# IB Biology DP

YOUR NOTES

## 8. Metabolism, Cell Respiration & Photosynthesis (HL Only)

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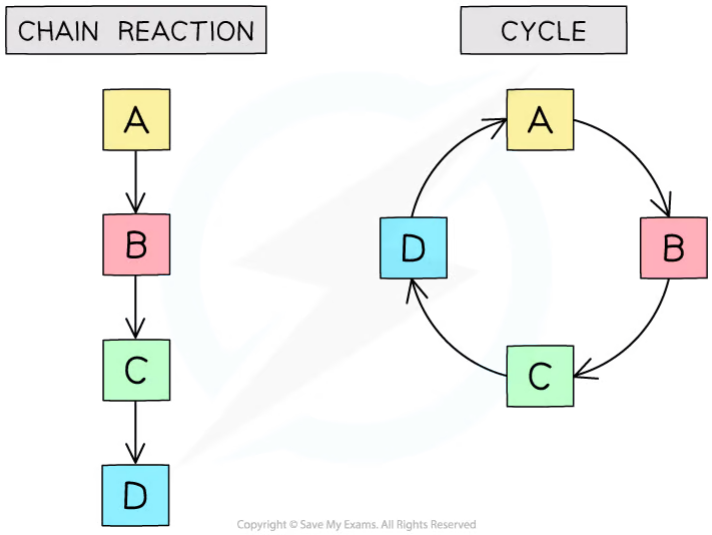
YOUR NOTES

# 8.1 Metabolism

## 8.1.1 Metabolic Pathways

### Metabolic Pathways

- Metabolic pathways involve a **series of small steps**, each step involves a **chemical change**
- The **enzyme-catalysed** reactions that make up metabolic pathways usually consist of **chains** or **cycles**:
  - Chain reactions are a linear sequence with a distinct beginning and end
    - Glycolysis, part of respiration, is an example of a reaction chain metabolic pathway
  - Cycles involve the end product starting the next cycle, these are less common than chain reactions
    - The Calvin cycle, part of photosynthesis, is an example of a cyclic metabolic pathway




***A chain metabolic pathway has a distinct start and finish, whereas in a cycle the end product feeds back into the starting reactant***

- Chemicals involved in metabolic pathways are called **metabolites** or **intermediates**
  - Some form new molecules within cells
  - Others breakdown molecules and involve an energy transfer

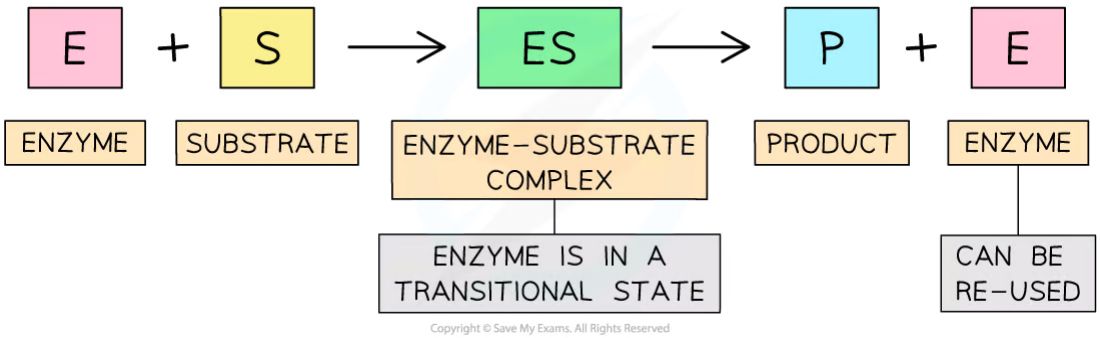
# Enzymes & Activation Energy

YOUR NOTES

- **Metabolic pathways** are controlled by **enzymes** in a biochemical cascade of reactions
  - Virtually every metabolic reaction within living organisms is catalysed by an enzyme
  - Enzymes are therefore essential for life to exist
- Enzymes are **biological catalysts**
  - 'Biological' because they function in **living systems**
  - 'Catalysts' because they **speed up** the rate of chemical reactions without being used up or undergoing permanent change

## The Enzyme-Substrate Complex

- The starting point of a metabolic pathway is a **substrate** which is converted to an end product
- The enzyme works by binding to the substrate at a special site on the enzyme called the **active site**
  - The active site of an enzyme has a specific shape to fit a specific substrate
- Substrates **collide** with the enzyme's active site and this must happen at the **correct orientation** and speed in order for a reaction to occur
- An **enzyme-substrate complex** is formed, temporarily, when the substrate binds to the active site
  - The substrate is said to be in a **transitional state** at this moment
- The product is formed and enzyme is released to take part in another reaction
- The reaction can be shortened to a simple equation





***The simple equation can show how an enzyme reaction proceeds***

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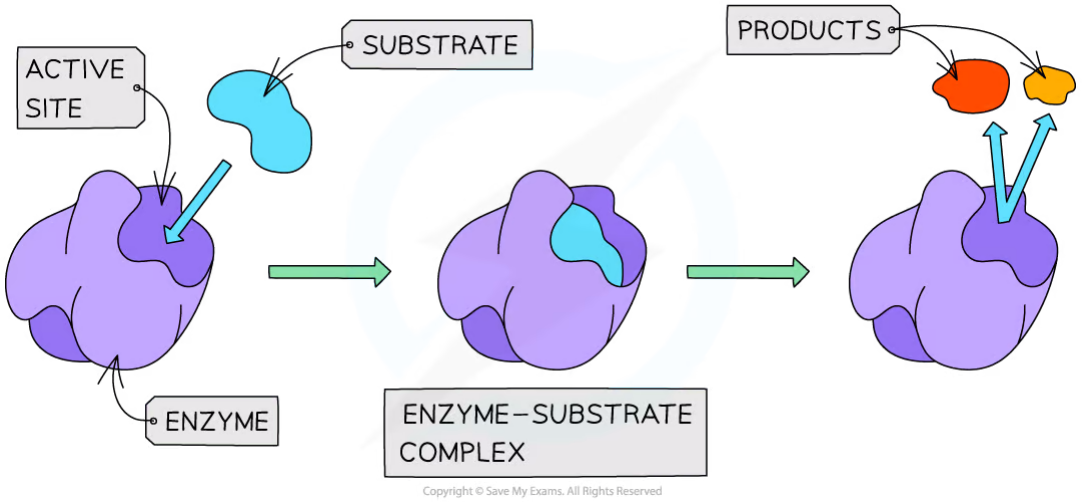
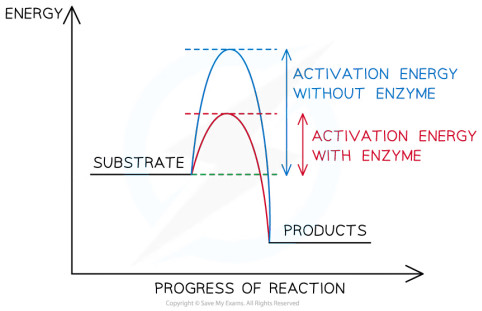



***The formation of the enzyme-substrate complex where the substrate is said to be in a transitional state, before forming the product(s)***

## Enzymes and the lowering of activation energy

- All chemical reactions, including metabolic pathways, are associated with **energy changes**
- Energy may either be released or absorbed during a reaction
  - If energy is released to the surroundings it is an **exergonic** reaction
  - If energy is absorbed from the surroundings it is an **endergonic** reaction
- For a reaction to proceed there must be enough **activation energy**
- Activation energy is the amount of **energy** needed by the substrate to become **unstable** enough for a reaction to occur and for **new products** to be formed
- Enzymes **speed up** chemical reactions because they reduce the **stability of bonds** in the substrate
- Enzymes **lower the activation energy** needed to catalyse a reaction
  - The energy released is unchanged but the activation energy required is lowered
  - The rate of reaction is therefore quicker




***The graph shows how an enzyme lowers the activation energy required for a reaction***

## Exam Tip

Don't forget that enzymes are **proteins**, meaning that anything that could **denature** a protein and make it non-operational (such as extremes of heat, temperature, pH etc.) would also denature an enzyme.

**Endergonic** and **exergonic** reactions are defined by the net the intake or output of energy (respectively) this differs from endo**thermic** and exo**thermic** reactions which are defined by the intake or output of **thermal energy** only.

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## Enzyme Inhibitors

- **Inhibitors** are chemical substances that can bind to an enzyme and **reduce its activity**
- Inhibitors can be formed from **within the cell** or can be introduced from the **external environment**
- An enzyme's activity can be **reduced** or **stopped,** temporarily, by an inhibitor
- There are two types of inhibitors: **competitive** and **non-competitive**

### Competitive inhibitors

- Competitive inhibitors have a **similar shape** to that of the substrate molecules
- They **bind to the active site** of the enzyme, interfering with it and **competing** with the substrate for the active site
- The substrate, therefore, cannot bind to the active site if a competitive inhibitor is already bound

### Non-competitive inhibitors

- Non-competitive inhibitors bind to the enzyme at an **alternative site**, which **alters the shape** of the **active site**
- This therefore prevents the substrate from binding to the active site

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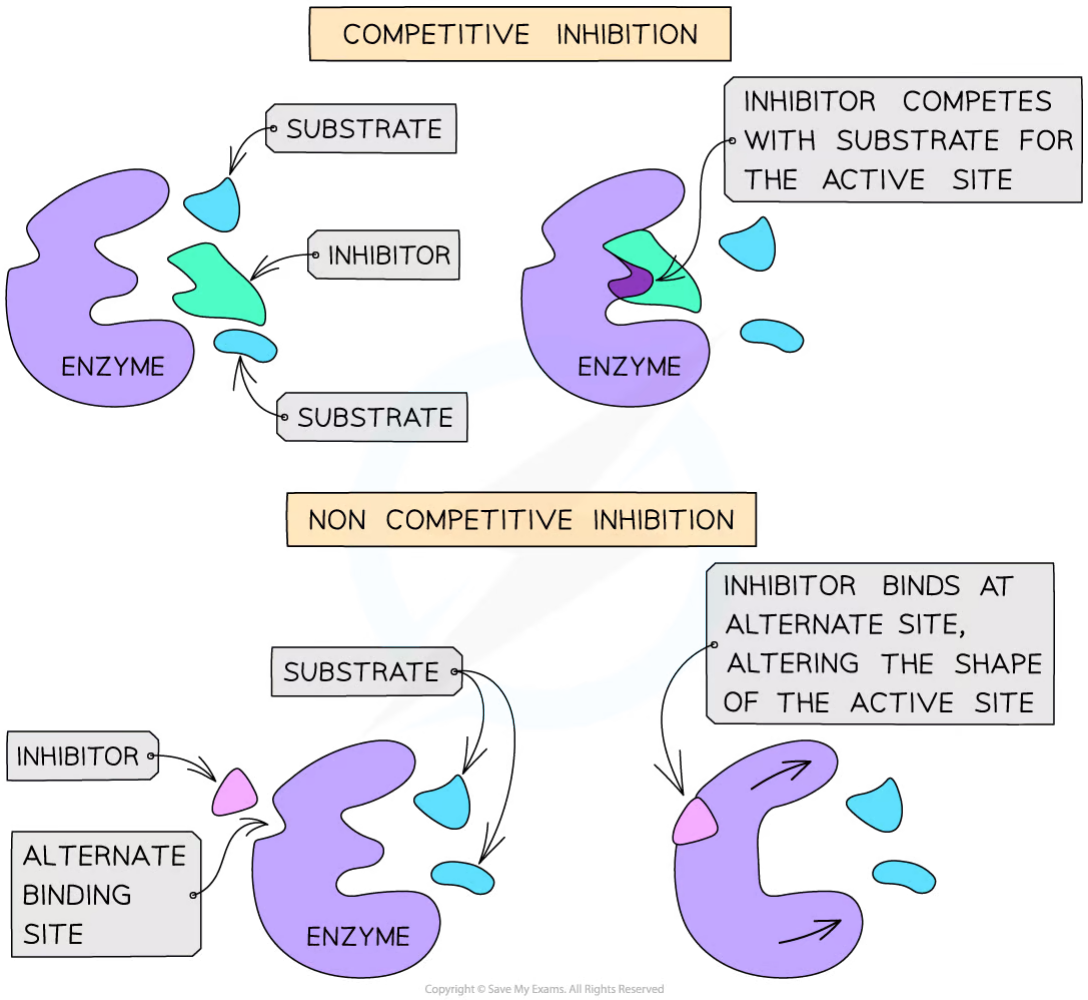


***Competitive and non-competitive inhibition***

## Examples of competitive and non-competitive inhibitors

- An example of a **competitive** inhibitor involves the **enzyme RuBisCo**, an important carbon fixation enzyme in **photosynthesis**
  - **Oxygen** is a competitive inhibitor to this enzyme and blocks the active site for carbon dioxide
  - Therefore carbon dioxide cannot bind to RuBisCo and reactions involved in **photosynthesis slow down or cease to occur**
  - This can be **fatal** to the plant
- An example of a **non-competitive** inhibitor involves the enzyme **cytochrome c oxidase**, a mitochondrial enzyme that catalyses one of the key reactions in aerobic respiration
  - **Cyanide ions** are a non-competitive inhibitor that binds to a site on the enzyme and **change the shape of the active site**
  - Therefore cytochrome c oxidase cannot carry out its functions in respiration
  - This can be **fatal** as it takes too long to produce new enzymes and the organism will die before this can occur
  - Cyanide is known as a **metabolic poison** because it interferes with metabolic pathways

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**Table comparing competitive and non-competitive inhibitors**

| Competitive Inhibitors | Non-Competitive Inhibitors |
|---|---|
| Bind to the active site | Bind to alternative site on the enzyme |
| Chemically resemble the substrate | Chemically unlike the substrate |
| Block the active site | Change the shape of the active site |
| Low concentration allows high substrate concentration to overcome inhibitors | Low concentration doesn't allow high substrate concentration to overcome inhibitors |



**Exam Tip**

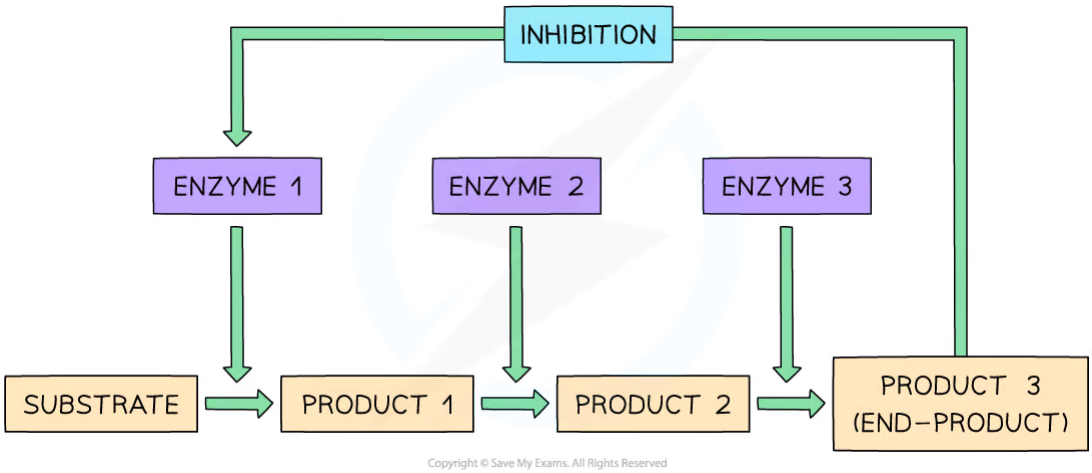
You need to be able to give a named example for competitive and non-competitive inhibition

## End-product Inhibition

YOUR NOTES

- Enzymes can be **regulated** by chemical substances that bind to a site on the enzyme away from the active site, known as the **allosteric site**
- Binding to this site, away from the active site forms an **allosteric interaction** leading to a reversible change in the **shape and activity**
- Chemicals that regulate the metabolic pathway like this are termed **allosteric regulators**

- **End-product inhibition** occurs when the end product from a reaction is present in excess and itself acts as a **non-competitive inhibitor** of the enzyme
- The end product binds to an **allosteric site** on the enzyme and causes inhibition of the pathway, so they are referred to as **allosteric inhibitors**
- Allosteric inhibitors are important to prevent the build-up of **intermediate products** in a metabolic pathway, as each small step of the pathway may produce a new product
- The product therefore does not accumulate and the pathway can continue
    - An outline of the process is as follows:
        - As the enzyme converts substrate to an end product, the process is itself slowed down as the **end-product** of the reaction chain binds to an allosteric site on the original enzyme, changing the shape of the active site and preventing the formation of further **enzyme-substrate complexes**
        - The inhibition of the enzyme means that product levels fall, at which point the enzyme begins catalysing the reaction once again; this is a continuous **feedback loop**
            - The end-product inhibitor eventually detaches from the enzyme to be used elsewhere; this is what allows the active site to **reform** and the enzyme to return to an **active state**





