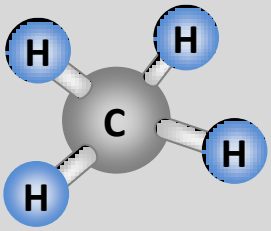
Biological Molecules

3.1 Biochemistry and chemical bonds

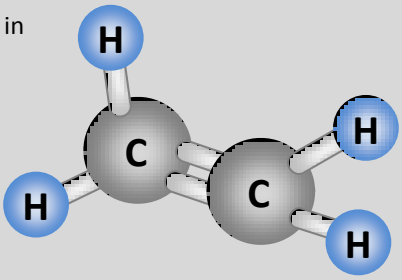
Biochemistry is the study of biology at a molecular level. The emphasis is on the biological significance on chemical molecules. The ones which are studied in this unit are carbohydrates, lipids, proteins, water, nucleic acids and enzymes. The unit is centred around *organic chemistry*, so only molecules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

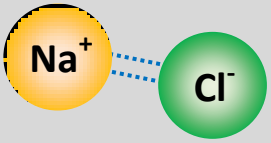
There are four main types of bond which you should know about as basic chemistry knowledge:



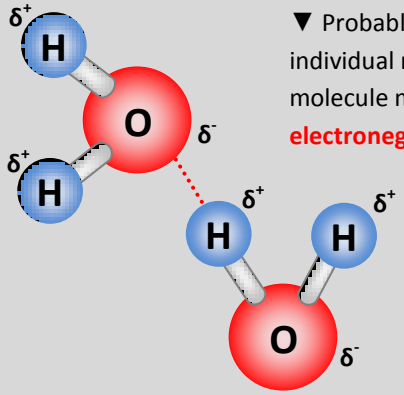
◄ The most stable atoms are those with a completed outer energy level of electrons, and for carbon, as with many other elements, this number is eight. As carbon has four naturally, it can form four **covalent bonds** with other carbon atoms or other atom types

► 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

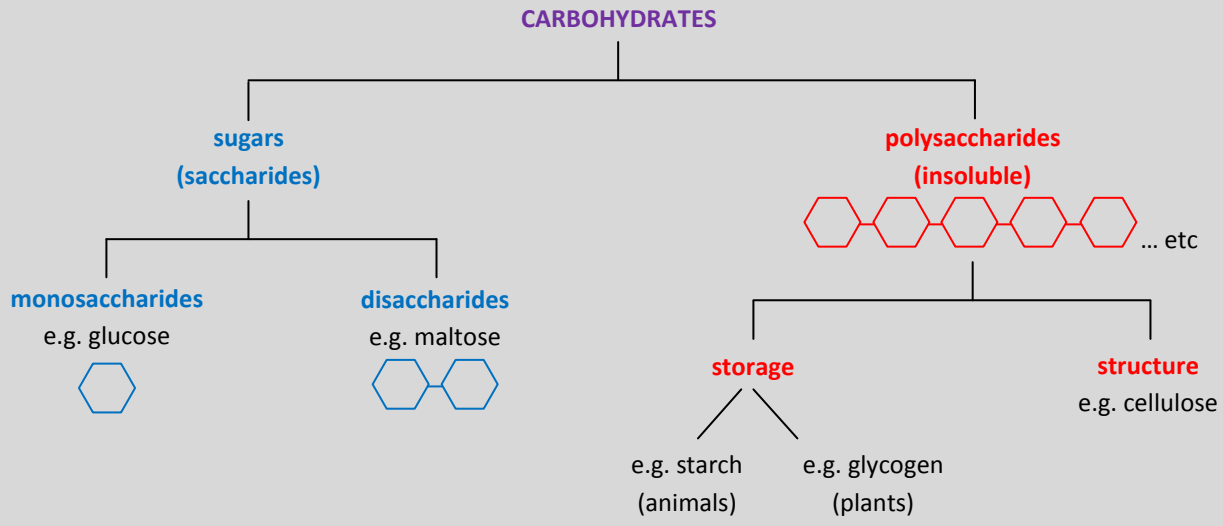


▼ Probably the most important bond in this module, the **hydrogen bond** is used to hold together individual monomers into larger groups (polymers). They form where *slightly* positive parts of a molecule meet *slightly* negative parts of another. We use the Greek letter delta (δ) to denote this **electronegativity**,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

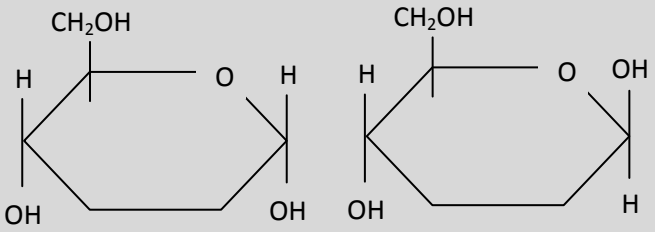
3.2 Carbohydrates

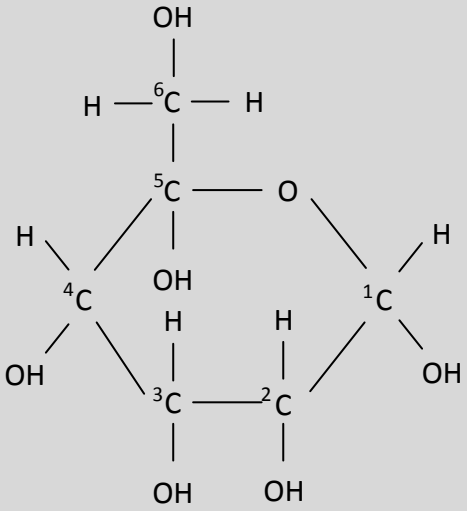
A **carbohydrate** is a biological molecule made of carbon, hydrogen and oxygen atoms. Their primary functions are to be used as an *energy source*, as *energy storage* or to have *structural properties*. The general formula is $C_x(H_2O)_y$



