

Cambridge AS & A Level · Biology 9700 · Notes

Photosynthesis A2

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Read first: [Cell structure](#), [Enzymes](#), [Transport in plants](#), [Energy and respiration](#)

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What you need to know

By the end of this topic you should be able to:

Photosynthesis as an energy transfer process (13.1)

1. **Link the structure of a chloroplast to its function**, from diagrams and electron micrographs.
2. **Explain that ATP and reduced NADP** made in the light-dependent stage are used in the light-independent stage (the **Calvin cycle**) to make complex organic molecules.
3. **State where each stage happens**: the **thylakoids** (thylakoid membranes and thylakoid spaces), stacked into **grana**, are the site of the light-dependent stage; the **stroma** is the site of the light-independent stage.
4. **Describe the role of the chloroplast pigments** (chlorophyll a, chlorophyll b, carotene and xanthophyll) in absorbing light in the thylakoids.
5. **Interpret absorption spectra** of the pigments and **action spectra** for photosynthesis.
6. **Describe and use chromatography** to separate and identify chloroplast pigments, using **Rf values**.
7. **State that cyclic and non-cyclic photophosphorylation** both happen in the light-dependent stage.
8. **Explain cyclic photophosphorylation**: only photosystem I (PS I), photoactivation of chlorophyll, ATP made.
9. **Explain non-cyclic photophosphorylation**: PS I and PS II, photoactivation of chlorophyll, the **oxygen-evolving complex** catalysing the **photolysis of water**, ATP and reduced NADP made.
10. **Explain photophosphorylation** itself: energetic electrons release energy along the electron transport chain; the energy moves protons across the thylakoid membrane; protons return to the stroma by facilitated diffusion through **ATP synthase**, giving the energy to make ATP. (You don't need the names of the carriers or the details of ATP synthase.)
11. **Outline the three stages of the Calvin cycle**: **rubisco** fixes CO₂ onto **RuBP** (5C) to give two **GP** (3C); GP is **reduced** to **TP** using reduced NADP and ATP; **RuBP is regenerated** from TP using ATP.
12. **State what the intermediates make**: GP makes some amino acids; TP makes carbohydrates, lipids and amino acids.

Investigation of limiting factors (13.2)

13. **State that light intensity, carbon dioxide concentration and temperature** are limiting factors of photosynthesis.

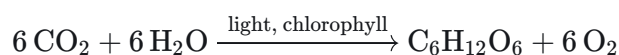
14. **Explain how changing each of them** affects the rate of photosynthesis.
15. **Describe and carry out investigations with redox indicators** (DCPIP, methylene blue) and a **chloroplast suspension**, to find the effect of light intensity and light wavelength.
16. **Describe and carry out investigations with whole plants**, including aquatic plants, to find the effects of light intensity, CO₂ concentration and temperature.

C4 plants (maize, sorghum) appeared in 2021 questions, but they are **not** in the 2025–2027 syllabus.

Understand it

The big picture

Photosynthesis moves energy from light into chemical bonds. Overall,

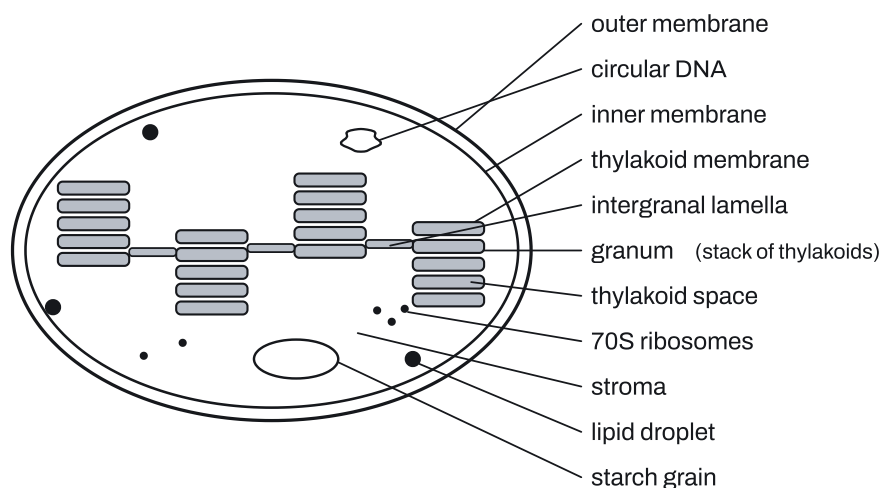


It happens in two linked stages:

- the **light-dependent stage**, on the **thylakoid membranes**: light energy makes **ATP** and **reduced NADP**, and water is split, releasing **oxygen**;
- the **light-independent stage** (the **Calvin cycle**), in the **stroma**: ATP and reduced NADP are used to turn CO₂ into **triose phosphate**, the sugar that everything else is built from.

The second stage needs the products of the first. Stop the light-dependent stage (darkness, or a herbicide that blocks photophosphorylation) and the Calvin cycle soon stops too: without ATP and reduced NADP, GP is not reduced to TP, RuBP is not regenerated, and no carbohydrate is made.

The chloroplast



Each part has a job, and questions usually ask you to link the two:

Structure	Function
Envelope (two membranes)	Keeps the reactions and enzymes of photosynthesis separate from the rest of the cell
Thylakoid membranes	Hold the photosystems (pigments), electron carriers , ATP synthase and the oxygen-evolving complex : the site of the light-dependent stage
Grana (stacks of thylakoids) and intergranal lamellae	Many membranes give a large surface area for pigments, so more light is absorbed
Thylakoid space (lumen)	Small space where protons build up , making the proton gradient
Thylakoid membrane is impermeable to protons	Keeps the gradient; protons can only return through ATP synthase
Stroma	Fluid with the Calvin cycle enzymes (including rubisco) and RuBP: site of the light-independent stage, where TP is made
Circular DNA and 70S ribosomes	Code for and make some chloroplast proteins (including part of rubisco)
Starch grains and lipid droplets	Store the products of photosynthesis

In an electron micrograph the grana show as stacks of dark lines (the membranes), the stroma as a paler granular background, and starch grains as large pale shapes. Chloroplasts do **not** contain mitochondria, and the stroma is not "cytoplasm".

