AS

OCR Biology

Module 2: Molecules, Biodiversity, Food and Health

UNIT 3
Biological Molecules

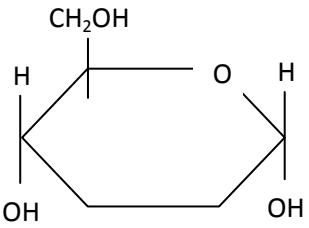
AS Revision 101

3.1 Biochemistry and Chemical Bonds

Biological Molecules

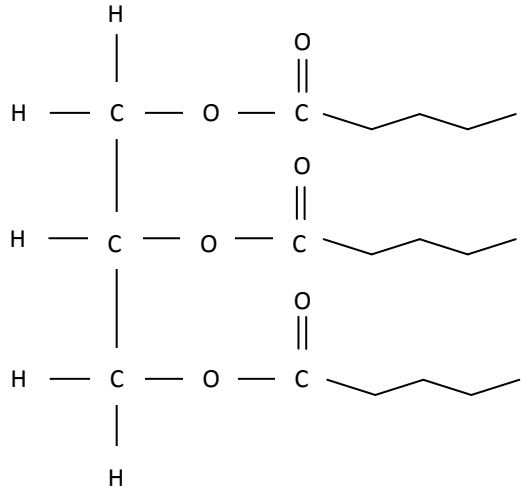
An introduction to the chemistry behind biomolecules

What is biochemistry? Well, it's the study of biology at a molecular level. So the emphasis of this unit is the biological significance of chemical molecules. As part of the course, there are *six* biological molecules that you need to know about:



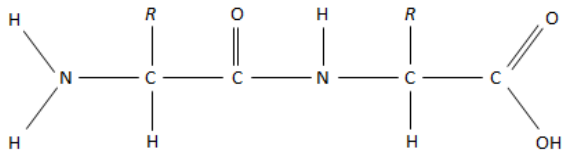
Carbohydrates

These molecules are one of many vital to life. They are used for energy (for both storing and supplying energy), and in some cases can be used structurally, such as *cellulose*



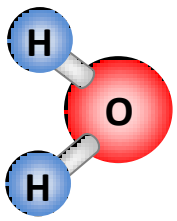
Lipids

These come in many varieties: fats, oils, cholesterol, steroids, and more, and have uses in cellular membranes, insulating and protecting, and also act as a minor energy supply



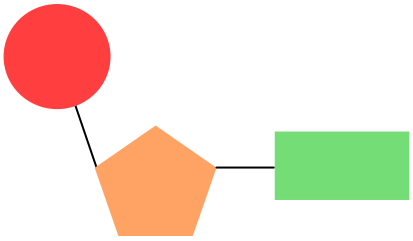
Proteins

Proteins have several uses, such as for transport and structure; but they are also the basic components of all enzymes, hormones, antibodies, haemoglobin, ribosomes, and many more materials



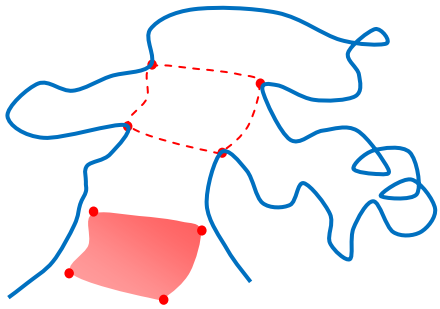
Water

Another essential life component, this is the most important content of many reactions forming most of these molecules, and also metabolic reactions; water is also an essential structural component in plants, and in the diet of animals



Nucleic acids

These are responsible for the formation of both DNA and all forms of RNA molecules, consisting of individual *nucleotides*



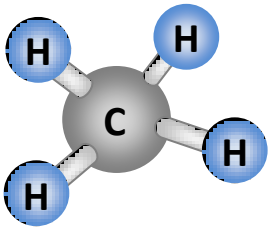
Enzymes

These are *proteins* which are used in many reactions – their function is to *catalyse* metabolic reactions in the vast majority of living organisms

There is a lot of chemistry knowledge in the Biological Molecules section of this module, which is why it is important that you are aware of a few chemistry basics, such as the types of **chemical bond**. This unit on Biological Molecules is centred around **organic chemistry** (organic being 'involving carbon'). All of the molecules studied are *carbon-based*, with the exception of water, which only contains the elements hydrogen and oxygen.

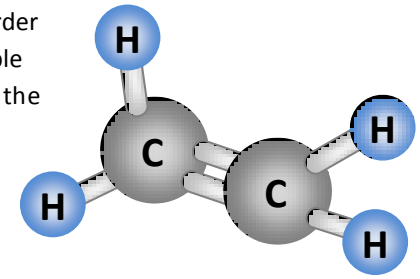
Covalent bonds

As you may well know from GCSE chemistry, a stable atom is one with a completed outer **energy level** (**shell**). For the majority of elements, this number is eight, which goes for carbon too. Carbon, however, naturally has *four* electrons in its outer energy level: so to stabilise it must share four electrons with other atoms, which forms **covalent bonds**. So it can form four covalent bonds – and these can be with other carbon atoms, or other atom types.



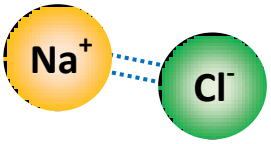
Double bonds

These types of covalent bond also exist, where atoms share multiple electrons in order to stabilise where there is a lack of available atoms. Common examples of the double bond are found within carbon and oxygen atoms (the carbon C=C double bond and the carbon-oxygen C=O double bond).



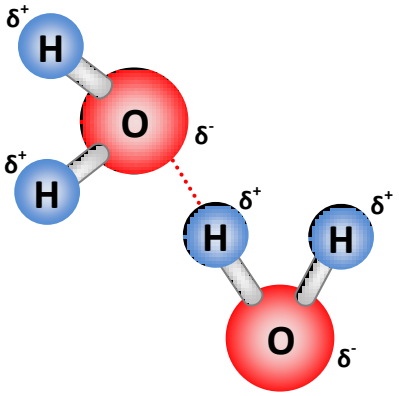
Ionic bonds

An **ionic bond** occurs between two *oppositely* charged **ions**. This will always take place between a *metal* and a *non-metal* ion. This involves the *donation* of electrons from the outer energy level, rather than the sharing which happens in covalent bonds. The metal ion will donate one or multiple electrons to the non-metal, which causes the metal to become **positively charged** (due to the loss of a *negative* electron) and the non-metal to become **negatively charged**. The two **polar** ions then are brought together due to the opposite charges. These bonds are much weaker than covalent bonds.



Hydrogen bonds

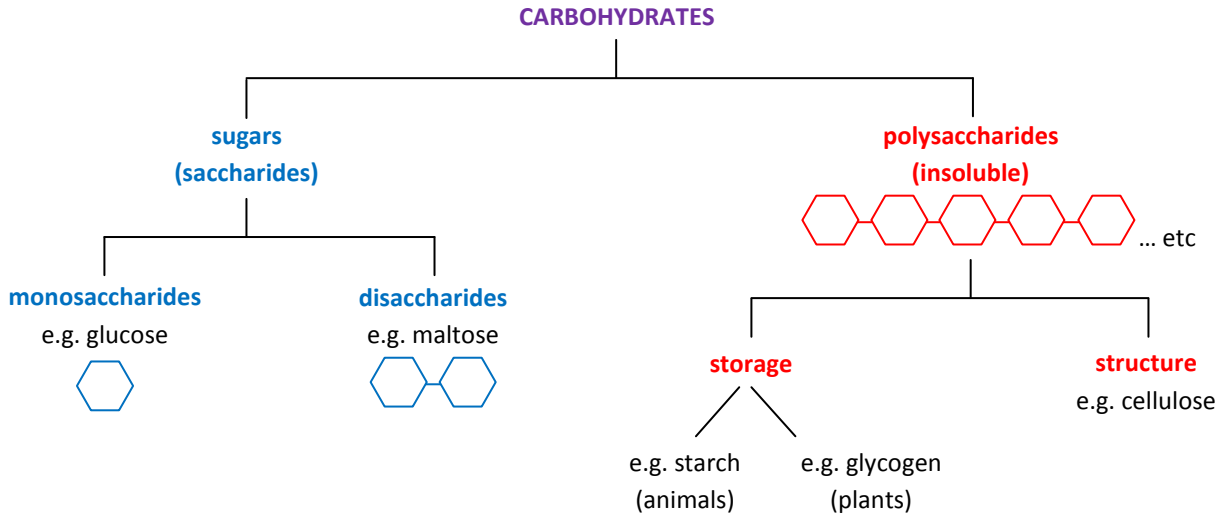
This is possibly the most important type of bond studied in this unit. It is found in just about every molecule you could think of. **Hydrogen bonds** are used to hold together individual **monomers** into large groups, called **polymers**. They form where a *slightly* positively charged part of a molecule meets a *slightly* negatively charged part of another molecule. We use the denotation of **δ** to represent **electronegativity**, where δ^- denotes a slight negative charge, and δ^- denotes a slight positive charge. These bonds are extremely weak, often describe merely as "interactions" – but when thousands of these bonds form in a polymer to hold the structure together, they are enough to stabilise a large polymerised structure.



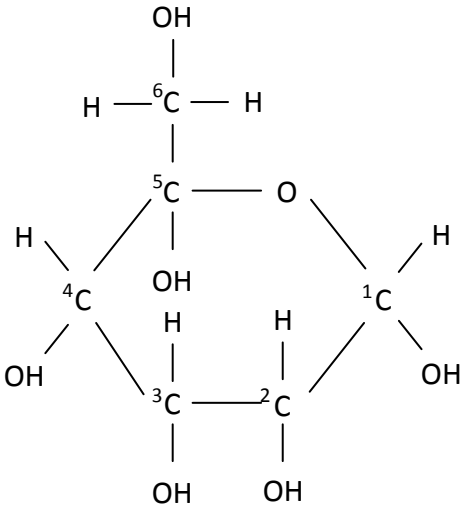
2.2 CARBOHYDRATES

Carbohydrate sugars, energy and storage; monosaccharides and polysaccharides

A **carbohydrate** is made up of *carbon*, *hydrogen* and *oxygen* atoms. Carbohydrates make up approximately one tenth of organic material inside a cell. They have the general chemical formula of $C_x(H_2O)_y$ and have three primary functions. They are mainly used as an **energy source** (released from glucose during respiration) or as **energy storage** (e.g. starch). They also have **structural** uses, such as *cellulose*.



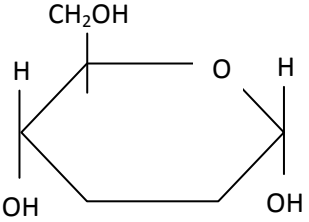
The simplest **sugars** are the **monosaccharides**. A monosaccharide is a **monomer** (single unit). One of the most common monosaccharide is **glucose**. This has the molecular formula $C_6H_{12}O_6$ and is the first product of photosynthesis, and the most commonly-used respiratory substrate.



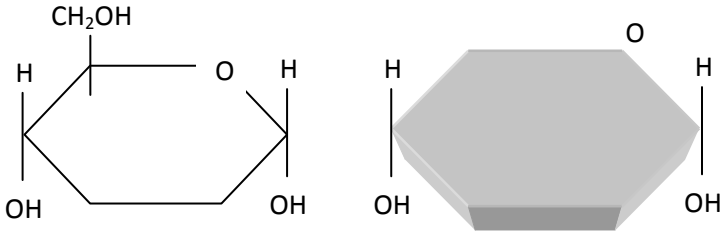
The diagram shows glucose drawn as a *ring structure*. The glucose molecule shown consists of a ring of five carbon atoms and one oxygen atom. Oxygen atoms make *two* bonds, carbon atoms make *four* bonds and hydrogen atoms make *one* bond. This can be seen in this structure. The carbon atoms are numbered to make them identifiable.

The oxygen atom in the ring has two bonds (with two C atoms). Four of the carbon atoms in the ring have four bonds, two with another two C atoms, one with an H atom and one with a **hydroxyl group**. A hydroxyl group is just an oxygen and hydrogen group. The fifth carbon atom also has four bonds: one with a C atom in the ring, one with an O atom in the ring, one with an OH hydroxyl group and another with a C atom outside the ring. This carbon atom has its own four bonds, as seen. It is an **alcohol group**. An alcohol group is a carbon atom attached to two hydrogen atoms and one hydroxyl group.

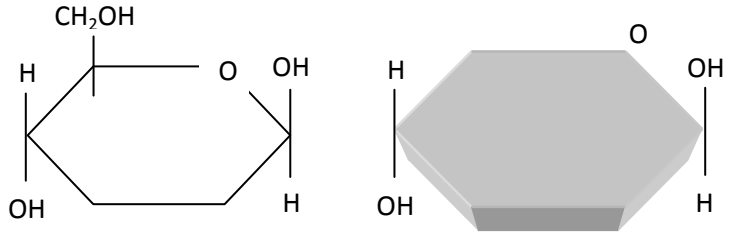
Alternatively, the molecule can be drawn as shown to the right. This version shows the ring containing the oxygen, and the carbons are assumed, but not written in. Carbon ^{2} and Carbon ^{3} are ignored, as they are thought to be unimportant. The alcohol group is written as CH_2OH to simplify it.



There are two forms of glucose. The diagram below is drawn to represent a 3D molecule. In this form of glucose, the Carbon [1] bonds with a hydrogen on top and the hydroxyl group below. This is called **α-glucose**.



In the other form of glucose, the hydroxyl OH group of the Carbon [1] is *above* the plane of the carbon ring and the hydrogen is below. However, the Carbon [2], Carbon [3] and Carbon [4] remain the other way round. This is called **β-glucose**.



POLYMERISATION

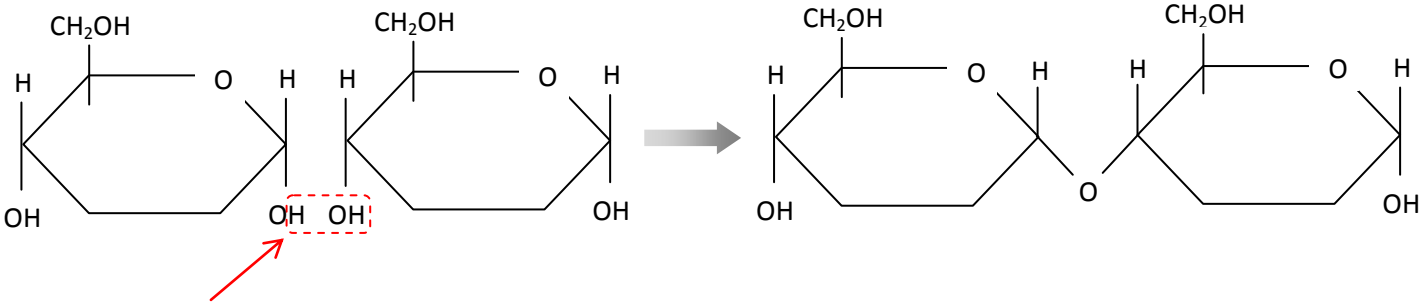
When α-glucose **polymerises** it becomes either *starch* (plants) or *glycogen* (animals). When β-glucose polymerises it becomes cellulose. Two monosaccharides will join together in a **condensation reaction** to form a **disaccharide**. Two glucose molecules join together to produce **maltose**. The two molecules are held together by a covalent bond called a **glycosidic bond**.

2 α-glucose → maltose + water

$2C_6H_{12}O_6 \rightarrow C_{12}H_{22}O_{11} + H_2O$

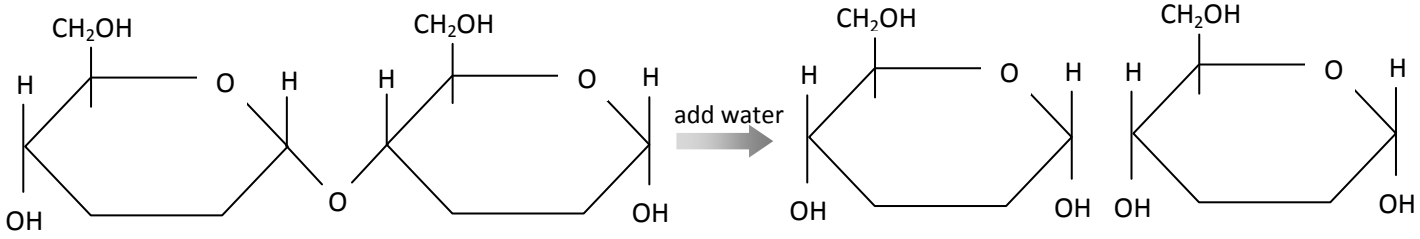
Condensation Reaction - a reaction where molecules join together which also produces water

Hydrolysis - splitting a molecule or breaking a bond by adding water



When the two molecules join, the hydroxyl group of one glucose molecule, and the hydrogen of the other bond to become water. The two molecules then join by forming a covalent *glycosidic* bond with the remaining oxygen.

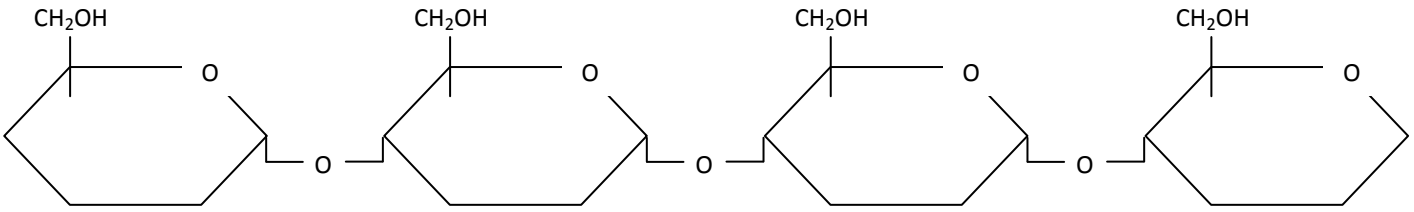
This reaction is *reversible*. A disaccharide can be split via **hydrolysis**. This is the breaking down of a bond by adding water, the opposite of the previous reaction.



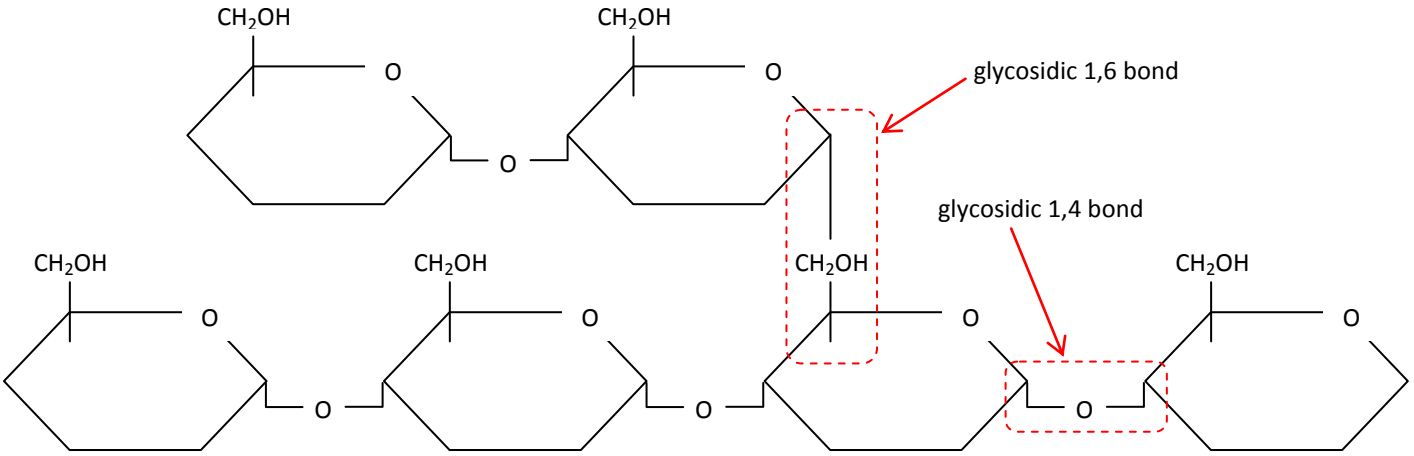
CARBOHYDRATES IN ENERGY STORAGE

Two α-glucose molecules will join together to form a disaccharide called *maltose*. When more and more of these glucose molecules join together to form a longer chain, a **polysaccharide** is formed. **Starch** is an energy store used by plants, which is a polysaccharide. It consists of two different types of molecules formed primarily of glucose…

The first of the molecules is **amylose**. Amylose is a long chain of α-glucose molecules joined together. Each glucose molecule is joined together by a covalent glycosidic **1,4** bond. This means they bond between the Carbon[1] and Carbon [4] of adjacent molecules. Amylose is formed in a condensation reaction, and it forms a **helix** shape because of the **hydrogen bonding** in sugar molecules between the hydroxyl group.



The second molecule is **amylopectin**. This is a *branched* molecule which forms a glycosidic **1,6** bond. Structurally, the basic arrangement of amylopectin is the same as amylose, it consists of a long chain of glucose molecules bonded by the oxygen of the hydroxyl group. However, amylopectin has separate branches coming off of it which make it more 3D.



Starch is a mixture of amylose (approximately 20%) and amylopectin (80%) and is used as an energy store. It can be broken down back into glucose molecules, which can be used in **respiration** which releases energy.

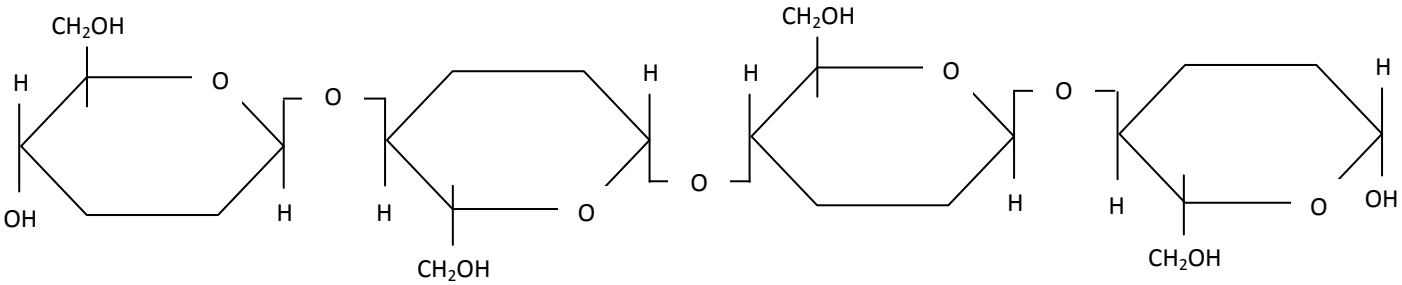
Starch is the polysaccharide product of glucose molecules in *plants*. In animals, however, **glycogen** is formed, and this is used as the energy store for animals. It is found especially in muscle cells and liver cells. The structure of glycogen (also named "animal starch") is the same as plant starch in that it is composed of α-glucose subunits, and it can also be broken down into glucose to be used in respiration to release energy. The only real structural difference with glycogen is that the glycosidic 1,4 chains are a lot shorter, and there are more branches of 1,6 bonds on each chain, so the molecule is more compacted.

Both glycogen and starch share a couple of features:

- ✓ They do not dissolve, so the stored glucose does not affect the water potential of the cell. This feature is vital in both animals and plants because glucose stored in a cell as free molecules would dissolve, reducing the water potential
- ✓ They hold glucose molecules in chains that can be easily broken (hydrogen bonds are weak) so the individual glucose molecules can be used in respiration to release energy

CARBOHYDRATES AS STRUCTURAL COMPONENTS

It is not only α-glucose molecules which can bond together. β-glucose molecules bond together in a very similar way, but instead of forming long coiled and branched chains, they form long, straight chains. The β-glucose molecules join in a series of condensation reactions.



Each alternate glucose molecule flips 180° to allow the bonding of the hydroxyl groups. This means that the CH_2OH alcohol group of every other molecule is above the carbon ring, and the others are below. When these molecules bond, the OH hydroxyl groups on Carbon^1 and Carbon^4 condense to form β1,4 glycosidic bonds. The rotation of each alternate glucose molecule means that they do not fit together like a helix, but instead remain in a straight chain.

These chains can consist of thousands and thousands of molecules. Such chains are called **cellulose** chains. Cellulose is a structural carbohydrate, which, like amylose, forms hydrogen H-bonds with each other to form **microfibrils**. Each microfibril consists of about 50 to 100 molecules. Microfibrils then bond together to form **macrofibrils** which are woven in layers to form structures, such as cell walls.

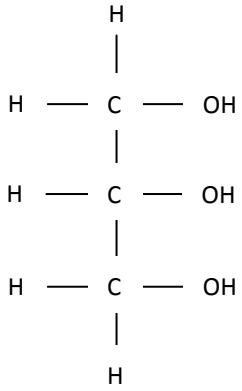
3.3 Lipids

Triglycerides, phospholipids, cholesterol and other chemically-similar biomolecules

The **lipid** group is a variable group. Lipids tend to be insoluble in water and all contain the elements carbon, hydrogen and oxygen, like carbohydrates, except the ratio of hydrogen:oxygen is not a 2:1 ratio as with carbohydrates. Lipids have a H:O ratio which is much higher than this. Some lipids will also contain other chemicals as well. Some of the most important groups of lipids are **fats** and **oils**, **waxes**, **steroids** and **phospholipids**.

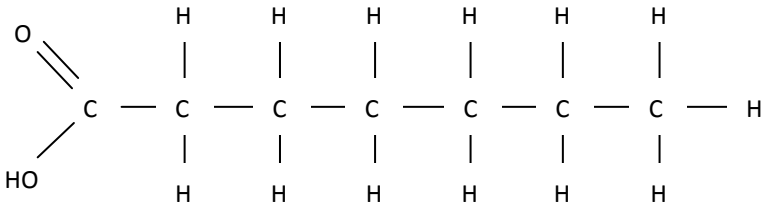
A **triglyceride** is a lipid which falls under the fats and oils group. Chemically, they are all very similar, as in they all only contain 6 atoms of oxygen (e.g. $C_{54}H_{98}O_6$ and $C_{44}H_{84}O_6$). Physically, they are insoluble in water, but soluble in *ethanol*, and they are less dense than water (they float). Oil is liquid at room temperature, whereas a fat is a solid. Both oils and fats act as good insulators – both electrical and thermal.

One triglyceride molecule contains the molecule **glycerol** and three **fatty acids**.

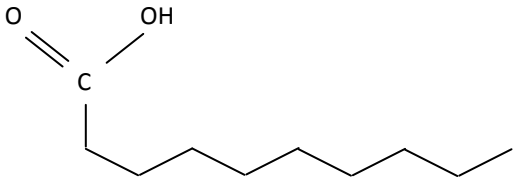


A glycerol molecule consists of three **alcohol groups** and the elements carbon, hydrogen and oxygen only. The glycerol molecule is *always the same* in any triglyceride, whereas the fatty tails can vary. The diagram to the left shows a glycerol molecule.