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

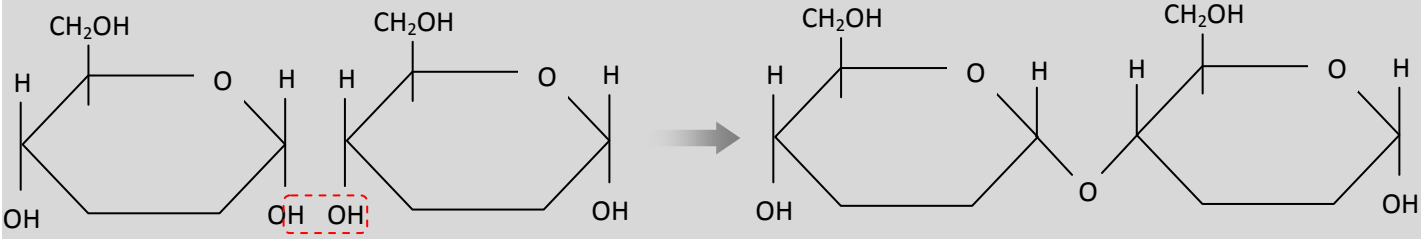
The diagram shows glucose's molecular structure, shown as a *ring structure*. The molecule consists of a ring of five carbons and one oxygen. Carbon atoms can make four bonds, oxygen two bonds and hydrogen only one. Any OH groups are called **hydroxyl groups**. There is also an **alcohol group** attached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

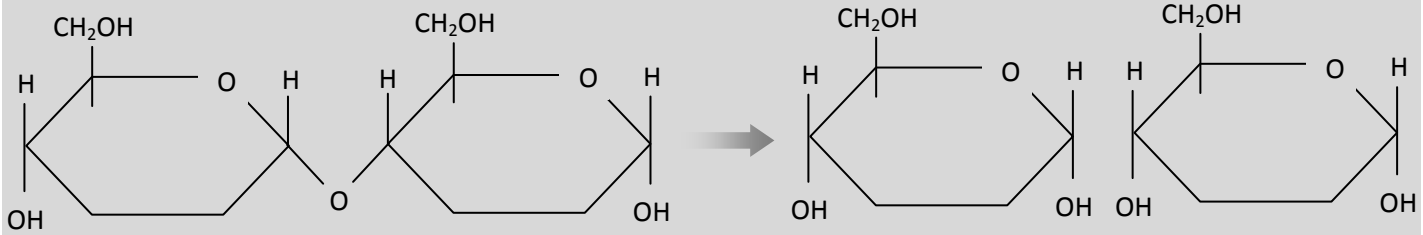
Two monosaccharides polymerise in a **condensation reaction** (one which produces water). Two α-glucose molecules join together to become **maltose**. This bond holding the molecules together is called a **glycosidic bond**



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

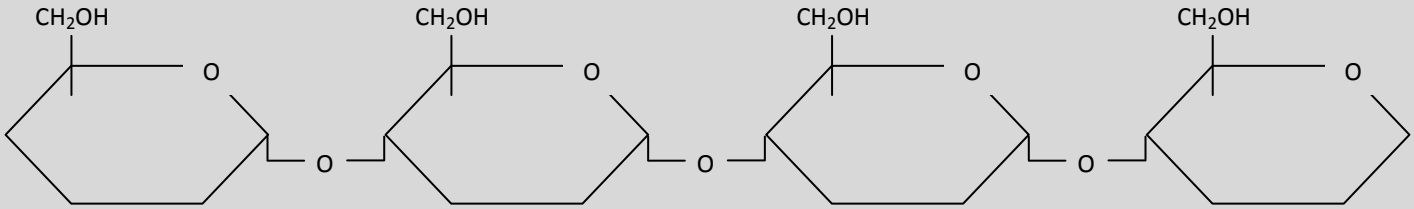
In this reaction, the hydrogen of one hydroxyl group bonds with the hydroxyl group of another α-glucose molecule to produce water, leaving the glycosidic bond (C-O-C) behind

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



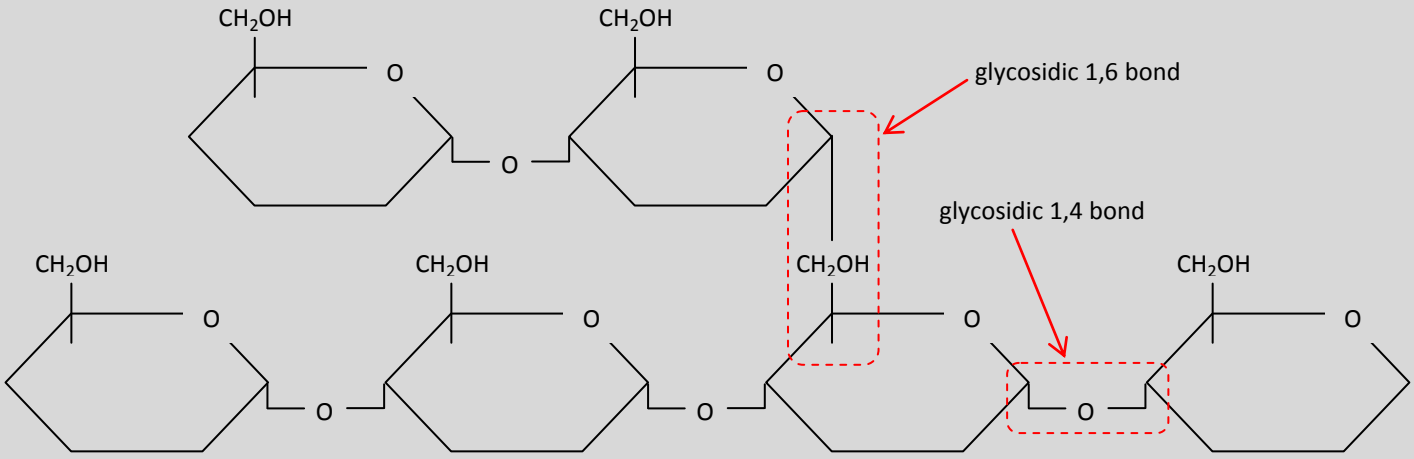
▲ *Hydrolysis reaction showing the breaking of a glycosidic bond to produce two α-glucose molecules*

