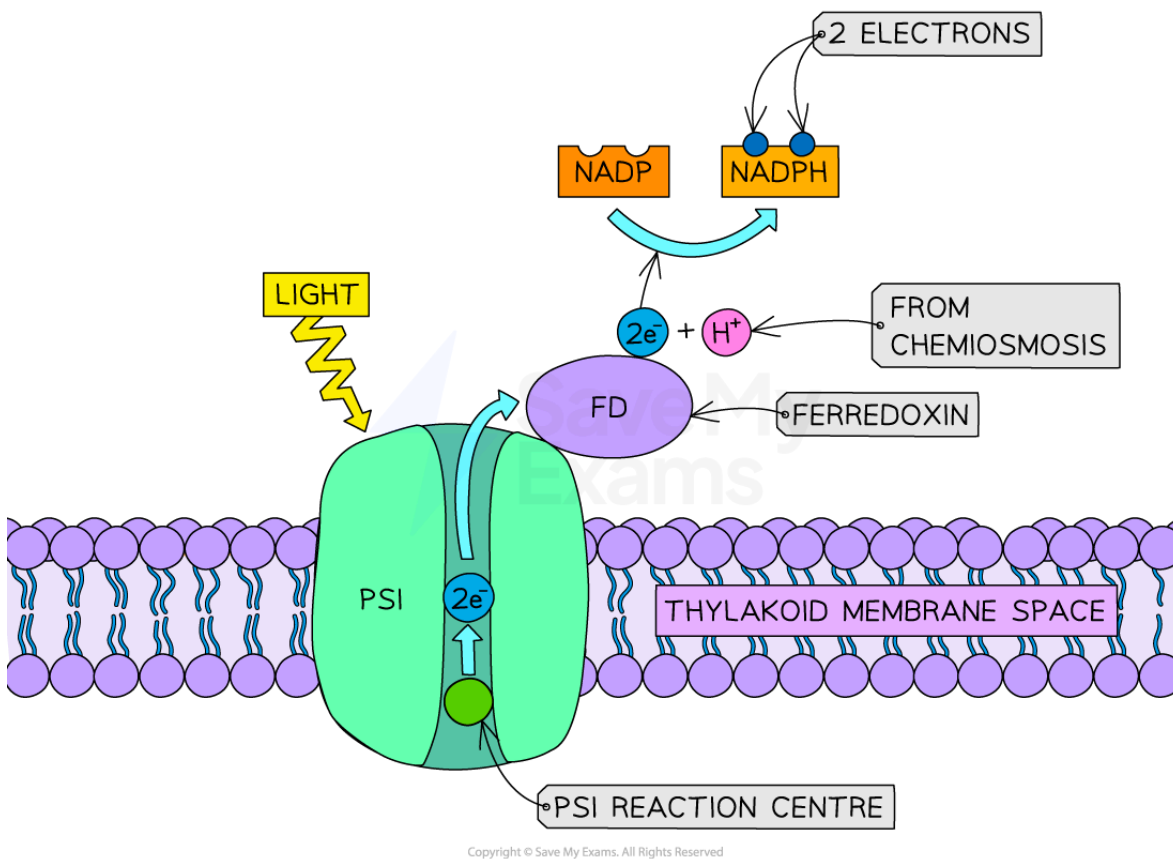


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## Reduction of NADP

- **Photosystem I** is involved in the reduction of NADP which is a key molecule used in the light-independent reaction
  - Chlorophyll molecules in the reaction centre **absorb photons** of light energy
  - **Electrons** within the reaction centre are **photoactivated** to a higher energy level
  - They are passed to a **protein** on the outside of the thylakoid membrane (called ferredoxin) and reduce it
  - The reduced ferredoxin, along with **protons** that have passed through ATP synthase during chemiosmosis, are used to reduce NADP<sup>+</sup> to NADPH
    - $\text{NADP}^+ + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{NADPH} + \text{H}^+$
  - Reduced NADP now carries a pair of electrons and can be passed into the light-independent reactions of photosynthesis

Diagram to show the reduction of NADP in the light dependent stage of photosynthesis



**Reduction of NADP in Photosystem I**



Your notes

## Light Independent Reactions (HL)

### Carbon Fixation

#### Location of the light-independent reactions

- The light-independent reactions of photosynthesis take place in the **stroma** of the chloroplasts
- The stroma is within the double membrane and is a thick protein rich environment containing the **enzymes** needed for the light-independent reactions