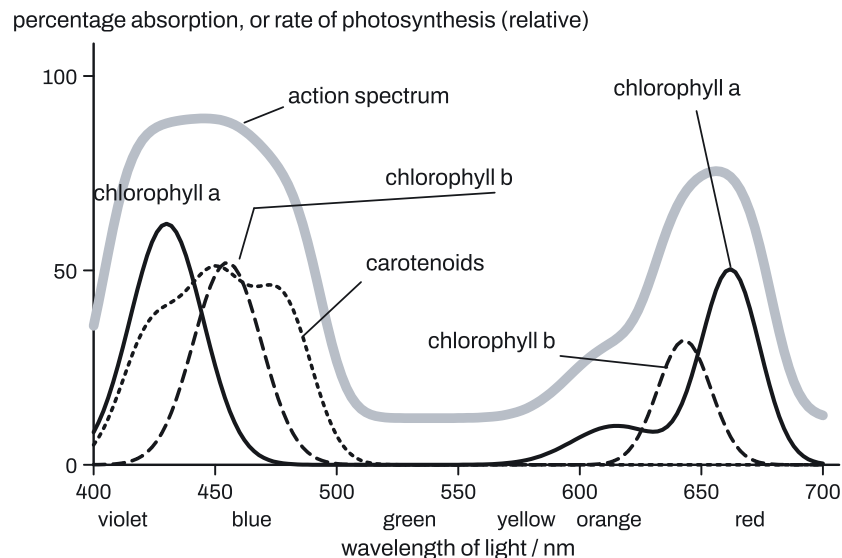
Chloroplast pigments

Pigments absorb light. In the thylakoid membranes they are grouped into **photosystems**: a **light-harvesting (antenna) complex** of many pigment molecules around a **reaction centre** containing chlorophyll a.

- **Chlorophyll a** is the **primary pigment**. Its reaction-centre molecules give off electrons when they absorb energy. PS II's reaction centre absorbs best at 680 nm (P680), PS I's at 700 nm (P700).
- **Chlorophyll b**, **carotene** and **xanthophyll** are **accessory pigments**. They absorb **wavelengths that chlorophyll a absorbs poorly**, so the plant uses a **wider range** of wavelengths, and they **pass the energy** (not the light) to chlorophyll a at the reaction centre. This makes the light-dependent stage more efficient. Carotene and xanthophyll together are the **carotenoids** (yellow and orange); chlorophylls are green.

Absorption and action spectra

- An **absorption spectrum** shows **how much light of each wavelength a pigment absorbs**.
- An **action spectrum** shows the **rate of photosynthesis at each wavelength**.



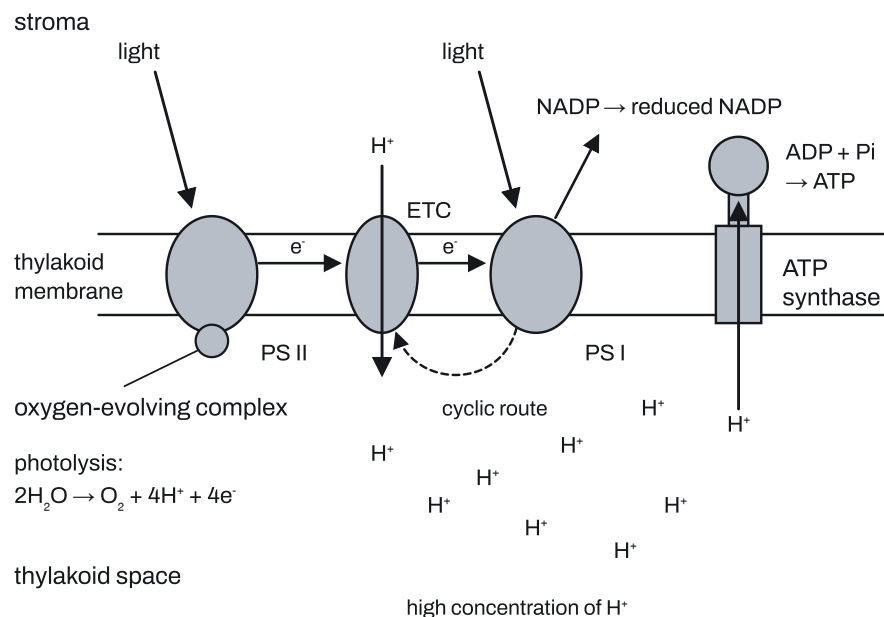
Read the graph like this:

- The chlorophylls absorb mainly **blue-violet and red** light. Almost nothing absorbs much **green and yellow** light (about 530–600 nm): it is reflected or passes through, which is why leaves look green.
- The carotenoids absorb only **blue-violet** light (about 400–520 nm), with several small peaks and **no red peak**.
- The action spectrum has the **same shape** as the combined absorption: wavelengths that are absorbed well give a high rate. More light absorbed means more photoactivation, so more light-dependent reaction.
- Between about 450 and 550 nm the action spectrum is **higher than chlorophyll a's absorption alone**. The extra comes from the **accessory pigments**, which absorb there and pass the energy on. An action spectrum that reaches wavelengths one pigment does not absorb shows that **other pigments** must be present.
- **Whole chloroplasts** absorb more than an extract of their pigments, because they hold more pigment, arranged in stacked membranes that absorb light better.

Organisms in deep water, such as red algae, have extra accessory pigments that absorb the **green** light that reaches them, which chlorophyll a absorbs poorly.

Photophosphorylation: how light makes ATP

Photoactivation: when a photosystem absorbs light, the energy is passed to chlorophyll a at the reaction centre, and an electron is **excited** to a higher energy level and **emitted**. The electron passes to an **electron acceptor** and along the **electron transport chain** (ETC) of carriers in the thylakoid membrane.



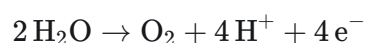
Chemiosmosis makes the ATP:

1. As electrons pass along the ETC they **release energy**.
2. The carriers use the energy to **pump protons (H^+) from the stroma into the thylakoid space**.
3. Protons build up in the thylakoid space, making a **proton gradient** (the membrane is impermeable to them).
4. Protons return to the **stroma** by **facilitated diffusion** through **ATP synthase**.
5. This flow gives the energy to join **ADP and Pi** into **ATP**.

This is the same idea as oxidative phosphorylation in mitochondria, but here the energy comes from **light**, the electrons come from **water** (via chlorophyll), the protons go into the **thylakoid space**, and the last electron acceptor is **NADP** (making reduced NADP), not oxygen (making water). Because protons leave the stroma in the light, the **stroma's pH rises** in the light and falls again in the dark.

Non-cyclic photophosphorylation (makes ATP **and** reduced NADP, releases O_2):

1. Light is absorbed by **both PS II and PS I**; electrons are excited and emitted from both.
2. Electrons from **PS II** pass along the ETC (making ATP by chemiosmosis) and **replace the electrons lost by PS I**.
3. Electrons from **PS I** go to **NADP**, which also takes a proton from the stroma: $\text{NADP} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{reduced NADP}$.
4. PS II gets its electrons back from **water**. The **oxygen-evolving complex** of PS II catalyses the **photolysis** of water:



The oxygen is released (as O_2 , not "O"), the protons add to the gradient in the thylakoid space, and the electrons refill PS II. So the **oxygen comes from PS II**.