***End-product inhibition where the end-product of an enzyme controlled pathway inhibits the starting enzyme and limits the reactions***

### Worked Example

Show, with a **diagram**, the end-product inhibition of the pathway that converts **threonine** to **isoleucine**

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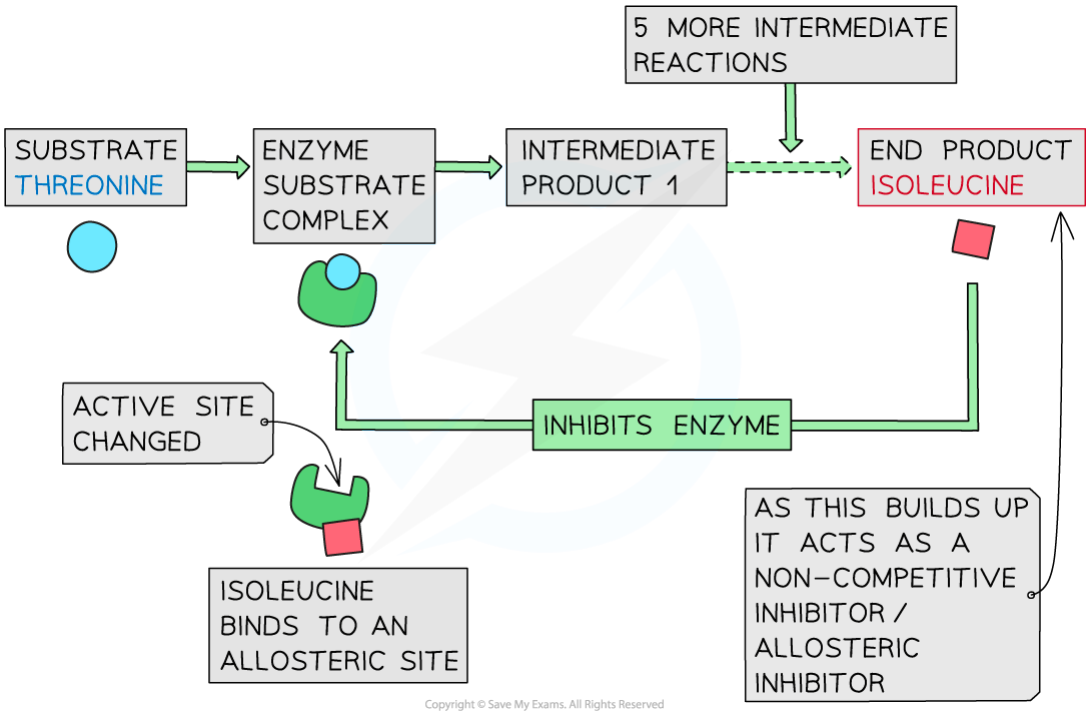


***Example of end-product inhibition between threonine and isoleucine***

**Exam Tip**

You need to know the specific example of end-product inhibition of **theonine** and **isoleucine**

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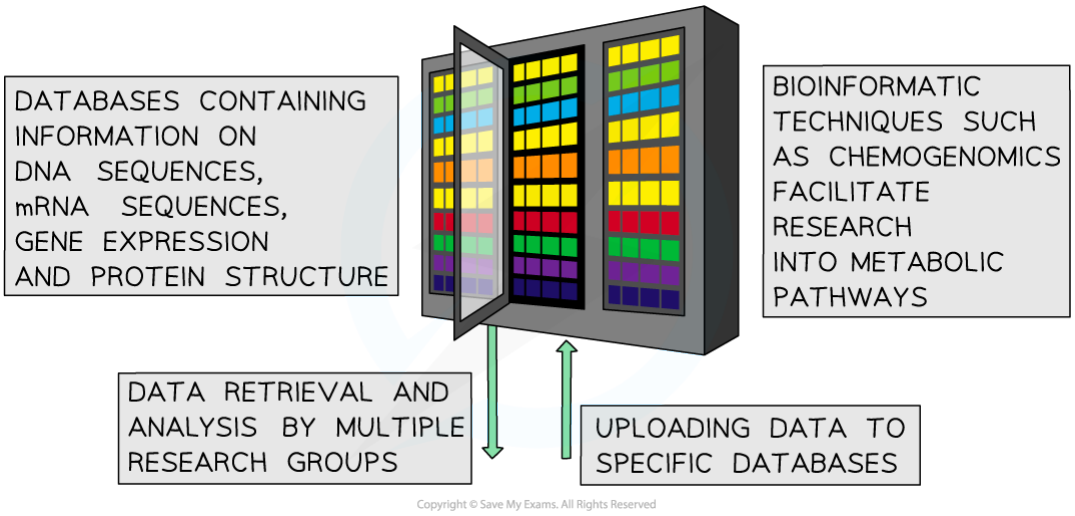
## Bioinformatics: Investigating Metabolism

NOS: Developments in scientific research follow improvements in computing: developments in bioinformatics, such as the interrogation of databases, have facilitated research into metabolic pathways

- **Bioinformatics** is the use of **computers to analyse and sequence data** in biological research
- It has led to the creation of massive **databases** of information on molecules such as proteins, genes and DNA sequences
- Bioinformatics involves multiple scientific research groups contributing into central databases; other groups can then analyse the research and raise queries
- There are a number of different applications of bioinformatics
  - **Testing commercially available drugs** on diseases that the drugs have not been originally targeted for
  - Theoretical molecular chemicals can be developed to **screen databases for new compounds** with the potential for targeting specific diseases, such as malaria
  - **Gene function** can be studied using model organisms with similar sequences
  - When **developing new drugs** scientists can test whole libraries of chemicals individually on a range of model organisms





***The use of bioinformatics by scientists***

- One bioinformatics technique has specifically facilitated research in **metabolic pathways** and is called **chemogenomics**
- Chemogenomics focuses on finding chemicals that target enzyme binding sites in order to alter metabolic pathways

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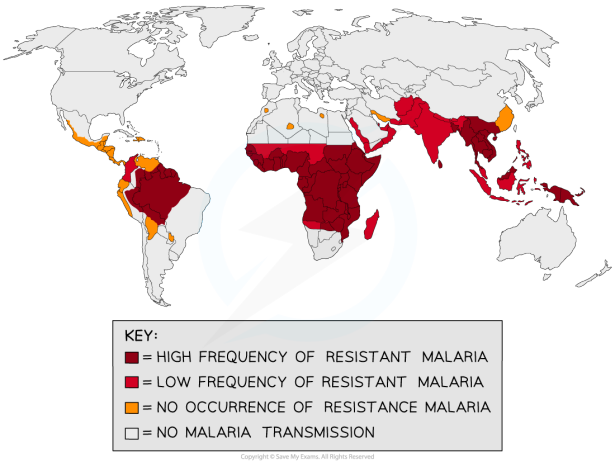
## Bioinformatics: Identifying Anti-malarial Drugs

- **Malaria** is a disease caused by the parasitic **protozoans** of the genus ***Plasmodium***
- Some *Plasmodium* protozoa have become **resistant** to many of the available drugs currently used to treat the disease, such as chloroquine
- The development and life cycle of the parasite is governed by specific **enzymes** and **metabolic pathways**
- A global research effort is in place to determine **new methods of treatment** for malaria
- The use of **bioinformatics** has a crucial place in this research by targeting the enzymes and metabolites within the parasite




***Map showing the occurrence of resistant malaria parasites across the globe***

### The use of bioinformatics in identifying malarial inhibitors

- Scientists have sequenced the proteome of the parasitic *Plasmodium falciparum*
- Consequently the enzymes involved in the parasite's metabolism have been **identified** and can be **targeted** for **inhibition**
- Targeting these enzymes and metabolic pathways by inhibition can facilitate the development of **new anti-malarial drugs and medications**
- Bioinformatics can be used to **screen** the parasite's enzymes against a **database of chemicals** to identify potential **enzyme inhibitors**
  - Molecular models of the target enzymes can be **tested against computer designed models of inhibitors**
  - So far over 300,000 chemicals have been screened against resistant malaria strains to identify **19 new chemicals** that can inhibit the parasite's enzymes

### The use of bioinformatics in finding treatments for malaria

- Aside from targeting malarial inhibitors, bioinformatics has also played a key role developing other **new treatments** for malaria, including
  - Chemical **modification of current anti-malarial drugs** to create hybrid drugs
  - Screening databases for **new compounds** with potential anti-malarial activity
  - **15 new chemicals** have been identified that bind to 61 malarial proteins creating new lines of investigation for scientists to follow in the search for anti-malarials

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## Calculating & Plotting Rates of Reaction

- Enzyme catalysed reactions can be affected by changes in **pH, temperature or substrate concentration**
- The **rate of reaction** can be determined by measuring the **rate of disappearance of a substrate** or the **rate of product accumulated** in a **given time period**
- This may be shown as a change in quantity (usually volume or mass) of substrate or product over a measured time period:

$$\text{RATE OF A REACTION} = \frac{\text{CHANGE IN AMOUNT OF REACTANTS OR PRODUCTS (mol dm}^{-3}\text{)}}{\text{TIME (s)}}$$

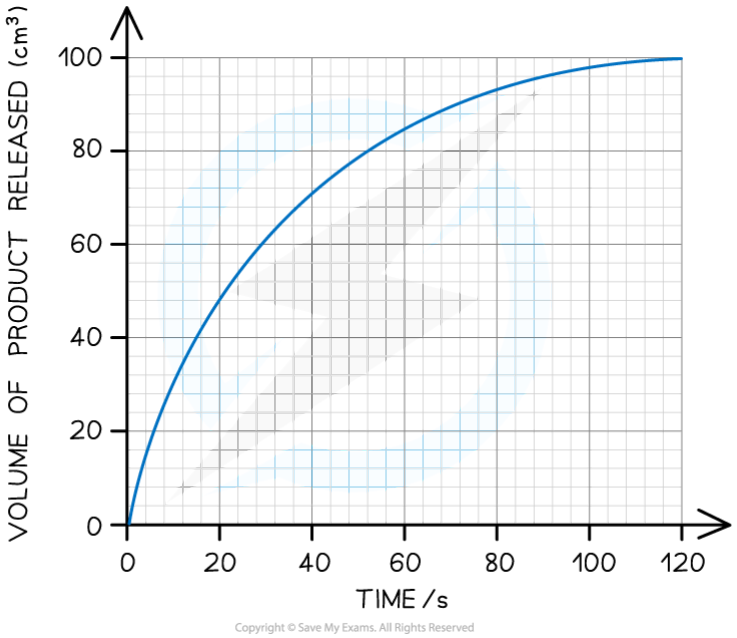


- **Or,** if we cannot collect quantitative data on the amount of substrate or product, we can calculate the rate of reaction **based on the time measured** using the following equation:

$$\text{RATE OF REACTION} = \frac{1}{\text{TIME TAKEN (s}^{-1}\text{)}}$$



  - **1 ÷ time taken (seconds)** and should include the units $s^{-1}$
- A **high** rate of reaction is when the reaction happens in **less time** i.e. it is faster
- A **low** rate of reaction is when the reaction happens in **more time** i.e. it is slower
- The rate of a reaction is likely to change throughout a reaction as the **substrate concentration will decrease** as the reaction proceeds
  - This leads to a graph that starts out as a **directly proportional** straight line (the value on the X increases at the same rate as the value on the Y) but then **plateaus as the reaction slows down**
- The **steeper the line the faster the rate of reaction**
- The rate of reaction can be calculated from a graph plotted where the reaction **time** is shown on the X-axis and the **quantity of product or substrate** is shown on the Y-axis






***Graph produced when plotting the volume of a product produced against time***

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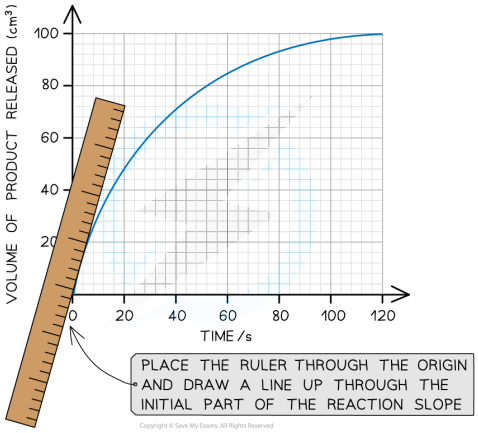
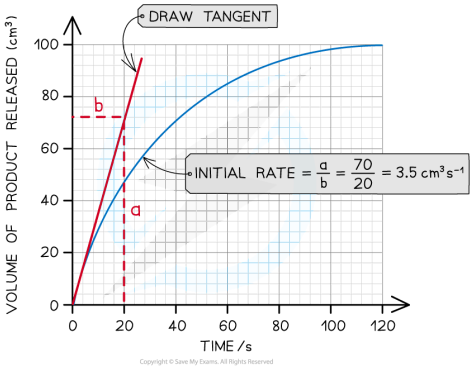
- The gradient is calculated from a point on the graph and used as a measure of the rate of reaction at that point in time
- A **tangent** must be drawn to calculate the change in x and y so the rate of reaction can be calculated
  - E.g. if calculating the initial rate of reaction
    - Place a ruler on the point of **origin** and draw a line that corresponds to the curve during the early part of the reaction
    - **Extend the line** as far as is convenient to perform the calculations e.g. to 60 seconds




***Drawing a tangent to the graph to calculate the initial rate of reaction***

## Calculating the rate of reaction

- Once the tangent is drawn you can calculate the **gradient** of the line which is equal to the rate of the reaction
  - Initial rate = a ÷ b
  - Where
    - a = change in volume and
    - b = change in time
  - The units will be **$cm^3\ sec^{-1}$** (this means volume per sec)




***Calculating the rate of reaction from the tangent***

## Identifying Types of Inhibition

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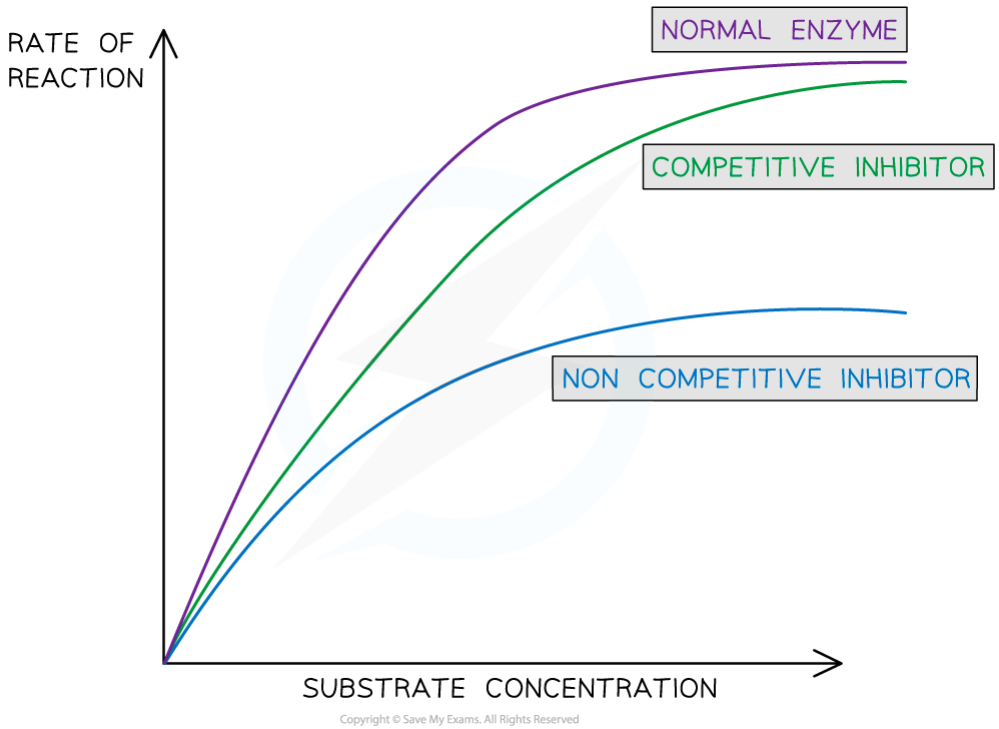
- The effect of competitive and non-competitive inhibitors on enzyme controlled reactions can be represented graphically
- Both types of inhibitors **slow down** or **stop** enzyme activity, decreasing the rate of reaction
- **Increasing** the **concentration** of an inhibitor **reduces** the rate of reaction and eventually, if inhibitor concentration continues to be increased, the reaction will **stop completely**
  - For **competitive inhibitors** countering the increase in inhibitor concentration, by increasing the substrate concentration, **can increase** the rate of reaction but the substrate needs to reach a high enough concentration in order to displace the inhibitor (more substrate molecules mean they are more likely to collide with enzymes and form enzyme-substrate complexes)
  - For **non-competitive inhibitors** increasing the substrate concentration **cannot increase** the rate of reaction, as the shape of the active site of the enzyme remains changed and enzyme-substrate complexes are still unable to form