#### Light-independent reactions: Carbon fixation

- The light-independent reactions of photosynthesis are also known as the **Calvin cycle**
- There are three main steps within the Calvin cycle:
  1. **Carbon fixation**: The enzyme **Rubisco** catalyses the **fixation of carbon dioxide** by combination with a molecule of ribulose biphosphate (RuBP), a 5C compound, to yield two molecules of glycerate 3-phosphate (GP), a 3C compound
  2. **Reduction**: **GP is reduced** to triose phosphate (TP) in a reaction involving reduced NADP and ATP
  3. **Regeneration**: **RuBP is regenerated** from TP in reactions that use ATP
- **Carbon dioxide** is converted into carbohydrates, namely **glucose**, during the cycle in a series of **anabolic** reactions
  - **Anabolic reactions** require energy in order to build large complex molecules from smaller simpler ones
- The Calvin cycle relies on the **products of the light-dependent reactions** namely ATP and reduced NADP
- During the cycle **endergonic** reactions take place that involve the hydrolysis of ATP and oxidation of reduced NADP
  - An **endergonic reaction** requires energy to be absorbed before the reaction can proceed

#### Carbon fixation details

- Carbon dioxide is the source of carbon for all organisms that carry out photosynthesis
- Carbon fixation involves carbon dioxide (1C) being removed from the external environment and becoming part of the plant, and is then said to be “fixed”
  - It is transformed into a three-carbon compound (3C) called **glycerate-3-phosphate** (sometimes shortened to as GP)
- During the fixation step of the Calvin cycle **carbon dioxide** is combined with a five-carbon compound (5C) called **ribulose biphosphate (RuBP)** to make an **unstable** six-carbon (6C) compound that splits into **two** molecules of **glycerate-3-phosphate**
- This reaction is catalysed by the enzyme **Rubisco**
  - This is the most abundant enzyme on Earth
  - It works relatively slowly, therefore high concentrations of it is needed in the stroma
  - It is not effective in low carbon dioxide concentrations
- Glycerate-3-phosphate is then used in the next step of the cycle

## Synthesis of Triose Phosphate

- Energy from **ATP** and hydrogen from **reduced NADP** (from the light-dependent reactions) are used to **reduce glycerate-3-phosphate** to a phosphorylated three-carbon molecule called **triose phosphate** (sometimes shortened to TP)
- After the reduction step **one sixth** of the triose phosphate is converted into **usable products** for the plant:
  - Hexose phosphates which can be used to produce carbohydrates such as **starch, sucrose or cellulose**
  - **Glycerol** and **fatty acids** which join to form cell membranes
  - Production of **amino acids** for protein synthesis
- It is important that **not all** the triose phosphate is converted to **alternative compounds** for the plant, or the supplies of ribulose biphosphate would run out
- The remaining triose phosphate is used to **regenerate RuBP**

### Examiner Tip

For the Calvin cycle to continue it needs a constant supply of RuBP and carbon dioxide. As much RuBP must be produced as is consumed. If three RuBP molecules are used then this generates six triose phosphates. Five of the triose phosphate molecules are needed to regenerate the three RuBP molecules. So there would only be one left over to convert into other usable molecules for the plant (such as starch). To produce just one molecule of glucose, six turns of the Calvin cycle are needed.



Your notes

## Regeneration of RuBP

- **One sixth** of the triose phosphate that is generated will be used in the synthesis of organic compounds, such as glucose
- The remaining **five sixths** of triose phosphate are used to **regenerate** the four-carbon compound ribulose biphosphate (**RuBP**)
  - Five molecules of triose phosphate are converted to three molecules of RuBP
- This process requires **ATP** (from the light-dependent reaction)
- Once RuBP has been regenerated it can go on to fix further carbon dioxide and the cycle can begin again