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*

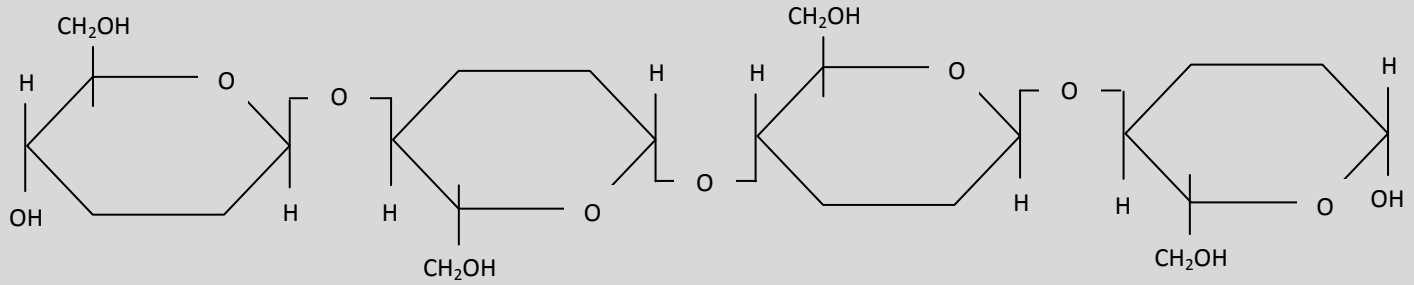
The second type of molecule is **amylopectin**, which structurally is the same as amylose. However, amylopectin does not only form straight chains, it is a *branched* molecule. To be branched, amylopectin forms glycosidic 1,6 bonds, giving it a 3D structure



▲ *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



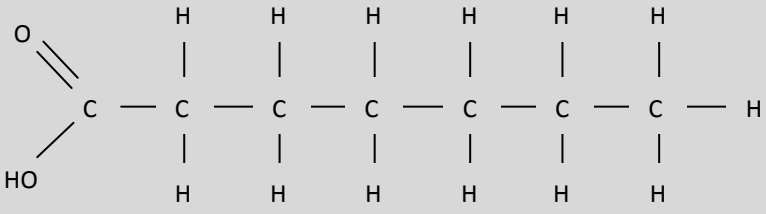
▲ *Cellulose*

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

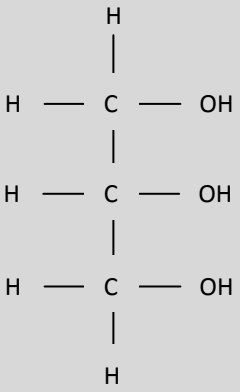
The **lipid** group is a variable group – it contains many different types of molecule with different properties. They contain the same elements (carbon, hydrogen and oxygen) as carbohydrates by don't have a hydrogen:oxygen of 2:1 like carbohydrates, the ratio is much higher. Some lipids may contain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



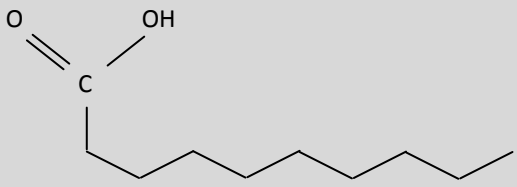
▲ *Fatty acid*

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*

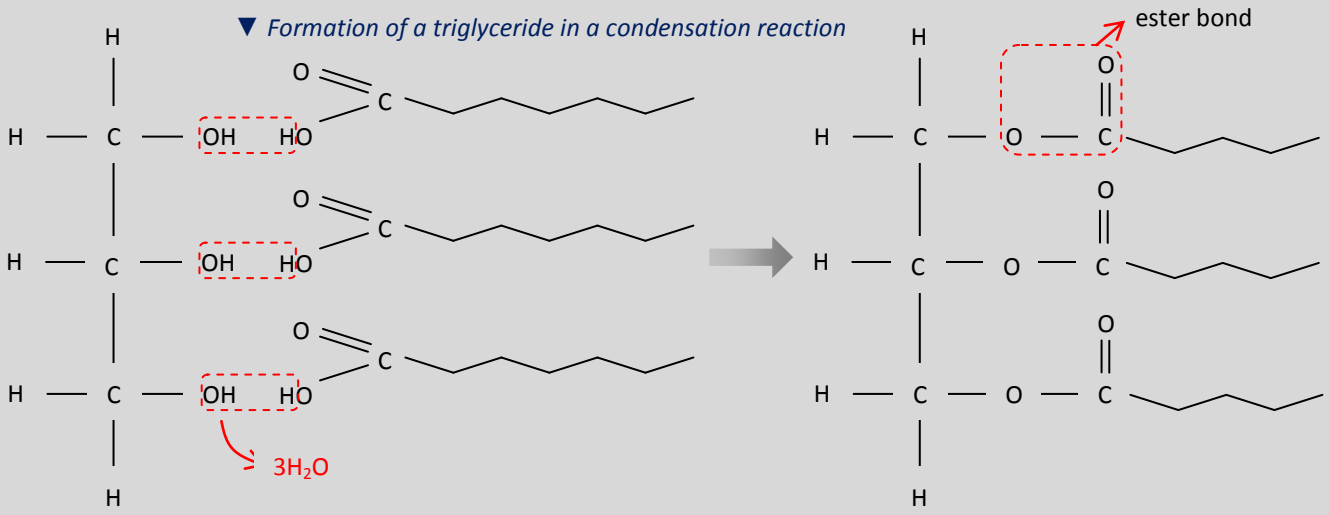
A glycerol molecule contains three alcohol groups and is always the same in any triglyceride. The diagram to the left shows a glycerol molecule, and the diagram on the right shows a simplified fatty acid which may be used for diagrammatic purposes