- A graph can be used to distinguish between the two different types of inhibitors and their effect on the rate of reaction
- The patterns shown are **notably different for each type of inhibitor** and also for an **uninhibited enzyme**



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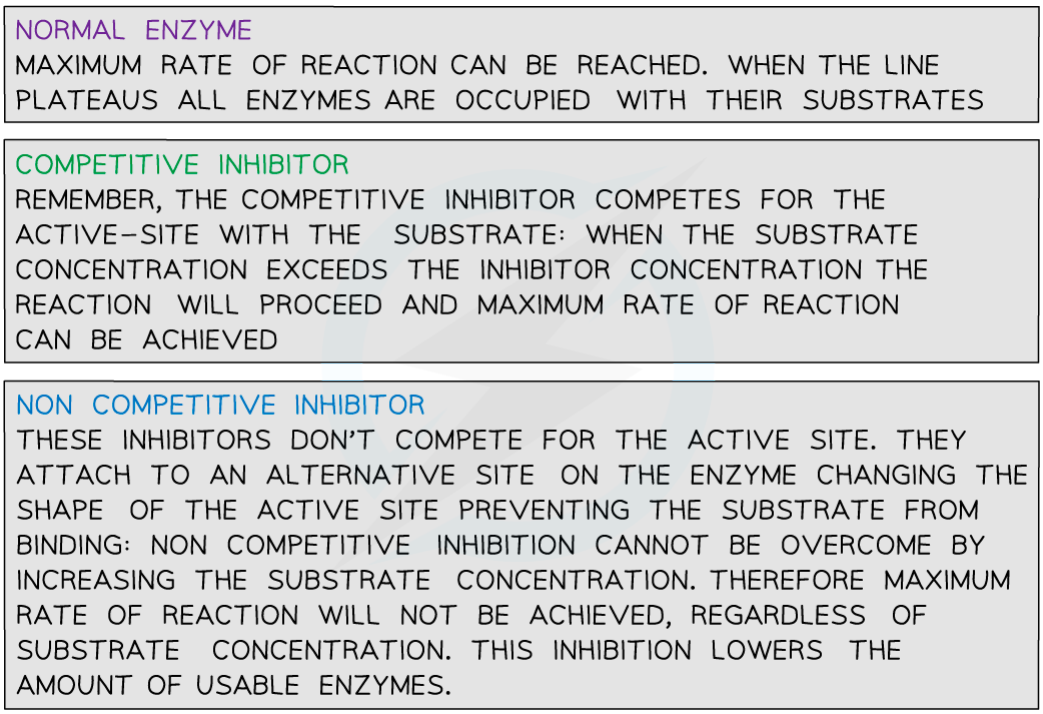



***Graph showing different types of inhibitors and their effect on rate of reaction***

- A competitive inhibitor will lower the initial rate of reaction (by occupying some of the available active sites), whilst the maximal rate is not affected
  - **Eventually,** the same amount of product will be produced as would have been produced without the competitive inhibitor
- Non-competitive inhibitors lower the initial rate of reaction **and the maximal** rate of reaction
  - A lower amount of product is produced than would normally be produced

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## 8.2 Cell Respiration

### 8.2.1 Oxidation, Reduction & Phosphorylation

## Oxidation & Reduction

- Oxidation and reduction are commonly known as **redox reactions**
- These reactions occur at the same time and involve the transfer of electrons between molecules
  - **Oxidation** is the **loss of electrons**
  - **Reduction** is the **gain of electrons**
- Redox reactions also involve hydrogen, oxygen and energy transfer
  - Oxidation is also the **loss of hydrogen**, **gain of oxygen** and **releases energy** to the surroundings (exergonic)
  - Reduction is also the **gain of hydrogen**, **loss of oxygen** and **absorbs energy** from the surroundings (endergonic)
- Molecules that have a strong tendency to lose/donate their electrons, are known as **reducing agents**
- Molecules that that have a strong tendency to gain electrons, are known as oxidising agents
- Oxidation and reduction reactions feature in **cellular respiration** and **photosynthesis**

**Table comparing oxidation and reduction**

| Oxidation | Reduction |
|---|---|
| Loss of electrons | Gain of electrons |
| Loss of hydrogen | Gain of hydrogen |
| Gain of oxygen | Loss of oxygen |
| Exergonic (releases energy) | Endergonic (absorbs energy) |



### Oxidation and reduction in cell respiration

- Respiration involves a group of molecules called **electron carriers** which accept or donate their electrons
  - **$NAD^+$** is the primary electron carrier involved in respiration
  - **FAD** is another electron carrier used in respiration
- Both NAD and FAD serve as **oxidising agents**:
  - $NAD^+$ and FAD gain electrons and also gain one or more hydrogen ions (from molecules involved in respiration), switching to a slightly different form called **reduced NAD** and **reduced FAD**
  - $NAD^+ + 2e^- + 2H^+ \dashrightarrow NADH + H^+$

- $FAD + 2e^- + 2H^+ \dashrightarrow FADH_2$

- These electron carriers are used to transport the electrons they have gained to other reactions in respiration
- When they lose these electrons they return to their original form releasing their electrons in the process
  - $NADH \dashrightarrow NAD^+ + 2e^- + 2H^+$
  - $FADH_2 \dashrightarrow FAD + 2e^- + 2H^+$
- This is an example of a **redox reaction**

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## Exam Tip

To help you remember which way around loss and gain of electrons is from redox reactions, think OILRIG:

- **O**xidation **I**s **L**oss
- **R**eduction **I**s **G**ain

NAD is a collective term for the different forms NAD takes; NAD exists in an oxidised and a reduced form:

- $NAD^+$ is the oxidised form and acts as an oxidising agent
- NADH is the reduced form and acts a reducing agent

## Phosphorylation

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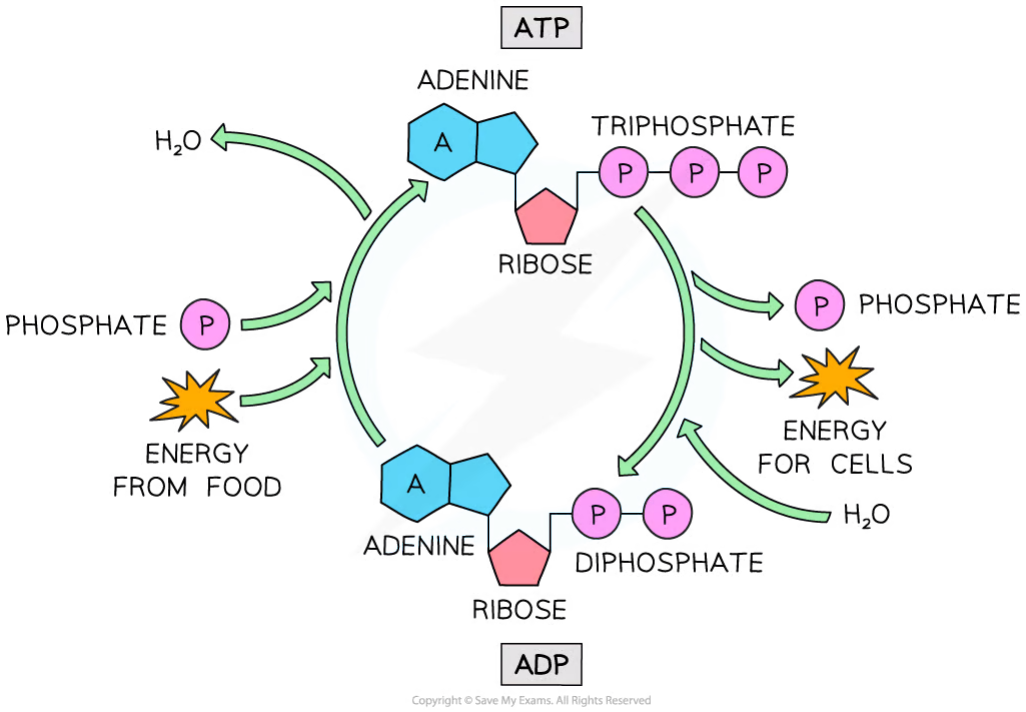
- Phosphorylation occurs when a **phosphate ion is added** to a molecule
  - E.g. the phosphorylation of ADP to make ATP
- This makes the molecule **less stable** and therefore **more likely to react**
  - We can say that phosphorylation **activates** a molecule because it makes it more reactive
- There are two main types of phosphorylation:
  - **Substrate level phosphorylation** where the phosphate ion is transferred from a donor compound
    - This takes place in glycolysis and the Krebs cycle
  - **Oxidative phosphorylation** where phosphorylation is coupled with oxidation
    - This takes place in the the electron transport chain
- Phosphorylation is an **endergonic** reaction whereas the removal of the phosphate ion by hydrolysis in dephosphorylation is an exergonic reaction
  - Remember that the hydrolysis of ATP to ADP releases energy, therefore dephosphorylation of ADP is exergonic
- The addition of a phosphate ion to one molecule occurs at the same time as the removal of a phosphate from another; this is known as the **coupling** of reactions
  - E.g. the exergonic dephosphorylation of ATP is coupled with the endergonic phosphorylation of glucose at the start of glycolysis





***The cyclic formation of ATP from ADP by phosphorylation***

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# Stages of Respiration

## An overview of respiration

- Respiration involves the transfer of **chemical potential energy** from nutrient molecules (such as carbohydrates, fats and proteins) into a usable energy form (through the synthesis of ATP) that can be used for work within an organism
- It is a vital process that takes place in the cells of all living organisms
- There are two forms of respiration depending on the oxygen availability of the cell:
  - **Aerobic** respiration
  - **Anaerobic** respiration
- **Aerobic** respiration is the process of breaking down a **respiratory substrate** in order to produce ATP **using oxygen**
- **Anaerobic** respiration takes place **in the absence of oxygen** and also breaks down a respiratory substrate but produces **less ATP** for the cell
- The main **respiratory substrate** involved in respiration is **glucose**

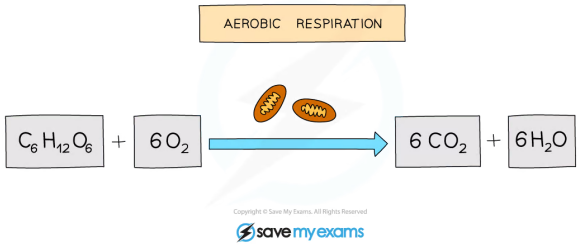
**Comparison of Aerobic & Anaerobic Respiration Table**

| | Aerobic respiration | Anaerobic respiration |
|---|---|---|
| **Stages** | Glycolysis<br>Link reaction<br>The Krebs cycle<br>Oxidative phosphorylation | Glycolysis<br>Fermentation |
| **Oxidation of glucose** | Complete | Incomplete |
| **Total ATP produced** | High (~36) | Low (2) |
| **Location** | Cytoplasm and mitochondria | Cytoplasm |
| **Products** | $CO_2$, $H_2O$ | Yeast: $CO_2$, ethanol<br>Mammals: Lactate |



## Aerobic respiration

- Aerobic respiration can be summarised by the following equation
  - Glucose + oxygen → carbon dioxide + water + energy






## The stages of aerobic respiration

- The process of aerobic respiration using glucose can be split into four stages
- Each stage occurs at a particular location in a eukaryotic cell:
  - **Glycolysis** takes place in the cytoplasm
  - The **link reaction** takes place in the matrix of the mitochondria

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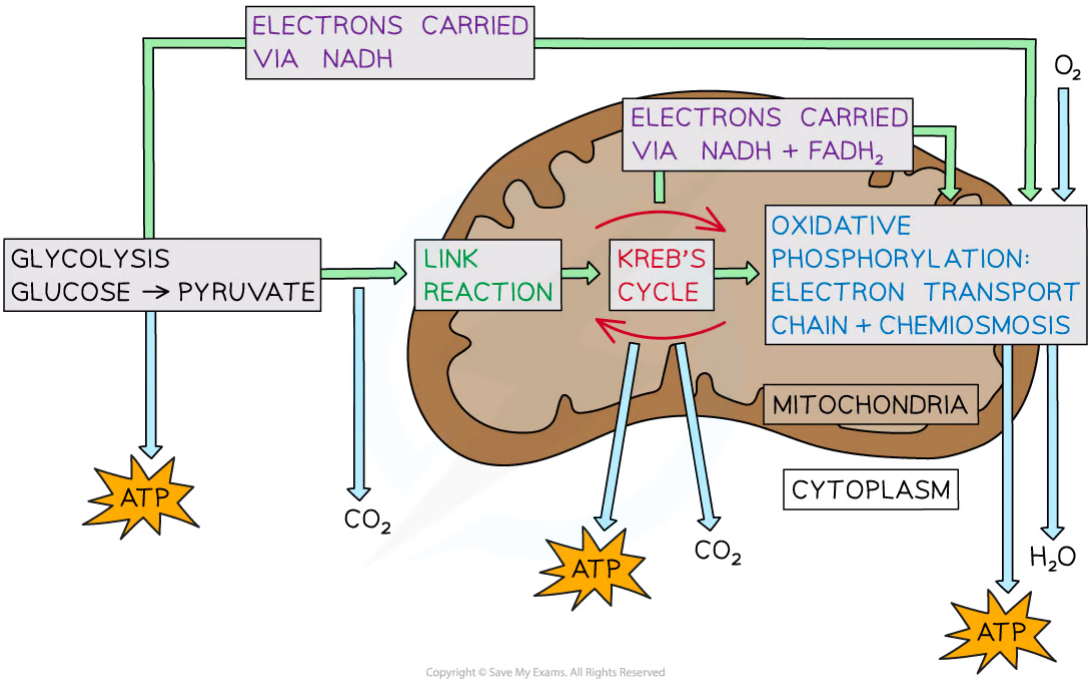
- The **Krebs Cycle** takes place in the matrix of the mitochondria
- **Oxidative phosphorylation** which involves the **electron transport chain** and **chemiosmosis** and occurs at the inner membrane of the mitochondria





*Overview of the four stages of aerobic respiration*

**Four Stages of Respiration Table**

| Stage | Description | Location |
|---|---|---|
| **1. Glycolysis** | Phosphorylation and splitting of glucose | Cell cytoplasm |
| **2. Link reaction** | Decarboxylation and dehydrogenation of pyruvate | Matrix of mitochondria |
| **3. Krebs cycle** | Cyclical pathway with enzyme–controlled reactions | Matrix of mitochondria |
| **4. Oxidative phosphorylation** | Production of ATP through oxidation of hydrogen atoms | Inner membrane of mitochondria |

### Exam Tip

It's important to know the exact locations of each stage. It is not enough to say the Krebs cycle takes place in the mitochondria, you need to say it takes place in the **matrix** of the mitochondria.