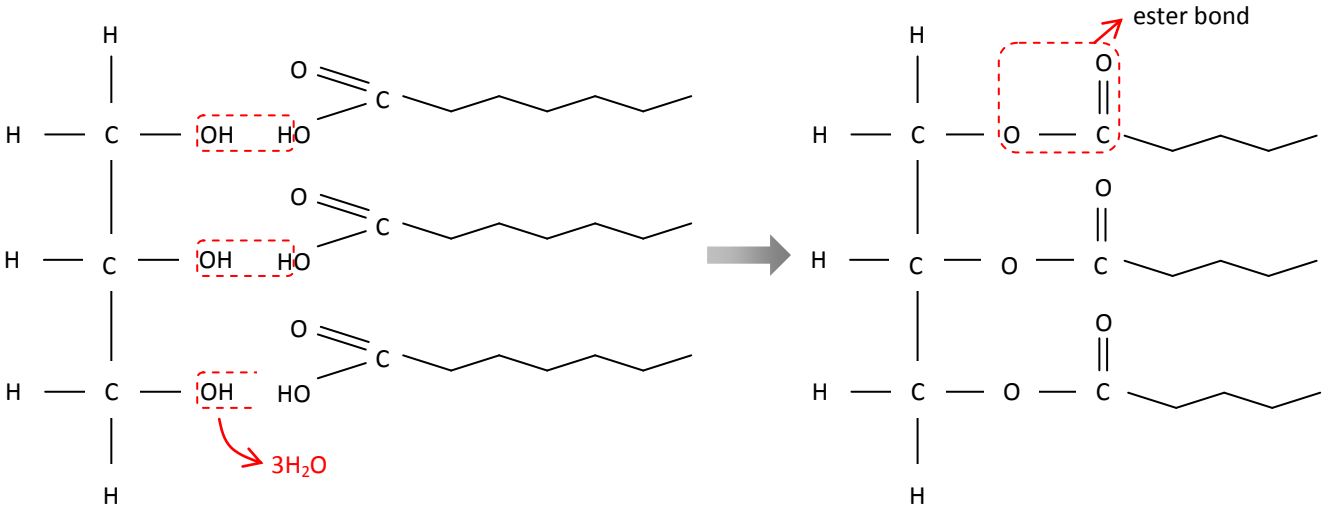
A fatty acid molecule consists of two parts: an **acid group** at one end, and a **hydrocarbon chain** at the other. The acid group is made from one carbon atom bonded with a hydroxyl group and a sole oxygen atom. The hydrocarbon chain is a long chain of purely carbons bonded together and with hydrogen atoms.



A fatty acid can also be drawn in a more simplified version for diagram purposes:



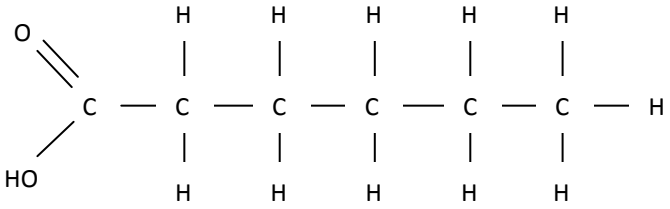
Three fatty acids and one glycerol molecule join together in a *condensation* reaction. Three molecules of water are released in this reaction, and the resultant product is one O – C – O covalent bond for each carbon (called an **ester bond**). This attaches one fatty acid to each carbon atom.



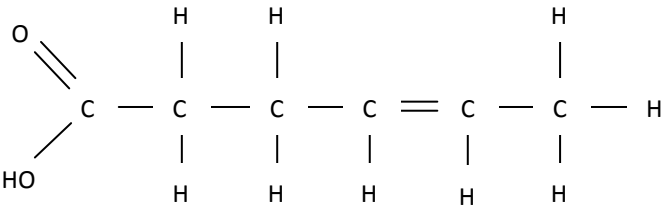
The reaction can be reversed using **hydrolysis** to turn the triglyceride back into its individual molecules. This is done using three water molecules to complete the reaction, and the enzyme **lipase** to hydrolyse the reaction.

The hydrocarbon chain which is a part of the fatty acid can be altered. Generally, it consists of a long linkage of carbons which each have two bonds with other carbons, and two bonds with hydrogens, completing their total four bonds which a carbon atom likes to make. When every carbon atom in the hydrocarbon chain is like this, the molecule is **saturated**, because it is fully hydrogen-saturated. An **unsaturated** fatty acid will have carbon-to-carbon (C=C) double bonds, meaning fewer hydrogen atoms can be bonded with them.

saturated



unsaturated

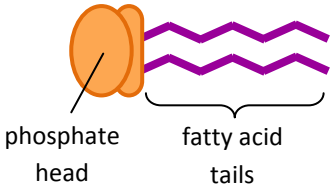


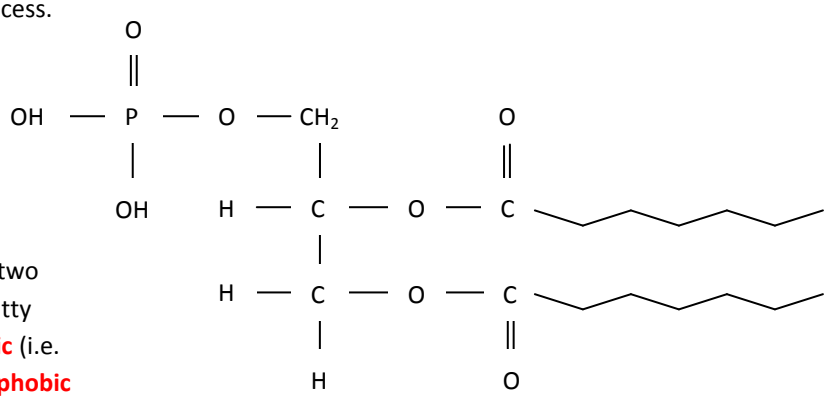
Having the C=C double bonds alters the shape of the hydrocarbon chain. It makes the molecules in the lipid push apart and so makes them more fluid. This will mean that lipids which are unsaturated tend to be oils, and saturated lipids are usually fats.

PHOSPHOLIPIDS

These biochemical structures form the basis of every biological membrane. A **phospholipid** is a molecule which is structurally almost identical to that of the triglyceride, in that there is one glycerol molecule and fatty acid molecules bonded to it from condensation reactions, producing these ester bonds.

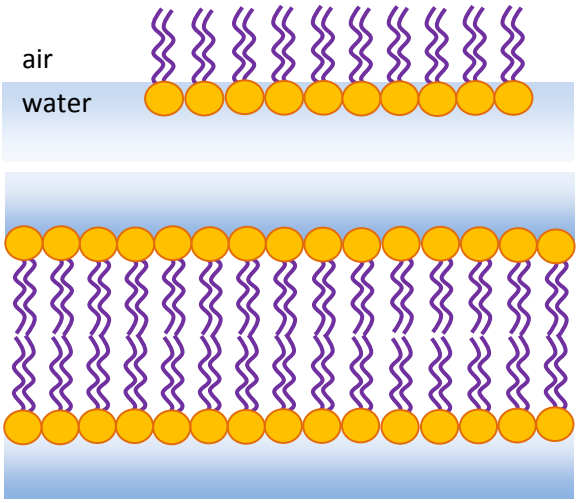
However, with phospholipids, there is no third fatty acid bonded to the glycerol molecule – instead, a **phosphate group** is bonded **covalently** to the third hydroxyl group on the glycerol. Again, this is all done by condensation reactions, so a water molecule is released in the process.





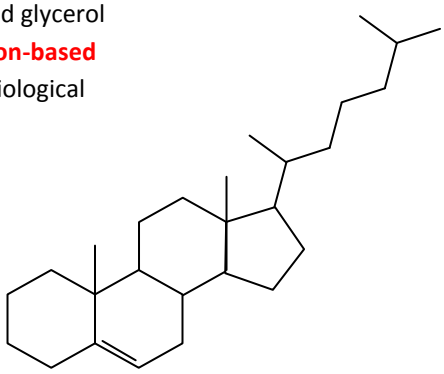
A phospholipid therefore consists of two parts: the phosphate head and the fatty acid tails. The head is very **hydrophilic** (i.e. water-loving) and the tails are **hydrophobic** (i.e. water-hating).

When you place a group of phospholipids in water, they will arrange themselves so that their water-loving phosphate heads are in the water, leaving their hydrophobic tails sticking out. When immersed inside water, they can form two layers, which is called the **phospholipid bilayer**. This is designed to leave the hydrophobic tails inside the bilayer so as they are protected from the water.



This bilayer forms the basis of any biological membrane, whether it be a cellular plasma membrane, or an internal organelle membrane and it acts as a barrier to certain substances trying to get into or out of a cell, or cell component (see 1.5 Biological Membranes).

CHOLESTEROL



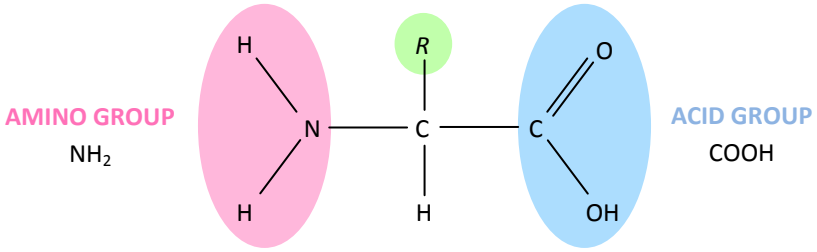
Cholesterol is also a class of lipid, even though it does not form from fatty acids and glycerol like triglycerides and phospholipids do. It is a small molecule made from *four* **carbon-based rings**. Cholesterol is a small structure which is very hydrophobic. It is found in all biological membranes, and these features allow it to sit nicely between the fatty acid tails.

This molecule is vital to living organisms, so many cells (especially those in the liver) can manufacture it. Excess cholesterol can cause health problems. A condition known as **familial hypercholesterolemia** (**FHC**: high blood cholesterol levels that run in families) is a genetic disorder, where cells manufacture and secrete cholesterol even though there is already sufficient in the blood to provide for the organism's requirements. This happens because the cells do not obey the signals to stop cholesterol production, as they lack a particular cell surface **receptor**.

3.4 Amino Acids and Proteins

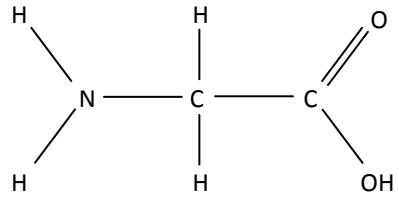
Proteins being made from amino acids

A **protein**, which is a **polymer**, is made up of many **amino acids** (which are individual **monomers**). Proteins are also made up of the elements carbon, oxygen and hydrogen, but unlike carbohydrates and lipids, also contain *nitrogen*. Some of them also contain the element *sulphur*. A protein is formed from many amino acids joined end-to-end.



The basic structure of an amino acid is shown. At one end of the molecule is an **amino group** (NH_2), and a **carboxylic acid group** (COOH) is at the other end. They are separated by another carbon, which has one hydrogen bonded to it and also a **variable group** (*R*).

The *amino* (or amine) *group* is acidic, as it releases H^+ ions into solution. The *acid group* is basic (the opposite of acidic). The variable group, which is always written as *R* can be a wide range of different amino acids.

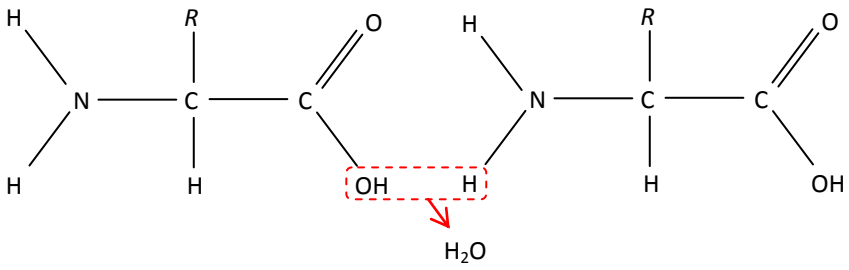


The diagram shows the structure of **glycine**. This is the simplest amino acid. The variable group *R* is another H atom. But there are 20 different amino acids which are naturally-occurring, and they all have different structures around the *R* group. There are other amino acids (in fact, thousands more) but these have all been manufactured artificially, and only those 20 occur naturally. Some *R* groups are very large – larger than the H_2-N-C-C-OHO base of the structure.

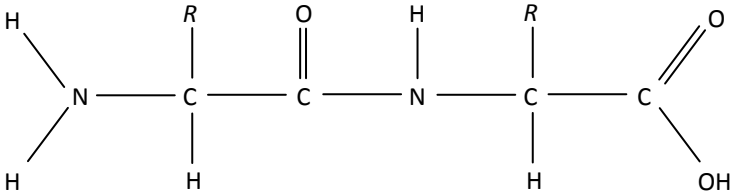
Amino acids join together end-to-end to form a long chain, similarly to glucose molecules. The *R* groups that they contain do not affect how they bond: it is always in one long chain. A **condensation reaction** joins the molecules, whereby water is released in the process. The bond formed is a covalent bond (so it is quite stable and strong, simply heating it will not break the bond), called a **peptide bond**.

When you put two amino acids next to each other, it is immediately obvious how the reaction is going to take place: the OH (hydroxyl) group from the carbon of one molecule and the hydrogen atom from the nitrogen of the other molecule will supply the ingredients for the condensation reaction.

This new molecule which is produced (see below) is called a **dipeptide**. Of course the reaction is reversible using a **hydrolysis reaction** – this will break the peptide bond. This reaction will *use up* one water molecule (replaces) in order to form the OH and H groups once again.



This process of making and breaking peptide bonds is important in animals, for example in digestion, where it is important to break down **polypeptide** molecules into individual amino acids.



A *polypeptide* is a molecule which is made up of multiple amino acids, but might not necessarily be a protein…

Peptide bond - a bond formed between two amino acid molecules

Polypeptide - the name given to a larger structure consisting of multiple amino acids

A *polypeptide* is a chain of amino acids, but a *protein* is something made of amino acids which has a distinct biological function. A protein is made of *one or more* polypeptides (therefore, one polypeptide may be a protein, but not all polypeptides are proteins).

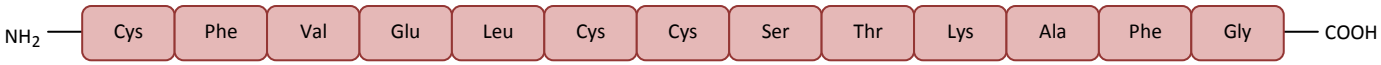
Examples of biological functions which make proteins include:

- ✓ structural uses (such as proteins of muscle or bone)
- ✓ membrane carriers and pores (used for movement of substances across a membrane)
- ✓ all **enzymes** are proteins
- ✓ many **hormones** are proteins

Primary Structure of Proteins

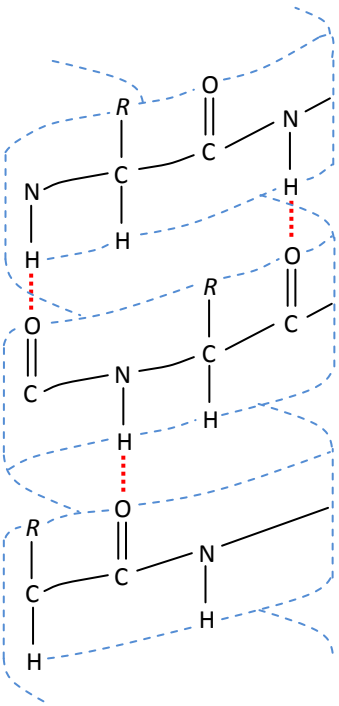
Proteins have different levels of complexity in their structure. They can be five amino acids long, or they can be hundreds of them long. Either way, they will have the amino NH_2 group on one end of the chain and the carboxylic acid group on the other end.

The diagram below shows a chain of amino acids. Each box represents an individual amino acid, symbolised by three letters, for example, **glycine** (see the previous page) is one amino acid, where the *R* group is an H atom, and **cysteine** where the *R* group is an S atom.



The function of a protein is determined by its **primary structure**. The primary structure refers to the unique sequence of amino acids which make the polypeptide chain. For example, if a protein consists primarily of a sequence of amino acids with hydrophobic *R* groups, then it may be a protein found within a membrane (e.g. a transport protein).

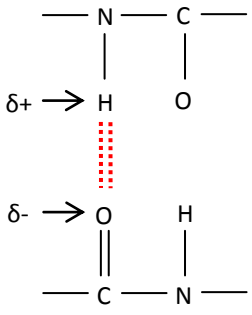
Secondary Structure of Proteins



The **secondary structure** of a protein refers to its formation of a 3D structure when a chain of amino acids coils, or folds. The most common secondary structure of proteins is the **alpha helix** (**α-helix**) which forms when the chain *coils*. There are **hydrogen bonds** which hold the coils in place. These are not very strong bonds, but they keep the helix structure quite stable because there are so many of them.

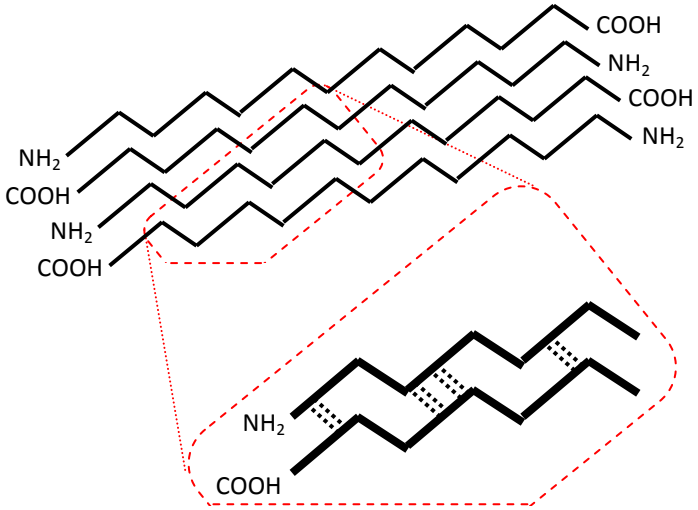
The helix shape and structure is shown to the left. The hydrogen bonds holding together the amino acids in the α-helix occur are shown in this diagram below:

Hydrogen bonding can only take place between **polarised** substances (charged). This means it will happen between the hydrogen atom attached to the N of one amino acid and the oxygen atom of the secondary C of another amino acid, one of which will be positively charged, and the other negative. In this case, the hydrogen ion is positive (a positive charge is written as **δ+**) and the oxygen ion is negative (**δ-**).



This hydrogen bonding therefore takes place between the *polar groups of the peptide bonds*.

The alpha helix is not the only secondary structure that can be formed by proteins. There is a far less common structure, called the **beta pleated sheet**. A *pleat* is an angular fold within the polypeptide chain. A **beta pleat** (**β-pleat**) is the simple structure, formed by multiple polypeptides joining together side-by-side in a pleated chain (see the diagram on the following page). These pleats associate with each other and join together to form the beta pleated sheet (a series of beta pleats fixed together, resulting in a very thin but tall, almost 2D shape).



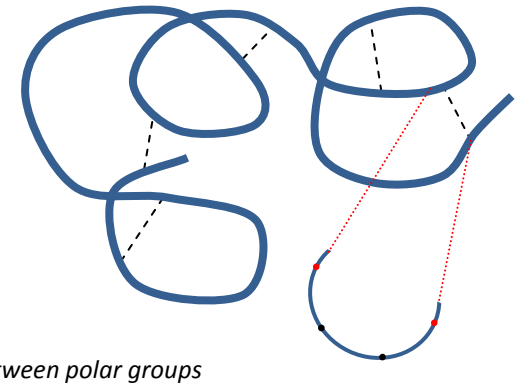
The polypeptide chains form a sheet and not a helix because they do not have the amino coding necessary, which is possessed by those amino acids that do form a helix.

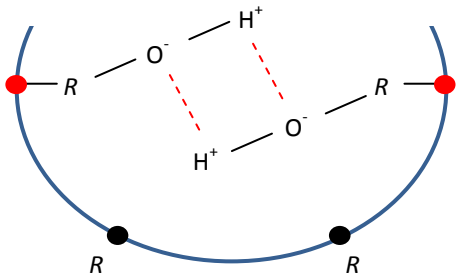
Just like with the helices formed, a β-pleated sheet is held together by multiple hydrogen bonds.

TERTIARY STRUCTURE OF PROTEINS

An α-helix can wrap itself into a 3-dimensional complex shape. Polypeptides that do this form a **globular protein**. Their shape is maintained by bonding between the *R* groups of individual amino acids.

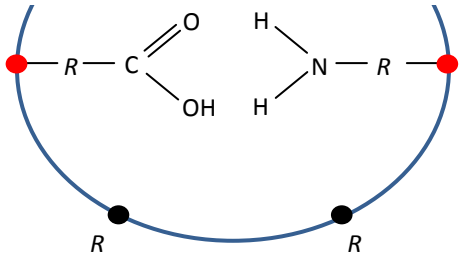
There are four different types of bonds that can be found between the *R* groups which maintain this globular **tertiary structure**…





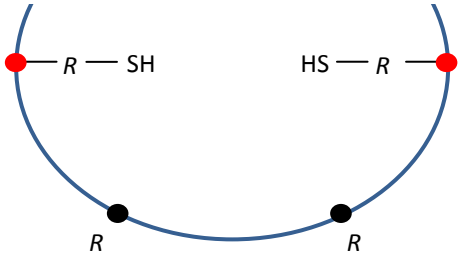
A – *Hydrogen bonds between polar groups*

Hydrogen bonds can form between oppositely charged ions in the groups which are polar, such as this hydroxyl group. Hydrogen bonds, as always, are very weak and easily broken; temperatures above approximately 40°C will cause a loss in their tertiary structure as the bonds become broken – this is called **denaturation**



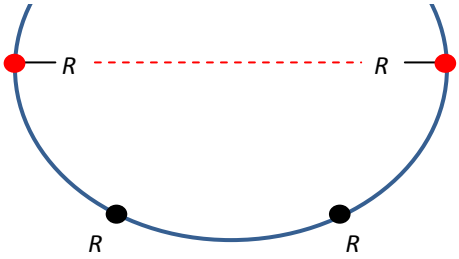
B – *Ionic bonding of oppositely-charged R groups*

When amino acids bond with each other, the COOH (carboxylic acid group) shown in the diagram no longer exists, as the OH is lost, but some amino acids have an extra acid group as their *R* group, like shown. The amino acid on the left is therefore an **acidic amino acid** and it *donates* H^+ ions in solution, leaving an oxygen δ- and the nitrogen of the other amino acid (which is a **basic amino acid**) *accepts* the H^+ ion. An **ionic bond** then forms between the O^- and the H^+ ions



C – *Disulphide bridges*

When two cysteine amino acids are found near each other, they can form a very strong covalent bond called a **disulphide bridge** (or disulphide bond). They lose their hydrogen ions in an **oxidation** reaction, and the two sulphurs can then form the bond. This bond can be broken by reversing the reaction (i.e. in a **reduction** reaction)



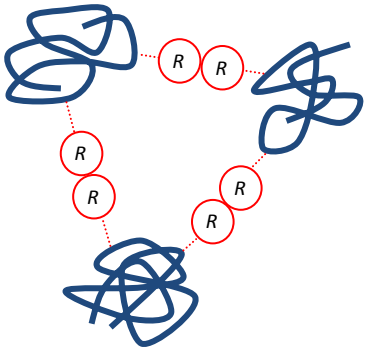
D – *Hydrophobic, non-polar bonding*

Non-polar *R* groups are **hydrophobic** (i.e. hate water). Two amino acids close to each other with hydrophobic *R* groups will bond together, clustering to exclude water. The bond formed is a **hydrophobic bond** which is a very strong type of bond and hard to break.

All globular proteins will roll up to form balls in 3D. But there are also **fibrous proteins**, which form fibres when they become three-dimensional. These are formed from regular and repetitive sequences of amino acids, and are usually insoluble in water, whereas globular proteins are most often soluble in water. An example of a fibrous protein is **keratin**. This is protein found in fingernails and hair. Fibrous proteins have only a primary and secondary structure, very few have this tertiary structure, as a fibre is a 2D shape. All globular proteins have a tertiary structure when in globular form, and also have a *quaternary structure*.

QUATERNARY STRUCTURE OF PROTEINS

A protein's **quaternary structure** (if they have one) refers to its state when it **polymerises** – i.e. when more than one globular protein joins together.



They bond together in exactly the same ways as the *R* groups join in the tertiary structure development: through ionic bonding, hydrogen bonding, hydrophobic bonding and forming disulphide bridges.

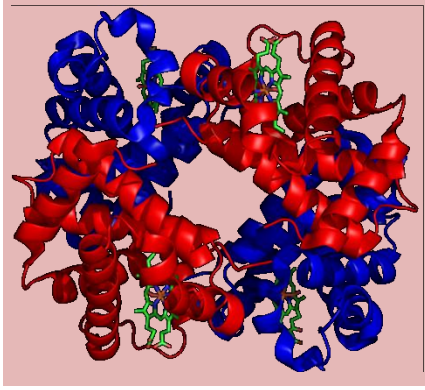
Examples of proteins which have a quaternary structure are **haemoglobin** and **antibodies**. You are required to know about *haemoglobin* and **collagen** for the exam.

COLLAGEN

Collagen is a fibrous protein found in skin, bones, cartilage, tendons, teeth and the walls of blood vessels. It is an important **structural protein** found in most animals. Collagen consists of three polypeptide chains, each in the shape of a helix. The three helices wind around each other to form a *rope*. Almost every third amino acid in each chain is *glycine*. The small size of glycine allows the three strands to lie close together and form a tight coil. The strands are held together by *hydrogen bonds*. *R* groups of individual collagen molecules form bonds with other collagen molecules

These cross-links form **fibrils**. Many *microfibrils* bond together to form larger *macrofibrils*. These associate together to form much bigger bundles called **fibres**. Collagen, a fibrous protein, has a tremendous amount of **tensile strength**, i.e. can withstand a high pulling pressure

HAEMOGLOBIN



A haemoglobin molecule is made of four polypeptide chains. Each chain is wrapped around a group of atoms, called a **haem group** (see 2.8 Haemoglobin) which holds an **iron Fe^{2+} ion** in the centre, as shown in the diagram. Each iron ion is able to bond with two oxygen atoms (one oxygen molecule), so the haemoglobin molecule as a whole can carry up to eight oxygen atoms (or four molecules of oxygen)

The usual bonds are responsible for giving the haemoglobin molecule its quaternary structure: hydrogen bonds, hydrophobic bonds, ionic bonds and disulphide bridges. The molecule consists of two α-chains and two β-chains

3.5 Testing for Biochemical Substances

Practical biochemistry: testing for the presence of sugars, proteins and lipids

Below is a list of chemical tests for different substances covered throughout chapters 3.2 to 3.4. This builds the *How Science Works* section of the course, and it is important that you know the test for each of them.