### Light-independent stage of photosynthesis diagram

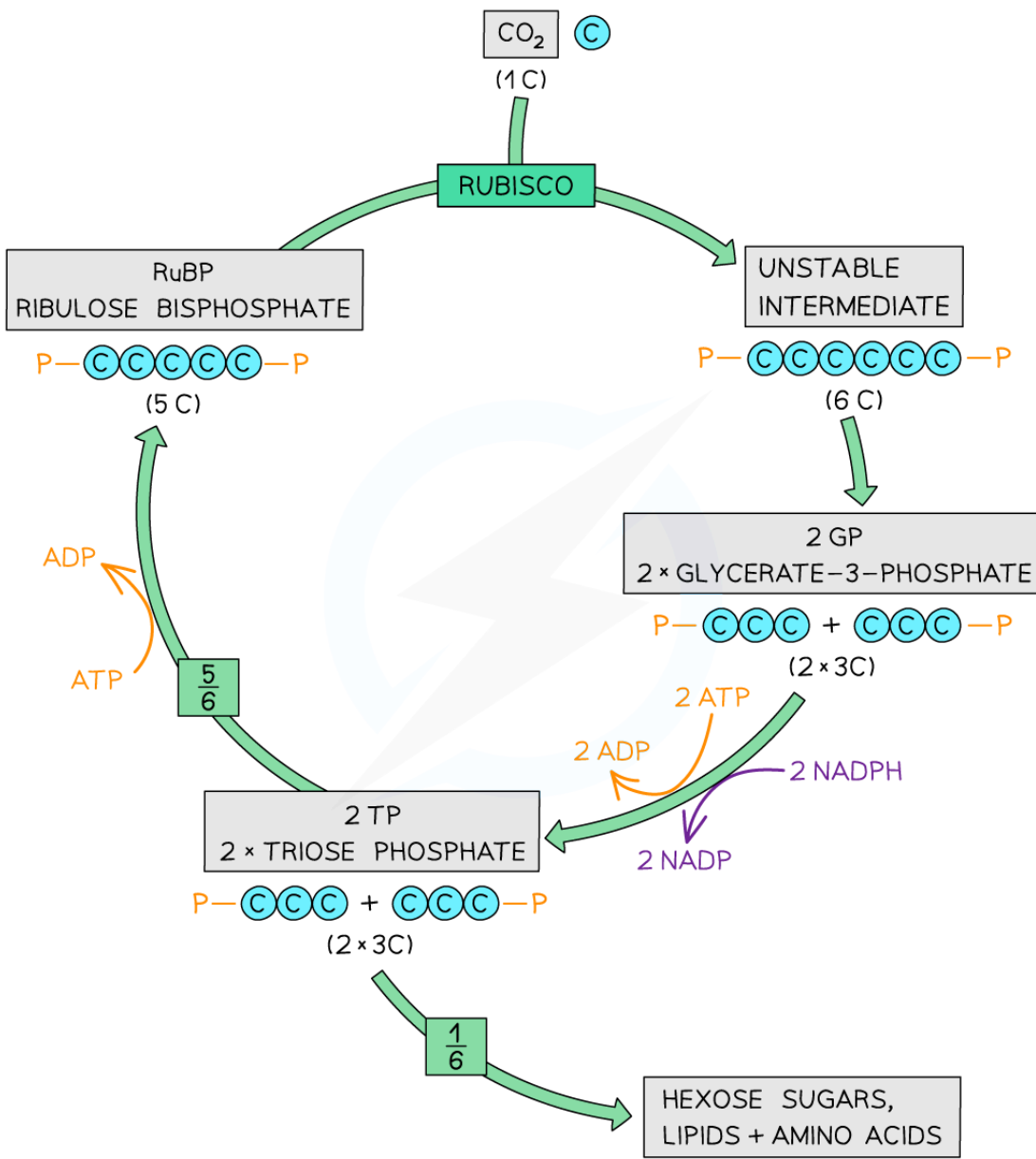


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**The Calvin cycle of the light-independent reactions showing the regeneration of RuBP**

## Synthesis of Carbon Compounds

- Carbon containing compounds, to include carbohydrates, proteins and lipids, all rely on the **products of the Calvin cycle** in combination with other mineral nutrients
- Whilst glucose is the key respiratory substrate which sustains the metabolism of green plants, other required substances can be produced from the further metabolism of TP, for example:
  - Triose phosphate can be sent through the **glycolysis and link reaction** pathways to produce acetyl coenzyme A which can then be conjoined to make **fatty acids**
  - Triose phosphate can be used to make **glycerol**
  - Glycerol can then be joined to fatty acids to make **triglycerides**
  - All 20 **amino acids** can be produced in plants using nitrate or ammonium ions



Your notes



Your notes

## Interdependence of Photosynthetic Reactions (HL)

### Interdependence of Photosynthetic Reactions

- The light dependent reaction and light independent reaction are **interdependent**
- This means that one cannot occur without the other
  - Products from the light dependent reaction (reduced NADP and ATP) are **directly used** in the Calvin cycle to produce carbohydrates
    - This means that in low light intensity, the products are produced at a slower rate which limits the conversion of GP to TP
  - Once the reduced NADP has been oxidised in the Calvin cycle, NADP is returned to the **light dependent** stage to accept electrons at the end of the electron transport chain
    - In high light intensity, the light dependent reactions occur more quickly, providing more reduced NADP and ATP to drive the Calvin cycle
    - However, if NADP is not returned to the light dependent stage quickly enough, then the process will be restricted
- Carbon dioxide, in the form of **hydrogen carbonate ions** ( $\text{HCO}_3^-$ ), **accepts protons** from photosystem II when water is split during photolysis
  - Hydrogen carbonate also plays an important role in the functioning of the **electron transport chain**
- A lack of carbon dioxide (and thus hydrogen carbonate) will not only prevent carbon fixation from occurring but also **prevent photosystem II** from functioning