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# Glycolysis

- **Glycolysis** is the first stage of respiration
- It takes place in the **cytoplasm** of the cell and involves:
  - **Trapping glucose** in the cell by phosphorylating the molecule
  - **Splitting the glucose molecule in two**
- It results in the production of
  - Two **pyruvate** (3 carbon/3C) molecules
  - Net gain two **ATP** (Four ATP are produced in total but two are used during the reactions of glycolysis)
  - Two reduced NAD

## Steps of glycolysis

- **Phosphorylation**: glucose (6C) is activated by phosphorylation from two ATP to form fructose-1,6-bisphosphate (6C)
  - This makes the 6C molecule less stable and therefore more **reactive**

**Glucose + 2ATP → Fructose-1,6-bisphosphate**

- **Lysis**
  - Fructose-1,6-bisphosphate (6C) splits into two molecules of triose phosphate (3C)

**Fructose-1,6-bisphosphate → 2 Triose phosphate**

- **Oxidation**:
  - Hydrogen is removed from each molecule of triose phosphate by dehydrogenase enzyme and transferred to coenzyme NAD to form two reduced NAD
  - Triose phosphate is oxidised to for another 3C molecule glycerate-3-phosphate

**2 Triose phosphate →2 Glycerate-3-phosphate**
**$4H + 2NAD \rightarrow 2NADH + 2H^+$**

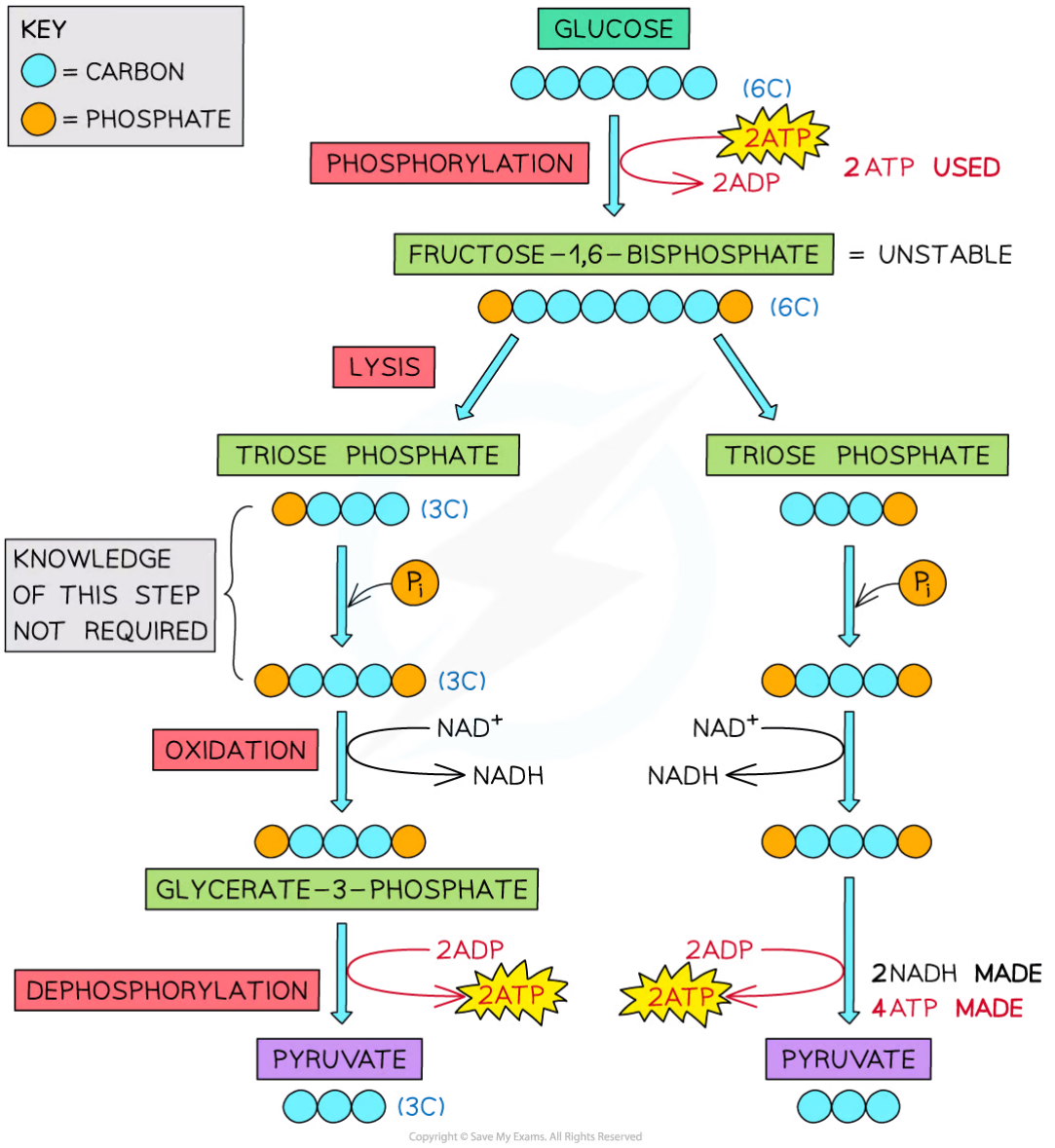
- **Dephosphorylation**
  - Phosphates are transferred from the intermediate substrate molecules to form four ATP through **substrate-linked phosphorylation**

**$4P_i + 4ADP \rightarrow 4ATP$**

- **Pyruvate is produced**
  - The end product of glycolysis which can be used in the next stage of respiration

**2 Glycerate-3-phosphate → 2 Pyruvate**

YOUR NOTES




***Glycolysis, the formation of two pyruvate molecules from one glucose sugar molecule***

## Exam Tip

It may seem strange that ATP is used and also produced during glycolysis. At the start ATP is used to **make glucose more reactive** (it is usually very stable) and to **lower the activation energy** of the reaction.

You may see 4H (four hydrogens) also written as $2H^+ + 2e^-$

You do not need to know all the **intermediate** compounds of glycolysis apart from TP and G3P. The starting reactant, fructose-1,6-bsiphosphate, and the product, pyruvate, in this chain reaction you do need to be able to name also.

YOUR NOTES

# Link Reaction

## Entering the link reaction

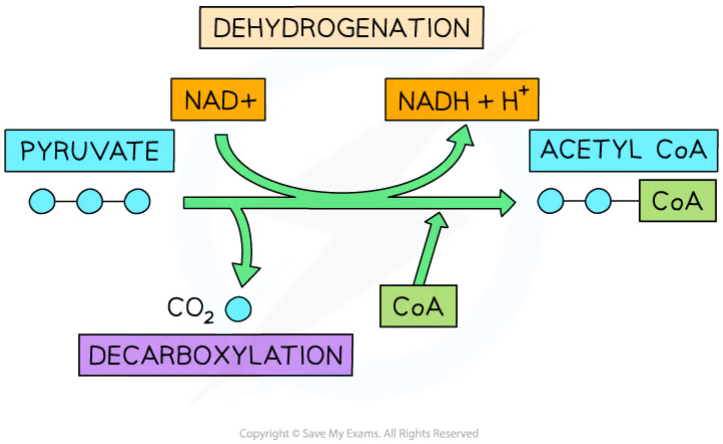
- The end product of glycolysis is **pyruvate** (3C)
- Pyruvate contains a substantial amount of chemical energy that can be further utilised in respiration to produce more ATP
- When **oxygen is available** pyruvate will **enter the mitochondrial matrix** and **aerobic** respiration will continue
- Once in the matrix pyruvate takes part in the link reaction

## The link reaction

- The link reaction takes place in the matrix of the mitochondria
- It is referred to as the link reaction because it **links glycolysis** to the **Krebs cycle**
- The steps are:
  - **Oxidative carboxylation** reaction in which:
    - **Carbon dioxide is removed** to produce a 2C molecule
    - **This 2C molecule is then oxidised** (loss of hydrogen and 2 high energy electrons) to produce an **acetyl compound** and thereby reducing NAD to NADH
  - **Combination of the acetyl compound with coenzyme A** to form acetyl coenzyme A (acetyl CoA)
- It produces:
  - Acetyl CoA
  - Carbon dioxide ($CO_2$)
  - Reduced NAD (NADH)

**pyruvate + NAD + CoA → acetyl CoA + carbon dioxide + reduced NAD**

- **Acetyl coenzyme A is supplied to the Krebs cycle** where aerobic respiration continues






***The link reaction occurs in the mitochondrial matrix. It dehydrogenates and decarboxylates the three-carbon pyruvate to produce the two-carbon acetyl CoA that can enter the Krebs Cycle.***

### Exam Tip

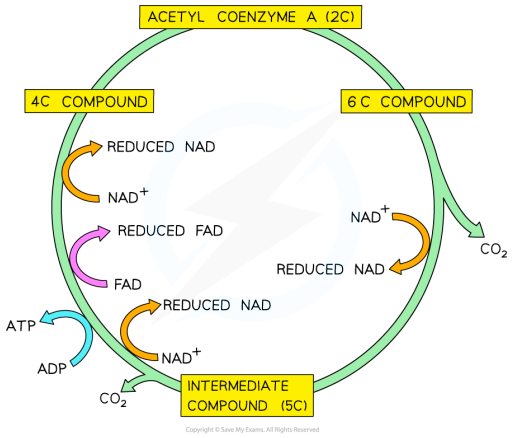
Remember that there are two pyruvate molecules produced per glucose molecule so you need to **multiply everything by 2** when thinking about what happens to a single glucose molecule in aerobic respiration.

YOUR NOTES

YOUR NOTES

## Krebs Cycle

- The Krebs cycle (sometimes called the citric acid cycle) consists of a **series of enzyme-controlled reactions**
- The **Krebs cycle** takes place in the matrix of the mitochondria
- **Two carbon (2C) Acetyl CoA** enters the circular pathway from the **link reaction**
- A four carbon compound (4C) accepts the 2C acetyl fragment from acetyl CoA to form a six carbon compound (6C)
  - Coenzyme A is released in this reaction to be reused in the link reaction
- The 6C **compound is then converted back to the 4C compound** through a series of **oxidation-reduction (redox) reactions**




***The Krebs Cycle uses acetyl CoA from the link reaction to produce reduced carbon dioxide, reduced NAD, reduced FAD and ATP***

### The reactions involved in the Krebs cycle

- The 4C compound is regenerated in the Krebs cycle through a series of **redox reactions**
- **Decarboxylation** of the 6C compound
  - Releasing **two $CO_2$** as waste gas
- **Oxidation** (dehydrogenation) of the 6C compound releases hydrogen atoms
- **Reduction** of coenzymes NAD and FAD (by the released H atoms)
  - 3 $NAD^+$ and 1 FAD → **3NADH + $H^+$ and 1 $FADH_2$**
- **Substrate-level phosphorylation**
  - A phosphate is transferred from one of the intermediates to ADP, forming one **ATP**

- As the link reaction produces two molecules of acetyl CoA (one per each pyruvate), the **Krebs cycle occurs twice**
- **Per glucose molecule**, the Krebs cycle produces:
  - 4 $CO_2$
  - 2 ATP
  - 6 NADH + $H^+$ (reduced NAD)
  - 2 $FADH_2$ (reduced FAD)

## Exam Tip

The Krebs cycle is often referred to as cyclical or circular. This is because the 4C acceptor molecule is **regenerated** throughout the reaction so that it can start all over again by adding another acetyl CoA.

The names of the intermediate molecules in the Krebs cycle and the link reaction are not required.

YOUR NOTES

YOUR NOTES

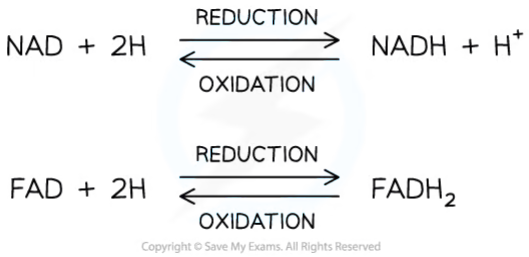
# NAD & FAD

## Summary of oxidative phosphorylation

- Oxidative phosphorylation is the **last stage of aerobic respiration**
- It takes place at the **inner mitochondrial membrane**
- This is the most efficient producer of ATP in the process of aerobic respiration
- It also is the stage that produces water from oxygen
- Oxidative phosphorylation is comprised of the **electron transport chain** and **chemiosmosis**
  - During the electron transport chain, electrons are passed along carrier molecules forming an electrochemical gradient
  - Chemiosmosis describes the formation of ATP using this gradient
- **Coenzymes $NAD^+$ and FAD** play a critical role in oxidative phosphorylation by **transferring electrons** (from hydrogen) from the previous stages of aerobic respiration through a series of carrier molecules

## NAD and FAD

- Coenzymes NAD and FAD play a critical role in aerobic respiration by transferring hydrogen through different stages of respiration
- When hydrogen atoms become available at different points during respiration NAD and FAD accept these hydrogen atoms
  - A hydrogen atom consists of a **proton** (hydrogen ion/$H^+$) and an **electron** ($e^-$)
- When the coenzymes gain a hydrogen they are '**reduced**'
- They **transfer the hydrogen atoms** (**protons** and **electrons**) from the different stages of respiration to the **electron transport chain** on the inner mitochondrial membrane, called the cristae (the site where hydrogens are removed from the coenzymes)
- When the hydrogen atoms are removed the coenzymes are '**oxidised**'





***The reduction and oxidation of NAD and FAD***

## Sources of reduced NAD & FAD

- A certain amount of reduced NAD and FAD is produced during the aerobic respiration of a single glucose molecule
- **Reduced NAD:**
  - 2 x 1 = 2 from Glycolysis
  - 2 x 1 = 2 from the Link Reaction
  - 2 x 3 = 6 from the Krebs cycle
- **Reduced FAD:**

YOUR NOTES

- 2 x 1 = 2 from the Krebs cycle

### Exam Tip

Note at all stages there is a doubling (2×) of reduced NAD and FAD. This is because one glucose molecule is split in two in glycolysis and so these **reactions occur twice per single molecule of glucose.**

Remember NAD can also be written as **$NAD^+$**

## The Electron Transport Chain in Respiration

### The electron transport chain

- The electron transport chain is made up of a **series of redox reactions** that occur via membrane proteins (also known as electron carriers) embedded into the inner mitochondrial membrane
- The chain is used to transport electrons and move protons across the membrane
  - Electron carriers are positioned close together which allows the **electrons to pass from carrier to carrier**
  - The cristae of the mitochondria are **impermeable to protons** so the electron carriers are needed to pump them across the membrane to establish a **proton (or electrochemical) concentration gradient** that can be used to power oxidative phosphorylation
- All of the electrons that enter the transport chain come from **reduced NAD** and **reduced FAD** molecules produced during the earlier stages of cellular respiration

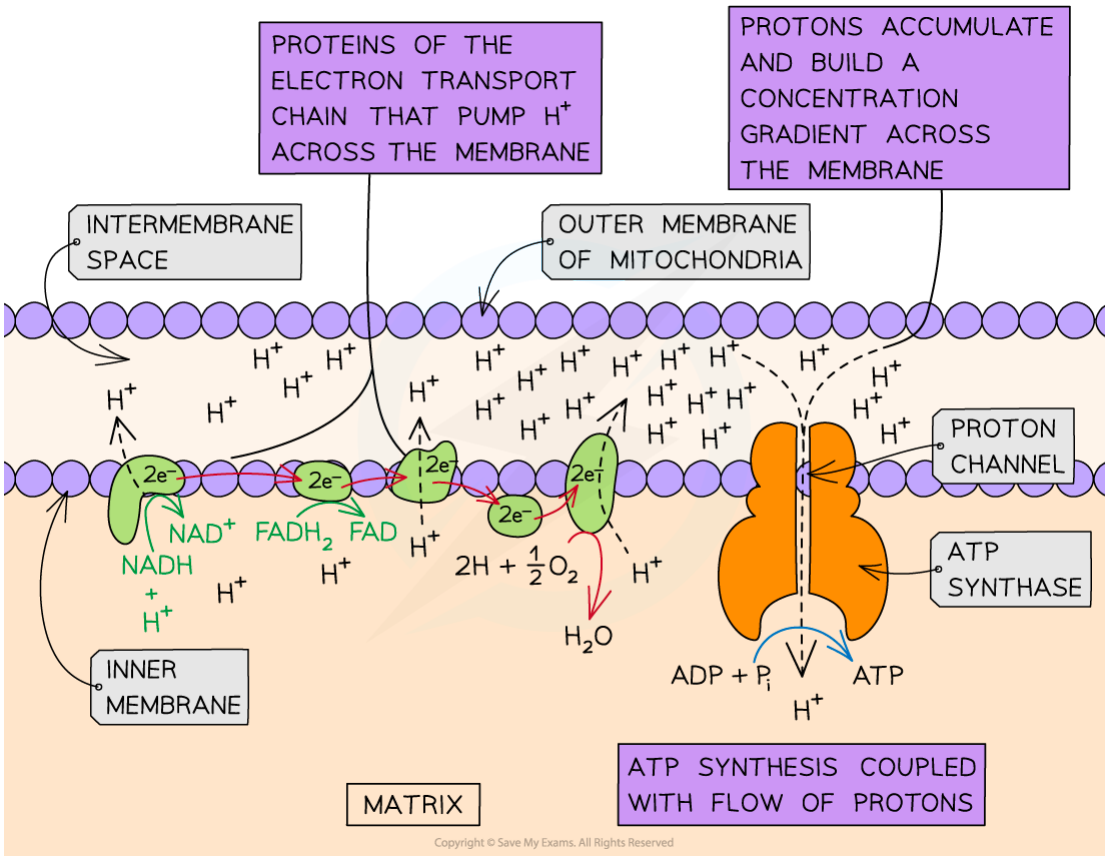
### The importance of protons and electrons

- **Protons** and **electrons** are important in the electron transport chain as they play a role in the synthesis of ATP
  - **Electrons** are **given to the electron transport chain** (from reduced NAD and reduced FAD)
  - **Protons** (from reduced NAD and reduced FAD) are r**eleased when the electrons are lost**
  - The electron transport chain drives the movement of these protons across the cristae into the intermembrane space, **creating a proton gradient** (more hydrogen ions in the matrix)
  - Returning the protons down the gradient, back into the mitochondrial matrix, **gives the energy required for ATP synthesis**

YOUR NOTES

## Chemiosmosis in Respiration

- Movement of electrons through the electron transport chain causes a **proton or electrochemical gradient**
  - Positively charged protons accumulate in the **intermembrane space**
  - The movement of protons back into the matrix is then used to power ATP synthesis
- Protons that have built up in the intermembrane space can only pass through the phospholipid bilayer by facilitated diffusion through a membrane-embedded protein called **ATP synthase**
- ATP synthase acts a lot like a water wheel; it is turned by the flow of the protons moving through it, down their electrochemical gradient.
- As ATP synthase turns, it catalyses phosphorylation of ADP, generating ATP
- This process, in which energy from a proton gradient is used to make ATP, is called **chemiosmosis**.