Testing for Starch

(See 3.2 Carbohydrates) Starch is an important carbohydrate which is used for energy storage in animals. The test for starch is very simple: prepare the sample you are testing, and add **iodine solution**. If starch is present in the sample, it will turn from a yellow-orange colour to a blue-black colour

Testing for Reducing Sugars

(See 3.2 Carbohydrates) All *monosaccharide* and *disaccharide* sugars are called **reducing sugars**: meaning that a molecule of this sugar can react with other molecules by giving electrons to them – **reduction**

When a reducing sugar is heated with *alkaline copper sulphate* (**Benedict's solution**) the solution will change colour from blue to an orange-red. This is called **Benedict's test**

Benedict's test is a test used frequently in this series of practicals. The result of using Benedict's test is either the same as it was before, or there might be a change. In the test for a *reducing sugar* described above, if it changes to a red-orange colour is it called a **precipitate** because it comes out of the solution and forms solid particles dispersed around the water. Below is a reduction scale used to describe the amount of reducing sugar in a sample:

(nothing) blue → green → yellow → orange → red (lots)

Testing for Non-Reducing Sugars

(See 3.2 Carbohydrates) If the reducing sugar test comes out as negative (no colour change), the **non-reducing sugar** test can be done

If a substance does not react with Benedict's solution, this test is used:

- Boil the sample with hydrochloric acid – this hydrolyses any sucrose present, splitting sucrose molecules to give glucose and fructose (see below)
- Cool the solution and neutralise it by adding sodium carbonate solution (an alkali solution)
- Carry out the reducing sugar test (Benedict's test) again: if there were non-reducing sugars present in the original sample, the test will now come out as positive (as they have been broken down into reducing sugars glucose and fructose)

The sugar *sucrose* is a non-reducing sugar. It is formed in a condensation reaction making a *glycosidic bond* between a glucose molecule and a fructose molecule. Fructose and glucose are both monosaccharides, and sucrose a disaccharide. The glycosidic bond formed in sucrose is different from that one found in, for example, maltose (maltose being a reducing sugar). It is this difference which prevents the sucrose from reacting with the Benedict's solution.

The non-reducing sugar test works because if there is any sucrose present (which is a non-reducing sugar, that we are testing for), it is broken down into those monosaccharides, which can be tested for using the ordinary reducing sugar test. A positive result therefore means non-reducing sugars are present on the original sample. The same scale applies:

(nothing) blue → green → yellow → orange → red (lots)

The table below shows some of the expected results for the two tests for glucose and sucrose:

	Reducing sugar test	Non-reducing sugar test
glucose	green	green
sucrose	blue	green
mixture	green	orange

If you test a sample for both reducing and non-reducing sugars, and the colours produced are the same, the conclusion is that there are *no non-reducing sugars*. If the colour in the non-reducing sugar test is more towards the *red* end of the spectrum, then *non-reducing sugars are present*.

(nothing) blue → green → yellow → orange → red (lots)

We call the tests for reducing and non-reducing sugars **semi-qualitative tests** because they produce results which indicate what *type* of molecule is present, not a specific amount, although the colour spectrum above does give a rough idea of comparison of how much is present. But it is also important that we can do **quantitative tests** to identify exact amounts of *how much* of a molecule is present.

USING BENEDICT'S SOLUTION

- Using Benedict's test will reveal the presence of reducing sugars
- It results in an orange-red precipitate
- The more reducing sugar there is present, the more precipitate will be formed, and the more Benedict's solution (copper sulphate) will be used up
- The precipitate is filtered out then the concentration of the remaining solution can be measured
- This will tell you how much Benedict's solution has been used up allowing you to estimate the concentration of reducing sugar in the original sample

A device called a **colorimeter** can be used to make more accurate measurements. This device shines a beam of light through a prepared sample, and a reading is measured of percentage light transmission.

USING A COLORIMETER

- The solution is places in a clear plastic cuboid called a **cuvette** which then goes into a small chamber in the colorimeter
- The colorimeter shines a beam of light through the sample
- A **photoelectric cell** picks up the light that is passed through the sample (on the other side) and will provide you with a reading of the amount of light that was passed through – transmitted

The more copper sulphate that has been used in the Benedict's test, the less light will be blocked out in the sample, and more transmitted. Therefore the reading gives a measure of the amount reducing sugar based on the Benedict's reaction

Whilst using a colorimeter alone will provide a measure, it doesn't specify an exact amount: in order to **quantify** the amount, a **calibration curve** must be made…

CALIBRATION PLOTTING

- Take a range of known concentrations of reducing sugar, carry out a Benedict's test on each one, then filter out the solution; use a colorimeter to give readings of the amount of light passing through the solutions
- Plot the readings in a graph to show the amount of light getting through (transmission) versus reducing sugar concentration
- Then you can take the reading of an unknown concentration – use the graph to make a precise measurement

TESTING FOR LIPIDS

(See 3.3 Lipids) Testing for the presence of a lipid uses the **ethanol emulsion test**:

- Mix the sample with *ethanol*: this dissolves any lipid present, because they are soluble in alcohols
- Pour the mixture into water contained in another test tube
- If there is lipid present, a cloudy white *emulsion* will form near the top of the water

TESTING FOR PROTEINS

(See 3.4 Amino Acids and Proteins) A protein test uses the **biuret test**. Biuret reagent, which contains sodium hydroxide and copper sulphate, and is pale blue in colour, is added to the sample. These chemicals react with the *peptide bonds* found in proteins, which results in a colour change to lilac

There is a summary of all of these semi-qualitative tests (also known as **food tests**) in the below table:

Test for…	**Description**	**Result (colour changes)**
Starch	Add a few drops of iodine solution	Orange to blue-black
Reducing sugars	Add Benedict's solution, heat to 80^{o}C in a water bath	Blue to orange-red
Non-reducing sugars	(If reducing sugar test is negative) boil with hydrochloric acid, cool and neutralise with sodium carbonate solution, repeat Benedict's test	Initially no change, repeated Benedict's test will turn blue to orange-red
Lipids	Add ethanol, pour mixture into water in another test tube	White emulsion forms in water
Proteins	Add biuret reagent	Pale blue to lilac

3.5 Testing for Biochemical Substances

Practical biochemistry: testing for the presence of sugars, proteins and lipids

Use the information provided in the various boxes for each test to answer the following questions.

A: The non-reducing sugar test

Fill in the following table to identify potential colours for results of the reducing and non-reducing sugar tests, and the conclusions which can be drawn from the results of each. The first two have been done for you.

Solution	Colour in reducing sugar test	Colour in non-reducing sugar test	Conclusions
A	blue	blue	No sugars at all: neither reducing sugars or non-reducing sugars are present
B	blue	orange	No reducing-sugars are present Quite a large amount of non-reducing sugars present
C	yellow	yellow	
D	green	red	
E	yellow	orange	
F	green	yellow	

B: Testing for lipids

What are the conclusions that can be drawn from the following?

Solution	Result after alcohol + sample are mixed with water sample	Conclusions
G	a clear mixture	
H	a white emulsion forms as a layer on top of the water	

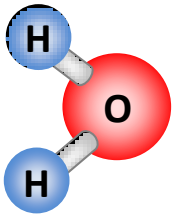
C: Testing for proteins

What conclusion can be drawn about the following results? Explain, in the table, why the result is as it is.

Solution	Resultant colour after having added the biuret reagent	Conclusions
I	pale blue to lilac	

3.6 Water

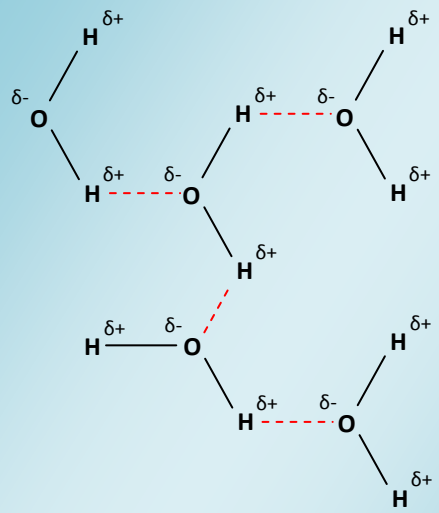
The biological significance of the molecule water



Water is a very special substance, and this is all down to their **hydrogen bonds** (for a detailed explanation of hydrogen bonding and how it works, see 3.1 Biochemistry and Chemical Bonds). The molecule consists of *one oxygen* atom covalently bonded to *two hydrogen* atoms. However, the electrons are not shared perfectly evenly: the oxygen atom is capable of pulling them towards itself and further away from the hydrogen atoms. The result is that the oxygen part of the molecule becomes slightly *negatively* charged, and the hydrogen atoms slightly *positively charged*. Water is therefore described as a **polar** molecule (polar means charged internally).

Within liquid water, it is the many thousands of these hydrogen bonds which allow the polymerised molecules to exist as they do, and the fact that they are constantly making and breaking bonds that give it its properties. The network they form allows the molecules to slide over each other as the new bonds form and then break. This makes it more difficult for the water molecules to escape and become a gas, explaining the need to heat it up to 100°C to make it boil.

Hydrogen bonding in water



Hydrogen bonds form between slightly positively charged and slightly negatively charged areas of separate molecules. This happens in water between an oxygen of one water molecule and a hydrogen of another, because the oxygen part of a water molecule tends to drag the shared electrons towards itself, leaving the hydrogen atoms slightly more positive, and causing the oxygen atom to become slightly more negative

At lower temperatures, water has less **kinetic energy** and so they move less readily. Hydrogen forms are forming, but not breaking very frequently – this is because it takes energy to break bonds, but energy is *released* in the making of bonds. When water becomes a solid (**ice**), the hydrogen bonds hold the structure in a **semi-crystalline** form.

The **solubility** of a substance in water depends on whether or not water molecules can interact with the substance. Any molecule which is *polar*, like water is, will dissolve in water. This is because the **solute** (substance being dissolved) has slightly positive and slightly negative parts which can interact with the water molecules. The water molecules will then cluster around the slightly charged parts of solute molecules, which separates solute molecules, so they are dissolved.

Water has many various other properties. One is called **cohesion**, which can be shown if you place a drop of water onto a waxy surface, such as the cuticle of a leaf. It forms a spherical perfect drop. This is because the hydrogen bonds pull water molecules in at the surface.

Its properties in its solid form, *ice*, also are unusual. Strangely, ice is less dense than water. As water cools, its density increases until the temperature reaches 4°C, and then the density decreases again. This property would mean that ice is able to float on liquid water, which it is able to do. When this happens, it insulates the water below, allowing organisms to live under the ice.

Q1 Biological Molecules

Questions on Units 3.1 – 3.6 on Biological Molecules

1 The molecule glucose can occur in two different forms: α-glucose and β-glucose.

(a) Explain the differences between the two forms of glucose. You may use the space below to show your answer in a diagram also.

……

……

……

……

……

(3 marks)

(b) Explain how two α-glucose molecules may join to form a disaccharide.

Use the space provided to give an annotated diagram as part of your answer.

……

……

……

……

……

……

(4 marks)

(c) Explain how two or more β-glucose molecules may join to form a polysaccharide.

You may use the space provided to give an annotated diagram.

……

……

……

……

……

……

……

……

(5 marks)

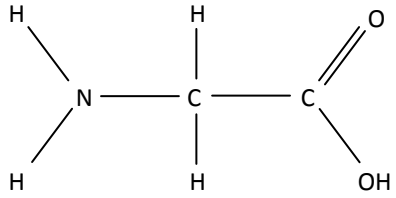
(d) Give the name of an example of a structure formed from long chains of what you have described in part (c).

……

(1 mark)

Total: 13 marks

2 (a) The diagram below shows the amino acid glycine.

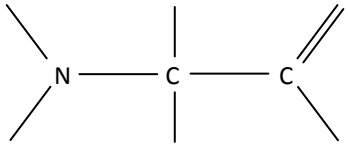


(i) Identify the following parts of the amino acid in the diagram.

carboxylic acid group, **amine group**, **variable group**

(3 marks)

(ii) The following diagram has some atoms missing. Complete the diagram to represent the amino acid **cysteine**.



(2 marks)

(b) Proteins are made from one or more polypeptide chains. The tertiary structure of proteins is maintained by several different chemical bonds.

(i) What is meant by the term *tertiary structure* of a protein?

……

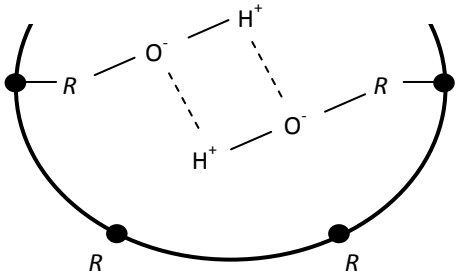
……

……

……

(2 marks)

(ii) Identify and explain the following chemical bonds holding together a protein, protein **A**.



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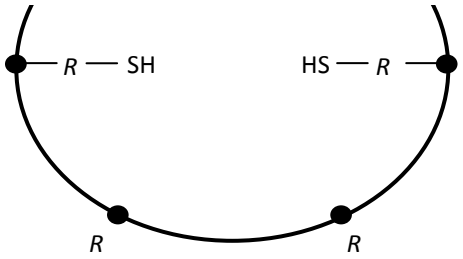

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(7 marks)

(c) All polypeptide chains have a *primary structure* and a *secondary structure*.

(i) Name the two possible secondary structures of polypeptides that can be formed:

1 ……

2 ……

(2 marks)

(ii) Explain the formation of **one** of the structures you identified in (c)(i).

……

……

……

……

……

……

……

……

(4 marks)

Total: 20 marks

3 A *triglyceride* is a type of lipid.

(a) Outline **two** key differences between lipids and carbohydrates as biological molecules.

1 .....

.....

2 .....

.....

(2 marks)

(b) State all of the element(s) found in a triglyceride molecule.

.....

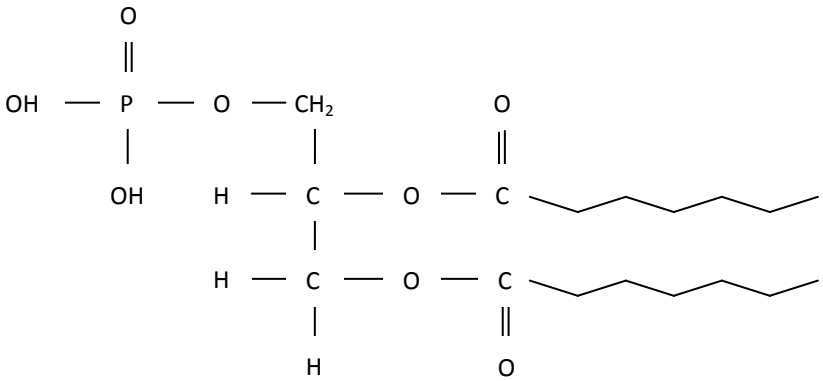
(1 mark)

(c) Complete the following paragraph by filling in the gaps, about triglycerides.

One triglyceride molecule contains one ..... molecule attached to three .... tails. The latter consists of an acid group at one end of each tail, and at the other end a ..... chain. This can be either saturated or unsaturated. The components join together in a ..... reaction, which releases ..... in the process. We call the O-C=O bond produced an ..... bond. A ..... reaction can be used to reverse the reaction. Any tail which has C=C double bonds is more ..... as a structure, and therefore less stable.

(8 marks)

(d) A phospholipid is another type of lipid. It can be drawn in the following way.



(i) In the space below, draw and annotate a diagram to show a more simplified version of the diagram on the previous page.

(3 marks)

(ii) On the diagram on the previous page, identify the following features using labels.

fatty acid tail, **phosphate group**, **covalent bond**, **ester bond**

(4 marks)

(iii) Phospholipids can arrange themselves to become a *phospholipid bilayer* when in water.

Describe and explain their arrangement, focusing on the biological significance of this layering.

……

……

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……

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……

……

(5 marks)

Total: 23 marks

4 A student is testing some chemical substances labelled chemical **V**, chemical **W**, chemical **X**, chemical **Y** and chemical **Z**.

The information below displays the various results for the chemical tests.

Identify for each chemical:

- If there is reducing sugar present
- If there is non-reducing sugar present
- If there are lipids present
- If there are proteins present

Fill in your answers in the answer boxes on the following page.

Chemical **V** (originally a blue solution) was heated with Benedict's solution, but did not change colour. After boiling with hydrochloric acid and then neutralising with sodium carbonate solution, it was again heated with Benedict's solution, where the colour changed to orange

Chemical **W** (originally a blue solution) was heated with Benedict's solution, but did not change colour. After boiling with hydrochloric acid and then neutralising with sodium carbonate solution, it was again heated with Benedict's solution, and again there was no colour change

Chemical **X** (originally a blue solution) was heated with Benedict's solution, and it changed to a yellow colour. After boiling some more of chemical **X** with hydrochloric acid and then neutralising with sodium carbonate solution, it was again heated with Benedict's solution, and the colour changed from blue to green

Chemical **Y** had biuret reagent added to it, and the solution became pale blue

Chemical **Z** (originally orange in colour) had iodine solution added to it, and the colour change observed took the solution to blue-black

Use the following spaces to identify the biological molecules present in each chemical.

You can answer with a **tick** (✓) if it is present, a **cross** (✗) if it is not present, or a **question mark** (?) if there is insufficient data to conclude whether or not it is present.

Chemical **V**: Is there reducing sugar present? .....

Is there non-reducing sugar present? .....

Is there lipid present? .....

Is there protein present? .....

Chemical **W**: Is there reducing sugar present? .....

Is there non-reducing sugar present? .....

Is there lipid present? .....

Is there protein present? .....

Chemical **X**: Is there reducing sugar present? .....

Is there non-reducing sugar present? .....

Is there lipid present? .....

Is there protein present? .....

Chemical **Y**: Is there reducing sugar present? .....

Is there non-reducing sugar present? .....

Is there lipid present? .....

Is there protein present? .....

Chemical **Z**: Is there reducing sugar present? .....

Is there non-reducing sugar present? .....

Is there lipid present? .....

Is there protein present? .....

(20 marks)

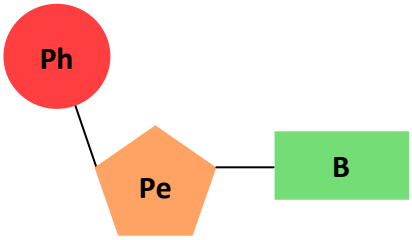
Total: 20 marks

3.7 Nucleic Acids

The coding molecules nucleotides and their polymerisation into nucleic acids

A **nucleic acid** comes in two different forms: as **DNA** (**deoxyribose nucleic acid**) and as **RNA** (**ribose nucleic acid**). They are both the *macromolecules* which are formed by the *polymerisation* of molecules known as **nucleotides**.

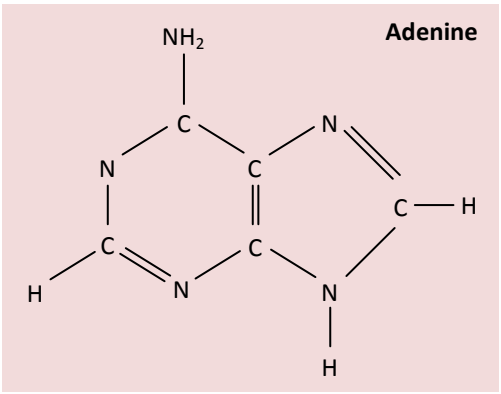
A single nucleotide is made up of three individual sections.

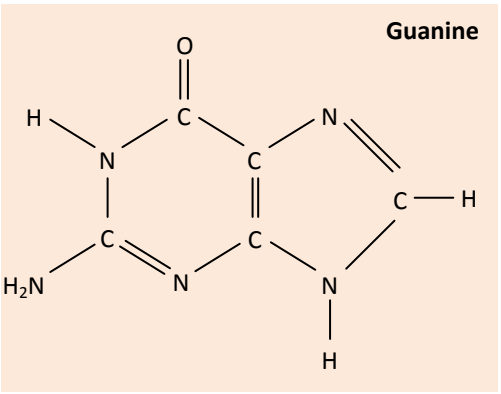


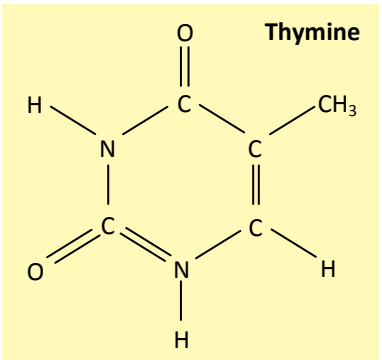
One subunit is a single **phosphate group** (*Ph*). Another is a sugar molecule, **pentose** (*Pe*), which is a five-carbon sugar – this will be either *deoxyribose* or *ribose*. The third subunit is an *organic* **nitrogenous base** (*B*), of which there are five possible bases it can be, which are covered below.

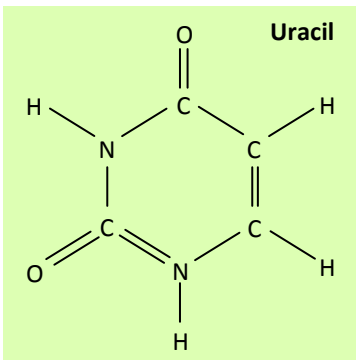
The three subunits join together, forming covalent bonds, to form a single nucleotide molecule.

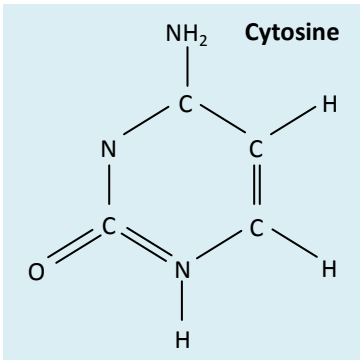
The five bases which can be found in a nucleotide are **adenine**, **cytosine**, **guanine**, **thymine** and **uracil**. Their chemical structures are drawn below. The two larger molecules are called **purines**. The three small molecules are **pyrimidines**.







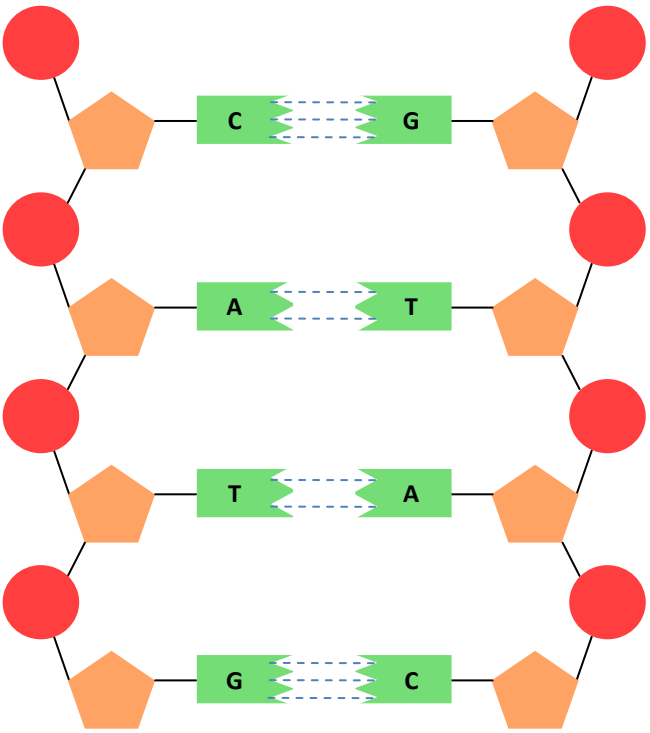




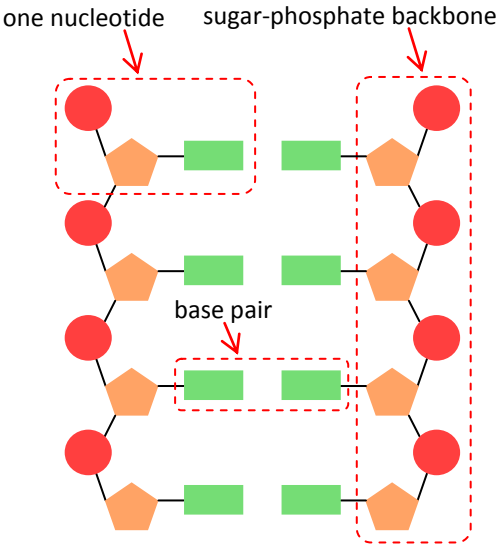
It is a **condensation reaction** which joins the phosphate group of one nucleotide to the sugar of another. As more and more nucleotides join together, they begin to form a **sugar-phosphate backbone**. This structure consists of a long chain of the nucleotides, composed purely of the sugar groups and phosphate groups – the nitrogenous bases stick in towards the middle.

A chain of nucleotides bonded together is called a *nucleic acid*. Only molecules carrying the same sugar are able to bond together, therefore only nucleotides of all ribose or all deoxyribose sugars can join together.

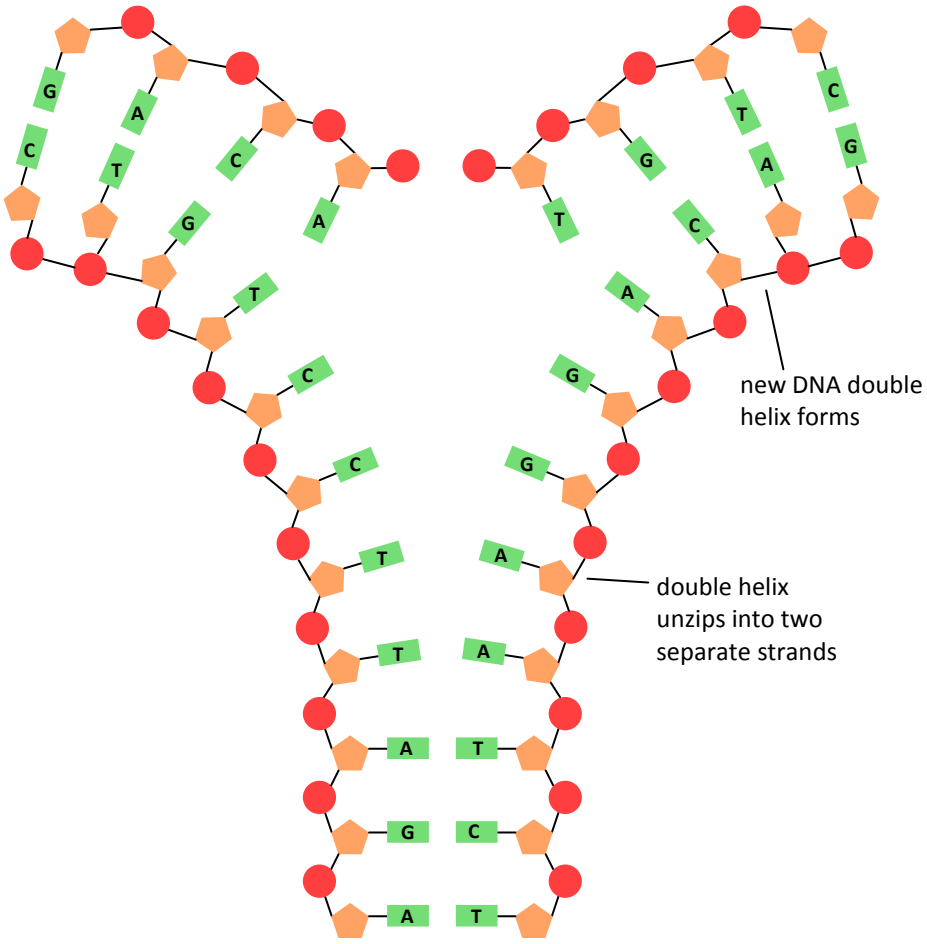
This means that a nucleic acid is described as DNA when the nucleotides contain the sugar deoxyribose, and RNA when they contain the sugar ribose.