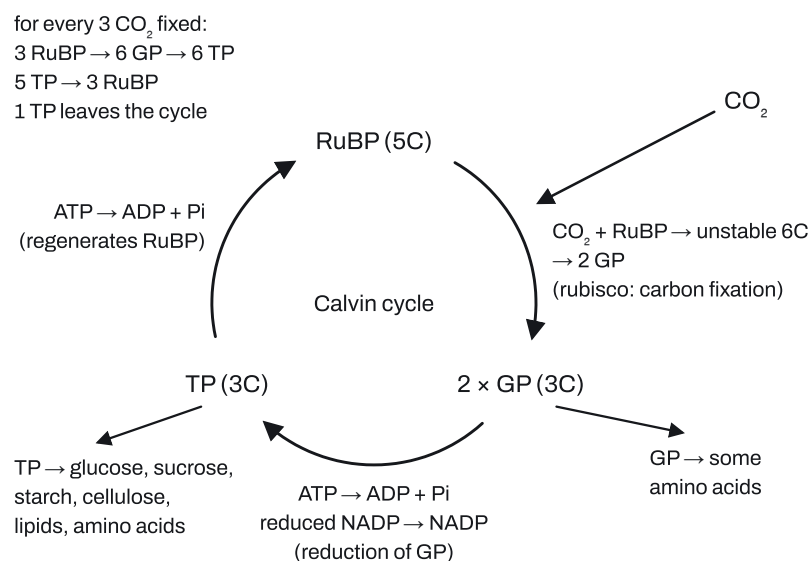
Cyclic photophosphorylation (makes **ATP only**):

1. Only **PS I** is involved; light excites and emits its electrons.
2. The electrons pass along the ETC (protons are pumped, ATP is made) and **return to PS I**.
3. No photolysis, no oxygen and no reduced NADP.

It tops up ATP, because the Calvin cycle uses more ATP than reduced NADP.

	Cyclic	Non-cyclic
Photosystems	PS I only	PS I and PS II
Electrons emitted from PS I	return to PS I	go to NADP
Source of electrons	PS I (recycled)	water, via PS II
Photolysis, oxygen-evolving complex, O ₂	none	yes
Products	ATP	ATP and reduced NADP (and O ₂)
In both	photoactivation of chlorophyll, ETC, proton gradient, ATP synthase, on the thylakoid membrane	

The Calvin cycle



1. **Carbon fixation.** CO₂ combines with **ribulose biphosphate (RuBP, 5C)**, catalysed by the enzyme **rubisco**. The **unstable 6C** compound splits at once into **two glycerate 3-phosphate (GP, 3C)**.
2. **Reduction.** GP is **reduced** to **triose phosphate (TP, 3C)**. **Reduced NADP** gives the hydrogen and **ATP** gives energy. (GP is reduced, not "broken down" and not oxidised.)
3. **Regeneration.** Most TP is used to **regenerate RuBP**, using more **ATP**. It is a **cycle** because RuBP, the molecule that accepts CO₂, is made again, and all the intermediates are always present.

Counting carbons. Three turns fix 3 CO₂: $3 + 3 \times 5 = 18$ carbons, which is 6 GP (6 × 3C). These become 6 TP. Five TP (15C) remake three RuBP (3 × 5C), and **one TP leaves** the cycle. So 10 TP make 6 RuBP, and two TP (six turns) are needed for one hexose.

What the intermediates make:

- **TP** → glucose and other hexoses → **sucrose** (for transport), **starch** (storage) and **cellulose** (cell walls); TP → **glycerol** and fatty acids → **lipids** (membranes, storage); TP → **amino acids**.
- **GP** → some **amino acids**.

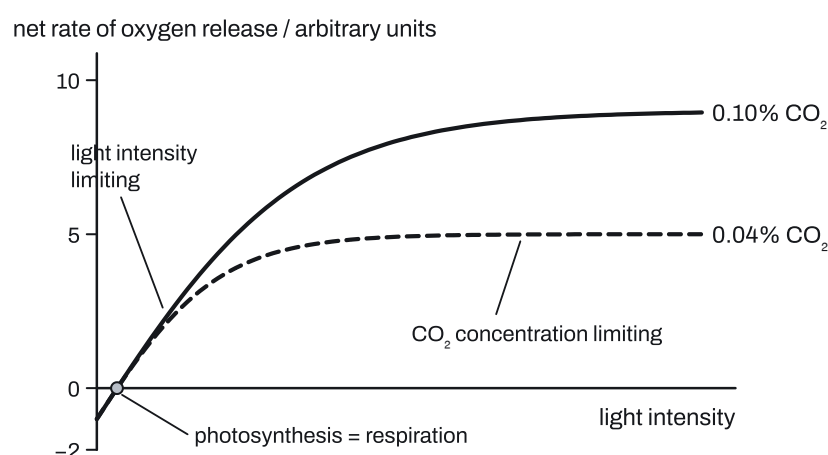
Amino acids (and so proteins and enzymes such as rubisco) also need **nitrogen**, from **nitrate** ions absorbed by the roots.

What changes the amounts of RuBP, GP and TP:

- **Less CO₂**: RuBP is still made from TP but is used more slowly, so **RuBP rises**; **GP falls** (less fixation), and **TP falls** too.
- **Darkness**: no ATP or reduced NADP, so GP cannot be reduced: **GP rises**, **TP falls** and **RuBP falls** (it is not regenerated).
- **Rubisco denatured** (for example at high temperature): **less or no fixation**, so **less GP and TP**, less RuBP regenerated and less hexose made.
- **More rubisco, or faster RuBP regeneration** (for example a genetically modified plant): more fixation, more GP and TP per second, more organic molecules, faster growth.

Limiting factors

When a process depends on several factors, the **limiting factor** is the one in shortest supply: increasing it increases the rate, increasing the others does not.



- **Light intensity** supplies the energy for the **light-dependent stage**: more light, more photoactivation and photolysis, so more **ATP and reduced NADP** for the Calvin cycle. It directly affects **photophosphorylation only**.

- **CO₂ concentration** is the substrate for **fixation by rubisco**. More CO₂, more RuBP fixed per second, more GP and TP. It directly affects the **Calvin cycle only**.
- **Temperature** changes the rate of **enzyme-controlled** reactions in **both stages** (rubisco and other Calvin cycle enzymes; ATP synthase and the oxygen-evolving complex). Warmer means more **kinetic energy**, more collisions and **enzyme-substrate complexes**, up to an **optimum**. Above it, enzymes **denature** (the active site changes shape), and the rate falls.

On a rate graph:

- On the **rising part**, the factor on the x-axis is limiting.
- Where the curve **levels off**, that factor is **no longer limiting**; something else is (CO₂ concentration or temperature on a light graph; light intensity or temperature on a CO₂ graph; or all of rubisco's active sites are full).
- If two curves at different CO₂ concentrations **overlap at low light**, light is limiting there; where they **separate**, CO₂ is limiting the lower one.