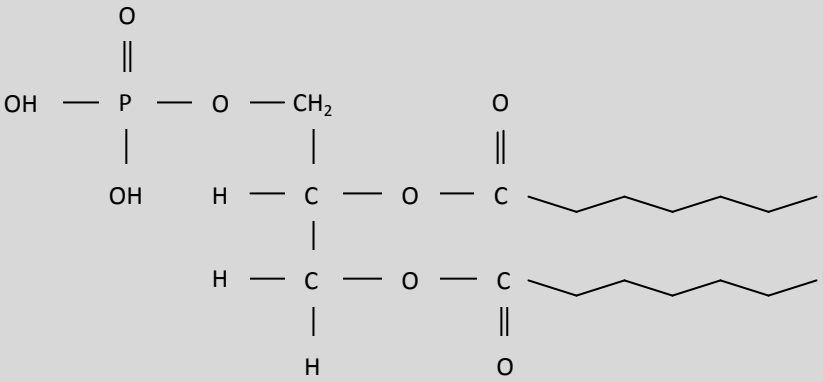
▲ *Fatty acid simplified*

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

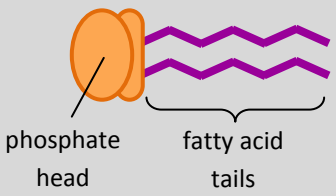
▼ *Formation of a triglyceride in a condensation reaction*



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*

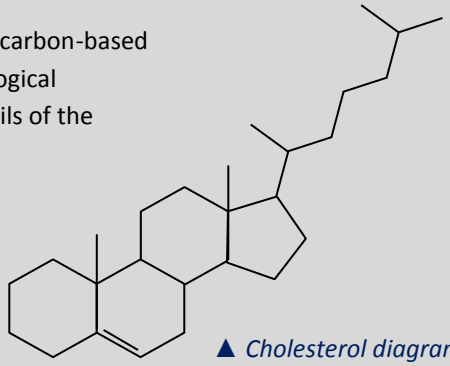


◄ *Chemical structure of a phospholipid*

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

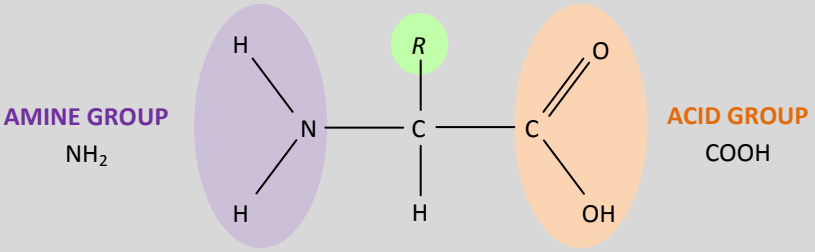
This molecule is vital to all living organisms, so many cells are able to make it, especially in the liver. However, excess cholesterol can cause health problems. An example is familial hypercholesterolemia, a genetic disorder whereby cells make and secrete cholesterol even though there are sufficient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



▲ *Cholesterol diagram*

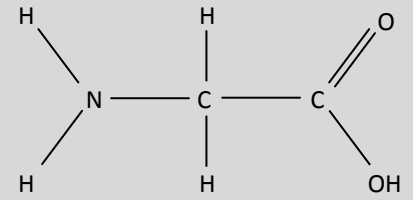
3.4 Amino acids and proteins

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

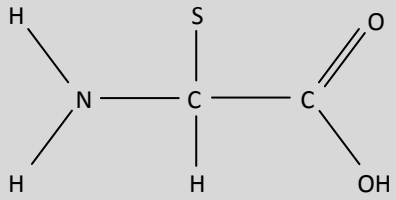


An amino acid consists of an **amine group** is basic (or alkaline) and has the formula NH_2 and the **carboxylic acid group** (COOH) is acidic, as it releases hydrogen ions into solution. These groups are separated by another carbon atom, which is also bonded to a hydrogen atom and a **variable group**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