The diagram shows some DNA. DNA is made of a double strand (**double helix**) where *hydrogen bonding* holds together the two antiparallel strands.



The two chains or "backbones" are always equidistant from each other. This is due to the way the **base pairs** form. As the different bases pair up, they follow set rules. The purine adenine always pairs up with the pyrimidine thymine (A-T) and the purine guanine always pairs up with the pyrimidine cytosine (C-G). As the strands come together, hydrogen bonds between the bases form. The term **complementary** is used to describe the preference of A joining to T and C joining to G.



When DNA replicates to form two separate DNA molecules from one original molecule, it is called **semi-conservative replication**.

This process involves a double-stranded molecule of DNA "unzipping" to become two single strands. Free nucleotides then join to the bases of the individual strands, and all of these new nucleotides form a new chain, creating two brand new DNA (double-helix) molecules.

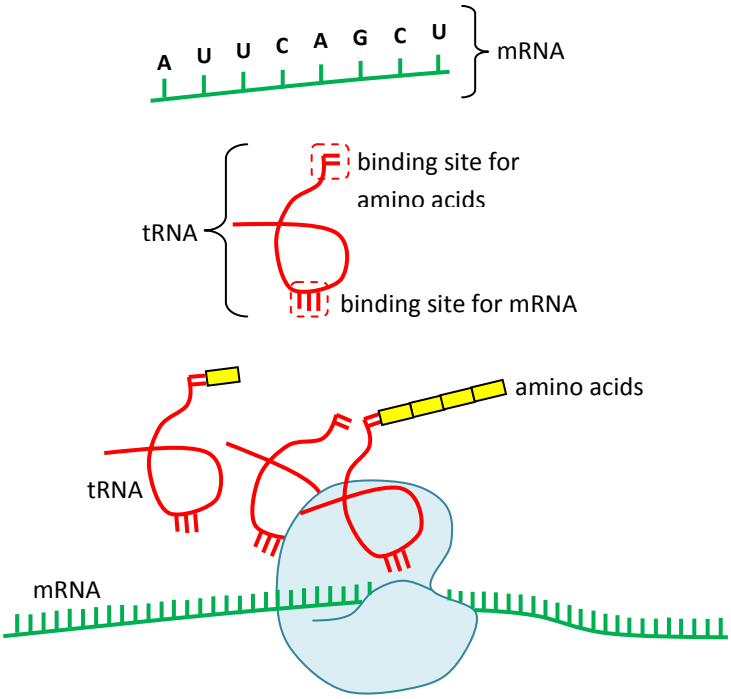
It is important to note that each of the two new DNA double-strands which are produced will be *identical*. This is because, as the original molecule unzips, there will be complementary bases on each strand, and complementary bases to those bases will bond to them. Therefore, as is shown in the diagram, each produced strand will be chemically identical.

Ribose nucleic acid (RNA) is different from DNA in three principal ways:

- the *pentose sugar* is **ribose**, instead of deoxyribose
- the molecule is single-stranded, whereas DNA is double-stranded
- the nitrogenous base **uracil** is found in RNA, instead of the base thymine

RNA only contains the nitrogenous bases adenine and guanine (purines) and cytosine and uracil (pyrimidines). Again, the bases cytosine and guanine are complementary to each other, and so hydrogen bonds between C-G can be made. Uracil, a base very similar to thymine, is able to make hydrogen bonds with adenine also, so with RNA, A-U bonds are made.

RNA is involved in a process called **protein synthesis**. This involves the production of proteins. RNA comes in three different forms, and these are all involved in individual parts of this process.



DNA is too large to escape from the nucleus – because it cannot fit through the **nuclear pores**. Instead, a new strand is developed which is small enough to fit through the pores. In this process, the double-stranded DNA molecule is unzipped (as with replication), and then a new nucleotide chain forms alongside it, which is complementary to the DNA sequence of nucleotides. Therefore, *adenine* in the DNA bonds to *uracil*, *thymine* in DNA bonds with *adenine*, and cytosine and guanine bond to each other. This process is called **transcription**. This new chain is called **messenger RNA** (or **mRNA**). This nucleotide chain is able to escape the nucleus. It is important that you know an mRNA strand is a *copy of one gene*. mRNA is delivered to the ribosomes, on the rough ER or dispersed around the cellular cytoplasm. The ribosomes are where proteins are synthesised, and ribosomes are made from **ribosomal RNA** (or **rRNA**) with proteins.

The third type of RNA is called **transfer RNA** (or **tRNA**). These have two binding sites: one for amino acids, and one for mRNA. Transfer RNA works by bringing in the correct amino acids to the ribosome in the correct sequence in order to be synthesised into proteins. But the binding site for the mRNA allows tRNA to attach to the mRNA so it can obtain those codings. The tRNA sequence will be *complementary* to the mRNA, so not identical. We call the sequence of amino acids produced for mRNA the **mRNA codon**, and the sequence for the ribosome use **tRNA anticodon**. We call the shape of the tRNA molecule a **hairpin loop**, which is shown in the above diagram.

The diagram above shows the mRNA entering the ribosome (these are normally simplified into two-lobed structures) and the tRNA binding to it. The tRNA molecule has brought the correct sequence of amino acids with it into the ribosome so that the protein can be synthesised. This process is called **translation**.

In the exam, you may be asked to provide the coding for various DNA and RNA molecules. If the DNA sequence is as follows, then we can work out what the mRNA and tRNA sequences will be:

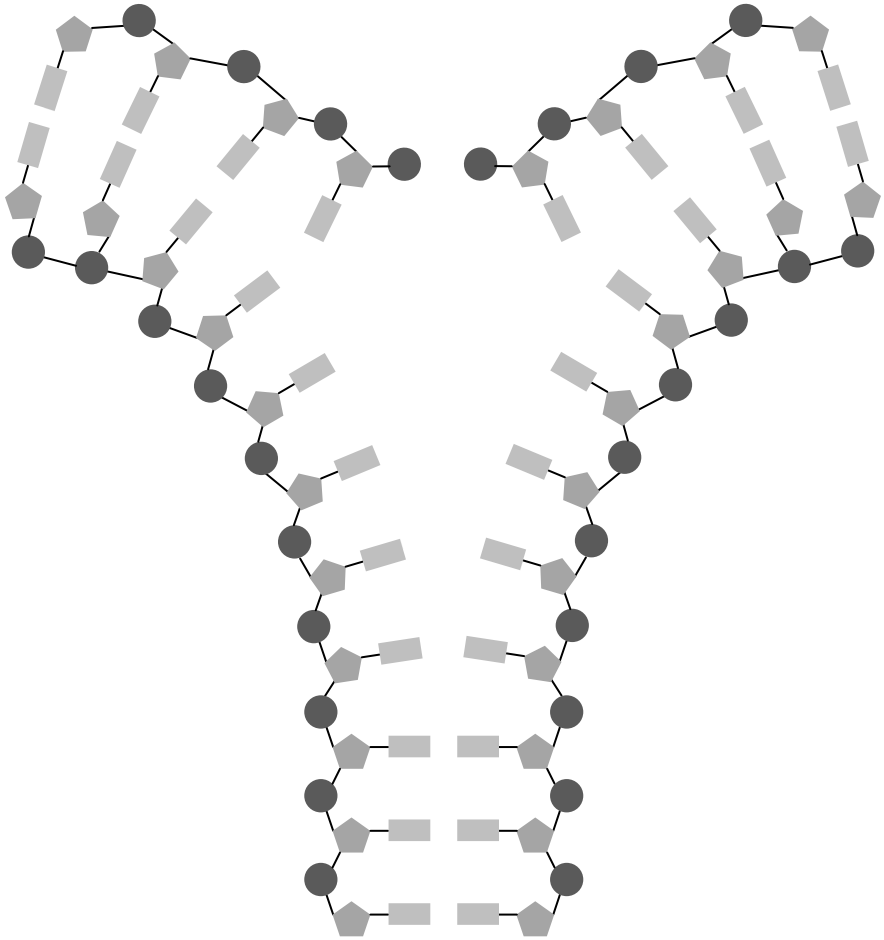
DNA	A	G	C	A	A	T	G	T	C	A	G	A	C	T	T
mRNA	U	C	G	U	U	A	C	A	G	U	C	U	G	A	A
tRNA	A	G	C	A	A	U	G	U	C	A	C	A	G	U	U

If we know the DNA sequence is as shown, the mRNA must be complementary to that, and then the tRNA to the mRNA.

Q2 Nucleic Acids

Questions on Units 3.7 on Nucleic Acids

1 The following diagram shows a DNA molecule which has been "unzipped" into two single strands.



(a) The diagram shows the DNA molecule undergoing **semi-conservative replication**. Explain the term semi-conservative replication.

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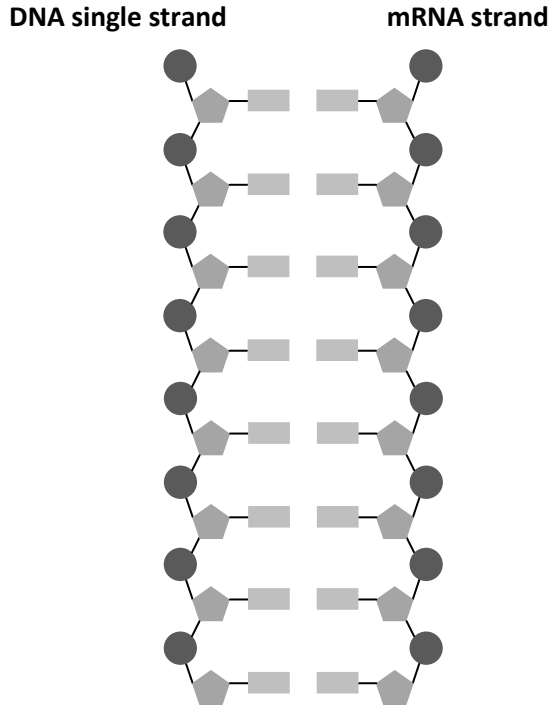
(3 marks)

(b) The same DNA molecule unzips to provide a template for mRNA. The simplified diagram below shows the DNA molecule and the mRNA strand being produced.



(i) The base sequence for part of the **DNA single strand** is shown below:

… **G C A** **C T G** **A T A** **C C G** **T A C** …

triplet 1 triplet 2 triplet 3 triplet 4 triplet 5

Complete the table below to identify the base sequence for the mRNA codon.

	triplet 1	triplet 2	triplet 3	triplet 4	triplet 5
DNA single strand	**G C A**	**C T G**	**A T A**	**C C G**	**T A C**
mRNA codon					

(2 marks)

(ii) Give the name to the specific length of the mRNA molecule.

……

(1 mark)

(iii) Complete the table below to identify the base sequence for the tRNA anticodon.

	triplet 1	triplet 2	triplet 3	triplet 4	triplet 5
DNA single strand	**G C A**	**C T G**	**A T A**	**C C G**	**T A C**
tRNA anticodon					

(2 marks)

(c) Explain the relationship between the sequence of the DNA triplets and the structure of a protein which is made in the ribosome.

……

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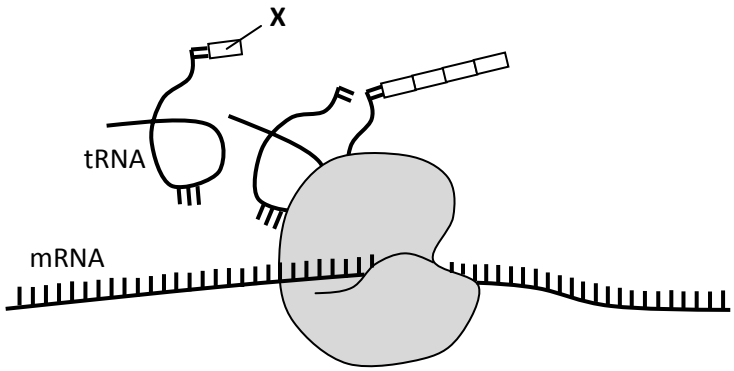
(5 marks)

(d) The diagram below shows the process of mRNA entering the ribosome and bonding with tRNA.



(i) Name the process that is taking place in the diagram.

……

(1 mark)

(ii) Identify material **X** from the diagram.

……

(1 mark)

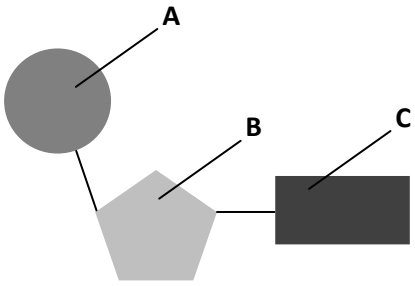
(iii) Name the material(s) which the ribosome is composed of.

……

(2 marks)

Total: 17 marks

2 The diagram below shows a simple nucleotide.



(a) Name the parts **A**, **B** and **C** in the diagram.

A .....

B .....

C .....

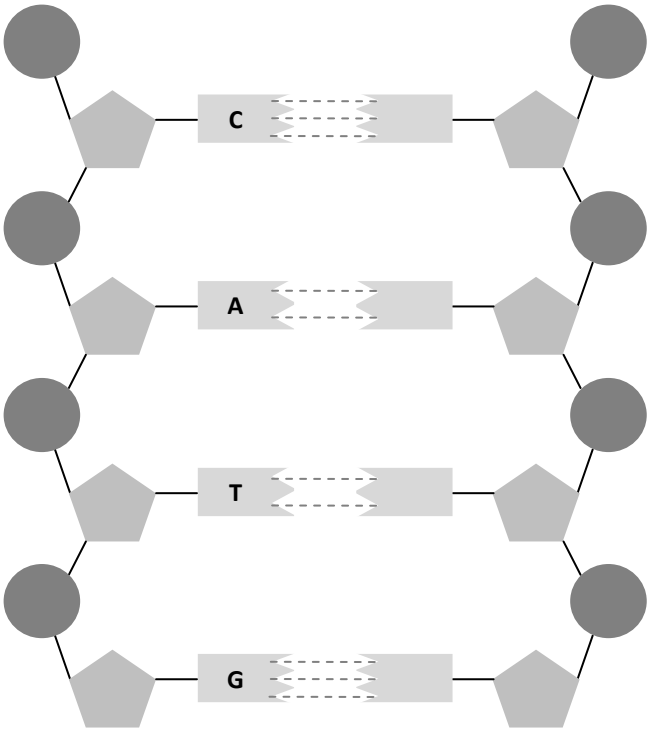
(3 marks)

(b) Which part of the nucleotide, **A**, **B** or **C**, contains the element nitrogen?

.....

(1 mark)

(c) The diagram below shows **eight** nucleotides from a DNA double-helix molecule.



(i) On the diagram, fill in the blank boxes to match the nucleotides correctly.

(2 marks)

(ii) The entire DNA molecule has 24% adenine and 20% cytosine.

State the percentages for the contents of each of the following in the DNA molecule:

guanine ..... %

thymine ..... %

uracil ..... %

(3 marks)

(d) State any **two** differences between DNA and RNA.

1 .....

.....

2 .....

.....

(2 marks)

Total: 11 marks

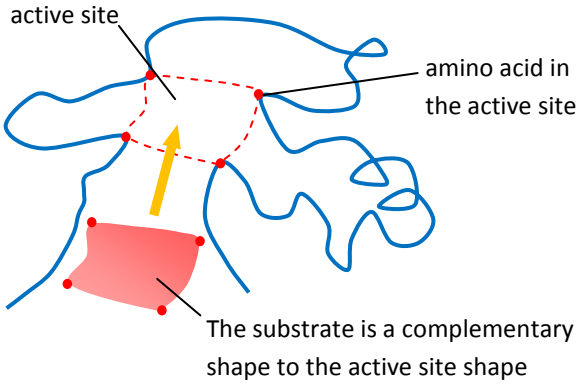
3.8 Enzyme Action

An introduction to enzyme structure and function

An **enzyme** is a protein. Each and every enzyme is a *globular protein* with a specific *tertiary structure* (see 3.4 Amino Acids and Proteins). They are **catalysts** – substances which speed up chemical reactions, but do not get used up in the process.

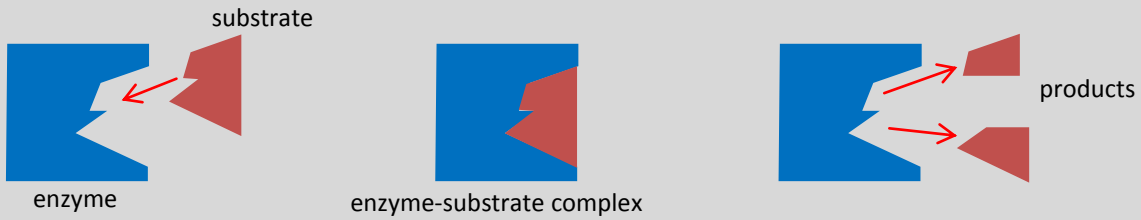
A single enzyme is quite a large molecule. The whole primary, secondary and tertiary structures are involved in giving an enzyme its specific shape. That special shape is needed to provide a certain shape for the **active site**. This is the area of an enzyme where the catalytic activity occurs, and is a 'pocket' or cleft in the enzyme's 3D structure.

Although there are hundreds, if not thousands, of amino acids found in a single enzyme, very few are responsible for maintaining the shape of the active site, usually less than ten, as shown in the diagram.



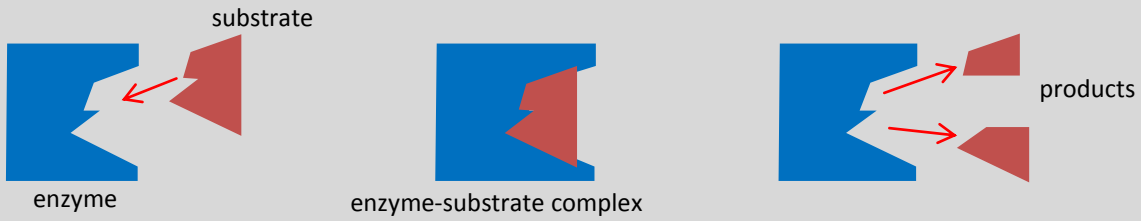
There is only one **substrate** which is specific enough to fit an enzyme. So each enzyme can catalyse a reaction involving only one type of substrate. Different enzymes use different substrates. The substrate will be shaped in a way which is *complementary* to the shape of the active site. The **lock and key theory** of enzymes states that the substrate is a "key" and the enzyme's active site a "lock" which can only be triggered by that one key that fits.

Lock and Key Theory



This is the generally-accepted model which suggests that only one substrate "the key" will fit one and only one active site belonging to an enzyme, "the lock". When the key is inside the lock, the reaction takes place with the substrate held inside the enzyme, and the result is the products released

Induced-Fit Hypothesis



A more recent explanation to the fitting of enzymes is the **induced-fit hypothesis**. This hypothesis still states that one substrate fits one active site, this is scientific fact. But this hypothesis suggests that the enzyme molecule *slightly* changes shape when it collides with substrate, making the active site fit more closely around the substrate molecule. The substrate is held in place, and the reaction takes place in the **enzyme-substrate complex**. The products made no longer fit the active site, so they are released, and this enables the enzyme to redo the whole process by taking in another substrate molecule

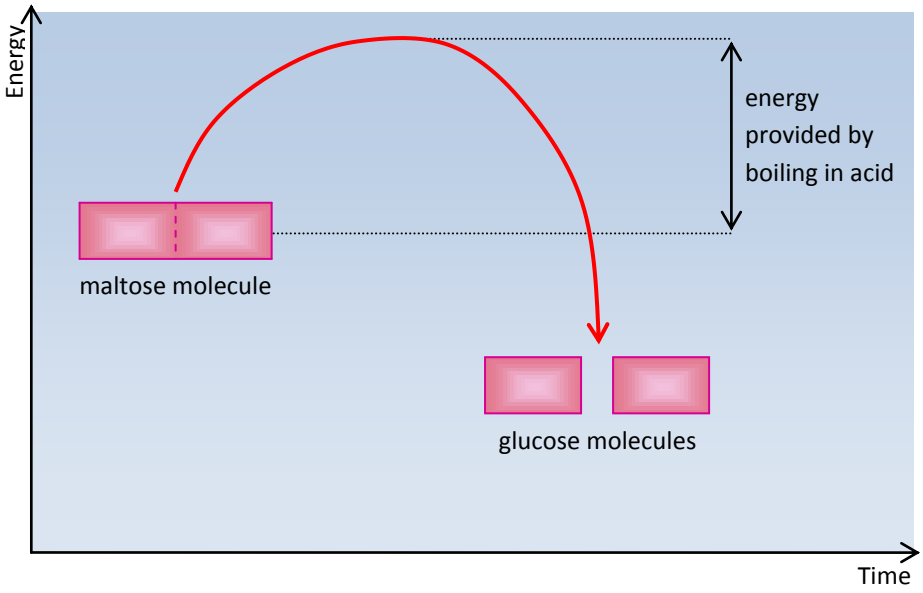
These two theories bring up the idea of *product*. In a chemical reaction, if an enzyme catalyses the reaction, *substrate* is turned into **product**. The general formula below shows this:

$$\text{substrate} \xrightarrow{\text{catalyst}} \text{product}$$

Take the example of the enzyme *maltase*. This is able to catalyse the conversion of maltose into glucose:

$$\text{maltose} \xrightarrow{\text{maltase}} \text{glucose + glucose}$$

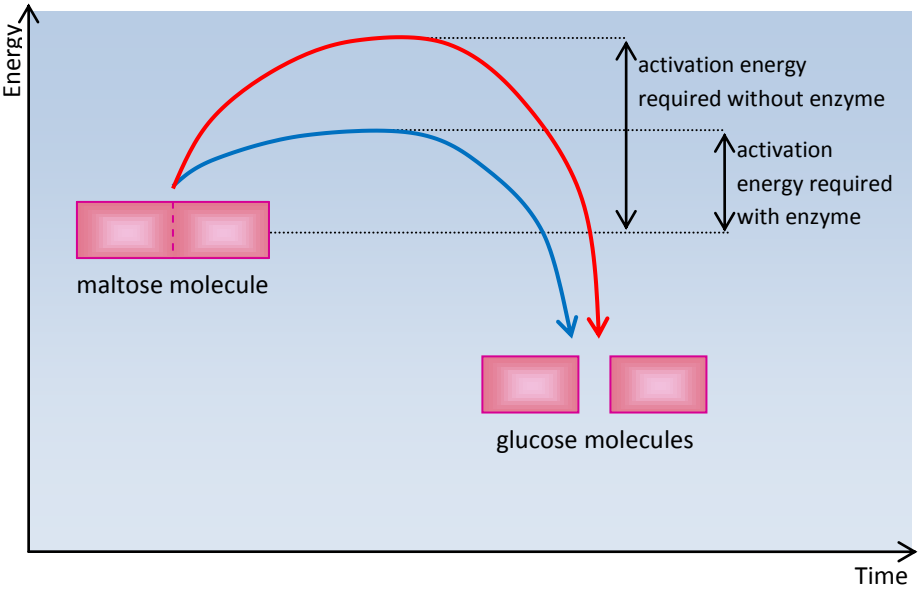
One maltose molecule is made up of two glucose molecules joined by a single **glycosidic bond** (see 3.2 Carbohydrates). If we want to break the component down back into two separate glucose molecules, we need to break that glycosidic bond up. We can boil maltose in hydrochloric acid to do this. It supplies the maltose molecules with enough energy to break the bond and collide with water molecules for the **hydrolysis** reaction to take place. This extra burst of energy required to initiate a reaction is called **activation energy**.



Many biological molecules, like maltose, are simply too stable to simply break their bonds out of thin air. These large amounts of energy are required to do so, because the bonds are strong, most of them covalent.

Boiling in hydrochloric acid provides the molecule with this energy, but it is very unlikely that cells do this naturally and even if they did, that they would survive. So how can these reactions be initiated without the need for such extremes?

A **catalyst** can be used to drive metabolic reactions. These work by enabling the same reactions more readily, because they reduce the amount of activation energy required to initiate the reaction. Enzymes are catalysts which reduce the amount of activation energy needed, so reactions can take place at lower temperatures and in a more diverse range of conditions. This is the case because the active site of the enzyme molecule can fit the substrate perfectly.



Without catalysts, the metabolic reactions which are essential to life would not be able to take place at the right amounts and at the right speeds, so they are essential substances.

It is important to remember that an enzyme is a catalyst, and that it is part of a chemical reaction, where it is used to speed up the reaction – but the enzyme does *not* get used up in the process: an enzyme can be used many times, but does eventually become less effective.

3.9 Factors Affecting Enzymes

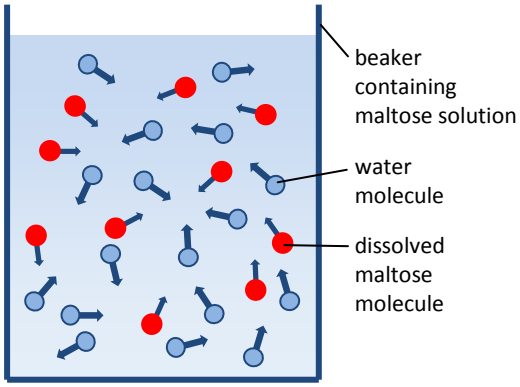
The effects of temperature, pH, concentration and inhibition on enzyme action

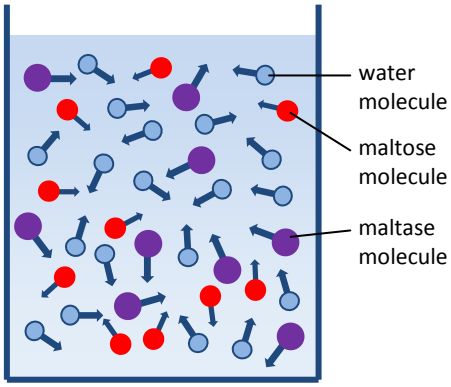
Enzymes do not function as simply as was explained in 3.8 Enzyme Action because there are a wide range of factors which affect their action. The main ones which you need to know about are discussed here.