***Oxidative Phosphorylation, involving the electron transport chain and chemiosmosis, generates a large amount of ATP***

## NOS: Paradigm shift; the chemiosmotic theory led to a paradigm shift in the field of bioenergetics

- A **paradigm shift** is a fundamental change in an approach or already existing assumption
- Some examples of paradigm shifts that have happened in the course of scientific history are:

YOUR NOTES

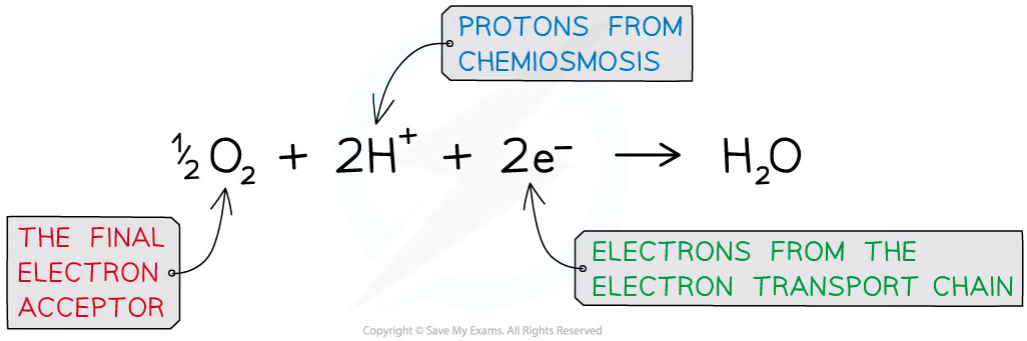
- **$CO_2$ emisions** - Emissions of $CO_2$ did not register as a contributing factor to climate change until late into the 20th century
- **Evolution** - Charles Darwin's theory of natural selection was a paradigm shift away from the traditional and religious views people held about the development of life on Earth
- **Parasites** - Scientists once believed that illnesses were caused by "bad air" called miasma; it wasn't until the 19th century that a paradigm shift in this thinking happened

- The **chemiosmotic theory** was proposed by Nobel Prize winner Peter Mitchell in 1961
- His idea was a paradigm shift away from the **existing theory** that the energy for electron transfer was stored as a stable chemical intermediate
- Mitchell's **chemiosmotic hypothesis** started a revolution which has led to this paradigm shift in the field of bioenergetics
- The idea was rejected at the time for being too novel and radical
- His theory has shaped understanding of the fundamental mechanisms of:
  - Biological energy conservation
  - Ion and metabolite transport
  - Bacterial motility
  - Organelle structure and biosynthesis
  - Membrane structure and function
  - Homeostasis
  - The evolution of the eukaryote cell

## Oxygen as the Final Electron Acceptor

YOUR NOTES

- The final link in the electron transport chain is **oxygen** and is referred to as the final or **terminal electron acceptor**
  - This is the **last acceptor of the electrons**
  - Oxygen is reduced by the **electrons**, forming **water**
- If oxygen is **not present** to accept electrons:
  - **Reduced NAD** and **reduced FAD** will not be oxidised to regenerate NAD+ and FAD, so there will be no further hydrogen transport
  - The **electron transport chain will stop**, and ATP will no longer be produced by chemiosmosis
  - Without enough **ATP,** cells can't **carry out the reactions** they need to function
- The electron transport chain is hugely efficient at generating energy in the cell but relies on an abundance of oxygen





### Exam Tip

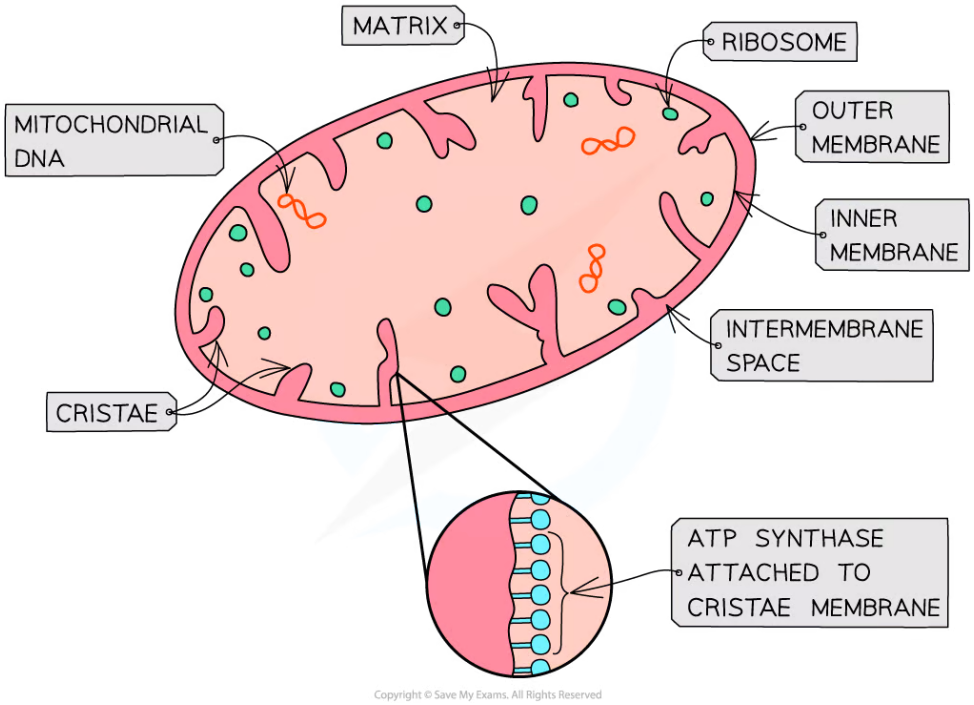
Examiners often ask why oxygen is so important for aerobic respiration, so remember the following:

- Oxygen acts as the final electron acceptor.
- Without oxygen, the electron transport chain cannot continue as the electrons have nowhere to go.
- Without oxygen accepting the electrons (and hydrogens) the reduced coenzymes NADH and $FADH_2$ cannot be oxidised to regenerate NAD and FAD, so they can't be used in further hydrogen transport.

YOUR NOTES

## Structure & Function of the Mitochondrion

- Mitochondria are rod-shaped organelles 0.5 - 1.0 μm in diameter
- They are the site of **aerobic respiration** in eukaryotic cells
- The function of mitochondria is to **synthesize ATP**
- Synthesis of ATP in the mitochondria occurs during the last stage of respiration called oxidative phosphorylation
  - This relies on membrane proteins that make up the 'electron transport chain' and the ATP synthase enzyme – the details of this are covered later in the notes



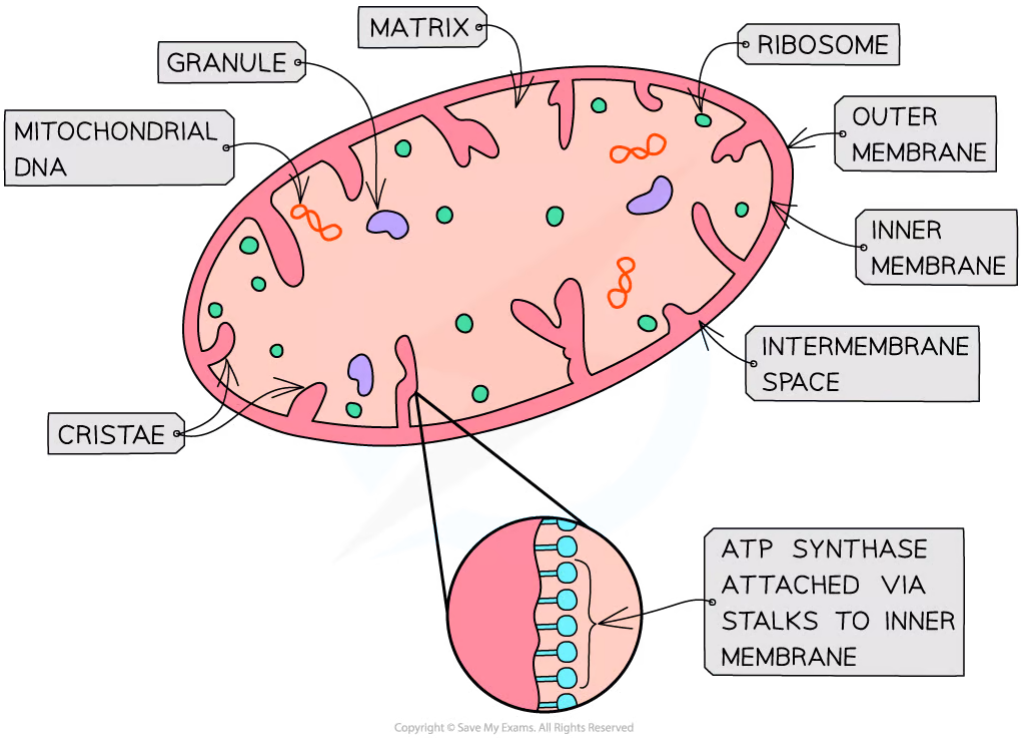

***The structure of a mitochondrion***

### Structure

- Mitochondria have **two phospholipid membranes**
- The outer membrane is:
  - Smooth
  - **Permeable** to several small molecules
- The inner membrane is:
  - **Folded** (cristae)
  - **Less permeable**
  - The site of the **electron transport chain** (used in oxidative phosphorylation)
  - Location of **ATP synthase** (used in oxidative phosphorylation)
- The intermembrane space:
  - Has a low pH due to the **high concentration of protons**
  - The concentration gradient across the inner membrane is formed during **oxidative phosphorylation** and is **essential for ATP synthesis**

YOUR NOTES

- The matrix:
  - Is an aqueous solution **within the inner membranes** of the mitochondrion
  - Contains ribosomes, enzymes and circular mitochondrial DNA necessary for mitochondria to function




***The structure of a mitochondrion***

## Relationship between structure & function

- The structure of mitochondria makes them well adapted to their function
  - They have a **large surface area** due to the presence of **cristae** (inner folds) which enables the membrane to hold many electron transport chain proteins and ATP synthase enzymes
  - More active cell types can have larger mitochondria with longer and more tightly packed cristae to enable the **synthesis of more ATP** because they have a **larger surface area**
  - The **number** of mitochondria in each cell can vary depending on cell activity
    - Muscle cells are more active and have more mitochondria per cell than fat cells

YOUR NOTES

## Exam Tip

Exam questions can sometimes ask you to explain how the structure of a mitochondrion helps it carry out its function effectively. Make sure to follow through with your answer.

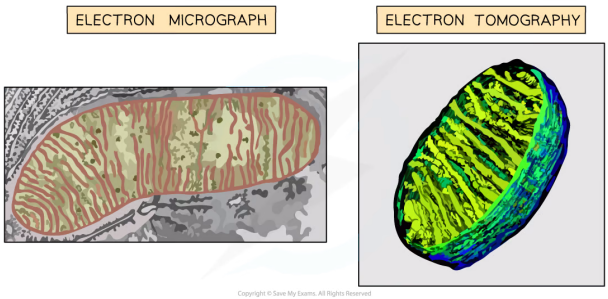
It is not enough to say that cristae increase the surface area of the inner membrane. You need to explain that an **increased surface area** of the inner membrane means there are **more electron transport chain** carriers and ATP synthase enzymes which results in **more ATP** being produced.

Be prepared to identify the different structures and locations in a mitochondrion from an electron micrograph.

## Electron Micrographs of Mitochondria

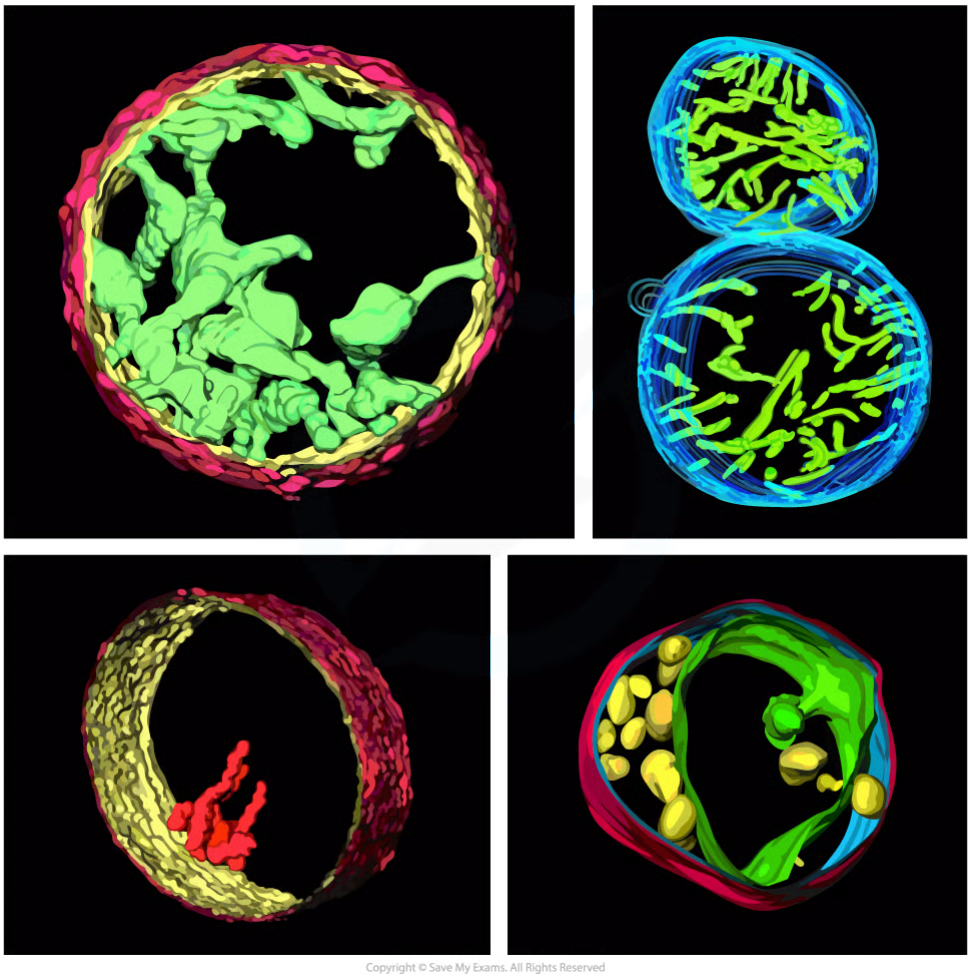
YOUR NOTES

- **Electron tomography (ET)** is a novel approach able to provide **three-dimensional (3D)** information on cells and tissues at molecular level
- The technique is an **extension of transmission electron microscopy**




***Transmission electron micrograph compared with electron tomography***

- **ET** has led to new discoveries about the mitochondria, including
  - The cristae are now known to be **dynamic in nature**, rather than static
  - The cristae have been seen to respond **physically and biochemically** to a changing environment with the mitochondria



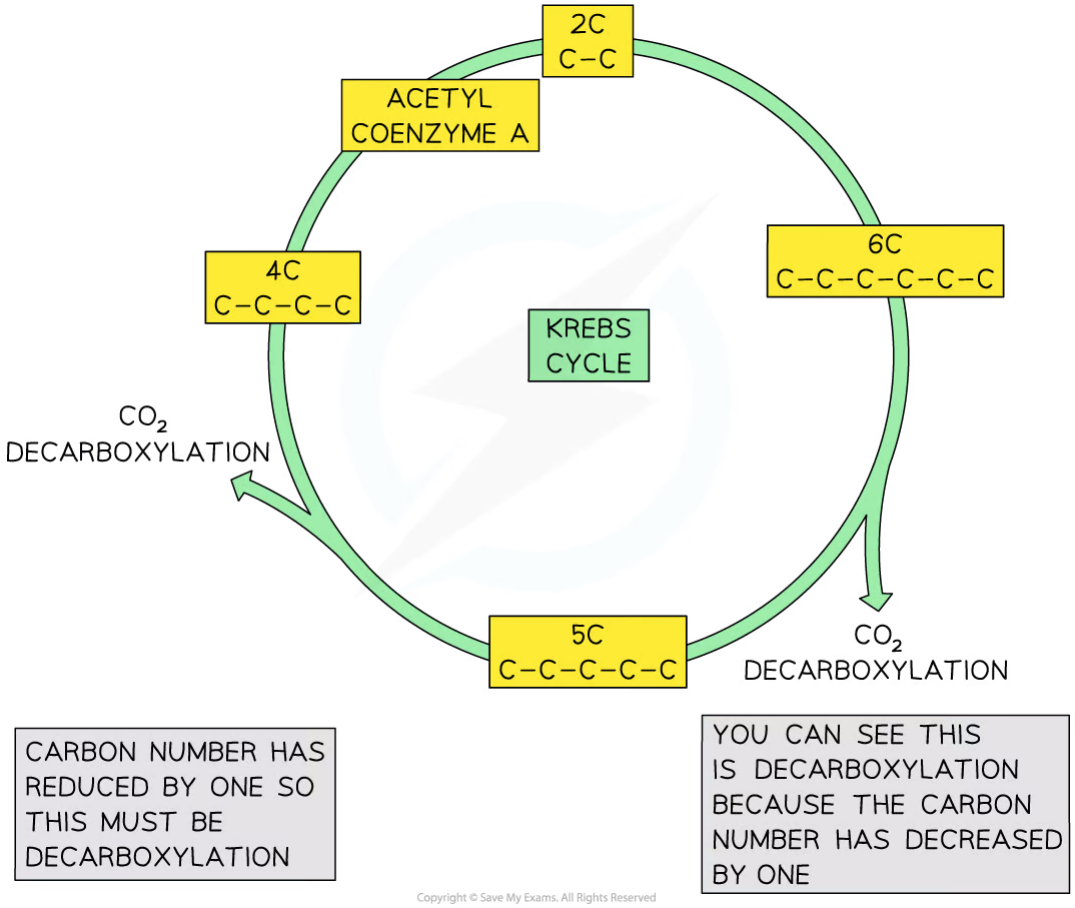
***Representation of EM images showing the dynamic nature of mitochondrial cristae***