Net and gross rates. Plants **respire all the time**, in light and dark. What you measure (O₂ given off, or CO₂ taken in) is the **net** rate:

$$\text{net rate} = \text{rate of photosynthesis} - \text{rate of respiration}$$

so the **gross** rate of photosynthesis = net rate + rate of respiration (measured in the dark). In the dark the net rate is **negative** (O₂ taken in, CO₂ given out) because there is no photosynthesis. In **dim light** the net rate can still be negative: photosynthesis **is** happening, but more slowly than respiration. Where the curve crosses zero, the two rates are **equal**.

In the field, hot dry conditions make **stomata close** (to save water), so less CO₂ diffuses into the air spaces, mesophyll cells and chloroplasts, and **CO₂ concentration** becomes limiting. Water shortage limits photosynthesis this way, not by running out of water for photolysis.

Investigations with a redox indicator and chloroplasts

DCPIP and **methylene blue** are blue when oxidised and turn **colourless when reduced**. Added to an illuminated suspension of chloroplasts, they accept the **electrons emitted from chlorophyll** (in place of NADP) and lose their colour. The faster the light-dependent stage, the faster the colour goes.

1. Grind leaves in a **cold, buffered, isotonic** solution, filter, and keep the chloroplast suspension cold (slows enzymes) and **in the dark** until use (no photoactivation before the timing starts).
2. Add DCPIP to tubes of suspension and put them in light of different **intensities** (lamp distances) or **wavelengths** (coloured filters of equal intensity).
3. Measure the **time to go colourless**, or follow the **absorbance** with a colorimeter (absorbance falls as DCPIP is reduced). Rate = $\frac{1}{t}$ or change in absorbance per minute.
4. **Controls**: a tube kept in the **dark** (stays blue: light is needed) and a tube with **no chloroplasts** (stays blue: the chloroplasts reduce the DCPIP).

In coloured light, **red** and **blue** tubes lose colour quickly (much light absorbed, many electrons emitted), and **green** tubes slowly (little green light absorbed).

Investigations with whole plants

Use an aquatic plant such as *Elodea* or *Cabomba*, or algae, in **sodium hydrogencarbonate** solution (a source of CO_2).

- **Light intensity:** move a lamp to different distances d . Light intensity is proportional to $\frac{1}{d^2}$: at 0.125 m it is $\frac{1}{0.125^2} = 64$, at 0.25 m it is 16, so doubling the distance gives **a quarter** of the light. Use an **LED** lamp (little heat) so temperature stays constant.
- **CO_2 concentration:** change the concentration of sodium hydrogencarbonate.
- **Temperature:** stand the tube in water baths at different temperatures.
- **Measure:** the **volume of oxygen** collected in a capillary tube or gas syringe in a set time (better than **counting bubbles**, which vary in size), the change in **dissolved oxygen**, or the colour of **hydrogencarbonate indicator** or **bromothymol blue**. For example, 36 mm^3 of oxygen in 30 minutes is $36 \div 30 = 1.2 \text{ mm}^3$ per minute.
- **Good practice:** let the plant **acclimatise** at each new condition; keep the other factors constant; **repeat** and find a **mean**; include a control (for example, the same set-up without the plant) to show the change is caused by the plant.

Indicator colours: when a plant in light takes up more CO_2 than it releases, the solution becomes **less acidic** (bromothymol blue turns blue; hydrogencarbonate indicator turns purple). In the dark, or with an animal, respiration adds CO_2 , the solution becomes **more acidic** (yellow). With a plant and an animal together in light, the CO_2 from respiration can be used in photosynthesis and the colour may not change.

Key facts and formulas

None of these are in the syllabus's list of mathematical formulae, so they are all "Learn it". A question that needs $\frac{1}{d^2}$ usually says so.

Formula	Status
$6 \text{ CO}_2 + 6 \text{ H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{ O}_2$	Learn it
Photolysis: $2 \text{ H}_2\text{O} \rightarrow \text{O}_2 + 4 \text{ H}^+ + 4 \text{ e}^-$ (in PS II, by the oxygen-evolving complex)	Learn it
$\text{RuBP (5C)} + \text{CO}_2 \xrightarrow{\text{rubisco}} \text{unstable 6C} \rightarrow 2 \text{ GP (3C)}$	Learn it
$\text{GP} \xrightarrow{\text{ATP, reduced NADP}} \text{TP}; \text{TP} \xrightarrow{\text{ATP}} \text{RuBP}$	Learn it
Per 3 CO_2 : $3 \text{ RuBP} \rightarrow 6 \text{ GP} \rightarrow 6 \text{ TP}; 5 \text{ TP} \rightarrow 3 \text{ RuBP}; 1 \text{ TP leaves}$	Learn it
$R_f = \frac{\text{distance moved by pigment from origin}}{\text{distance moved by solvent from origin}}$	Learn it
Gross rate of photosynthesis = net rate + rate of respiration	Learn it

Formula	Status
Light intensity $\propto \frac{1}{d^2}$ (d = distance from lamp)	Learn it
Rate = $\frac{\text{amount}}{\text{time}}$; redox indicator rate = $\frac{1}{t}$	Learn it
Percentage change = $\frac{\text{new} - \text{original}}{\text{original}} \times 100$	Learn it
Mean increase per unit = $\frac{\text{change in rate}}{\text{change in } x}$	Learn it

How to do it

We have tagged 40 questions on this topic (2021–2025), all structured questions from Paper 4. The types came up this often. 35 of the 40 questions mix more than one type, so the counts add up to more than 40.

Question type	Questions
Describe and explain limiting-factor graphs (light, CO ₂ , temperature)	18
Describe the Calvin cycle and uses of its intermediates	16
Link chloroplast structure to function or identify parts	11
Describe the light-dependent stage	11
Explain the role of accessory pigments	10
Interpret absorption and action spectra	9
Explain results of altered enzymes, GM or mutant plants	8
Calculate from photosynthesis data	8
Chromatography of chloroplast pigments	7
Compare cyclic and non-cyclic photophosphorylation	5
Photosynthesis and respiration: net gas exchange	5
Evaluate practical investigations	4
Compare photosynthesis with another process, or its importance	3
C4 leaf adaptations (old syllabus)	2

1. Describe and explain limiting-factor graphs (light, CO₂, temperature) (18 questions)

Worth 2–6 marks. Split **describe** from **explain**:

1. **Describe the shape**: "rate increases as light intensity increases, then levels off" or "increases to an optimum at 29 °C, then decreases".