Enzymes and temperature

Basic chemistry knowledge should remind you that molecules in a gas or liquid are constantly moving around, because they have natural *kinetic energy*. They continually collide with each other, which can initiate a chemical reaction. When the fluid is heated, their kinetic energy levels increase, and so they speed up, and the number of collisions become more frequent and with more force per collision.

An enzyme can only catalyse a reaction if the *substrate* collides with enough force into the *active site* of the enzyme, so that an **enzyme-substrate complex** is formed. It is the *random movements* of molecules which enable these collisions.



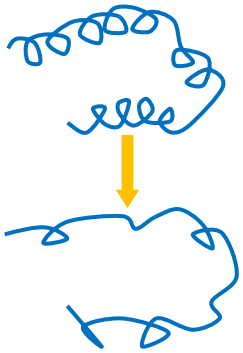


If we look at the beaker on the left, which contains only water and dissolved maltose, we know that the molecules will all continually and randomly move around and collide with each other – this is known as **Brownian motion**.

But when we add the maltose enzyme molecules (**maltase**), we enable reactions to take place more easily. When a water molecule and a maltose molecule both collide with a maltase molecule, the reaction incurs, and the product (glucose) will be produced. For details on the process of substrate-to-product, see 3.8 Enzyme Action.

So knowing that the higher the temperature, the more collisions and the more the reactions, and also knowing that the presence of enzymes will increase the number of reactions, we can assume that an increase in temperature will increase **reaction rate**?

Rate of reaction - the speed of a reaction, or how fast one reaction takes place



No – this is true up until a certain point, but too high temperatures can cause problems with enzymes. This is because not only do higher temperatures increase the speed of the molecules movement, but they also make them vibrate, which puts strain on the bonds holding them in place. Weak bonds, like the hydrogen and ionic bonds in enzymes are broken when they vibrate at high temperatures. The problem is that these are the bonds responsible for maintaining an enzyme's tertiary structure, and more importantly, its active site. As more and more bonds break, the enzyme loses its tertiary structure and the proteins unravel, so the enzyme can no longer function – this of course, reduces reaction rate. This is called **denaturation**.

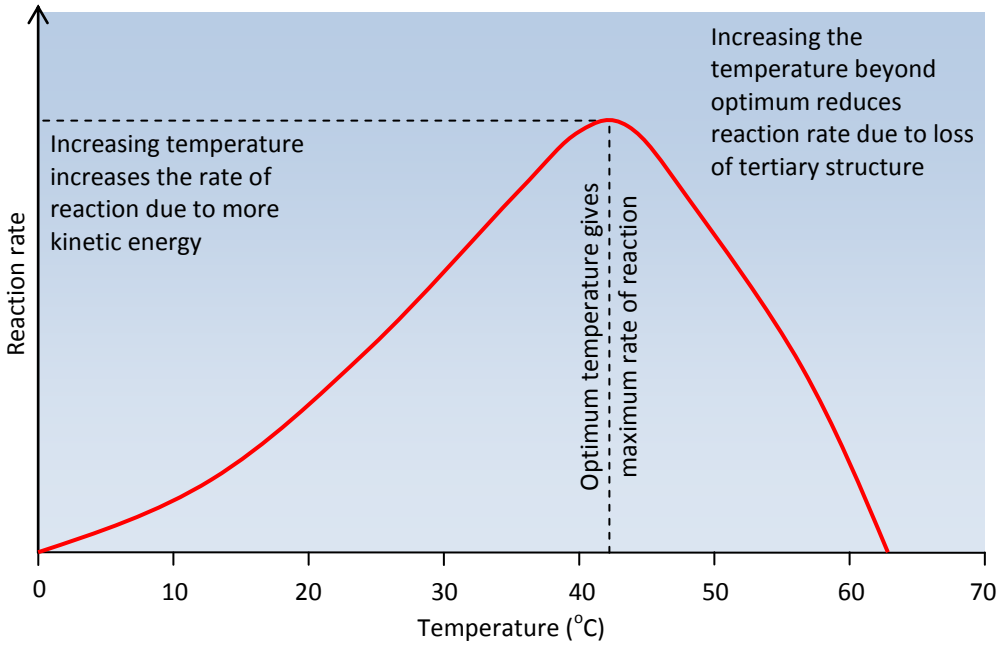
However, it is important to note that this does *not* affect the primary structure of the proteins which make up the enzyme. The peptide bonds which give it the primary structure are *covalent*, and so are not broken very easily.

Because increasing temperature improves the rate of reaction up to a certain point, but increasing it too much will decrease the reaction rate, we say that

Denaturation - when an enzyme is heated too much it loses its tertiary structure as the hydrogen and ionic bonds holding it together are broken, so the active site is destroyed – the enzyme is denatured

enzymes have an **optimum temperature** for operation (that is, the perfect temperature where they are at their highest efficiency).

The optimum temperature for the work of an enzyme will depend upon the enzyme, because they all have different functions, they all work best in different environments. The vast majority of enzymes have an optimum temperature of between 40°C and 50°C, but those enzymes which must be *heat-resistant*, such as in organisms where having such enzymes would be useless. The high temperatures of their environment would not allow enzymes to cope.

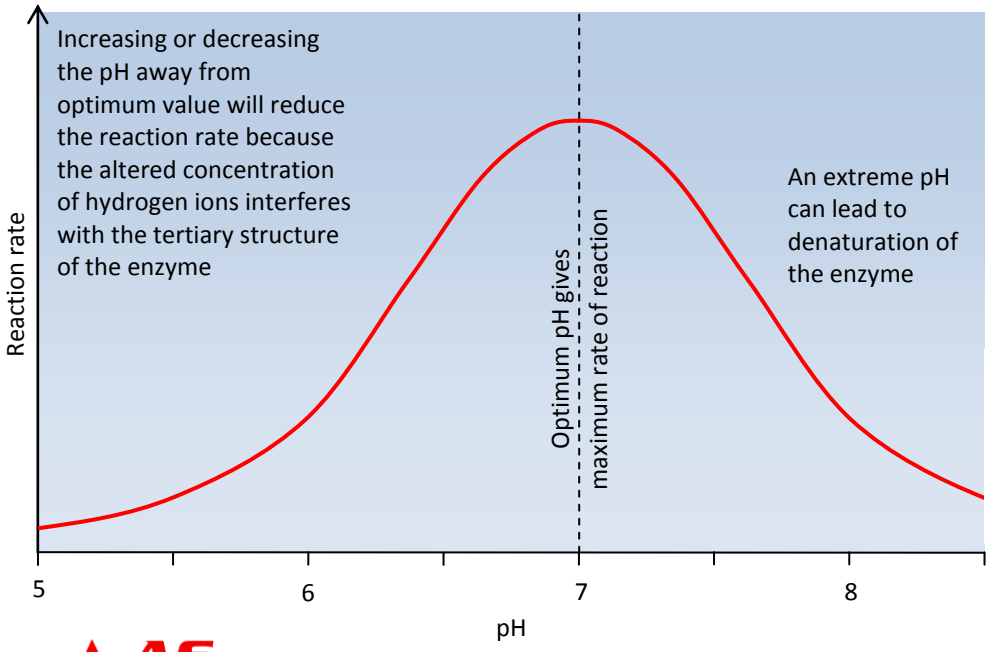


Enzymes and pH

pH is a measure of the concentration of H^+ ions, with a scale of numerical values from 1 to 14, where pH7 is **neutral**, anything below is **acidic**, and anything above is **alkaline** (or **basic**). An acid is known as a **proton donor** (because hydrogen ions are protons) and a base a **proton acceptor**.

Due to the positive charge of the hydrogen ion, it will be attracted to negatively-charged ions, molecules or parts of molecules, and repel positively-charged ions, molecules or parts of molecules. The bonds in enzymes which hold its tertiary structure and determine the shape of the active site are hydrogen and ionic bonds which both form according to *electronegativity*. It is down to the different charges each amino acid groups have in the polypeptide chains.

Obviously, the charge of these hydrogen ions therefore means that they are going to interfere with the bonds holding an enzyme's structure. So increasing or decreasing the pH will affect the concentration of the H^+ ions and in turn, alter the enzyme's tertiary structure. Again, this changes the shape of the active site, and also the rate of reaction.

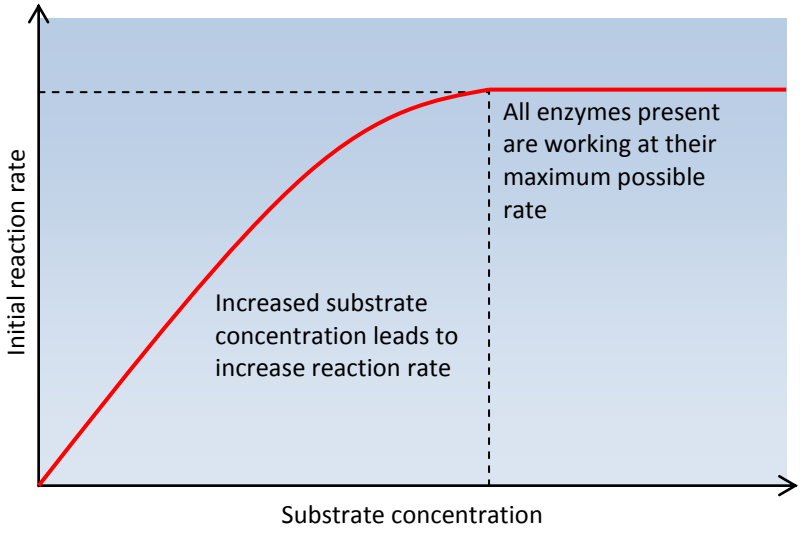


The optimum pH varies between individual types of enzyme. For many of them, however, it around neutral pH – pH7. Because enzymes normally work in a very narrow pH range environment, a sudden drop or increase in pH will cause the rate of reaction to decrease dramatically.

A minor change in the pH will not, however, cause an enzyme to denature. The bonds will still be disrupted, but not always to an extreme effect, they can be reformed. However, extreme pH changes may lead to denaturation.

Enzymes and concentration

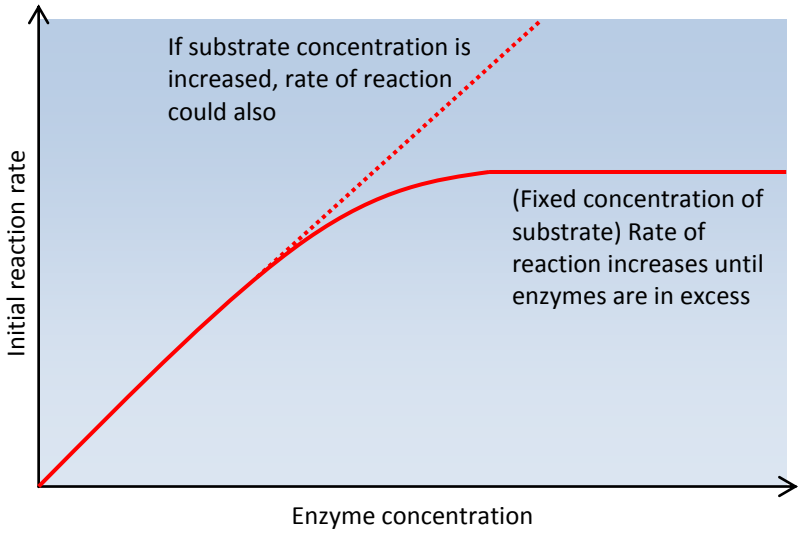
There are two things to consider here: we can change the concentration of the *substrate*, or change the concentration of the *enzymes*. Either way, the reaction rate will change…



▲ Graph to show the relationship between substrate concentration and reaction rate; note that the concentration of enzymes is fixed

First of all, let's consider changing the concentration of the substrate for a *fixed concentration of enzyme* molecules. It goes without saying, if there is no substrate present, the enzyme-substrate complex cannot form – no reaction. So quite obviously, the more substrate there is, the more collisions there will be and so there will be more reactions between the substrate and the active site. But there will be a point at where the rate of reaction reaches its top value (V_{max}). The rate cannot increase any more than this point, because *all* the enzymes present are working at their fastest possible rates, forming enzyme-substrate complexes and releasing product. We say that at this point, the substrate is in **excess**.

We can also examine what happens when the concentration of enzymes changes over a fixed concentration of substrate molecules. A similar relationship would be observed. As the enzyme concentration increases, more active sites become available to form enzyme-substrate complexes with substrates. As more and more of them form, the reaction rate increases. But again, adding more and more enzymes, you will eventually reach a point where all the substrate molecules are occupying enzymes' active sites, so the *enzymes are in excess*.



▲ Graph to show the relationship between enzyme concentration and reaction rate

The graph shows (with the *solid line*) this relationship. The line levels off when the enzymes are in excess, i.e. there are free enzymes laying around with no substrate to use.

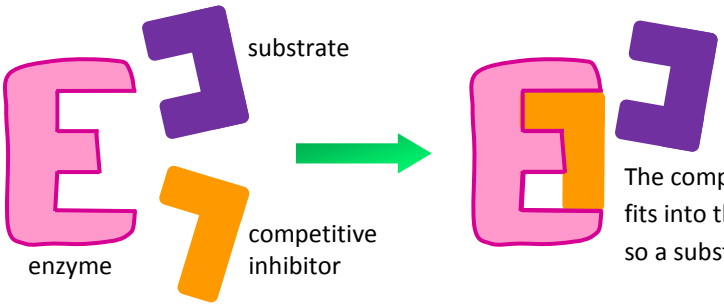
But the V_{max} of this can be increased by altering the substrate concentration. If you increase the number of available substrate molecules, the free enzymes will be able to react with them, therefore the rate of reaction will continue exponentially so long as the appropriate substrate concentration increases alongside it.

Because the concentration of substrates stops the reaction rate increasing in the graph, we call it the **limiting factor**.

Enzymes and inhibitors

There is one final factor to consider. An **inhibitor** is a substance which reduces the rate of an **enzyme-controlled reaction** by affecting the enzymes involved in some way. Some inhibitors change the shape of the active site, whereas others affect other parts of the molecules, which eventually will affect the active site, *inhibiting* its function.

A **competitive inhibitor** is a molecule which has a similar shape to the substrate required by an enzyme. This enables it to fill the active site, forming the **enzyme-inhibitor complex**. However, because the inhibitor is not identical to the substrate the enzyme is used to, no product is formed, and no reaction is induced. Inhibitors reduce reaction rate because when an inhibitor occupies the active site, a substrate cannot – so fewer reactions can happen.

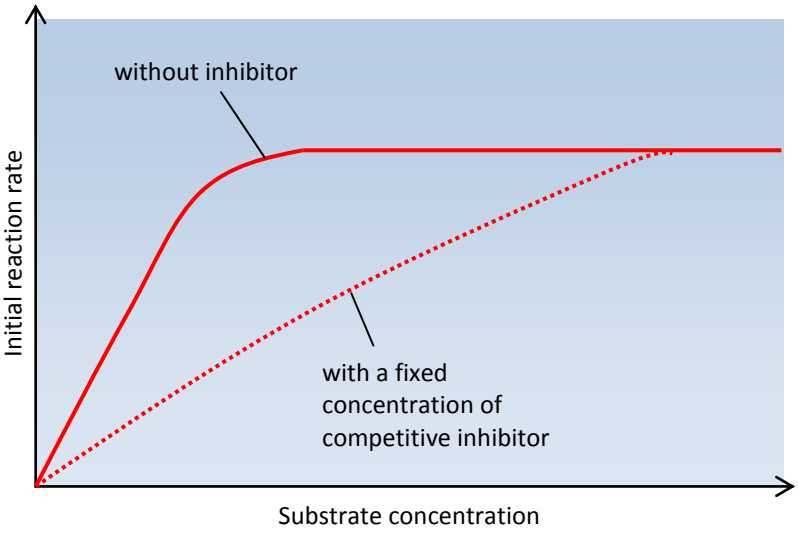


The competitive inhibitor fits into the active site and so a substrate cannot enter

The rate of inhibition depends on the relative concentration of substrate and inhibitor molecules – more inhibitor molecules present mean more collide with the active sites and inhibit substrates from entering them

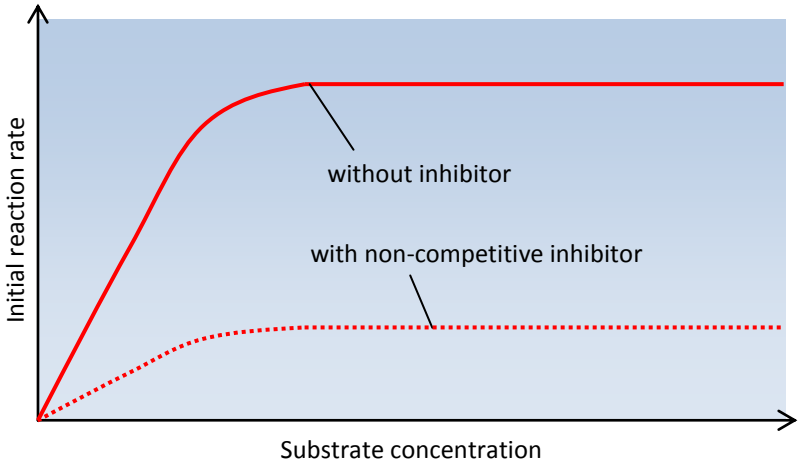
The diagrams show how competitive inhibitors work, and explain how the level of inhibition is caused by the concentration of inhibitor molecules, compared to the concentration of substrate molecules.

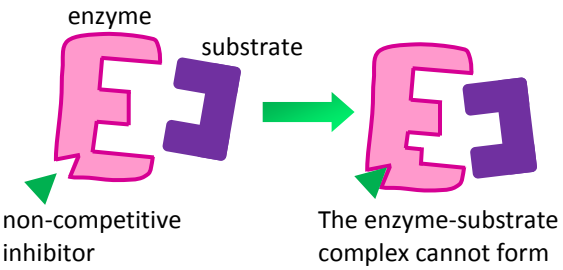
The graph displays the relationship of the competitive inhibitor on the rate of reaction. Increasing the concentration of the substrate would essentially *dilute* the effect of the inhibitor, because it increases the number of collisions between substrate and active site, and decreases the number of collisions between inhibitor and active site.



There is another type of inhibitor, called a **non-competitive inhibitor**. These, as the name suggests, do not compete with substrate molecules for a place in the active site. Instead, they attach to another region.

Non-competitive inhibitors attach themselves to a part of the enzyme away from the active site. This distorts the tertiary structure of the enzyme, and in turn the active site, preventing substrate molecules form being able to enter it. This of course, reduces the rate of reaction.





Most competitive inhibitors do not bind permanently to the active site of an enzyme. They will tend to remain there for a short period and then leave. However, many of the non-competitive inhibitors will bind to an enzyme permanently (**permanent inhibitors**).

If an enzyme is permanently-inhibited by an inhibitor, it is effectively *denatured*.

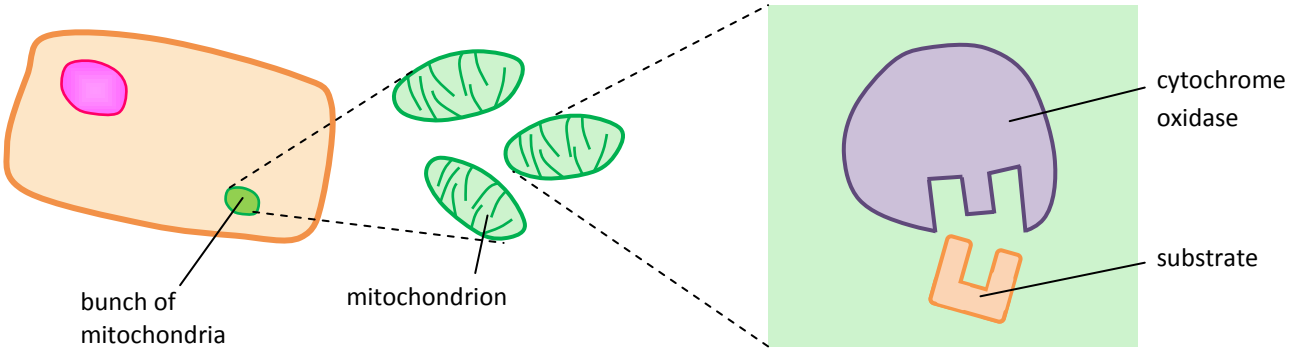
3.10 Poisons Interfering with Enzymes

The effect of a poison or drug inhibiting the function of enzymes

You learned in previous chapter how enzyme **inhibition** worked. Many poisons are in fact, enzyme inhibitors. For your course, you are required to know and understand one type of poison and how it works. You must also know about how medicinal drugs work by inhibiting the actions of certain enzymes.

The vast majority of deadly poisons inhibit the enzymes they encounter, but some of them can also overactivate enzymes. Your course suggests the poison **ethylene glycol**, found in antifreeze. However, the poison studied in detail here is **cyanide**.

Cyanide is a poison which inhibits **respiration**. It is a type of *non-competitive inhibitor*, which means that it binds to an enzyme in a place away from the active site, which causes the active site to alter its shape, ensuring that a substrate can no longer form the enzyme-substrate complex. The enzyme in question is **cytochrome oxidase**. This enzyme is found within the mitochondria of a cell, and when inhibited, oxygen usage decreases, which inhibits the production of **ATP** (*adenosine triphosphate*, the energy medium released in respiration).



Whilst a non-competitive inhibitor, cyanide is not a permanent inhibitor of enzymes. It is reversible. However, time is an important factor. For the average adult human, if even only 100mg of cyanide is absorbed, consciousness can be lost within ten-to-twenty seconds. If untreated, the body will slip into a coma in approximately 45 minutes, and death is inevitable within two-to-three hours. So whilst the action is reversible, and there are treatments available, it is very difficult to treat the problem before the effects are too serious.

The main biological effect of this inhibitor is of course the reduction in the usage of oxygen. This means that the infected cell or cells can no longer respire **aerobically**, but only can respire **anaerobically**. This leads to a build up of **lactic acid** in the blood stream.

Medicinal Drugs and Microorganisms

An **antibiotic** is a type of medicinal drug used to combat bacteria. Antibiotics can kill or inhibit the growth of microorganisms. They're used to treat diseases caused by bacterial infections. One example is **penicillin**. This inhibits a bacterial enzyme which forms cross-links in the bacterial cell wall of some bacteria. This means that the cell walls are not formed, and therefore bacterial production comes to an end.

One common problem in the treatment of bacterial infections is **resistance**. Bacteria can become *resistant* to antibiotics often due to one-off mutations and alterations within their enzymes. Due to natural selection and adaptation laws (see 5.7 Evolution), these will survive and pass on their mutations, making them a more resistant species.

3.11 Coenzymes and Prosthetic Groups

The importance of cofactors and coenzymes in enzyme-controlled reactions

Many enzymes rely on **cofactors** in order to function. These are *non-protein* substances which are present because some enzymes can only catalyse a reaction if they are present. A cofactor ensures that an enzyme-controlled reaction is taking place at an appropriate rate.

Cofactor - a substance which is present to ensure an enzyme-controlled reaction takes place at an appropriate rate

Coenzyme - a molecule which binds to the active site with the substrate and helps the reaction

A **coenzyme** is a non-protein molecule which also binds to the active site of an enzyme for a short period of time, either just before the substrate does or at the same time. A coenzyme has a part in the catalytic reaction, and (like the substrate) is changed in some way – but unlike a substrate, a coenzyme is recycled back to be used again after the reaction.

The role of coenzymes is to carry chemical groups between enzymes so that enzyme-controlled reactions may take place in sequence.

Vitamin B_3

You are required to know about one example of coenzymes in practice for the exam

Vitamin B_3 is used in the process of breaking down fats and carbohydrates to release energy. The vitamin is used to make a coenzyme that is required for **pyruvate dehydrogenase** (an enzyme which catalyses a reaction involved in respiration) to function properly. Normal growth and development cannot proceed without vitamin B_3 and a disease known as **pellagra** forms if there is a deficiency of this vitamin in the diet

A coenzyme which is a permanent part of an enzyme is called a **prosthetic group**. They are vital for the function of an enzyme, as with coenzymes, but a prosthetic group also contributes towards the protein's 3D shape and other properties.

Carbonic anhydrase

You are required to know about one prosthetic group and its enzyme for the exam

The enzyme **carbonic anhydrase** contains a zinc-based prosthetic group. This enzyme is a vital component in red blood cells, where it is involved in catalysing the combination of water and carbon dioxide to give carbonic acid. This is an important reaction that enables carbon dioxide to be transported in the blood

In a few enzyme-controlled reactions, it is the presence of certain **ions** that can increase the reaction rate. Ions may combine with the enzyme or the substrate. The ion binding makes the formation of an enzyme-substrate complex happen more easily, because it can affect the *charge distribution* or the end shape of the complex.

Amylase

You are required to know about one inorganic ion cofactor for the exam

Amylase catalyses the breakdown of maltose molecules. This enzyme will function properly only if **chloride ions** are present. Without the chloride ions, amylase cannot catalyse the reaction

2.12 Investigating Enzymes

Investigating the factors affecting enzymes experimentally

How Science Works

A practical involving investigating the effect of determinants of enzymes is a favourable way of completing your Biology coursework section (the Practical Skills Assessment)

The basics of enzyme action are that enzymes are used to *catalyse* a reaction, where *substrate* is turned into *product*. The substrate occupies the *active site* of an enzyme

A practical involving enzymes will usually ask you to find the relationship between enzyme action (and rate of reaction) versus one of the determinants affecting the reaction rate – these may be temperature, enzyme concentration, substrate concentration, or pH – and you will have to perform an experiment to manually calculate this relationship

It is important that when carrying out the practical, all *other* variables except for the **dependent variable** and the **independent variable** are controlled. The dependent variable (DV) is the variable which is being studied, and the independent variable (IV) is the one which you, as the experimenter, are manipulating. So, for example, in a study to see the effect of temperature on rate of reaction, the DV is rate of reaction, and the IV is temperature, and all other variables (**extraneous variables**, or **control variables**) must be *controlled* – i.e. remain constant.

Below is a table which explains how each type of variable would be kept constant. In your experiment, you will *not* need to control all of these variables, as one will be your IV and another your DV. But the other ones from the table must be.