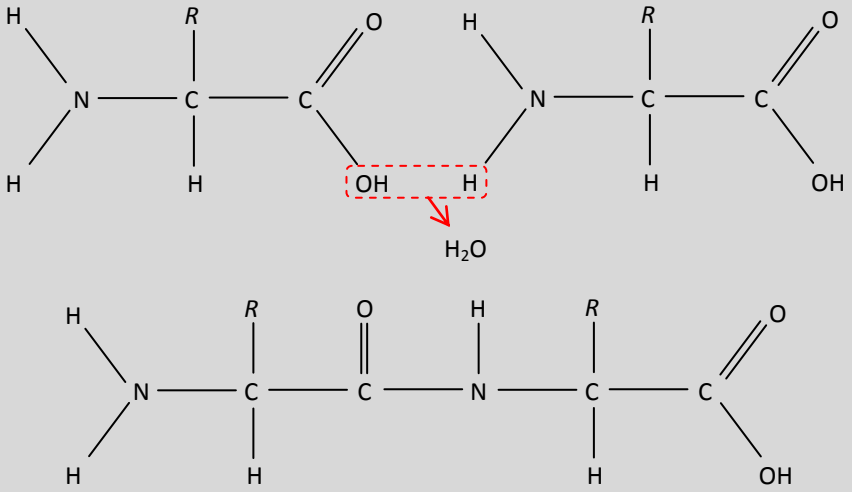
▲ *Glycine*



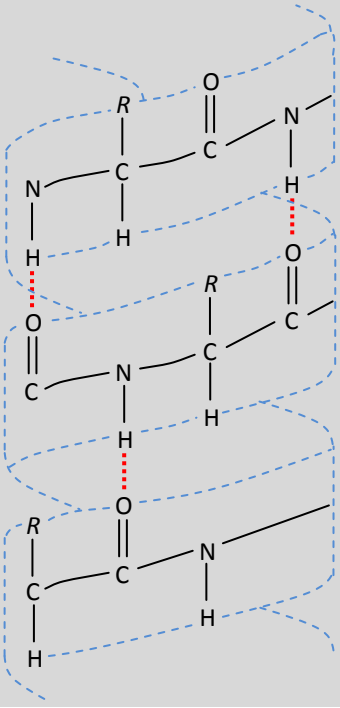
▲ *Cysteine*

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*

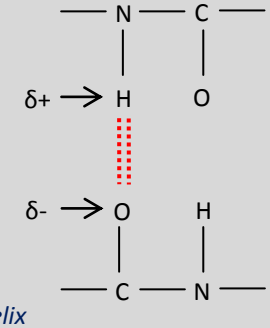


All proteins have a **primary structure**. This is the unique sequence of amino acids which makes the protein, and this will determine its main function

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

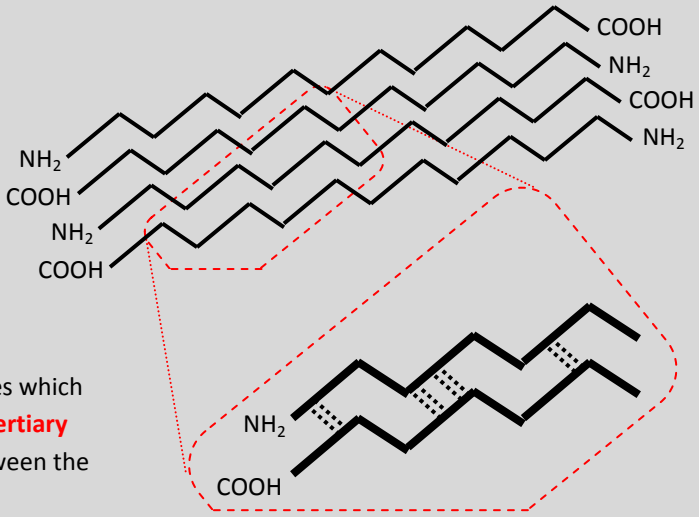
◄ *α-helix*



► *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*

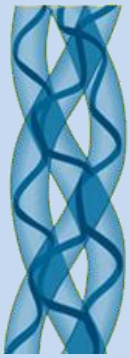
An α-helix can wrap itself into a 3D complex shape. Polypeptides which do this form a **globular protein**. Not all proteins will have this **tertiary structure**. Their shape is maintained by four types of bond between the variable *R* groups of different amino acids:

- 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

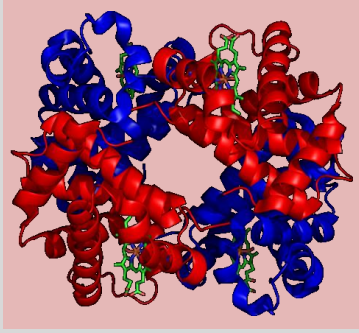
COLLAGEN



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)

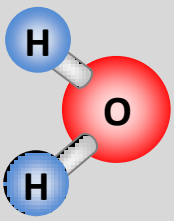
We call these tests semi-qualitative because they don't produce quantifiable results. We can use quantitative tests by using the following options:

- *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

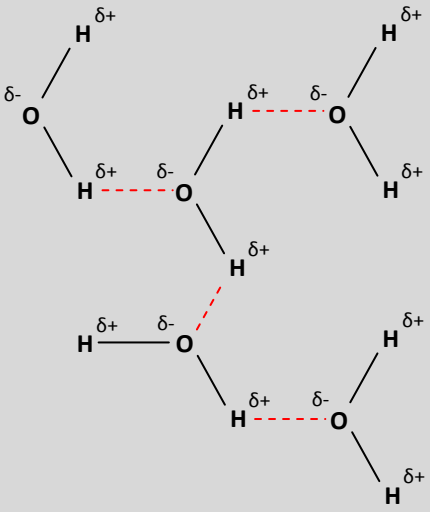
3.6 Water



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



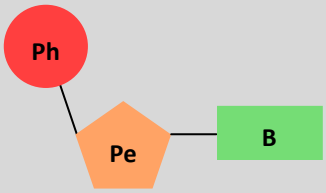
◀ *Hydrogen bonding in water*

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

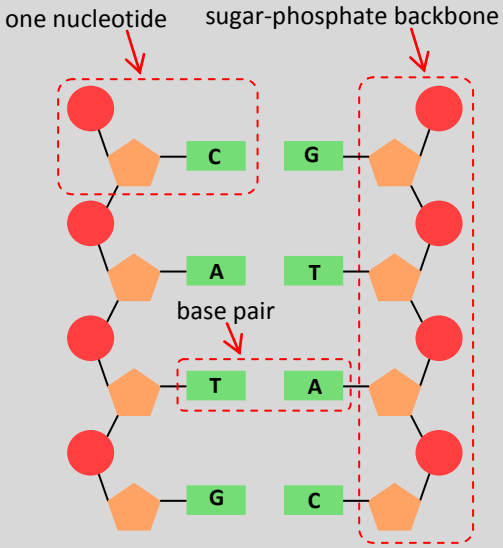
A **nucleic acid** comes in two different forms: as **DNA** and as **RNA**. They are both *macromolecules* formed by the individual monomers called **nucleotides**. A single nucleotide is made of three components