2. **Quote paired data with units:** both coordinates, read carefully, at the same x-value when comparing two curves ("at 400 lux, 3.1 au for mutant and 2.2 au for normal").
3. **Name the limiting factor on each part:** the x-axis factor on the rising part; on the plateau, "light intensity is **no longer** limiting; **CO₂ concentration** or **temperature** is limiting". Say "light **intensity**" and "CO₂ **concentration**", not just "light" or "CO₂".
4. **Give the mechanism:** light → more photoactivation, photolysis, ATP and reduced NADP; CO₂ → more fixation by rubisco, more GP; temperature → more kinetic energy, more enzyme–substrate complexes, then **denaturation** of rubisco, ATP synthase or the oxygen-evolving complex above the optimum.

Variations you will meet:

- **Mark a point where light is limiting** on a sketch graph: put it on the **rising** part, before the curve bends.
- **Low light only:** the rate is low and nearly constant because **light intensity** is limiting; less light is absorbed, so there is **less** (not no) photoactivation and photolysis.
- **Which stage does each factor directly affect?** CO₂ → Calvin cycle only; light intensity → photophosphorylation only; temperature → both.
- **Sketch a curve for a cloudy day:** same shape, **below** the sunny-day curve.
- **Dry conditions or deserts:** stomata close, less CO₂ diffuses in, CO₂ concentration limits; fewer, smaller plants absorb less light.
- **Ecosystems data:** compare only the ecosystems asked about; link higher rates to higher **temperature** and **light intensity** (and longer days).

2. Describe the Calvin cycle and uses of its intermediates (16 questions)

From 1-mark names to a 7-mark description. For the full description, write these points in order:

1. In the **stroma**; CO₂ **combines with RuBP** (5C);
2. catalysed by **rubisco**; this is **carbon fixation**;
3. forming an **unstable 6C** compound, which splits into **two GP** (3C);
4. GP is **reduced** to **TP**, using **reduced NADP** and **ATP** from the light-dependent stage;
5. most TP **regenerates RuBP** (using ATP), so the cycle continues;
6. some TP makes **hexoses**, sucrose, starch, cellulose, lipids and amino acids; GP makes amino acids.

Variations you will meet:

- **Fill in the gaps:** carbon dioxide, rubisco, reduced NADP, and a product of TP such as starch.
- **Name the process or reaction:** RuBP + CO₂ is **carbon fixation** (carboxylation); GP → TP is **reduction**.
- **The role of reduced NADP:** it **gives hydrogen** (reduces) GP to form TP.
- **Why "cycle"?** RuBP is regenerated; all intermediates are always present.
- **Numbers:** 10 TP → 6 RuBP; 3 CO₂ fixed for each TP that leaves.
- **Sketch RuBP and TP when CO₂ falls:** RuBP rises to a new plateau; TP (and GP) fall to a lower plateau.

- **"Explain how the Calvin cycle is affected"** (rubisco denatured, a herbicide, darkness): say which steps happen **less or not at all**, and name the first product lost (TP, then hexose).
- **Nitrogen**: an amino acid or protein needs nitrogen from nitrate, and comes from **GP** or **TP**.

3. Link chloroplast structure to function or identify parts (11 questions)

1. **Identify** from a diagram or micrograph: stacks of membranes = **granum** (thylakoids); space inside a thylakoid = **thylakoid space** (lumen); fluid = **stroma**; large pale body = **starch grain**.
2. **Match functions**: high proton concentration → **thylakoid space**; pumps protons, holds pigments, site of light-dependent stage → **thylakoid membrane**; makes TP, site of Calvin cycle → **stroma**; makes some proteins (such as rubisco) → **DNA** and **ribosomes**.
3. **Explain adaptations** as a pair each time, feature → function (use the table in "Understand it"): many stacked thylakoids → **large surface area** → **more light absorbed**; membranes hold pigments, ETC, ATP synthase, OEC; small thylakoid space → **proton gradient**; impermeable membrane → **keeps the gradient**.

Say "thylakoid **space**" or "thylakoid **membrane**", never just "thylakoid", and don't borrow mitochondrial words (intermembrane space, cristae).

4. Describe the light-dependent stage (11 questions)

Up to 7 marks, often from a diagram of the two photosystems. The points to cover:

1. Light absorbed by pigments in **PS I and PS II** → **photoactivation**: electrons **excited and emitted**.
2. Electrons pass along the **ETC**, **releasing energy**.
3. The energy **pumps H⁺ into the thylakoid space** → **proton gradient**.
4. H⁺ **diffuse back** into the stroma through **ATP synthase** → **ATP** from ADP + Pi (**chemiosmosis**).
5. PS II's **oxygen-evolving complex** catalyses **photolysis**: water → **O₂**, **H⁺** and **electrons**.
6. Electrons from PS II **replace** those lost by PS I; electrons from photolysis **replace** those lost by PS II.
7. Electrons from PS I, with H⁺, **reduce NADP** → **reduced NADP**.

Variations you will meet:

- **Products** needed by the Calvin cycle: **ATP and reduced NADP** (not reduced NAD).
- **Which photosystem releases oxygen?** PS II.
- **Roles of membrane proteins**: carriers transfer electrons and pump protons; the oxygen-evolving complex splits water; photosystem proteins hold the pigments; ATP synthase makes ATP.
- **Stroma pH rises in the light** (and falls in the dark): protons are pumped out of the stroma into the thylakoid space; this supports chemiosmosis.
- **Compare with chemiosmosis in mitochondria**: photophosphorylation vs oxidative phosphorylation; thylakoid membrane vs inner mitochondrial membrane; electrons from water (via chlorophyll) vs from reduced NAD and FAD; protons into the thylakoid space vs the intermembrane space; NADP vs oxygen as final acceptor; reduced NADP vs water formed. Both make ATP.

5. Explain the role of accessory pigments (10 questions)

Worth 2–4 marks. Four points, in this order:

1. Chlorophyll b, carotene and xanthophyll are **accessory pigments** (in the light-harvesting complex).
2. They **absorb** light of **wavelengths that chlorophyll a does not absorb** well.
3. So the **range of wavelengths** used is **wider**.
4. They **pass the energy** to **chlorophyll a** at the **reaction centre** (primary pigment), making the light-dependent stage **more efficient**.

If there is a spectrum, add its numbers: carotene absorbs about **400–500 nm**, peak near **450 nm**. Write "absorb", not "trap" or "capture", and "pass energy", not "pass light".

6. Interpret absorption and action spectra (9 questions)

1. **Definitions:** absorption spectrum = how much of **each wavelength** a **pigment absorbs**; action spectrum = **rate of photosynthesis at each wavelength**.
2. **Compare two spectra** with wavelengths: similarities first (both absorb blue; both absorb little green-yellow, about 525–600 nm), then differences (where each peaks, which absorbs at more wavelengths, which has a red peak).
3. **Explain a gap between action and absorption:** other (accessory) pigments absorb there and pass energy to chlorophyll a, so photosynthesis happens at wavelengths chlorophyll a absorbs little.