YOUR NOTES

YOUR NOTES

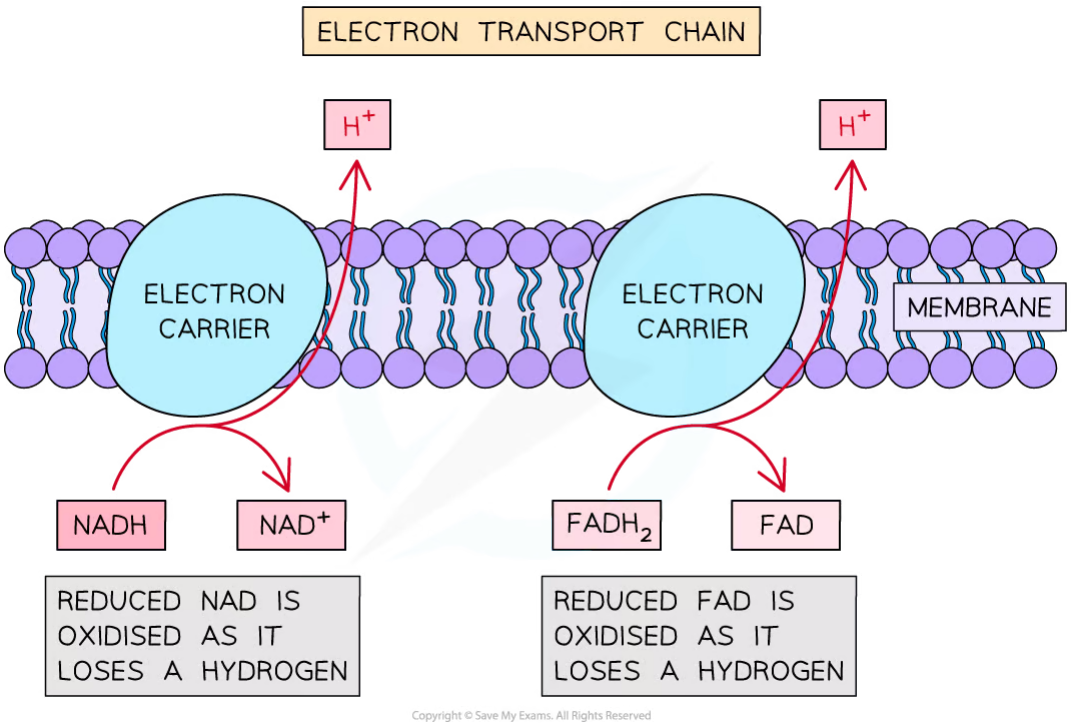
## Identifying Decarboxylation & Oxidation in Diagrams

- The stages of respiration can be shown and summarised by different reactions which are shown in diagrams involving chemical molecules
- The diagrams can be used to deduce where oxidation and decarboxylation occur
  - **Decarboxylation** is the removal of a carbon from a reaction, that releases carbon dioxide
  - **Oxidation** is either the gain of oxygen, or the loss of electrons or hydrogens




***Identifying decarboxylation in aerobic respiration***

YOUR NOTES

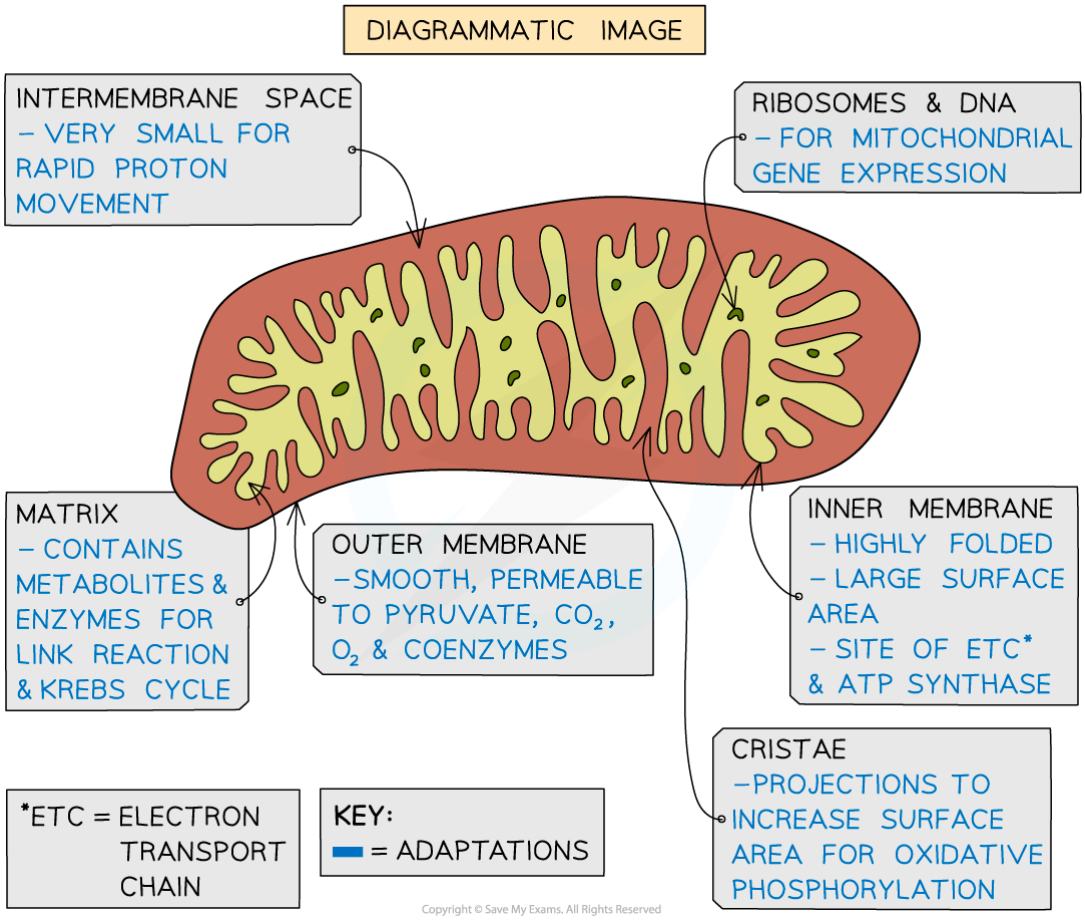



***Identifying oxidation in the electron transport chain***

## Annotating the Adaptations of Mitochondria

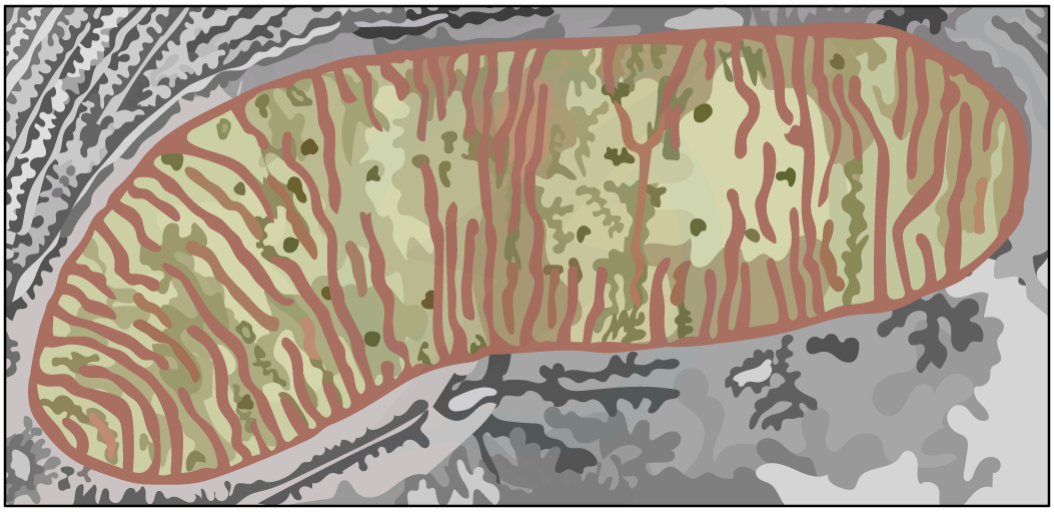
YOUR NOTES

- The structure of a mitochondrion can be investigated by **transmission electron microscopy**
- Electron micrographs of a mitochondrion may differ in appearance depending on where the cross-section occurs
- The clarity of electron micrographs make it possible to produce a line drawing to show the mitochondrion's double membrane around the matrix, and the way the inner membrane folds to form cristae
- Typically, mitochondrial drawings should show the following features:
  - Usually **sausage-shaped** in appearance
  - **Inner membrane** contains many internal protrusions (**cristae**)
  - **Intermembrane space** is **very small**
  - **Ribosomes and mitochondrial DNA** are usually **not visible** at standard resolutions and magnifications



YOUR NOTES

ELECTRON MICROGRAPH

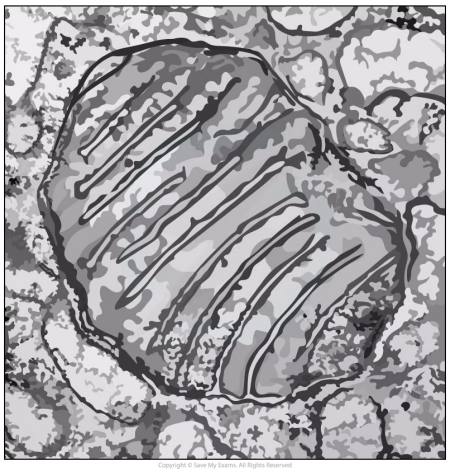



***Electron micrograph of a mitochondrion, with an interpretive drawing of the adaptations annotated***

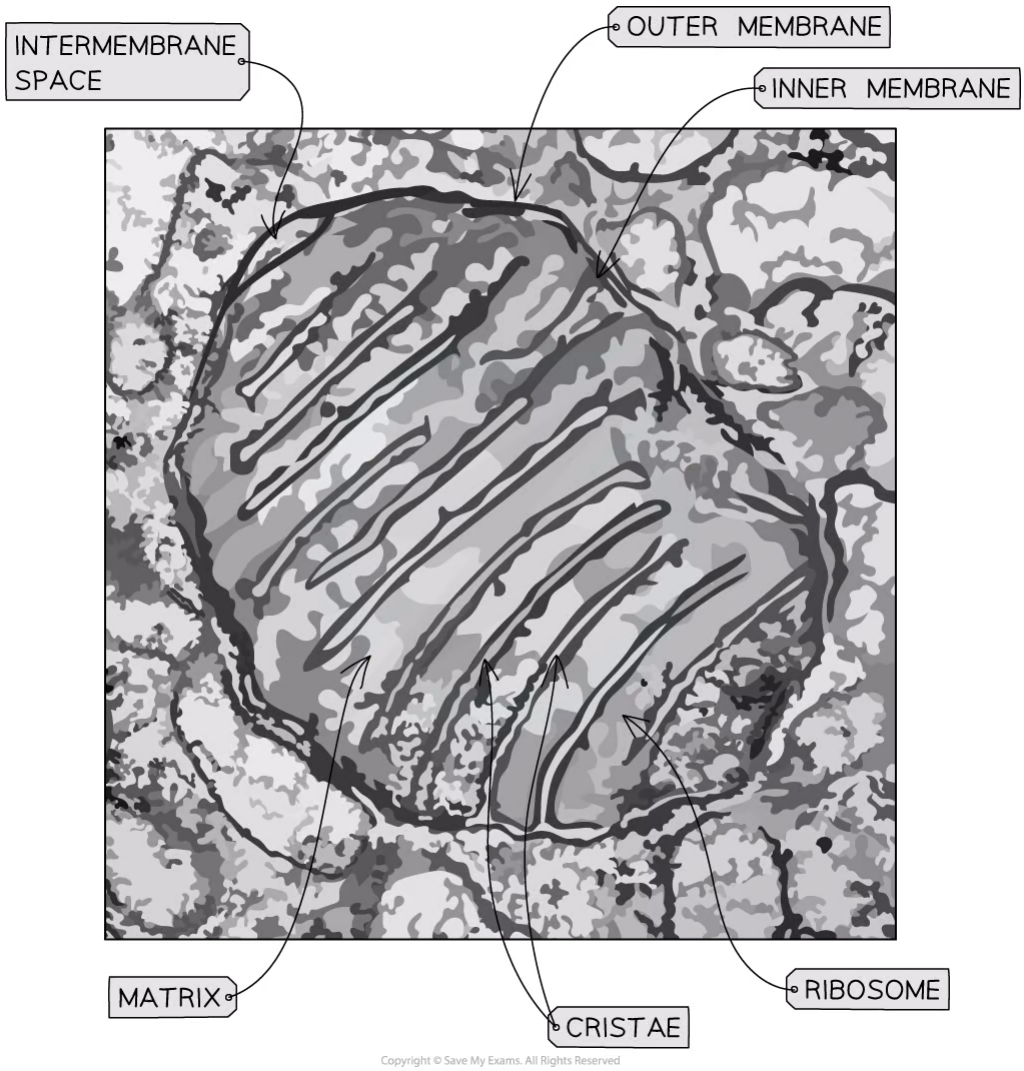
## Worked Example

Label the following structures on the electron micrograph of the mitochondria:

- Outer membrane
- Inner membrane
- Intermembrane space
- Cristae
- Matrix
- Ribosome



YOUR NOTES



YOUR NOTES

## 8.3 Photosynthesis

### 8.3.1 Light-dependent Reactions

#### 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
- Both these reactions 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

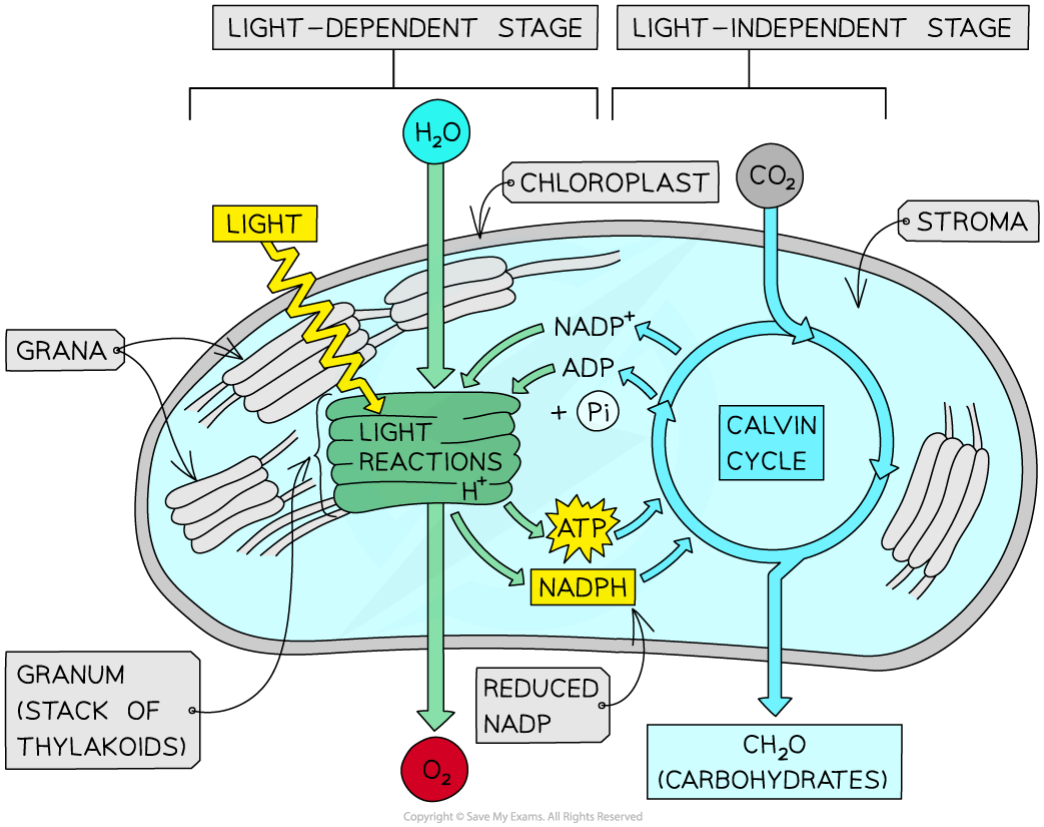
**Exam Tip**

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

YOUR NOTES

## Products of the Light-dependent Reactions

- During the light-dependent reaction light energy is converted into chemical energy in the form of **ATP** and **reduced NADP**
- ATP and reduced NADP are produced from the **photolysis** of water by light energy:
  - Water is split into protons, electrons and oxygen
  - The protons are picked up by the hydrogen acceptor $NADP^+$ thereby reducing it (NADPH, also called reduced NADP)
  - ATP is generated from the **phosphorylation** of ADP
- The useful products of the light-dependent reaction are transferred to the light-independent reaction within the chloroplast
- **Oxygen** is given off as a **waste product** of the light-dependent reaction




***The two stages of photosynthesis***

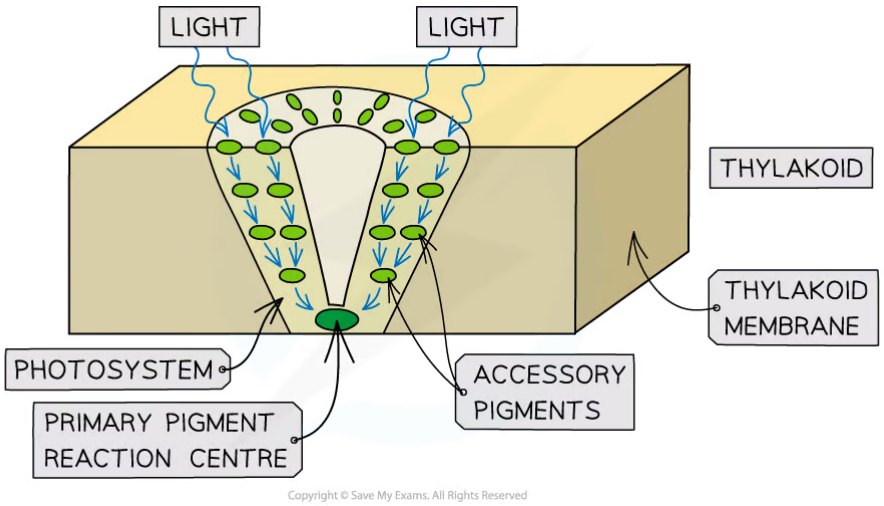
### Exam Tip

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. NADP can also be written as $NADP^+$.