Variable	Method of control	Reason for method of control
Temperature	Carry out the reactions in a water bath with a thermostat	Room temperature is never constant and is too variable, fluctuations in the temperature will affect your results
Enzyme concentration	Use accurately-measured volumes of enzyme in solution	Reaction rate depends on the concentration of enzyme, so having this kept constant will lead to valid results
Substrate concentration	Use accurately-measured volumes or masses of substrate	Reaction rate depends on the concentration of substrate, so having this kept constant will lead to valid results
pH value	Use a pH buffer (a solution which maintains pH at a set level by keeping the hydrogen ions – proton donors – in the solution at a constant level)	The pH of the solution will have an effect on the active site of an enzyme, and so it is important that this is controlled for accurate results

You may be asked to provide reasons for keeping the above variables constant in your chosen experiment as part of the written accompaniment of the practical exam, so it is important that you understand the biological and scientific reasons behind each of the above methods of controlling the variables.

You will also no doubt need to calculate **reaction rate**. Reaction rate is most often calculated using the formula:

$$\text{reaction rate} = {}^{1}/_{\text{time}}$$

But it can be any variation of that, such as 10/time, or 100/time. Most likely you will be given the formula you're expected to use in the exam paper.

It is also important that you understand the need to carry out **repeats**, in order to obtain *reliable* results, and identify any **anomalous** results.

Q3 Enzymes

Questions on Units 3.8 – 3.12 on Enzymes

1 (a) Explain the difference between the *lock and key theory of enzymes* and the *induced-fit hypothesis*

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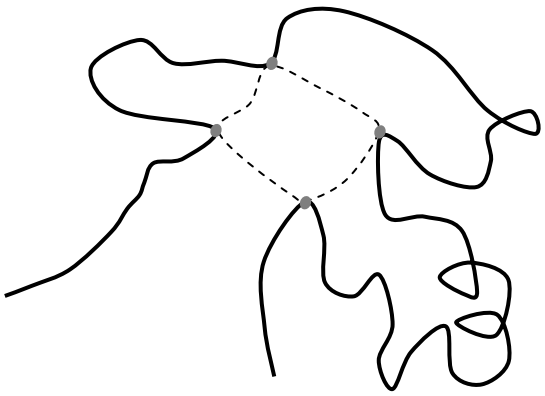
(3 marks)

(b) The diagram shows an enzyme, enzyme **A**.



(i) Annotate the diagram to identify the following:

amino acid, **active site**

(2 marks)

(ii) Enzymes are *globular proteins*. Explain what this means.

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(2 marks)

(iii) What can we say about the shape of the substrate for enzyme **A**?

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(1 mark)

(c) The purpose of enzyme **A** is to catalyse the conversion of starch into maltose. Explain why enzyme **A** will be unable to catalyse the conversion of a protein into amino acids.

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(3 marks)

Total: 11 marks

2 (a) Complete the following passage by filling in the blanks with the correct terminology.

Increasing the temperature condition in a reaction will …… the reaction rate, up to a certain point. If the temperature is too high, usually for most enzymes at …… ^{o}C, the enzyme will lose its …… structure, as the weak hydrogen bonds begin to break, and the enzyme will eventually …… becoming unable to function.

Another factor affecting enzyme action is pH. Similarly to temperature, having a pH that is too low or too high will cause the shape of the …… …… to change, so the enzyme becomes useless. This is because the bonds which hold together the enzyme's structure are mainly hydrogen bonds and …… bonds, which both form due to their electronegativity, and anything too acidic (which has many H^+ ions) will be attracted to the negative areas, and anything too basic (which has little H^+ ions) will be attracted to the positive parts. Generally speaking, the …… pH for enzymes tends to be around pH7.

(7 marks)

(b) The concentration of enzymes and the concentration of substrate are two factors which also affect the rate of enzyme action.

(i) Explain the term substrate.

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(2 marks)

(ii) Describe the relationship between the concentration of enzymes and reaction rate.

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(3 marks)

(iii) With regard to the effect on rate of reaction, explain what would happen in each of the following scenarios:

the substrate in a solution is in excess;

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the enzymes in a solution are in excess;

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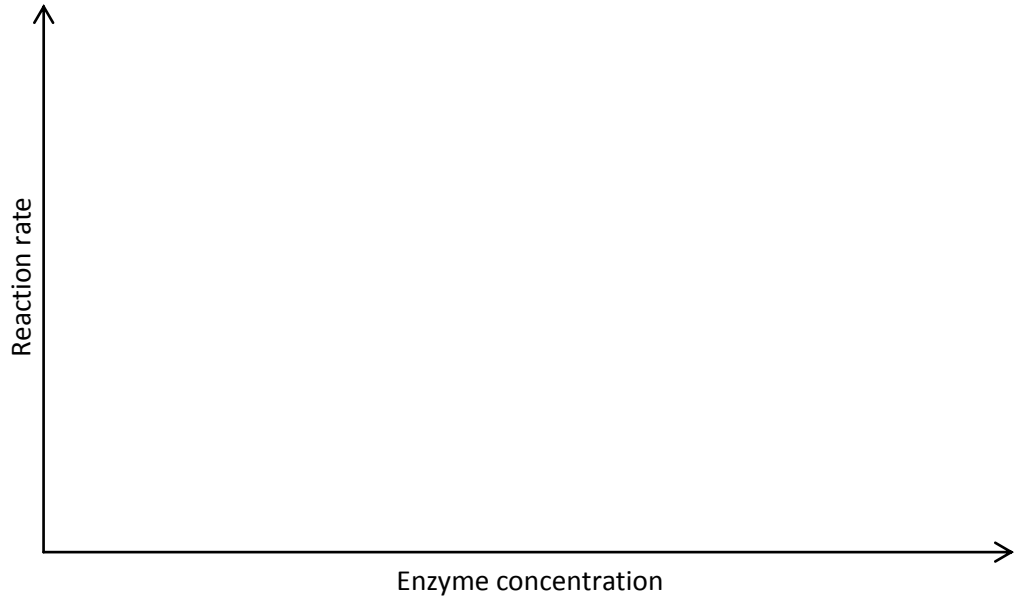
(4 marks)

(d) Below are a pair of axes plotting reaction rate against enzyme concentration.



Use the axes to sketch a line of best fit to show the relationship for the following:

(i) an experiment to measure reaction rate where the amount of substrate is kept constant

(1 mark)

(ii) an experiment to measure reaction rate where the increase in substrate concentration is exponentially growing

(1 mark)

(e) Identify the limiting faction in part (d)(i) …… *(1 mark)*

Total: 19 marks

3 Enzyme *inhibition* can involve a competitive inhibitor or a non-competitive inhibitor.

(a) Explain the process of enzyme inhibition, referring to both competitive and non-competitive inhibitors.

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(3 marks)

(b) Explain the difference between a permanent and a non-permanent inhibitor.

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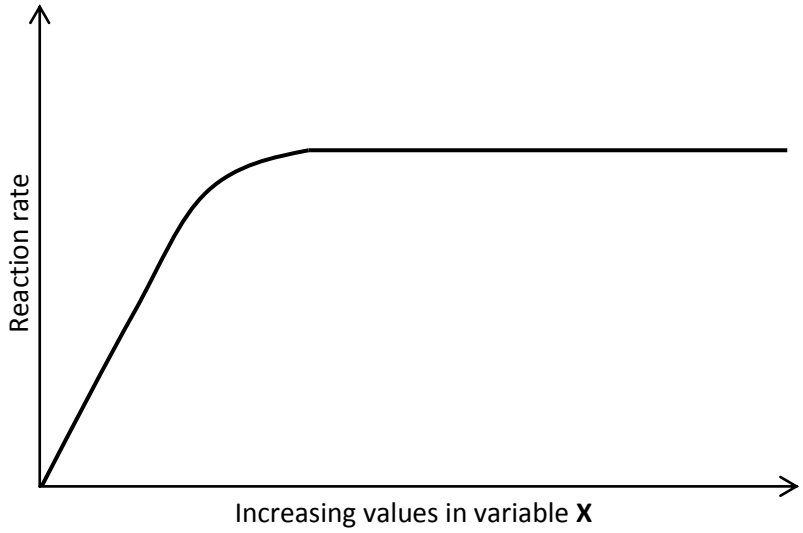
(2 marks)

(c) The graph below shows the reaction rate for an enzyme, affected by variable **X**.



(i) The line shows the effect of variable **X** on reaction rate, *without* an inhibitor.

Plot the line which shows the effect of variable **X** on reaction rate *with* a fixed concentration of competitive inhibitor. Label this line as line **Y**.

(1 mark)

(ii) Plot the line which shows the effect of variable **X** on reaction rate with a fixed concentration of a non-competitive permanent inhibitor. Label this line as line **Z**.

(1 mark)

(iii) Using the line already present on the graph for you, suggest what variable **X** might be.

……

(1 mark)

(iv) Suggest an explanation for your answer to part (iii).

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(2 marks)

(d) For your course you will have studied **one** type of poison.

Use the space to below to explain the effect of one named poison on the human body.

Name of poison: ……

Effect of poison: ……

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(5 marks)

Total: 15 marks

4 Many enzymes rely on *cofactors* to function.

(a) Explain the following terms:

(i) enzyme cofactor;

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(2 marks)

(ii) coenzyme;

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(2 marks)

(iii) prosthetic group.

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(2 marks)

(b) Give an example of where a prosthetic group is necessary for life.

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(2 marks)

(c) In some enzyme-controlled reactions, certain types of ion are required for reactions to take place. One enzyme, carbonic anhydrase, requires one such ion.

(i) State the function of carbonic anhydrase.

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(1 mark)

(ii) State the type of ion which is necessary for carbonic anhydrase to function.

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(1 mark)

(d) A disease known as **pellagra** can develop when there is a deficiency of the vitamin B_3 in the diet.

Explain why pellagra can lead to having respiratory problems.

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(3 marks)

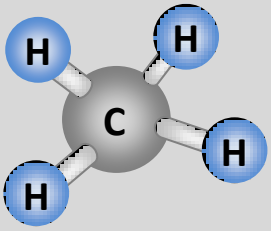
Total: 13 marks

Biological Molecules

3.1 Biochemistry and chemical bonds

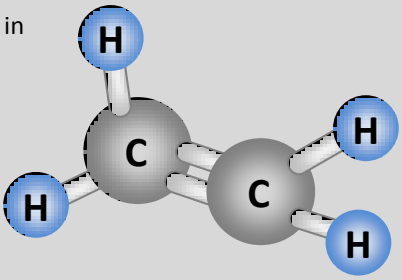
Biochemistry is the study of biology at a molecular level. The emphasis is on the biological significance on chemical molecules. The ones which are studied in this unit are carbohydrates, lipids, proteins, water, nucleic acids and enzymes. The unit is centred around *organic chemistry*, so only molecules which are carbon-based are studied, with the exception of water

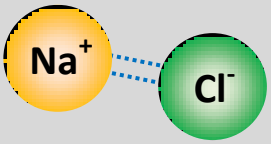
There are four main types of bond which you should know about as basic chemistry knowledge:



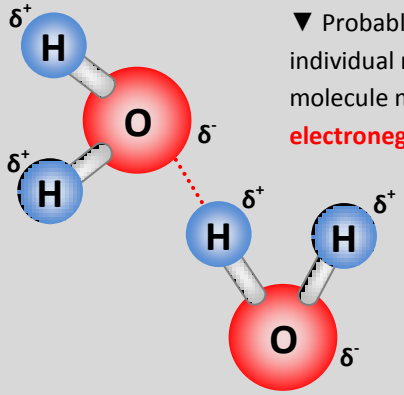
◀ The most stable atoms are those with a completed outer energy level of electrons, and for carbon, as with many other elements, this number is eight. As carbon has four naturally, it can form four **covalent bonds** with other carbon atoms or other atom types

► A **double bond** exists where atoms share multiple electrons in order to stabilise (a double bond is just two covalent bonds)





◀ An **ionic bond** occurs between two oppositely charged ions. This will always take place between one metal ion and one non-metal ion, and rather than the sharing of electrons, these bonds involve the *donation* of electrons to complete the outer energy level and stabilise the atoms

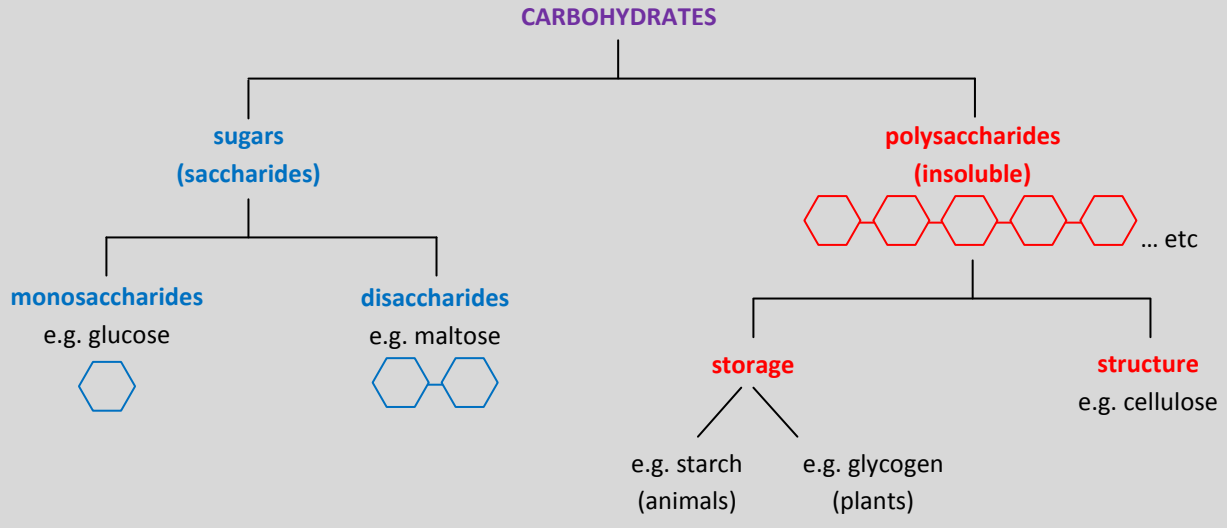


▼ Probably the most important bond in this module, the **hydrogen bond** is used to hold together individual monomers into larger groups (polymers). They form where *slightly* positive parts of a molecule meet *slightly* negative parts of another. We use the Greek letter delta (δ) to denote this **electronegativity**, where δ^+ means slightly positive and δ^- means slightly negative

Hydrogen bonds are extremely weak, often called 'interactions' rather than true bonds, but when thousands of these bonds form in a polymer to hold the structure together, they are strong enough to stabilise a large polymerised structure

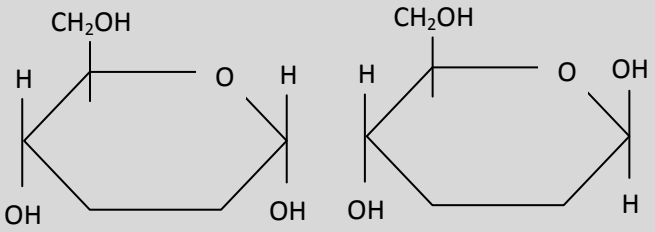
3.2 Carbohydrates

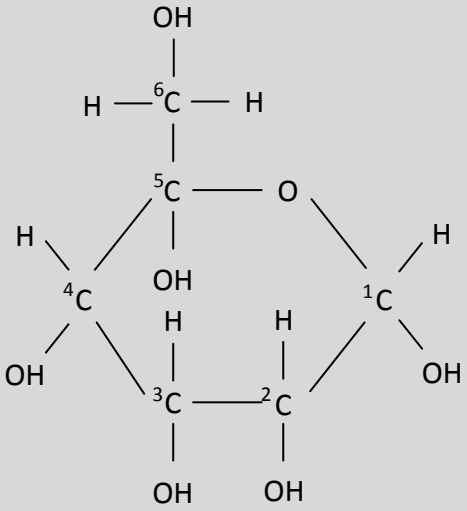
A **carbohydrate** is a biological molecule made of carbon, hydrogen and oxygen atoms. Their primary functions are to be used as an *energy source*, as *energy storage* or to have *structural properties*. The general formula is $C_x(H_2O)_y$



The simplest sugars are the monosaccharides, monomers. The most common is **glucose**. It has the molecular formula $C_6H_{12}O_6$ and is the first product of photosynthesis

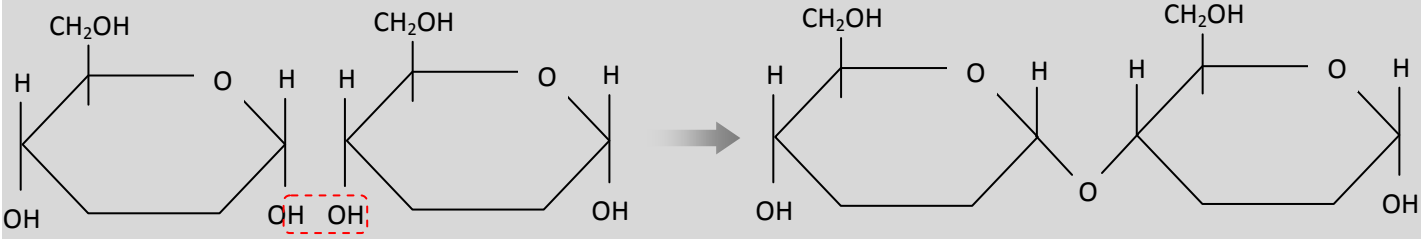
The diagram shows glucose's molecular structure, shown as a *ring structure*. The molecule consists of a ring of five carbons and one oxygen. Carbon atoms can make four bonds, oxygen two bonds and hydrogen only one. Any OH groups are called **hydroxyl groups**. There is also an **alcohol group** attached to the Carbon^5 atom (an alcohol group is a carbon attached to two hydrogens and one hydroxyl group)





The diagrams above show the simplified ways of drawing a glucose molecule. A glucose molecule can come in two forms: **α-glucose** (which has the hydrogen on the top and the hydroxyl group on the bottom of the first carbon) and **β-glucose** which has them the other way around

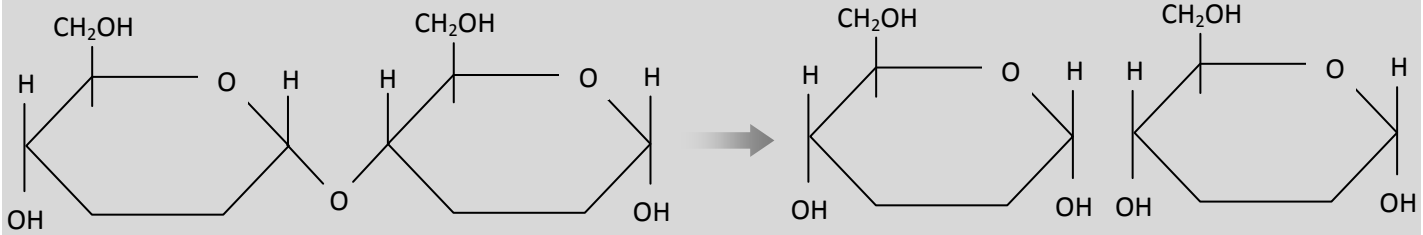
Two monosaccharides polymerise in a **condensation reaction** (one which produces water). Two α-glucose molecules join together to become **maltose**. This bond holding the molecules together is called a **glycosidic bond**



▲ *Formation of maltose from two α-glucose molecules in a condensation reaction*

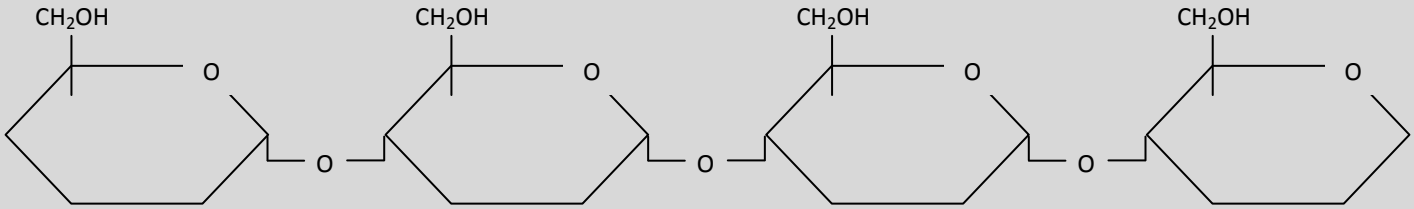
In this reaction, the hydrogen of one hydroxyl group bonds with the hydroxyl group of another α-glucose molecule to produce water, leaving the glycosidic bond (C-O-C) behind

A disaccharide splits in a **hydrolysis reaction** (one which uses adding water to break or split a bond)



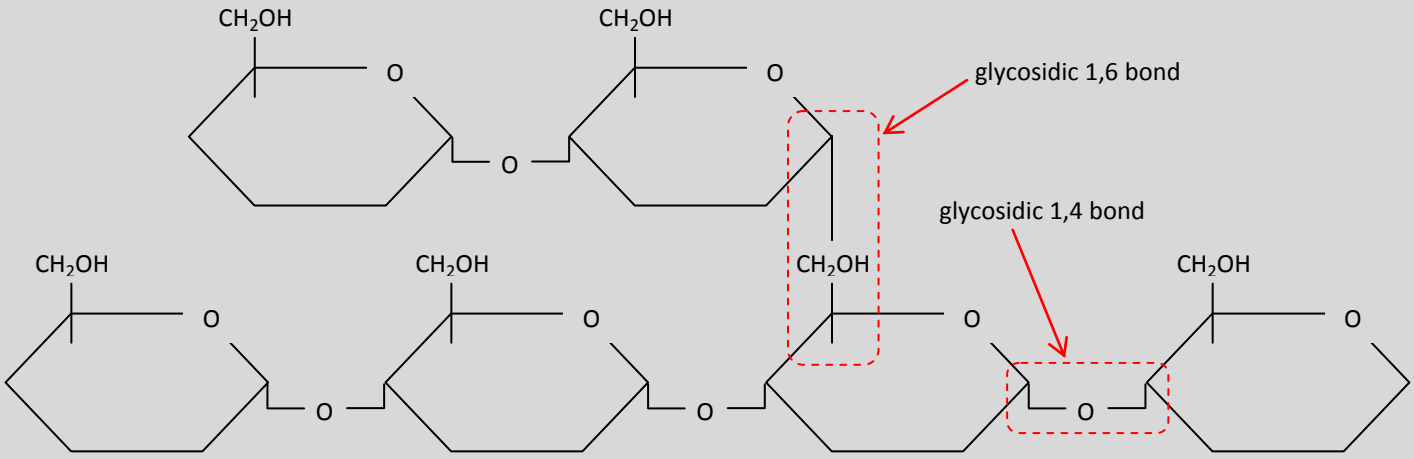
▲ *Hydrolysis reaction showing the breaking of a glycosidic bond to produce two α-glucose molecules*

When more and more α-glucose molecules join together, forming long chains, *polysaccharides* are formed. In plants, the energy store used is **starch**, which is made from two different molecules (both formed originally from glucose). The first molecule type found in starch is **amylose**, a long chain of α-glucose molecules joined by glycosidic 1,4 bonds



▲ *Amylose*

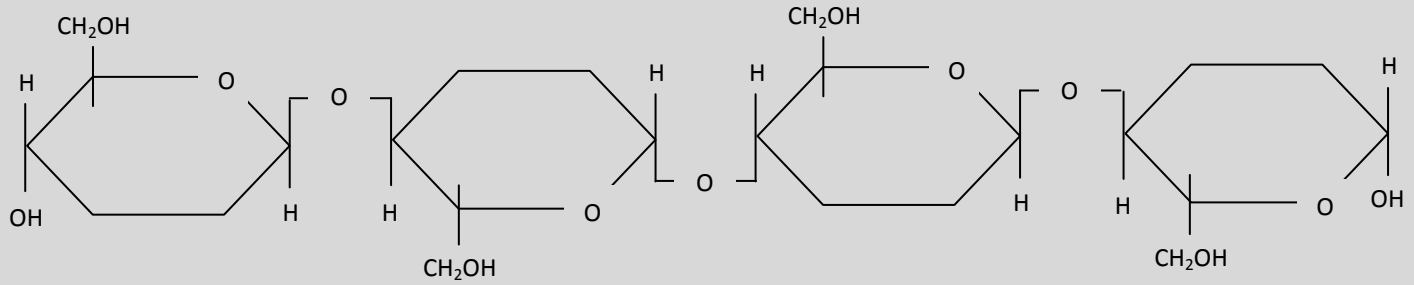
The second type of molecule is **amylopectin**, which structurally is the same as amylose. However, amylopectin does not only form straight chains, it is a *branched* molecule. To be branched, amylopectin forms glycosidic 1,6 bonds, giving it a 3D structure



▲ *Amylopectin*

Starch is a mixture of amylose (20%) and amylopectin (80%). Its purpose is to be an energy store. It can be broken down back into individual glucose monosaccharides and used in respiration which releases energy. However, in animals, starch is not formed – instead they use **glycogen**. Structurally the same as starch, the main difference with glycogen is that the glycosidic 1,4 chains are shorter and there are more 1,6 bonds per chain, making the molecule more compacted and more complex

When β-glucose molecules join together, they do so in a similar fashion to α-glucose molecules. However, they do not form coiled and branched chains, they form long, straight chains. The reaction bringing about the bond remains a condensation one. Every other β-glucose molecule flips 180° to allow the bonding of the hydroxyl groups, so the alcohol group of each alternate molecule is above the ring and for the ones in between it is below the carbon ring



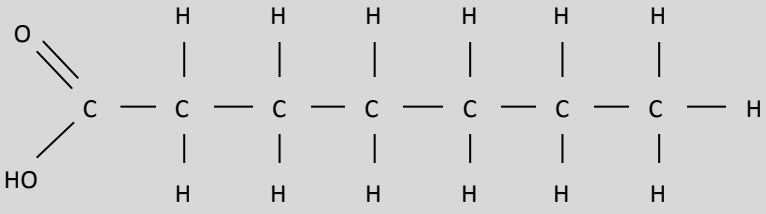
▲ *Cellulose*

These long chains can consist of thousands of β-glucose molecules, and are called **cellulose** chains. Amylose forms in a **helix** shape due to the hydrogen bonds between the molecules along the chain. Cellulose also has these hydrogen bonds, but cellulose remains in one long, straight chain – instead these hydrogen bonds form *microfibrils* which come together to form much larger *macrofibrils*

3.3 Lipids

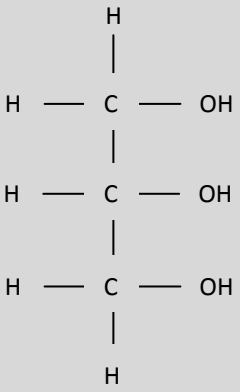
The **lipid** group is a variable group – it contains many different types of molecule with different properties. They contain the same elements (carbon, hydrogen and oxygen) as carbohydrates by don't have a hydrogen:oxygen of 2:1 like carbohydrates, the ratio is much higher. Some lipids may contain other elements too. The most common lipids are fats, oils, waxes, steroid and phospholipids