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

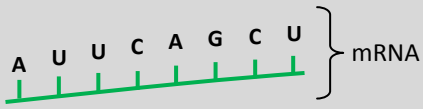


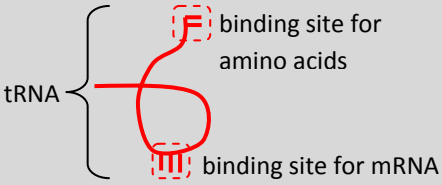
RNA (ribose nucleic acid) differs from DNA in three main ways:

- 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

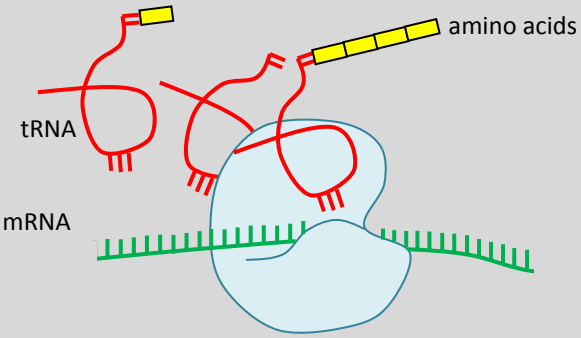
Because DNA is too big to escape from the nucleus through nuclear pores, a new single strand is developed inside the nucleus which can fit out. The DNA double-strand is unzipped (i.e. split down the middle), and then used as a *template strand*. A strand of **messenger RNA** (mRNA) is produced using 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**

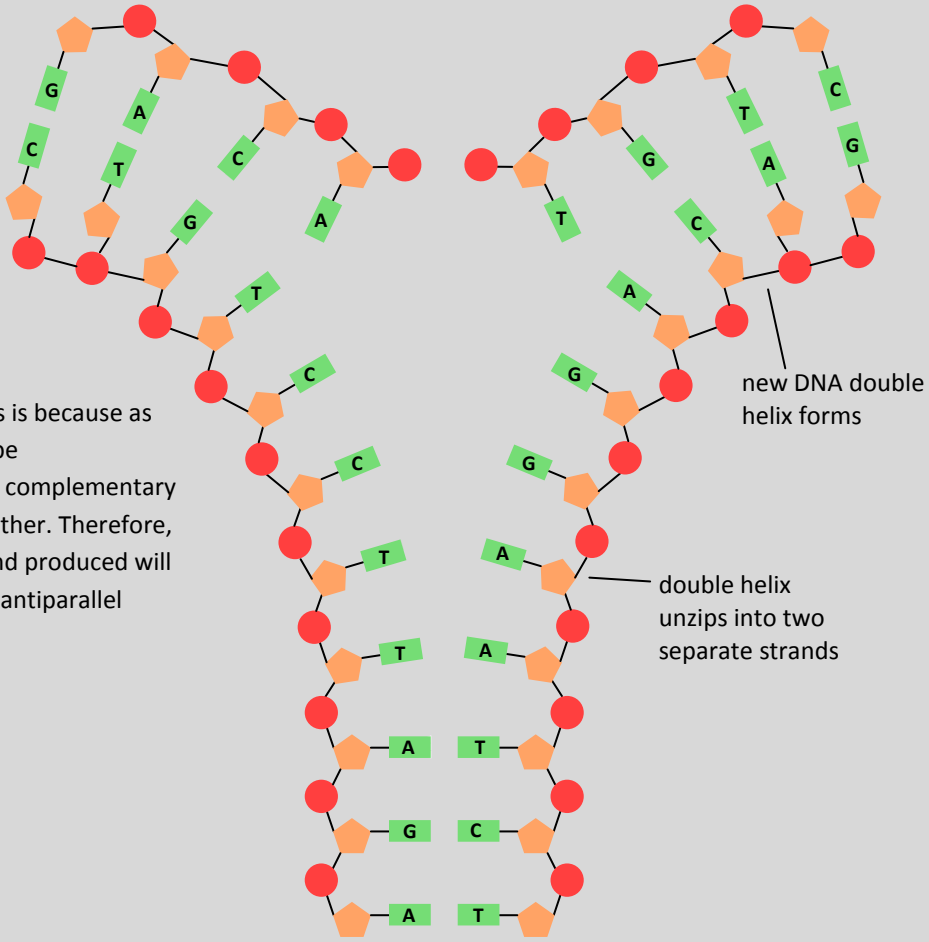


The shape of a tRNA molecule is often described as a *hairpin loop*. The staged diagram here shows the mRNA entering the ribosome and the tRNA binding to it. The tRNA molecule has brought the correct sequence of amino acids with it into the ribosome so that the protein can be synthesised: this stage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