Variations you will meet:

- **Relationship between them:** the curves have the same shape; more absorption, more photosynthesis.
- **Whole chloroplasts vs pigment extract:** chloroplasts absorb more at every wavelength (give the biggest difference with data); more pigment, arranged in stacked membranes.
- **Deep-water algae:** their extra pigments absorb the green and yellow light that reaches depth, which chlorophyll a absorbs poorly; more light absorbed → more light-dependent stage → more growth.
- **Coloured filters:** green light gives the lowest rate (least absorbed, mostly reflected); red and blue are well absorbed. Link more absorption to more **light-dependent stage**.
- **Prove another pigment is present:** the action spectrum continues at wavelengths where the named pigment absorbs nothing.

7. Explain results of altered enzymes, GM or mutant plants (8 questions)

Unfamiliar data about rubisco, rubisco activase, an enzyme that speeds RuBP regeneration, or a mutant with more of a pigment.

1. **Describe:** which is higher, whether both rise then level off, with a **paired quote** at one x-value.
2. **Explain as a chain**, stating what there is **more** of at each step: more light absorbed / more active rubisco / more RuBP regenerated → **more CO₂ fixed** → **more GP and TP** → more hexose (for **respiration**, starch, cellulose), amino acids (proteins) and lipids → **faster growth** or more biomass.

3. **Why a curve levels off:** another factor (light intensity, temperature) now limits, or rubisco's **active sites** are all occupied.

Variations you will meet:

- **Which part of rubisco was changed?** The **active site** (accepted: allosteric site).
- **Enzyme with a higher optimum temperature:** different gene/alleles, so a different primary and tertiary structure; transfer that gene into a crop to keep rubisco working at high temperature.
- **Nitrate shortage:** less nitrogen for amino acids (and nucleotides), so less enzyme made.
- **Mutant with more chlorophyll b:** absorbs more light → more ATP and reduced NADP → more turns of the Calvin cycle → more TP → growth.

8. Calculate from photosynthesis data (8 questions)

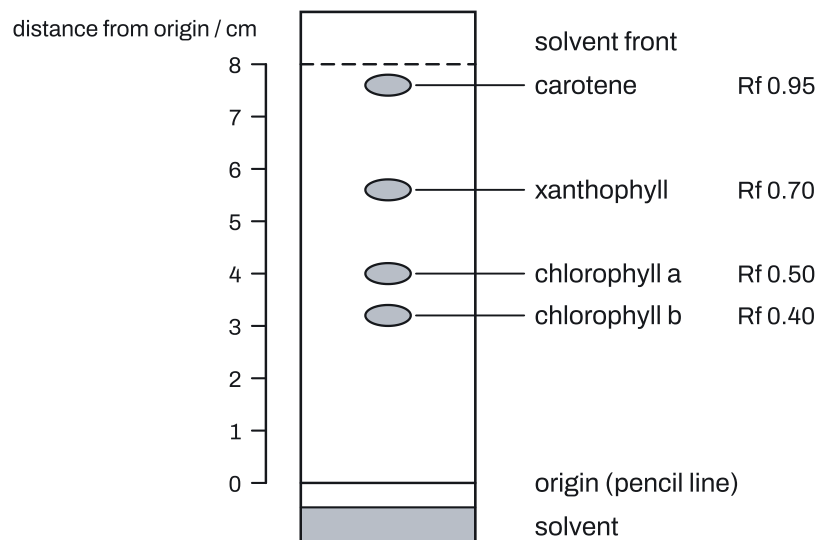
Show the working, give units, and round only at the end.

- **Rf value** = $\frac{\text{pigment distance}}{\text{solvent distance}}$, no units; rearrange to find a distance: $d = R_f \times \text{solvent distance}$.
- **Rate** = $\frac{\text{amount}}{\text{time}}$: 36 mm³ in 30 min → 1.2 mm³ min⁻¹.
- **Percentage change:** from 15 g to 24 g is $\frac{24-15}{15} \times 100 = 60\%$.
- **Mean increase per °C:** a rate rising from 11 to 35 au between 10 °C and 30 °C increases by $\frac{35-11}{30-10} = 1.2$ au per °C.
- **Gross rate** = net + respiration, both read at the same temperature or light intensity: net 5.2 plus respiration 0.8 gives 6.0.
- **Light intensity** = $\frac{1}{d^2}$.
- **Plot a graph:** independent variable on the x-axis, both axes labelled with units, points plotted exactly, a smooth **curve of best fit**; extend it only as the biology allows (it levels off when light stops limiting).

9. Chromatography of chloroplast pigments (7 questions)

Method:

1. Draw a **pencil origin line** near the bottom of the paper (ink would run).
2. **Spot** the pigment extract on it; dry and **repeat** to make a small, concentrated spot.
3. Stand the paper in **solvent**, with the solvent level **below** the origin line.
4. Remove it before the solvent reaches the top, and **mark the solvent front**.
5. Measure how far each pigment and the solvent moved **from the origin**; calculate **Rf**.
6. **Compare** each Rf with **known (reference) values** for the same solvent and paper to **identify** the pigment.



Variations you will meet:

- **Complete a table** of distances and Rf values; if one pigment is unnamed, it is the one of the four not yet listed.
- **Why Rf values differ** from published ones: a different **solvent, paper or TLC plate** (stationary phase), or **temperature**. The order of the spots depends on these too; **carotene** usually travels furthest, but the others can change places, so always identify pigments from reference Rf values, never from position alone.
- **Two chromatograms that differ**: one run for a shorter time, or a chemical that changes a pigment (removing magnesium turns chlorophyll into a different pigment with a different Rf).
- **Show a pigment is in one organism but not another**: run both extracts, calculate Rf values, and look for a spot (Rf) in one that is missing in the other, then match it to the reference value.

10. Compare cyclic and non-cyclic photophosphorylation (5 questions)

Use the comparison table in "Understand it". Write each difference as a **matched pair** in one sentence ("cyclic uses PS I only, **whereas** non-cyclic uses PS I and PS II"). Common pairs: photosystems; where PS I's electrons go (back to PS I vs to NADP); products (ATP only vs ATP and reduced NADP); photolysis and oxygen (none vs yes). If similarities are asked, give: both involve photoactivation, an ETC and ATP synthase, and both make ATP.

11. Photosynthesis and respiration: net gas exchange (5 questions)

1. Remember that plants **respire all the time**.
2. **Negative net uptake of CO₂** (or O₂ used up): respiration is faster than photosynthesis. In the dark there is no photosynthesis; in dim light there is **some**, just less than respiration. Don't write "no photosynthesis" for dim light.
3. **Gross** photosynthesis = measured net rate + respiration rate.

4. **Indicator tubes:** plant in light → CO₂ used → pH rises (bromothymol blue goes blue); animal → CO₂ released → pH falls (yellow); plant and animal in light → balance, little change; plant in the dark → only respiration → slightly more acidic.