A **triglyceride** is a lipid that covers fats and oils group. They all contain 6 oxygen atoms, e.g. $C_{54}H_{98}O_6$. One triglyceride molecule contains three **fatty acids** and one **glycerol** molecule



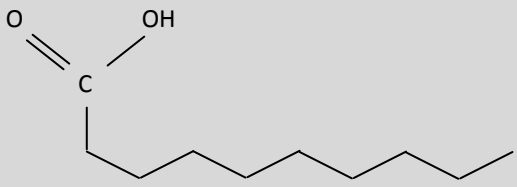
▲ *Fatty acid*

A fatty acid molecule consists of two parts: the **acid group** (COOH) at one end and a **hydrocarbon chain** at the other. A hydrocarbon chain is a long carbon chain with purely hydrogen atoms attached to it. A *saturated* hydrocarbon chain looks like the one to the left. An *unsaturated* one contains C=C double bonds which replace some hydrogens in the chain



▲ *Glycerol molecule*

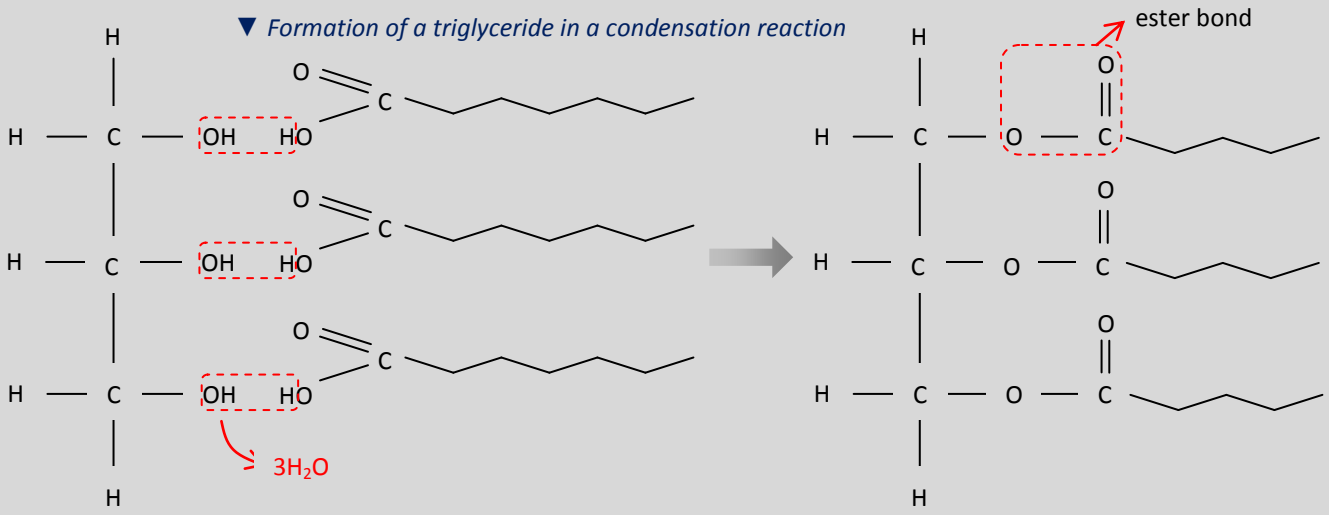
A glycerol molecule contains three alcohol groups and is always the same in any triglyceride. The diagram to the left shows a glycerol molecule, and the diagram on the right shows a simplified fatty acid which may be used for diagrammatic purposes



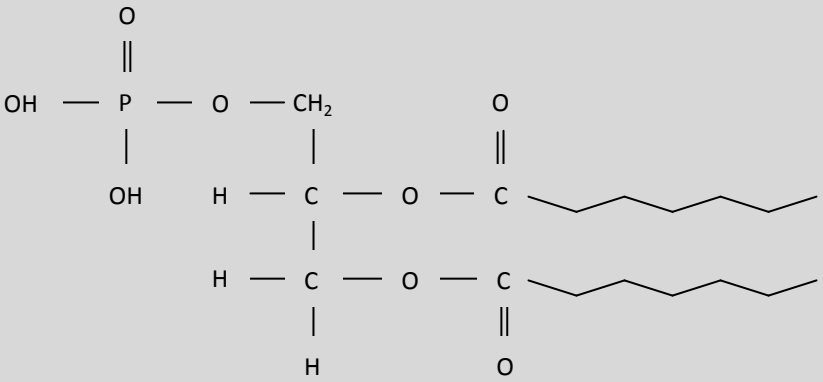
▲ *Fatty acid simplified*

Three fatty acids and one glycerol molecule bond in a *condensation reaction*. Three molecules of water are released, and the resultant bond holding the molecule together is a covalent O-C=O bond, called an **ester bond**. This attaches one fatty acid to each carbon atom of the glycerol molecule

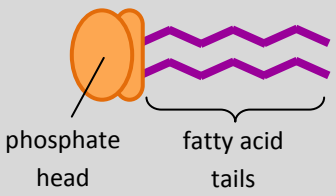
▼ *Formation of a triglyceride in a condensation reaction*



Phospholipids form the basis of all biological membranes. They are structurally similar to triglycerides in that they consist of a glycerol molecule and have fatty acids bonded to their carbon atoms, except there are only two fatty acids, the third carbon is occupied by a *phosphate group*. Again, these bonds are formed in condensation reactions



▼ *Simplified version of a phospholipid*

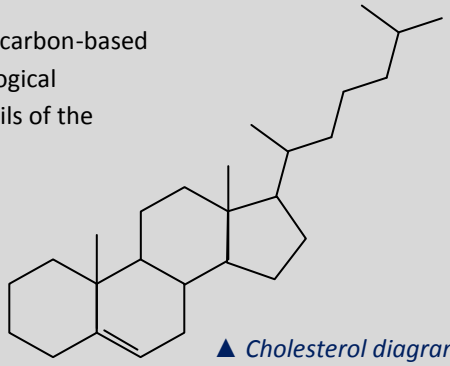


◄ *Chemical structure of a phospholipid*

The phosphate heads of phospholipids are very hydrophilic (water-loving) and the fatty acid tails are hydrophobic (water hating). The majority of the molecule is insoluble in water (as with most lipids). These characteristics are what allows phospholipids to form membranes

Cholesterol is a type of lipid formed not from fatty acids and glycerol, but from four carbon-based rings. It is a very small, hydrophobic structure. This molecule can be found in all biological membranes, and these characteristics allow it to fit in nicely in between fatty acid tails of the individual phospholipids in a bilayer

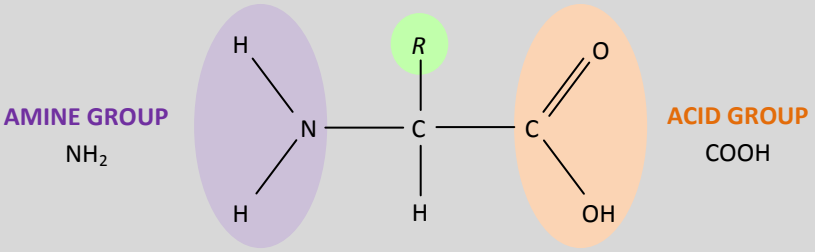
This molecule is vital to all living organisms, so many cells are able to make it, especially in the liver. However, excess cholesterol can cause health problems. An example is familial hypercholesterolemia, a genetic disorder whereby cells make and secrete cholesterol even though there are sufficient amounts of it in the blood already



▲ *Cholesterol diagram*

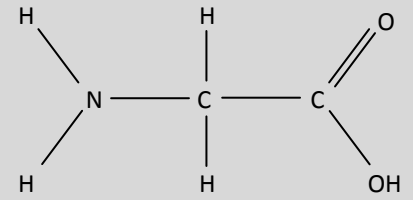
3.4 Amino acids and proteins

A **protein** (which is a polymer) is made up of many **amino acids** (individual monomers). Amino acids contain the elements carbon, hydrogen, oxygen and nitrogen, and some also contain sulphur

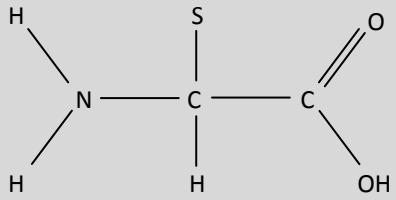


An amino acid consists of an **amine group** is basic (or alkaline) and has the formula NH_2 and the **carboxylic acid group** (COOH) is acidic, as it releases hydrogen ions into solution. These groups are separated by another carbon atom, which is also bonded to a hydrogen atom and a **variable group** (*R*)

There are 20 options as to what the *R* group can be, as there are 20 different natural amino acids. The amino acids shown below are **glycine** (the simplest amino acid) on the left, and **cysteine** (the simplest amino acid containing sulphur)



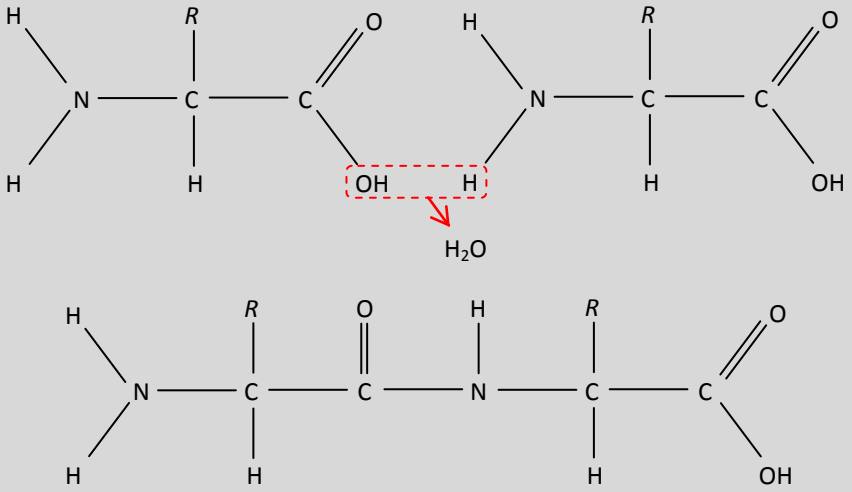
▲ *Glycine*



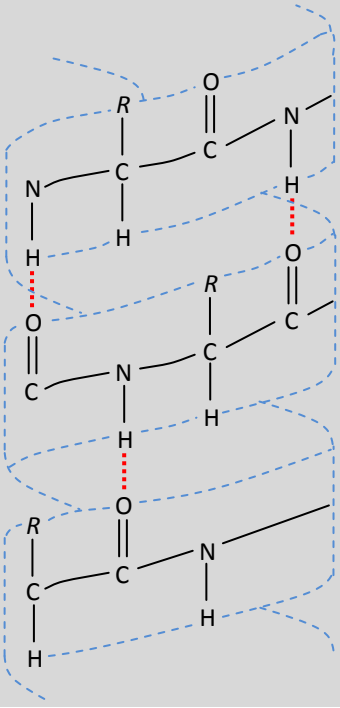
▲ *Cysteine*

Amino acids join end-to-end to form long chains in a condensation reaction. The bonds formed are nice and strong covalent bonds which we call **peptide bonds**. The diagram below shows how amino acids join together in a condensation reaction and break apart in a hydrolysis reaction

The molecule formed from two amino acids joining together is called a *dipeptide* which can become a **polypeptide** when multiple amino acids join together. Not all polypeptides are proteins, because although any chain of amino acids consisting of more than two amino acids is a *polypeptide*, a polypeptide is only a *protein* when that chain has a distinct biological function. A protein can be made from one or more polypeptides. It can be made from as little as five amino acids to as many as hundreds of them



▲ *Formation of a dipeptide from two amino acids*

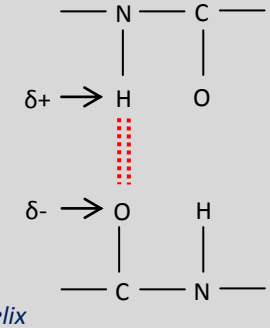


All proteins have a **primary structure**. This is the unique sequence of amino acids which makes the protein, and this will determine its main function

The **secondary structure** of a protein is its formation as a 3D structure. All proteins have a primary and secondary structure. Secondary structures arise where the polypeptide chain(s) coil or fold. The most common secondary structure for a protein is the **alpha helix** (α-helix) which forms as chains coil. Hydrogen bonds keep the helix structure, even though they are weak, because they are so abundant

The left diagram shows the α-helix and the right diagram shows how the hydrogen bonds exist. The bonds occur between various polarised parts of the molecules, so the slightly positively charged hydrogen atom from the nitrogen of one amino acid will be attracted to the slightly negatively charged oxygen atom from the carbon of a nearby amino acid

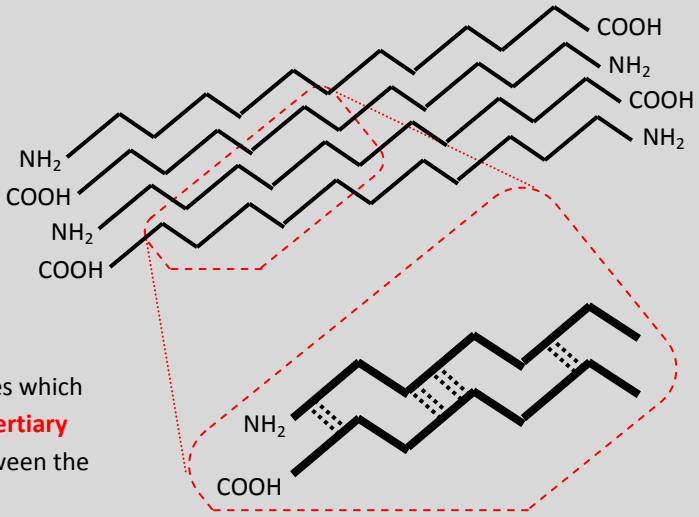
◄ *α-helix*



► *Hydrogen bonds in an α-helix*

The other type of secondary structure, although less common, is the **beta pleated sheet**. A *pleat* is an angular fold within the polypeptide chain. A *beta pleat* (β-pleat) is the simple structure formed by multiple polypeptides joining together side-by-side in a pleated chain. The individual pleats associate with each other to form a very tall but very thin 2D structure

The polypeptide chains which form a beta pleated sheet do so instead of a helix because they do not have the amino acid coding necessary which those chains which form a helix have



▲ *Beta pleated sheet*

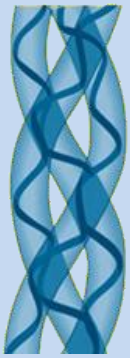
An α-helix can wrap itself into a 3D complex shape. Polypeptides which do this form a **globular protein**. Not all proteins will have this **tertiary structure**. Their shape is maintained by four types of bond between the variable *R* groups of different amino acids:

- hydrogen bonds between the polar groups
- ionic bonds of oppositely-charged *R* groups
- **disulphide bridges** – a very strong covalent bond between two sulphur atoms from different cysteine amino acids
- **hydrophobic bonding** – non-polar bonding between similar hydrophobic *R* groups coming together to exclude water

A protein has a **quaternary structure** when it *polymerises*. This occurs when more than one globular protein joins together. They do so using exactly the same four bonds as one protein uses to globule, and the bonding still occurs between the *R* groups of various amino acids on the outside of the globular proteins

A **fibrous protein** is one which forms fibres when it becomes three-dimensional, not globules. These are formed from regular and repetitive sequences of amino acids, and are normally insoluble in water, whereas globular proteins tend to be soluble

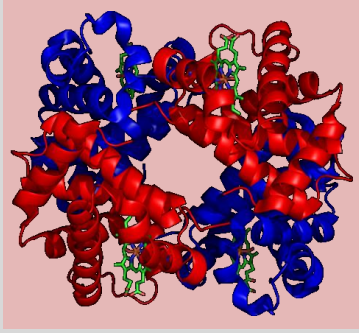
COLLAGEN



Collagen is a fibrous protein found in skin, bones, cartilage, tendons, teeth and the walls of blood vessels. It is an important **structural protein** found in most animals. Collagen consists of three polypeptide chains, each in the shape of a helix. The three helices wind around each other to form a *rope*. Almost every third amino acid in each chain is *glycine*. The small size of glycine allows the three strands to lie close together and form a tight coil. The strands are held together by *hydrogen bonds*. *R* groups of individual collagen molecules form bonds with other collagen molecules

These cross-links form **fibrils**. Many *microfibrils* bond together to form larger *macrofibrils*. These associate together to form much bigger bundles called **fibres**. Collagen, a fibrous protein, has a tremendous amount of **tensile strength**, i.e. can withstand a high pulling pressure

HAEMOGLOBIN



A haemoglobin molecule is made of four polypeptide chains. Each chain is wrapped around a group of atoms, called a **haem group** which holds an **iron Fe^{2+} ion** in the centre, as shown in the diagram. Each iron ion is able to bond with two oxygen atoms (one oxygen molecule), so the haemoglobin molecule as a whole can carry up to eight oxygen atoms (or four molecules of oxygen)

The usual bonds are responsible for giving the haemoglobin molecule its quaternary structure: hydrogen bonds, hydrophobic bonds, ionic bonds and disulphide bridges. The molecule consists of two α-chains and two β-chains

3.5 Testing for biochemical substances

Testing for starch: Add iodine solution to the sample. If starch is present, the solution will turn from a yellow-orange colour to a dark blue-purple colour

Testing for reducing sugars: A **reducing sugar** is a monosaccharide or a disaccharide. When a reducing sugar is heated with *Benedict's solution* (alkaline copper sulphate) the solution will change from blue to an orange red (**Benedict's test**)

Testing for non-reducing sugars: Used when the reducing sugar test is negative (no colour change). Boil the sample with hydrochloric acid, which hydrolyses any sucrose present and breaks it down into glucose and fructose. Cool it down and add sodium carbonate solution (an alkaline solution) to neutralise it. Repeat Benedict's test on the solution

The non-reducing sugars test works because sucrose is a non-reducing sugar, and so if there is a positive result on this test, we know that there *was* sucrose in the original solution because it had to have been broken down into glucose and fructose (both reducing sugars) to give the positive result

Benedict's test is used in both the reducing and non-reducing sugar test. The result is positive if there is a colour change and negative if not. The colour scale below is used as a "results range" for the test which is used to describe the amount of reducing sugar in a sample based on the strength of the colour and the colour change:

(nothing) blue → green → yellow → orange → red (lots)

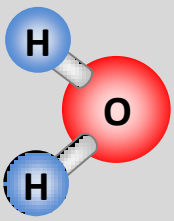
We call these tests semi-qualitative because they don't produce quantifiable results. We can use quantitative tests by using the following options:

- *Benedict's solution* – using Benedict's test reveals the presence of reducing sugars, resulting in an orange-red precipitate and the more reducing sugar there is the more copper sulphate (Benedict's solution) will have been used up, so the precipitate can be filtered and the concentration of the remaining solution measured telling us how much Benedict's solution has been used up allowing an estimate of the concentration of reducing sugar in the original sample
- *colorimeter* – a device which shines a beam of light through a sample calculating percentage light transmission; the sample is placed into a *cuvette* which goes into the colorimeter, and then a *photoelectric cell* picks up on the amount of light transmitted, and the reading gives a measure of the amount of reducing sugar, based on the principle that the more copper sulphate that has been used up the less light will be blocked out
- *calibration plotting* – taking a range of known concentrations of reducing sugar and using the colorimeter test to calculate readings, then plotting those results on a graph and using the calibration curve to take a precise measurement of unknown concentrations in solutions

Testing for lipids: Done using the **ethanol emulsion test**. Mix the sample with ethanol, which dissolves any lipids (lipids are soluble in alcohols). Pour the mixture into another test tube of water. If there is lipid present, a cloudy white *emulsion* forms at the top of the tube

Testing for proteins: Uses the **biuret test**. Add *biuret reagent*, which is blue in colour (containing sodium hydroxide and copper sulphate), to the sample, and if proteins are present this will react with the peptide bonds turning the solution to lilac colour

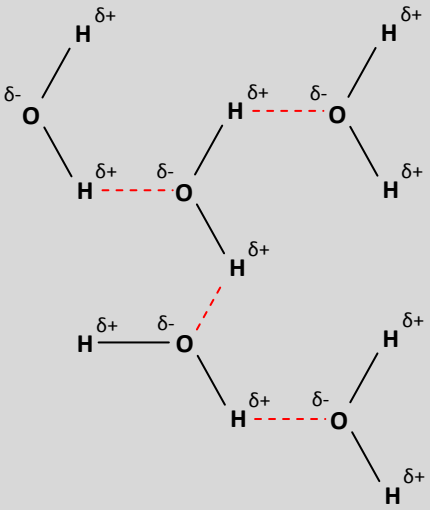
3.6 Water



One molecule of water consists of one oxygen atom covalently bonded with two hydrogen atoms. However, the electrons involved in these covalent bonds are not shared perfectly evenly, the oxygen acts more 'dominantly' and is capable of pulling the shared electrons closer to itself and away from the hydrogen atoms. The result of this is that the hydrogen atoms become slightly positive and the oxygen atom becomes slightly negative. It is this electronegativity that gives water its special properties which make it such an important substance. The electronegativity means that water is described as a **polar** molecule

It is because many thousands of hydrogen bonds exist between water molecules that water behaves as it does at room temperature. Its liquidity comes from the constant making and breaking of these bonds. The network the bonds make allow the molecules to slide over each other, making it difficult for water molecules to escape as a gas – which is why it has to be heated to 100^{o}C to make it boil

At lower temperatures, water has less *kinetic energy* and so the molecules move less readily. Hydrogen bonds form but don't break as frequently (as bond-breaking requires energy). When water solidifies, becoming *ice*, it is the hydrogen bonds which hold it in its semi-crystalline state



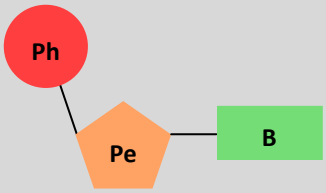
◀ *Hydrogen bonding in water*

The **solubility** of a substance in water is dependent upon whether or not water molecules can interact with the substance. *Any* molecule which is polar (as water is) will dissolve in water. This is because the **solute** (substance being dissolved) has slightly positive and slightly negative parts which can interact with water, as water molecules cluster around the charged parts of the solute molecules, which separates those molecules out – dissolving the substance

Water has many various other properties. One is called **cohesion**, which can be shown if you place a drop of water onto a waxy surface, such as the cuticle of a leaf. It forms a spherical perfect drop. This is because the hydrogen bonds pull water molecules in at the surface.

3.7 Nucleic acids

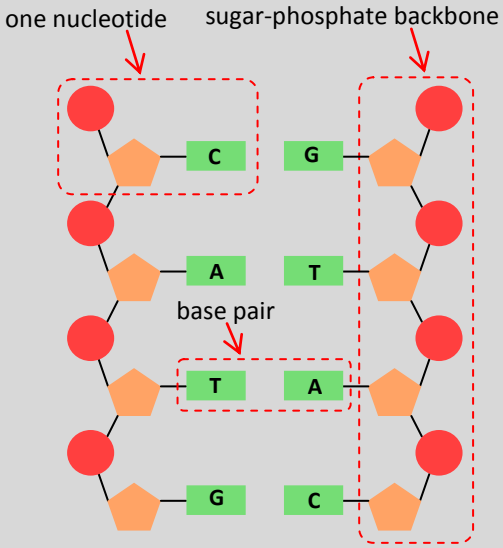
A **nucleic acid** comes in two different forms: as **DNA** and as **RNA**. They are both *macromolecules* formed by the individual monomers called **nucleotides**. A single nucleotide is made of three components



The *Ph* subunit represents a **phosphate groups**. The *Pe* subunit is the **pentose** sugar (named so because it is a five-carbon ring sugar). The pentose sugar will be either **deoxyribose** (in DNA) or **ribose** (in RNA). The *B* subunit represents the **nitrogenous base** – an organic sub-molecule, of which there are five possible options as to what it could be in a nucleotide: **adenine** (A), **cytosine** (C), **guanine** (G), **thymine** (T) or **uracil** (U).