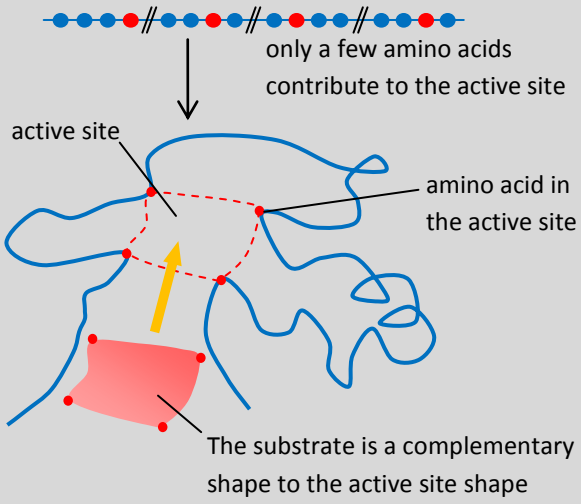
Each new DNA strand will be *identical*. This is because as the original nucleic acid unzips, there will be complementary bases on each strand, and complementary bases to those bases will bond with each other. Therefore, as is shown in the diagram, each new strand produced will be structurally identical,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

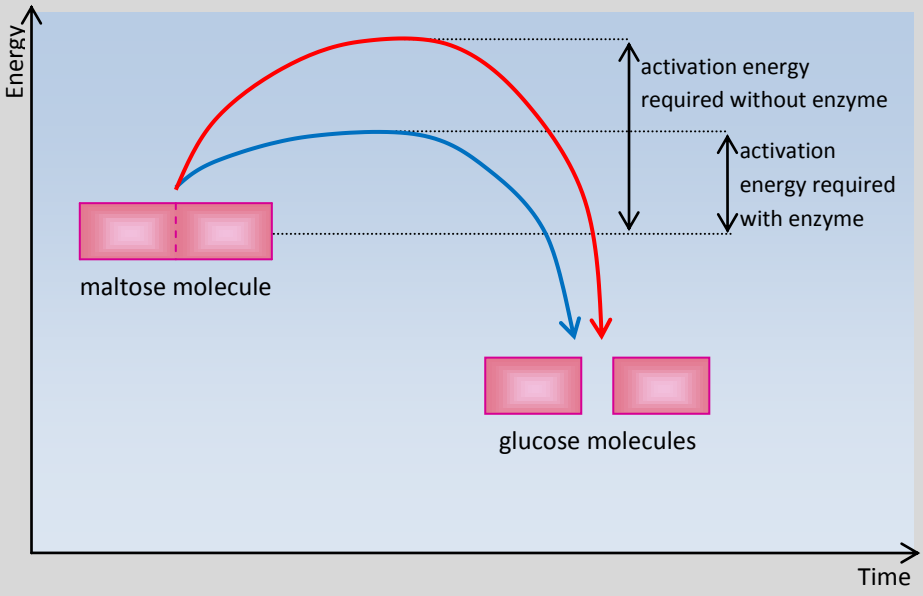


The **lock and key theory** shows how the substrate ('key') will fit one active site ('lock') only, and when the key is inside the lock, the reaction can take place and be catalysed

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

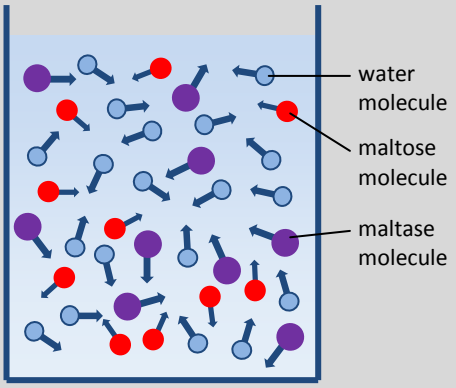


Boiling in hydrochloric acid provides sufficient energy, but this would never happen naturally. A *catalyst* can be used to drive metabolic reactions. They reduce the activation energy required. Enzymes are catalyst proteins which can do this, so reactions can take place in more diverse conditions, not 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*

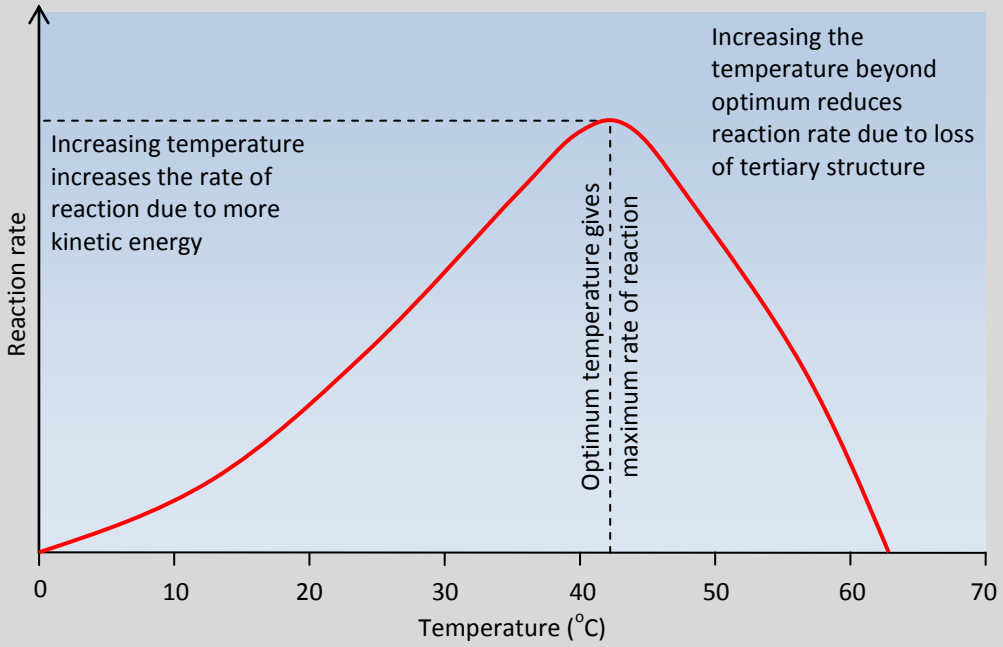
Without the enzyme present in the solution, there would be very few reactions taking place. But having the maltase molecules increases reaction rate because it means that whenever a maltose molecule and a water molecule collide with a maltase molecule, the hydrolysis reaction will be catalysed and take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