## Excitation of Electrons

YOUR NOTES

- Chloroplasts contains the pigment **chlorophyll**, plus other accessory pigments
- These are grouped together as structures called **photosystems** which are located in the thylakoids
- Photosystems contain many chlorophyll molecules and 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 within Photosystem II absorb light energy, in the form of photons, and pass it to the 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**
  - In the light-dependent reaction the electron acceptor is called **plastoquinone**
  - **Plastoquinone accepts two electrons** from Photosystem II and is reduced
  - It then moves to another position in the thylakoid membrane
- This process is repeated with another plastoquinone molecule
- In total **two plastoquinone** molecules are **reduced** and **four electrons are lost** from the reaction centre




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

### Exam 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.

## Photolysis

YOUR NOTES

- Photolysis occurs in Photosystem II during the **light-dependent reaction** of photosynthesis
- This occurs following the **reduction of plastoquinone** in Photosystem II:
  - 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 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 $2H_2O \rightarrow O_2 + 4H^+ + 4e^-$
- 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

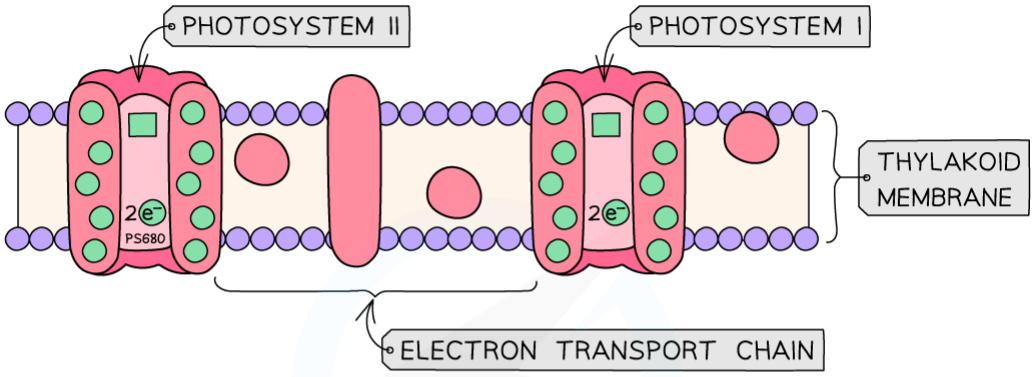
YOUR NOTES

## The Electron Transport Chain in Photosynthesis

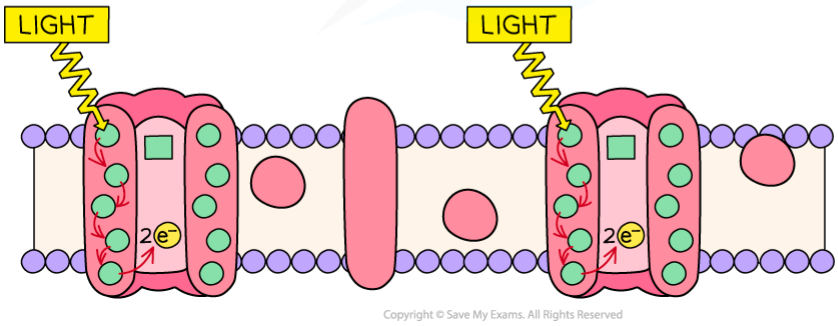
- Photophosphorylation is the term for the overall process of using **light energy** and the **electron transport chain** to **generate ATP** from ADP
- During photophosphorylation excited electrons (from Photosystem II) are passed down a series of **electron carriers** that form the **electron transport 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
- The reduced plastoquinone (an electron acceptor forming part of the electron transport chain) carries a pair of **excited electrons** from Photosystem II
- **Plastoquinone** carries the electrons 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

YOUR NOTES

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

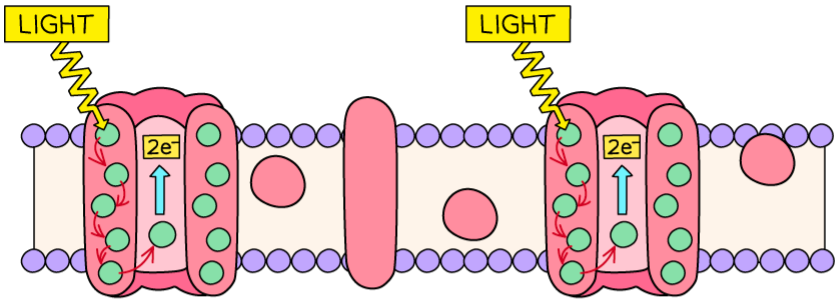



2 LIGHT IS ABSORBED BY BOTH PHOTOSYSTEMS. THE LIGHT IS PASSED TO THE PRIMARY PIGMENT OF EACH PHOTOSYSTEM

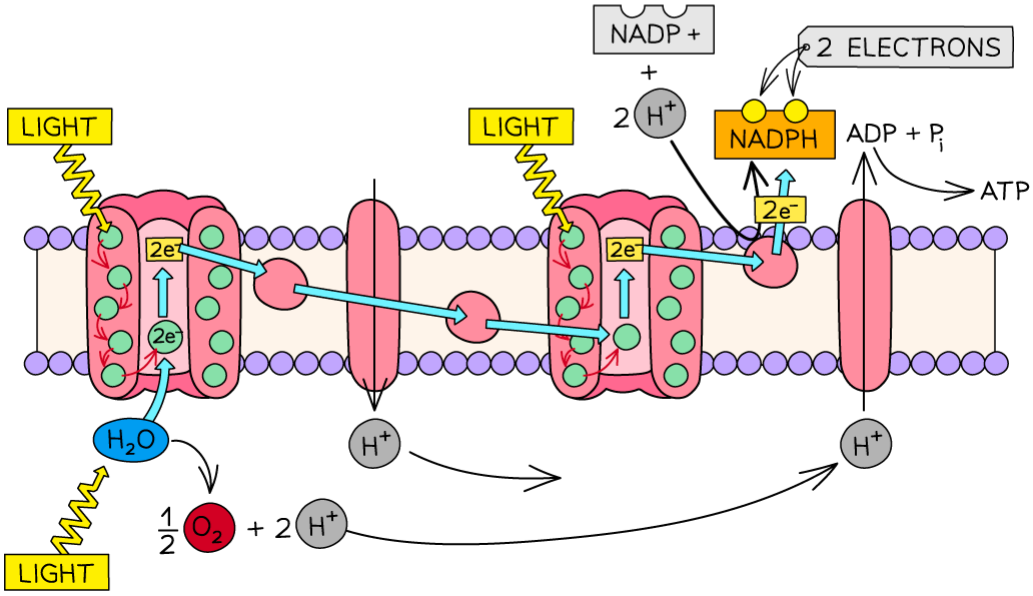


YOUR NOTES

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



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

YOUR NOTES

### Exam 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.

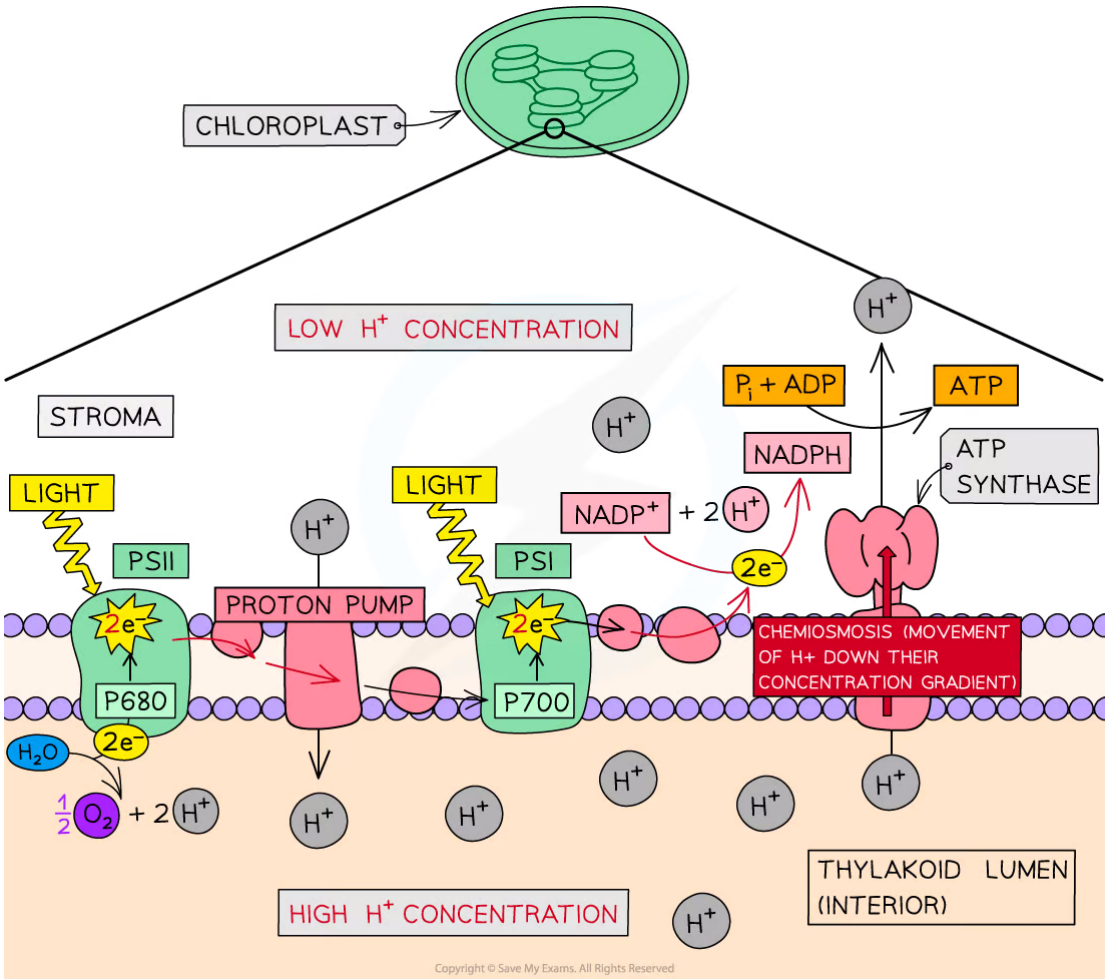
## Forming the Proton Gradient

- 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

## Chemiosmosis in Photosynthesis

YOUR NOTES

- 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

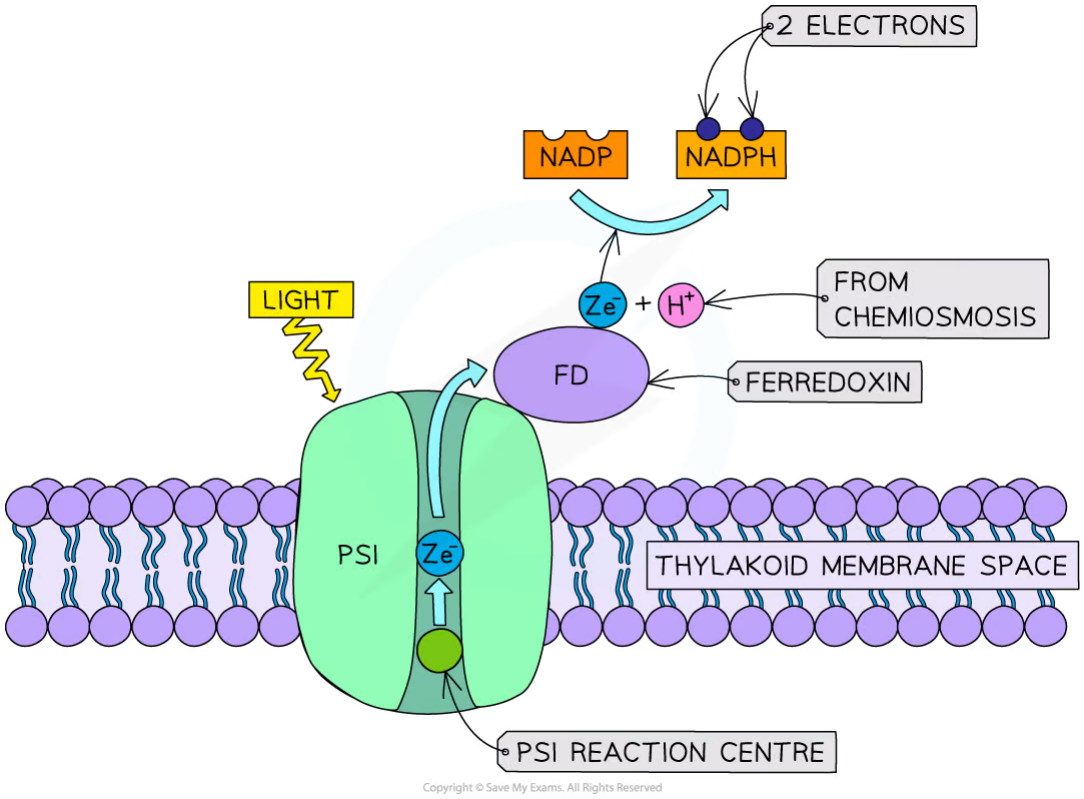



*Photophosphorylation and chemiosmosis*

## Reduction of NADP

YOUR NOTES

- **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 to NADPH (reduced NADP)
    - **$NADP + 2H^+ + 2e^- \rightarrow NADPH$**
  - The ferredoxin is now oxidised and free to be reused in this reaction again
  - Reduced NADP now carries a pair of electrons and can be passed into the light-independent reactions of photosynthesis




***Reduction of NADP in Photosystem I***

YOUR NOTES

## 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

## Carbon Fixation

- The light-independent reactions of photosynthesis are also known as the **Calvin cycle**
- **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
- There are three main steps within the Calvin cycle:
  - The enzyme **rubisco** catalyses the **fixation of carbon dioxide** by combination with a molecule of ribulose bisphosphate (RuBP), a 5C compound, to yield two molecules of glycerate 3-phosphate (GP), a 3C compound
  - **GP is reduced** to triose phosphate (TP) in a reaction involving reduced NADP and ATP
  - **RuBP is regenerated** from TP in reactions that use ATP

### Carbon fixation

- 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 bisphosphate (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**
- Glycerate-3-phosphate is then used in the next step of the cycle

## Role of Reduced NADP and ATP

YOUR NOTES

- **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 **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 bisphosphate would run out
- The remaining triose phosphate is used to regenerate RuBP