12. Evaluate practical investigations (4 questions)

- **Why keep chloroplasts in the dark before starting:** so no light is absorbed, no photoactivation and no DCPIP reduced before timing starts.
- **Why an LED lamp or a heat filter:** temperature is kept constant so light is the only variable; temperature changes enzyme activity (kinetic energy, denaturation).
- **Control tubes** (no plant, no chloroplasts, or in the dark): for **comparison**, to show the change is caused by photosynthesis.
- **Limitations:** few values of the independent variable, judging a colour end-point by eye, only one species, no repeats. Suggest improvements such as a colorimeter, more intermediate values, and repeats with a mean.
- **Describe DCPIP results:** absorbance falls with time in the light, fastest in the colour that is absorbed most, slowest in green; quote paired data.

13. Compare photosynthesis with another process, or its importance (3 questions)

- **Similarities** with an artificial "photosynthesis" system: uses CO₂, water and light energy; makes an organic product and oxygen; converts energy from one form to another; involves electron flow.
- **Is it more efficient than crops?** Give points on both sides: how much of the light is absorbed and which wavelengths are used, energy lost at each step, respiration, limiting factors, working in the dark, extra energy and resources needed, and how much of a crop is edible.
- **Importance to ecosystems:** oxygen for **aerobic respiration**; glucose as a **store of chemical energy** passed along **food chains**.

14. C4 leaf adaptations (old syllabus) (2 questions)

Both are 2021 essay questions on maize or sorghum. C4 plants are **not** in the 2025–2027 syllabus, so you will not be asked this; skip these two when practising.

Worked examples

The bold codes show where marks are earned, as in Cambridge mark schemes. The number is how many marks.

- **M1** method mark: for a correct method, even if the numbers slip.
 - **A1** accuracy mark: for a correct answer; it needs the M mark before it.
 - **B1** mark for a correct result on its own, with no method needed.
-

Example 1

A student used paper chromatography to separate the pigments in a leaf extract. The solvent front moved 12.5 cm from the origin. The four spots moved 11.9 cm, 8.8 cm, 5.5 cm and 4.4 cm.

(a) Calculate the R_f value of each spot, to 2 decimal places. **[2]**

(b) Reference R_f values for the same solvent and paper are: carotene 0.95, xanthophyll 0.71, chlorophyll a 0.45, chlorophyll b 0.36. Identify the four spots. **[1]**

(c) Describe the role of carotene and xanthophyll in photosynthesis. **[3]**

(a) $\frac{11.9}{12.5} = 0.95$, $\frac{8.8}{12.5} = 0.70$, $\frac{5.5}{12.5} = 0.44$, $\frac{4.4}{12.5} = 0.35$. **M1** for pigment distance ÷ solvent distance, **A1** for all four.

(b) Carotene (11.9 cm), xanthophyll (8.8 cm), chlorophyll a (5.5 cm), chlorophyll b (4.4 cm): each R_f is closest to one reference value. **B1**

(c) Any three of: they are **accessory pigments**; they absorb **wavelengths** (400–500 nm) that **chlorophyll a absorbs poorly**; so a **wider range** of wavelengths is used; they **pass the energy** to **chlorophyll a** at the reaction centre. **B1 B1 B1**

Example 2

A chloroplast suspension was mixed with DCPIP in four tubes. Three were lit by red, blue or green light of the same intensity; the fourth was kept in the dark. The times for the blue colour to disappear were: red 200 s, blue 210 s, green 750 s. The tube in the dark stayed blue.

(a) Explain why the tube in the dark stayed blue. **[1]**

(b) Calculate the rate for each colour as $\frac{1000}{t}$, to 1 decimal place. **[1]**

(c) Explain the difference between the rates in red and green light. **[3]**

(a) No light was absorbed, so there was no **photoactivation**: no electrons were emitted to **reduce** the DCPIP. **B1**

(b) Red $\frac{1000}{200} = 5.0$, blue $\frac{1000}{210} = 4.8$, green $\frac{1000}{750} = 1.3$ (arbitrary units). **B1**

(c) Red light is **absorbed** much more by **chlorophyll**; green is absorbed little (mostly reflected). **B1** So in red light there is more **photoactivation** and **more electrons are emitted** per second. **B1** So DCPIP is **reduced** (decolourised) faster, a rate $\frac{5.0}{1.3}$, almost four times as fast. **B1**

Example 3

(a) Describe the process of non-cyclic photophosphorylation. **[6]**

(b) When isolated chloroplasts are moved from the dark into the light, the pH of the stroma rises. Explain why. **[2]**

(a) Any six of:

- light is absorbed by **PS II and PS I**; **photoactivation**: electrons are **excited and emitted**; **B1**
- electrons pass along the **electron transport chain**, releasing **energy**; **B1**
- the energy **pumps protons into the thylakoid space**, making a **proton gradient**; **B1**
- protons **diffuse back** through **ATP synthase**, making **ATP** from ADP and Pi (**chemiosmosis**); **B1**
- the **oxygen-evolving complex** in PS II catalyses **photolysis** of water, into **oxygen, H⁺ and electrons**; **B1**
- electrons from PS II **replace** those lost by PS I, and electrons from water **replace** those lost by PS II; **B1**
- electrons from PS I and H⁺ **reduce NADP** to **reduced NADP**. **B1**

(b) In the light the electron transport chain **pumps protons out of the stroma** into the **thylakoid space**, **B1** so the **H⁺ concentration in the stroma falls** and its pH rises. **B1**

Example 4

An aquatic plant was kept at 20 °C. The net rate of oxygen release, in mm³ h⁻¹, was measured at different light intensities and two CO₂ concentrations. In the dark it took in 6 mm³ h⁻¹ of oxygen.

Light intensity / au	0	10	20	40	60	80
0.04% CO ₂	-6	12	26	38	41	41
0.10% CO ₂	-6	12	27	55	68	70

(a) Explain why the rate is negative at a light intensity of 0. **[2]**

(b) Calculate the gross rate of photosynthesis at 60 au with 0.10% CO₂. **[1]**

(c) Identify the limiting factor between 0 and 20 au, and at 60–80 au with 0.04% CO₂. Explain your answers. **[4]**

(d) Calculate the percentage increase in the net rate at 80 au when the CO₂ concentration is raised from 0.04% to 0.10%. Give your answer to 1 decimal place. **[2]**

(a) In the dark there is **no photosynthesis**, so no oxygen is released; **B1** the plant still **respires**, which **uses oxygen**. **B1**

(b) Gross = net + respiration = $68 + 6 = 74 \text{ mm}^3 \text{ h}^{-1}$. **B1**