It is in a condensation reaction that the phosphate group of one nucleotide joins to the sugar of another, which forms long chains of nucleotides, called a **sugar-phosphate backbone**. The nitrogenous bases stick into the middle of the backbone. In DNA (deoxyribose nucleic acid), two sugar-phosphate backbones run alongside each other in an *antiparallel* fashion, and the bases form hydrogen bonds with each other in twos from the equidistant backbones. These are called **base pairs** and always pair up C-G and A-T (in DNA) or A-U (in RNA)

The diagram on the right shows part of a DNA nucleic acid. This is made of a double strand (**double helix**). Hydrogen bonds hold together the antiparallel backbones via the base pairs. DNA contains only the bases adenine, cytosine, guanine and thymine. The bases adenine and guanine are described as **purines**. Cytosine, thymine and uracil are all **pyrimidines**. The pentose sugar in DNA is deoxyribose

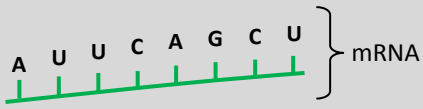


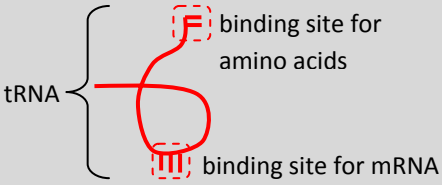
RNA (ribose nucleic acid) differs from DNA in three main ways:

- the pentose sugar is *ribose*, not deoxyribose
- the nitrogenous base *uracil* is found instead of thymine
- the molecule is single-stranded, not double-stranded

In RNA, adenine bonds with uracil, but cytosine still bonds with guanine. RNA is involved in protein synthesis. This involves the production of proteins. RNA comes in three different forms, each of which have a role to play in the production of proteins

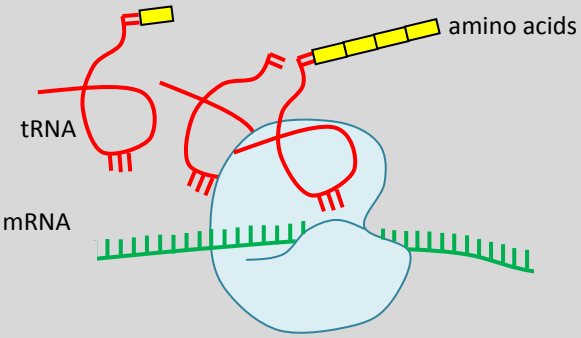
Because DNA is too big to escape from the nucleus through nuclear pores, a new single strand is developed inside the nucleus which can fit out. The DNA double-strand is unzipped (i.e. split down the middle), and then used as a *template strand*. A strand of **messenger RNA** (mRNA) is produced using the template strand as a template. Complementary nucleotides line up alongside the template strand and form a complementary sequence to the existing DNA so that the mRNA leaving the nucleus can provide an exact copy of the DNA sequence. This process of making the mRNA strand from the DNA strand is called **transcription** and the mRNA can leave the nucleus through the nuclear pores





An mRNA strand is *a copy of one gene*. It is delivered to ribosomes, which are made from **ribosomal RNA** (rRNA), where the proteins are synthesised

The third type is called **transfer RNA** (tRNA). There are two binding sites on a single molecule of tRNA, one for amino acids and one for the mRNA strand. Its role is to bring amino acids into the ribosome in the correct sequence to be synthesised into proteins. The mRNA binding site allows the tRNA to attach itself to the mRNA to obtain that coding. The sequence of amino acids on the mRNA strand is therefore called the **mRNA codon** and the sequence used by the ribosome to synthesise proteins is called the **tRNA anticodon**

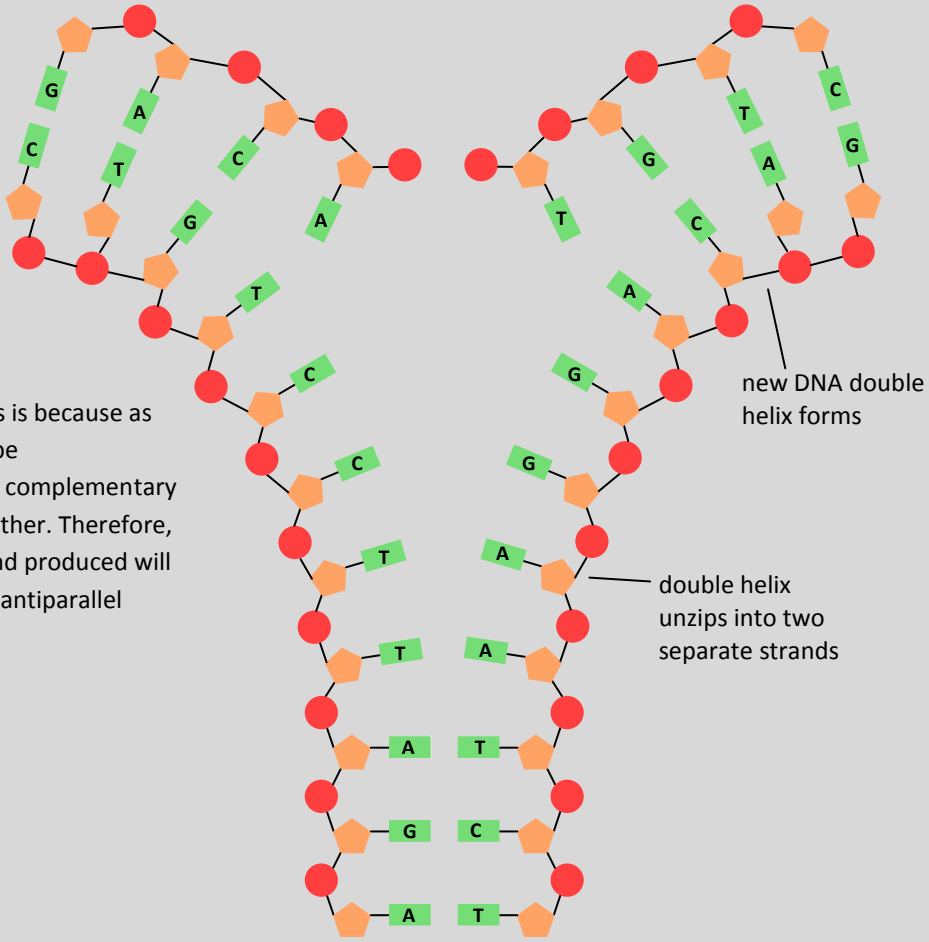


The shape of a tRNA molecule is often described as a *hairpin loop*. The staged diagram here shows the mRNA entering the ribosome and the tRNA binding to it. The tRNA molecule has brought the correct sequence of amino acids with it into the ribosome so that the protein can be synthesised: this stage is called **translation**

When DNA replicates, it begins with one DNA nucleic acid forming two new ones. This is called **semi-conservative replication**

This process involves a double-stranded molecule of DNA "unzipping" to become two single and separate strands. Free nucleotides then join to the bare bases on each single strand of the unzipped molecule. Each of these new nucleotides for a new strand alongside each existing strand to form two new DNA double helices

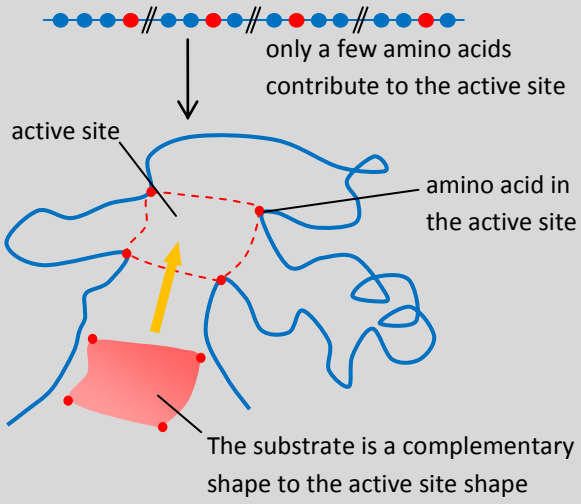
Each new DNA strand will be *identical*. This is because as the original nucleic acid unzips, there will be complementary bases on each strand, and complementary bases to those bases will bond with each other. Therefore, as is shown in the diagram, each new strand produced will be structurally identical, although running antiparallel



3.8 Enzyme action

An **enzyme** is a globular protein with a specific tertiary structure. It is a **biological catalyst** – a substance which speeds up a chemical reaction but does not get used up in the process. An enzyme is a large molecule, and its primary, secondary and tertiary structures denote its overall shape and function. These structures also determine its specific shape of the "pocket" or cleft area where the catalytic activity takes place – and this groove or cleft in the enzyme is called the **active site**

There may be thousands of amino acids in a single protein, but only a few of them contribute towards the shape of the active site, maybe no more than ten. Only one type of **substrate** can fit into the specific active site of an enzyme, so each enzyme can catalyse a reaction for only one substrate type. The substrate of an enzyme is shaped in a way which is *complementary* to the shape of the active site

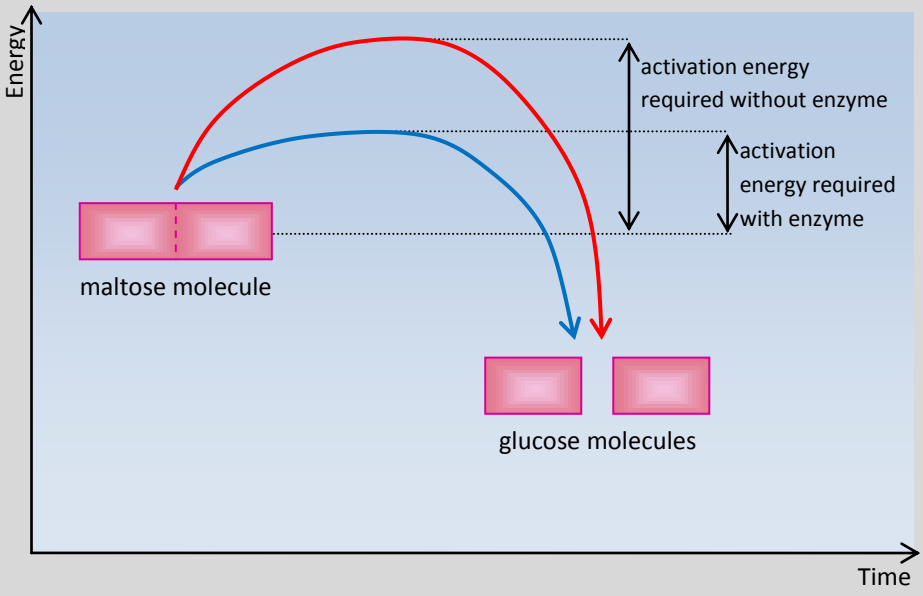


The **lock and key theory** shows how the substrate ('key') will fit one active site ('lock') only, and when the key is inside the lock, the reaction can take place and be catalysed

The **induced-fit hypothesis** is a more recent theory suggesting that in order for a specific substrate to be able to occupy the active site of its enzyme, the enzyme alters shape slightly to allow entry of the substrate and so it can hold it in place whilst the reaction takes place

When a substrate occupies the active site, an **enzyme-substrate complex** is formed. When this happens, the reaction takes place. The enzyme catalyses the reaction, and **products** are released at the end

An example of an enzyme is *lipase*. This has the job of breaking down one maltose molecule into two glucose molecules. To do this, the glycosidic bond holding the two glucose molecules together must be broken. This can be done in a *hydrolysis* reaction, but this requires energy. The energy to initiate that reaction is called **activation energy**. In this example, the energy can be obtained from boiling the maltose in hydrochloric acid to supply energy

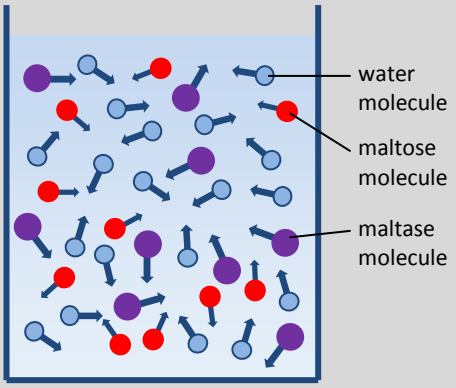


Boiling in hydrochloric acid provides sufficient energy, but this would never happen naturally. A *catalyst* can be used to drive metabolic reactions. They reduce the activation energy required. Enzymes are catalyst proteins which can do this, so reactions can take place in more diverse conditions, not just extremes such as boiling in acid. Without enzymes, essential-to-life metabolic reactions would not be able to take place. It is important to note that enzymes do not get used up in the reactions, but their effectiveness will deteriorate over time as they get used in reactions more and more

3.9 Factors affecting enzymes

Enzymes and temperature: If we look at the beaker below, we can see that the molecules continually move about bumping into each other because they have *kinetic energy*. This movement is called *Brownian motion*

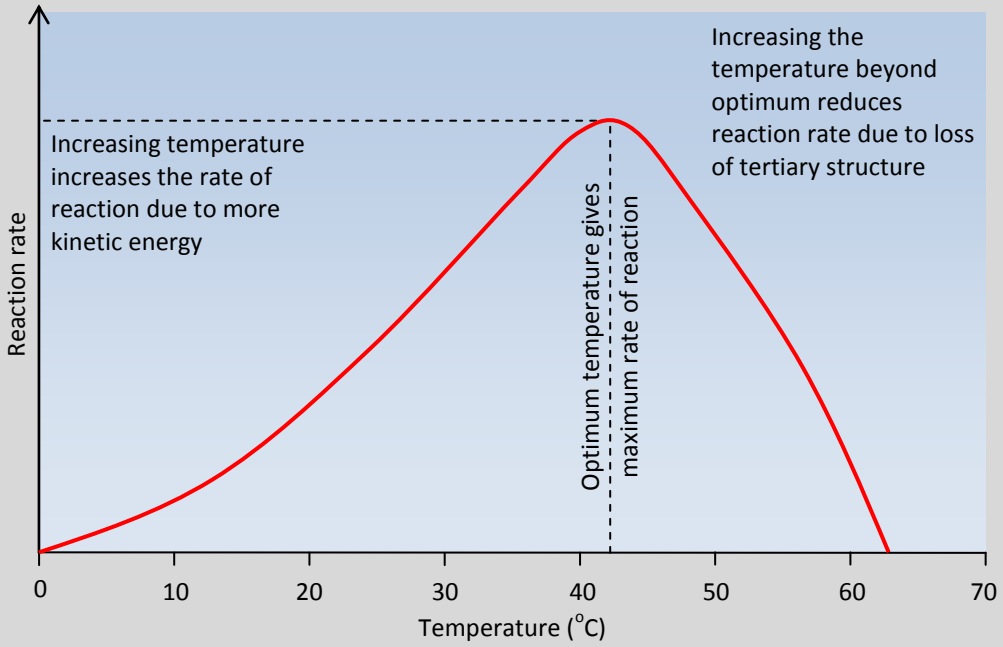
Without the enzyme present in the solution, there would be very few reactions taking place. But having the maltase molecules increases reaction rate because it means that whenever a maltose molecule and a water molecule collide with a maltase molecule, the hydrolysis reaction will be catalysed and take place



Surely therefore, increasing temperature increases this amount of kinetic energy so it will increase the number of collisions and therefore, reaction rate? Not quite, up to a certain point, yes it will, but after a certain temperature (usually around 40^{p}C) the bonds holding the tertiary structure of the enzymes together are broken and so the enzyme **denatures** (the shape of the active site changes and becomes useless)

We call the temperature where the highest rate of reaction is yielded the **optimum temperature**. It is important to note that temperatures which are too high only denatures the tertiary and secondary structures of the enzyme protein, the primary structure remains unaffected

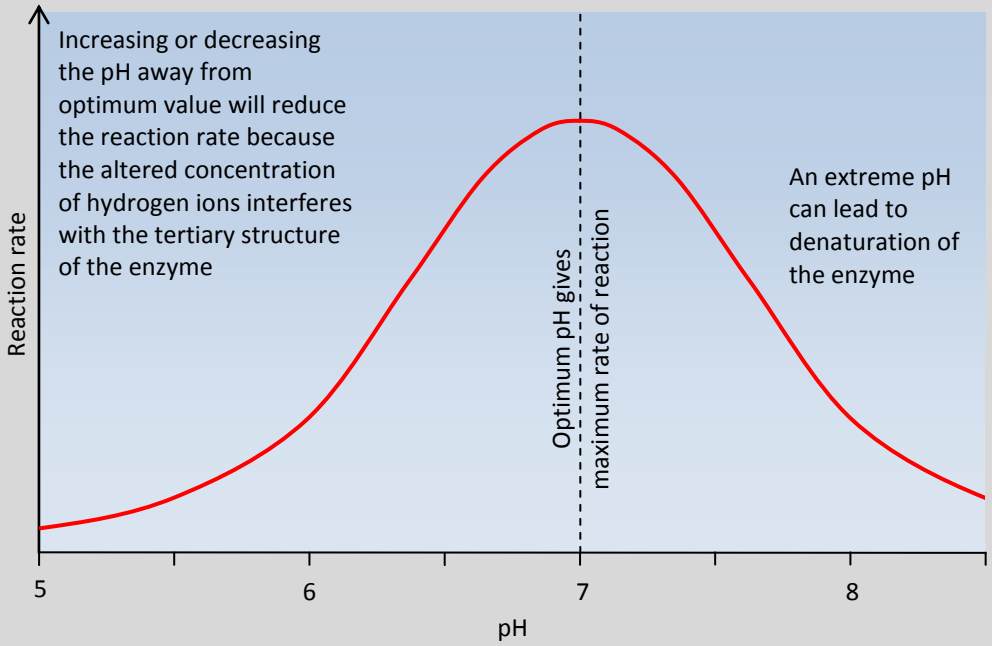
Enzymes tend to have fairly high *heat-resistance* so that they can operate in a diverse range of conditions, which is why optimum temperature is usually somewhere between 40 – 50^{p}C so they can survive fairly high temperatures

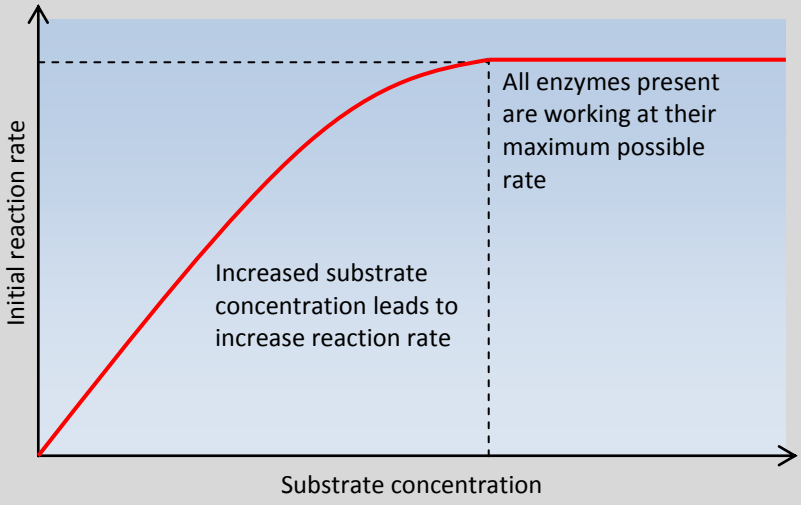


Enzymes and pH: pH is a measure of the concentration of H^+ ions (protons) – anything acidic is said to be a *proton donor* and anything basic – alkaline – is said to be a *proton acceptor*. Due to the positive charge of these ions, they are attracted to negatively charged parts of molecules and repel positively charged parts. The major bonds holding an enzyme's tertiary structure in place are hydrogen and ionic, which form due to *electronegativity*, so these ions interfere with this structure

Increasing/decreasing pH affects the concentration of hydrogen ions in a solution, so the larger the alteration in pH the more the enzymes' tertiary structures are affected

The optimum pH for enzymes varies, but for many of them it is pH7 (neutral). Most enzymes work in a fairly narrow range of pH values, so for most enzymes a sudden drop or increase in pH can cause the shape of the active site, and the whole enzyme, to completely change, rendering the enzyme useless – so this obviously *decreases* reaction rate





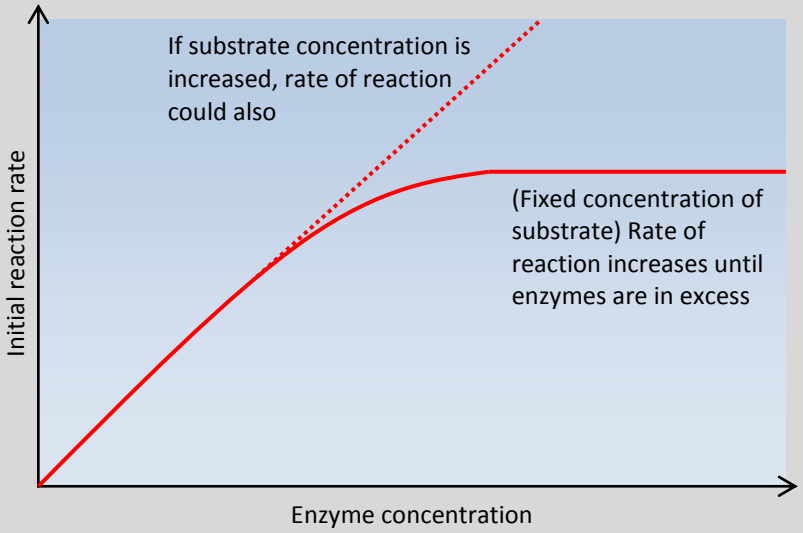
Enzymes and substrate concentration: Obviously, if there is a fixed concentration of enzymes, increasing substrate concentration will increase reaction rate. If there is no substrate present, there will be no reactions. The more substrates there are, the better the reaction rate – but only up to a certain point. This relationship is only true whilst enzymes are in *excess* (i.e. more active sites available than substrates). But when all the active sites that can be occupied are being occupied, no matter how much more substrate you introduce to the solution, the reaction rate cannot increase, so the rate levels off at a certain point

This point where the reaction rate reaches its top value is called **V_{max}** and this can only be increased by increasing the number of enzymes in the solution, otherwise there just aren't enough active sites available for the substrates to all be active

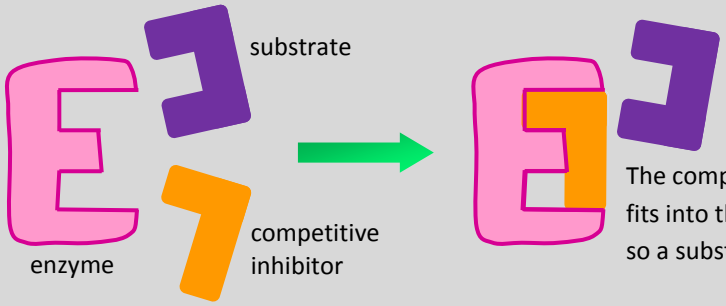
Enzymes and enzyme concentration: When there is a fixed concentration of substrate molecules, again the relationship is immediately obvious: more enzymes mean more reactions are possible and more likely as collisions will be more frequent

Similarly, the V_{max} can be increased by increasing the substrate concentration so that there aren't just enzymes waiting around idly. The rate of increase in reaction will continue to grow exponentially so long as there are sufficient substrate concentrations to match the level of enzyme concentration

We call the factor which stops the increase in reaction rate a **limiting factor** (so for enzyme concentration, substrate concentration can be a limiting factor)



Enzymes and inhibition: An **inhibitor** is a substance which reduces the rate of an enzyme-controlled reaction. A **competitive inhibitor** is a molecule which shares a similar shape to the substrate for a particular enzyme and so can occupy the active site to form the **enzyme-inhibitor complex**. But because the inhibitor is not identical to the substrate, no reaction takes place and no products are released

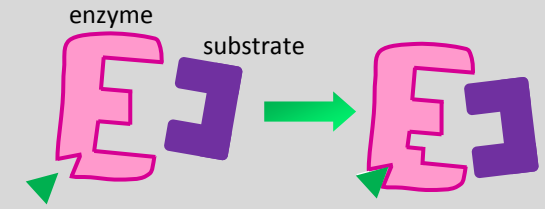


The competitive inhibitor fits into the active site and so a substrate cannot enter

The rate of inhibition depends on the relative concentration of substrate and inhibitor molecules – more inhibitor molecules present mean more collide with the active sites and inhibit substrates from entering them

▲ *Competitive inhibition*

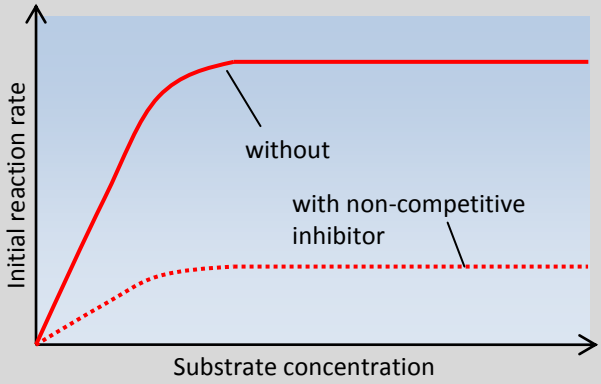
A **non-competitive inhibitor** does not compete with substrate for the active site of an enzyme. Instead, it attaches itself to another region of the enzyme

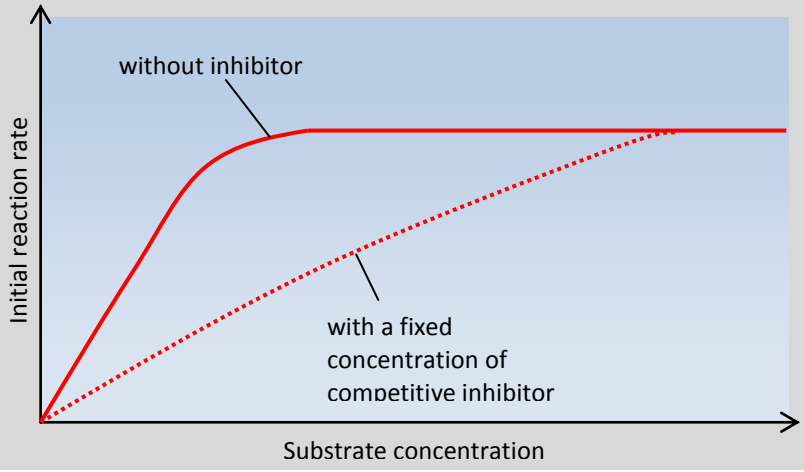


▲ *Non-competitive inhibition*

Non-competitive inhibitors attach themselves to a part away from the active site which distorts the tertiary structure, and in turn the active site, which prevents the substrate from being able to form the enzyme-substrate complex

Inhibitors can be **permanent inhibitors** or temporary inhibitors. One which permanently inhibits an enzyme effectively *denatures* it





3.10 Poisons interfering with enzymes

Many poisons are in fact enzyme inhibitors. Note: For your course you must know of *one named poison* and explain how it functions. The course recommends the poison *ethylene glycol*, but this guide has chosen to look at **cyanide**

Cyanide is a poison which interferes with respiration. It is a non-competitive inhibitor which binds to the enzyme (away from the active site) and alters the shape of the active site so the enzyme-substrate can no longer be formed. The enzyme it does this to is **cytochrome oxidase**. This enzyme is found abundantly inside mitochondria in a cell. It isn't a permanent inhibitor, but the strength of it can be deadly. 100mg of cyanide causes unconsciousness in about ten seconds. If untreated, the patient slips into a coma within 45 minutes and death within three hours. Cyanide decreases the usage of oxygen, so affected cells can no longer respire aerobically, they can only respire anaerobically which leads to a build up of lactic acid in the bloodstream

An **antibiotic** is a medicinal drug used to combat bacteria. They kill, or inhibit the growth of, bacteria. One example is *penicillin* which inhibits the bacterial enzyme that forms cross-links in the bacterium wall. This means that cell walls are not formed properly and so bacterial production comes to an end as the cells cannot survive

3.11 Coenzymes and prosthetic groups

Many enzymes rely on **cofactors** to operate – non-protein substances which must be present for the enzyme to catalyse a reaction and ensure the reactions are happening at an appropriate rate. In some enzymes, it is an inorganic *ion* cofactor which is used. This means a certain ion must be present for the reaction to take place

e.g. the enzyme lipase can only catalyse the breakdown of maltose into glucose if *chloride ions* are present

A **coenzyme** is a non-protein molecule which binds to the active site either just before or alongside the substrate. The coenzyme plays some part in the reaction, and helps to produce the products, but does not get used up in the reaction, it is recycled and released back out to be used again in the same reaction using a different substrate

e.g. vitamin B_3 is used in the process of breaking down fats and carbohydrates to release energy, and also to make the coenzyme *pyruvate dehydrogenase* (enzyme used in respiration), and a deficiency of the vitamin means normal growth and development cannot happen, and so a disease known as *pellagra* develops

A coenzyme which is a permanent part of an enzyme is known as a **prosthetic group**. These serve the same purpose as normal coenzymes, to help catalyse reactions, but also contribute towards the enzyme's overall 3D structure and other properties

e.g. the enzyme carbonic anhydrase is involved in the removal of carbon dioxide from red blood cells, and without it carbon dioxide would not be able to travel in the blood, and the enzyme relies on a zinc-based prosthetic group