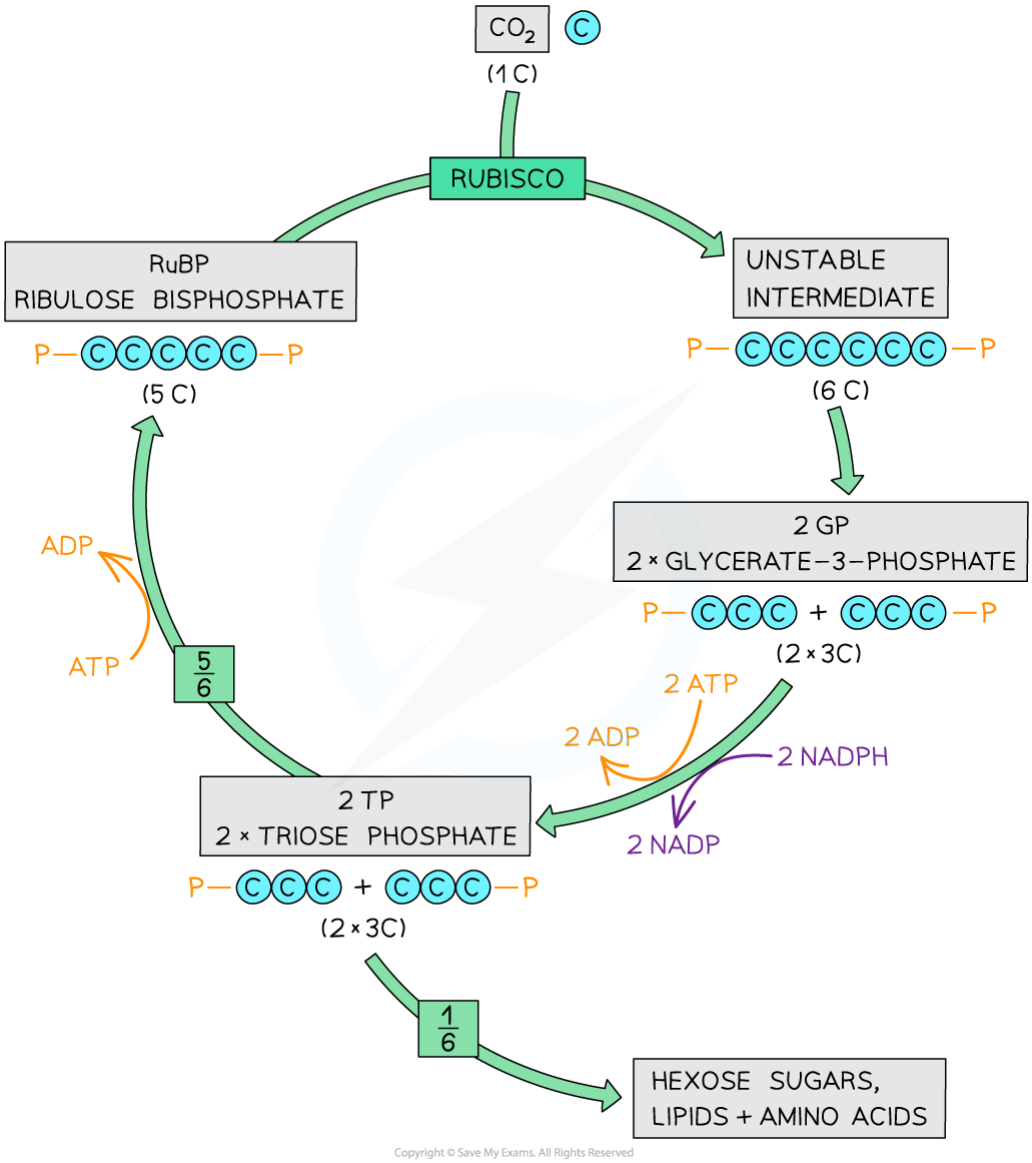
### Exam Tip

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 RuBPs 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

- The remaining five sixths of triose phosphate are used to **regenerate** the four-carbon compound ribulose bisphosphate **(RuBP)**
- This process requires **ATP** (from the light-dependent reaction)
- Once RuBP been has regenerated it can go on to fix further carbon dioxide and the cycle can begin again





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

YOUR NOTES

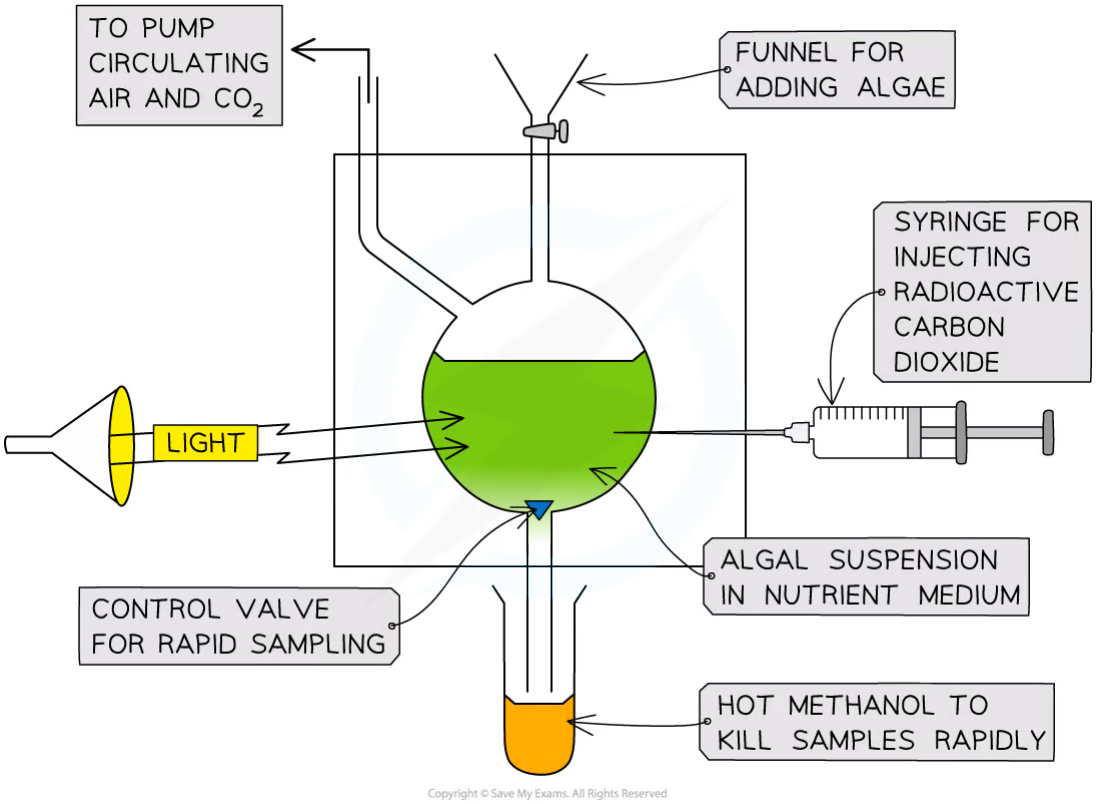
## Investigating Carbon Fixation in Photosynthesis

### NOS: Developments in scientific research follow improvements in apparatus: sources of $^{14}C$ and autoradiography enabled Calvin to elucidate the pathways of carbon fixation

- The Calvin cycle was named after American biochemist **Melvin Calvin** for his work in mapping the complete conversion of carbon dioxide to glucose
- The techniques used at the time were novel and showed developments in scientific research
- Calvin developed methods for growing algae in an apparatus he named "the lollipop" due to its shape
- This apparatus enabled Calvin to introduce radioactive carbon dioxide to the algae in order to study photosynthesis
- He also used paper chromatography and production of x-ray chromatograms to enable Calvin to identify compounds using in reactions during photosynthesis
- His approaches and methods were novel at the time and were only possible because of advancements in apparatus and technologies

- The experiments performed by Calvin show process of using **radioactive carbon dioxide** and **autoradiography** in explaining the reactions of the Calvin Cycle:
  - **Radioactively labeled carbon-14** ($^{14}C$) was introduced to the **algae *Chlorella*** in an apparatus called a lollipop (the experiments are sometimes referred to as the "lollipop experiment" due to the shape of the apparatus)
  - **Light** was shone on the lollipop vessel containing the *Chlorella* to **induce photosynthesis** and carbon-14 was **incorporated** into the algae
  - After varying time periods the **algae was killed** by heated alcohol which denatures proteins and enzymes within the cells and stops metabolic processes
  - The **pathway** of the radioactive carbon was **mapped and analysed** throughout the algae using two-dimensional **paper chromatography**
  - Chromatography **separated** out the different carbon compounds that had been made by the algae
  - Any **radioactive carbon-14 atoms** (that had been incorporated into either intermediates or products of photosynthesis) were **identified** using **autoradiography (x-ray)**
  - By **comparing** the different **time periods** in which the carbon compounds formed Calvin was able to map the order in which they were generated
- The results of the experiments showed that carbon was **converted** to carbohydrates during the **light-independent reactions** of photosynthesis
- Today, the method Calvin used is called "feeding experiments"

YOUR NOTES



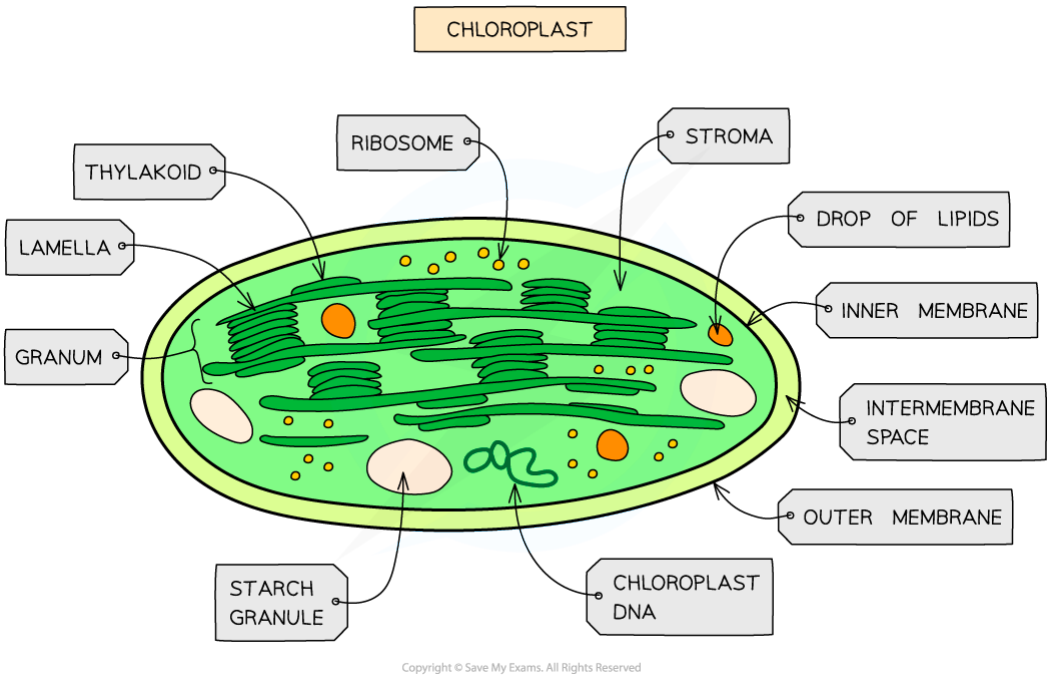

***Calvin's lollipop experiments for determining the reactions in the light-independent reaction***

YOUR NOTES

## Chloroplast Structure & Function

### Structure

- Chloroplasts are the organelles in plant cells where **photosynthesis** occurs
- These organelles are roughly **2 – 10 μm** in diameter (they are larger than mitochondria)
- Each chloroplast is surrounded by a **double-membrane envelope**
  - Each of the envelope membranes is a phospholipid bilayer
  - The **outer membrane** is permeable to a range of ions and small molecules
  - The **inner membrane** contains transport proteins that only allow certain molecules or ions to enter or leave the chloroplast
- Chloroplasts are filled with a cytosol-like fluid known as the **stroma**
  - $CO_2$, sugars, enzymes and other molecules are dissolved in the stroma
  - If the chloroplast has been photosynthesising there may be **starch grains** or **lipid droplets** in the stroma
- A separate system of membranes is found in the stroma
- This membrane system consists of a series of flattened fluid-filled sacs known as **thylakoids**
  - The **thylakoid membranes** contain pigments, enzymes and electron carriers
  - These thylakoids stack up to form structures known as **grana** (singular – granum)
  - Grana are connected by membranous channels called **stroma lamellae**, which ensure the stacks of sacs are connected but distanced from each other





*Chloroplast structure*

- The membrane system provides a large number of **pigment molecules** that ensure as much light as necessary is absorbed

YOUR NOTES ⬇

- The pigment molecules are **arranged** in light-harvesting clusters known as **photosystems**
  - In a photosystem, the different pigment molecules are arranged in funnel-like structures in the thylakoid membrane
  - Each pigment molecule passes energy down to the next pigment molecule in the cluster until it reaches the primary pigment reaction centre

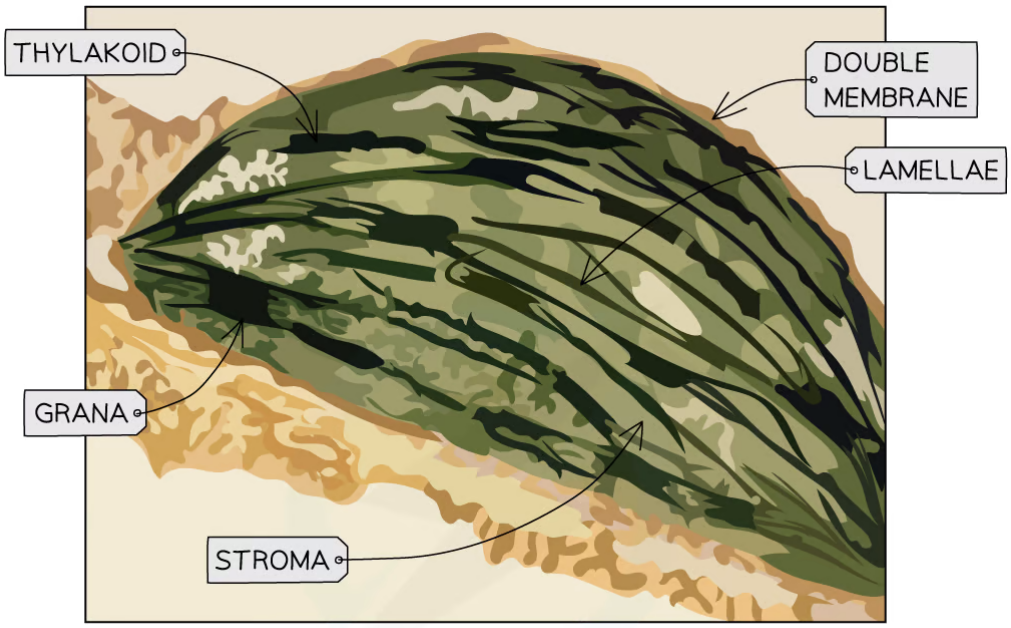
## Adaptations of chloroplasts to photosynthesis

- **Stroma**:
  - The gel-like fluid contains **enzymes** that catalyse the reactions of the light-independent stage
  - The stroma surrounds the grana and membranes, making the **transport** of products from the light-dependent stage into the stroma **rapid**
- **Grana**:
  - The granal stacks create a **large surface area** for the presence of many photosystems which allows for the **maximum** absorption of light
  - It also provides **more membrane space** for electron carriers and ATP synthase enzymes
- **DNA**:
  - The chloroplast DNA contains **genes** that code for some of the proteins and enzymes used in photosynthesis
- **Ribosomes**:
  - The presence of ribosomes allows for the **translation of proteins** coded by the chloroplast DNA
- **Inner membrane of chloroplast envelope**:
  - The selective transport proteins present in the inner membrane **control** the flow of molecules between the stroma and cytosol (the cytoplasm of the plant cell)
- **Thylakoid space**:
  - This is where a **proton gradient develops** (to generate ATP)
  - The space has a very small volume so a proton gradient can develop very **quickly**

YOUR NOTES

## Skills: Annotating Chloroplasts

- Electron micrographs may **differ** in shape and size depending on where the cross section of the organelle was taken
- Usually the following features should be notable and visible:
  - **Round** in shape
  - A **double membrane** exterior
  - Flattened discs, the **thylakoids**, arranged in stacks, the grana, **connected by** thin tubes, the **lamellae**
  - Ribosomes and DNA are not usually visible
  - **Starch granules** may be visible as dark spots within the stroma




**THYLAKOID** – SMALL INTERNAL VOLUME TO CREATE A PROTON GRADIENT QUICKLY

**LAMELLAE** – CONNECTS GRANA FOR MAXIMUM PHOTOSYNTHESIS

**GRANA** – LARGE SURFACE AREA FOR MAXIMUM ABSORPTION OF LIGHT

**STROMA** – CONTAINS ENZYMES FOR CALVIN CYCLE