(c) 0–20 au: **light intensity**, **B1** because the two CO_2 concentrations give the **same rates** (12 at 10 au, 26 and 27 at 20 au), so extra CO_2 has no effect; more light gives more ATP and reduced NADP. **B1** 60–80 au at 0.04%: **CO_2 concentration**, **B1** because the rate stays at $41 \text{ mm}^3 \text{ h}^{-1}$ as light increases, but raising the CO_2 gives $68\text{--}70 \text{ mm}^3 \text{ h}^{-1}$; more CO_2 means more fixation by rubisco. **B1**

(d) $\frac{70-41}{41} \times 100$ **M1** = 70.7%. **A1**

Common mistakes

- **Saying accessory pigments pass on light.** They pass on **energy** to chlorophyll a. Say what's special: they absorb wavelengths **chlorophyll a doesn't**, widening the range.
- **"Trap" or "capture" light.** Mark schemes want **absorb**.
- **Defining "limiting factor" when asked to explain.** Say **why** that factor limits the rate here (light → ATP and reduced NADP; CO_2 → fixation; temperature → kinetic energy, denaturation).
- **Writing "light" or " CO_2 " for light intensity and CO_2 concentration.** Both lose the mark.
- **Saying no light is absorbed** (or no photolysis) at low light intensity. There is **less**, not none; the plant is still making oxygen.
- **Saying a plant "respires instead" of photosynthesising.** It respires **all the time**. A negative net rate in dim light means respiration is **faster than** photosynthesis, not that photosynthesis has stopped.
- **Protons pumped "into the thylakoid" or "into the membrane".** They go **from the stroma into the thylakoid space (lumen)**, and back **into the stroma** through ATP synthase.
- **Mixing up thylakoid space and thylakoid membrane.** The **membrane** pumps protons; the **space** is where they build up.
- **Borrowing mitochondrial words:** "oxidative phosphorylation", "intermembrane space", "chloroplasts contain mitochondria", "ATPase". In chloroplasts it is **photophosphorylation**, the **thylakoid space**, **ATP synthase**. Chemiosmosis in chloroplasts **does** make ATP.
- **Reduced NAD instead of reduced NADP.** Photosynthesis uses **NADP**.
- **Photolysis products:** H^+ (not hydrogen), O_2 (not O), and electrons. Photolysis happens at **PS II**.
- **GP "breaks down" to TP, or is oxidised.** GP is **reduced**. GP is **glycerate** 3-phosphate, not glycerol or glucose phosphate.
- **Temperature and stomata.** If the question asks for the **direct** effect of temperature on the reactions of photosynthesis, explain the **enzyme-controlled reactions** (kinetic energy, enzyme–substrate complexes, denaturation), not stomata. If it asks why a **whole plant's measured rate** (such as CO_2 uptake on a graph) levels off or falls at high temperature, **stomatal closure** (to save water, so less CO_2 diffuses in) is also creditworthy, alongside enzyme denaturation, as in [9700/42/O/N/23 Q8](#).
- **Identifying pigments by position.** The order of spots depends on the solvent and paper; use **Rf values** compared with **reference values**.

- **Chromatography method:** drawing the origin in ink, letting the solvent cover the origin, forgetting to mark the solvent front.
- **Data quotes:** one number, or numbers for two curves at **different** x-values, or no units.

How to get the marks

- **"State" / "name" / "identify":** one precise term: "thylakoid space", "carbon fixation", "reduction", "PS II", "ATP synthase", "triose phosphate". Don't give a list; a wrong extra can cancel a right one.
- **"Describe" (a graph):** overall trend, where it changes (levels off, optimum), then a **paired data quote** with units. Don't explain unless asked.
- **"Explain":** link each step with "so": factor → which stage → which product (ATP, reduced NADP, GP, TP) → rate.
- **"Describe and explain":** both halves, usually a mark or two for each.
- **"Outline"** a process (Calvin cycle, non-cyclic photophosphorylation): the main steps in order, one mark each, with the key words (rubisco, fixation, GP, reduced, TP, regenerated; photoactivation, ETC, proton gradient, ATP synthase, photolysis, NADP).
- **"Compare" / "differences":** matched pairs with "whereas", or a two-column table. A negative on one side only ("cyclic makes no oxygen") needs the other half too.
- **"Suggest":** unfamiliar context; apply the principles (limiting factors, the Calvin cycle chain) to the data given. Refer to the figure.
- **Calculations:** show the working, use the decimal places asked, and give units (Rf has none). A mark can follow from your own earlier value.
- **Stay in context:** if the question is about the thylakoid membrane, don't describe the Calvin cycle; if it asks about three forests, don't discuss the grassland.

Quick check

1. Name the precise part of the chloroplast where (a) CO₂ is fixed, (b) protons reach a high concentration, (c) water is split.
2. The CO₂ supply to a leaf in bright light is suddenly cut. State and explain what happens to the concentrations of RuBP and GP.
3. How many molecules of RuBP can be regenerated from 25 molecules of TP? How many CO₂ molecules must be fixed for each TP that leaves the cycle?
4. A lamp is moved from 0.10 m to 0.20 m from a plant. Using $\frac{1}{d^2}$, by what fraction does the light intensity change?
5. A pigment moved 3.9 cm and the solvent 6.0 cm. Calculate the Rf value. A plant's net CO₂ uptake is 1.2 au at 10 °C and 3.0 au at 25 °C, and its respiration at 25 °C is 0.4 au. Calculate the mean increase in net uptake per °C, and the gross rate of photosynthesis at 25 °C.

Answers

1. (a) The **stroma**. (b) The **thylakoid space** (lumen). (c) At **PS II** in the **thylakoid membrane**, by the **oxygen-evolving complex** (on the thylakoid-space side).
2. **RuBP rises**: it is still regenerated from TP but is no longer used up in fixation. **GP falls**: less CO_2 is fixed, so less GP is made, while existing GP is still reduced to TP.
3. $25 \times 3 = 75$ carbons $= 15 \times 5$, so **15 RuBP**. **3 CO_2** per TP that leaves.
4. $\frac{1}{0.10^2} = 100$ and $\frac{1}{0.20^2} = 25$, so the intensity falls to **a quarter** of its value.
5. $R_f = \frac{3.9}{6.0} = 0.65$. Mean increase $= \frac{3.0-1.2}{25-10} = \frac{1.8}{15} = 0.12$ au per $^\circ\text{C}$. Gross rate at $25^\circ\text{C} = 3.0 + 0.4 = 3.4$ au.

Past questions to try

- 9700/42/O/N/24 Q7: identifying pigments by chromatography, the role of carotenoids, and describing the Calvin cycle.
- 9700/43/M/J/24 Q7: calculating and plotting rates against light intensity, then how photoactivation makes ATP.
- 9700/42/F/M/24 Q7: a DCPIP experiment with coloured light, and which stage each factor directly affects.
- 9700/42/M/J/22 Q2: light intensity as a limiting factor, sketching RuBP and TP when CO_2 falls, and a GM plant's biomass.