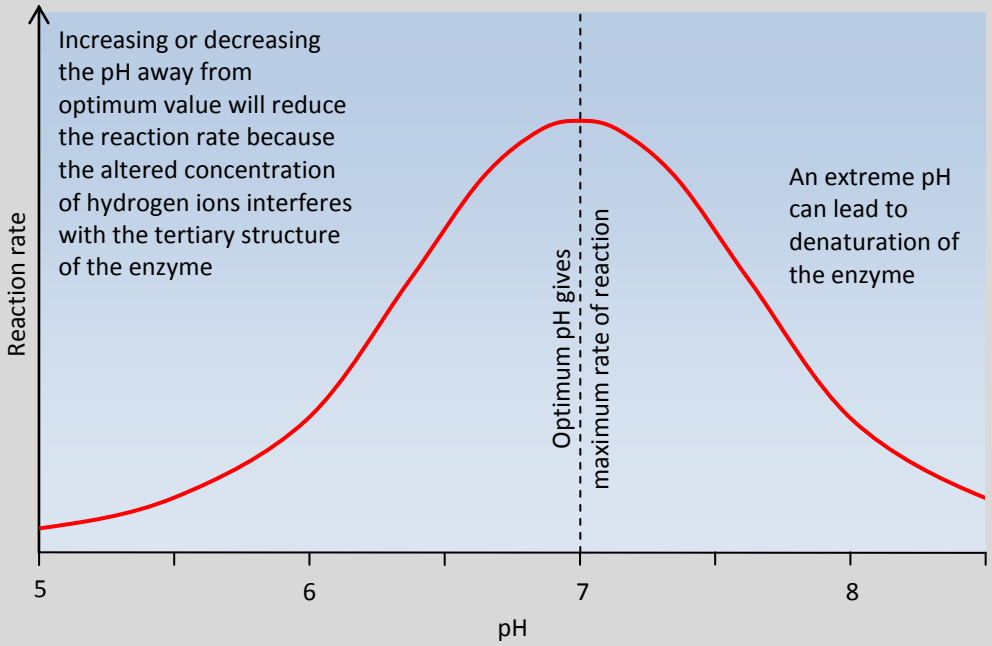
Enzymes tend to have fairly high *heat-resistance* so that they can operate in a diverse range of conditions, which is why optimum temperature is usually somewhere between 40 – 50^{p}C so they can survive fairly high temperatures

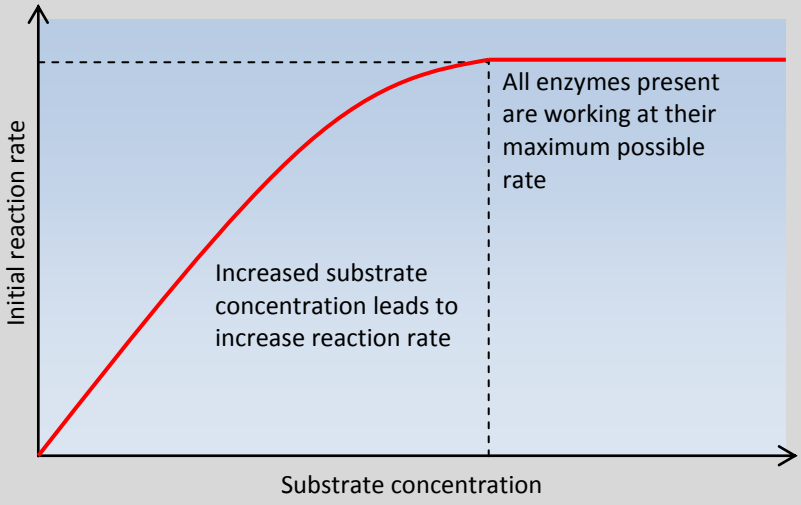


Enzymes and pH: pH is a measure of the concentration of H^+ ions (protons) – anything acidic is said to be a *proton donor* and anything basic – alkaline – is said to be a *proton acceptor*. Due to the positive charge of these ions, they are attracted to negatively charged parts of molecules and repel 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





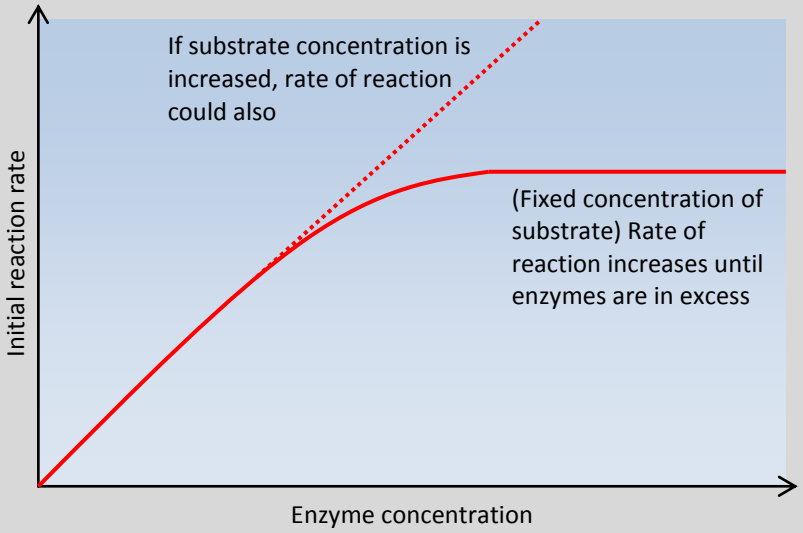
Enzymes and substrate concentration: Obviously, if there is a fixed concentration of enzymes, increasing substrate concentration will increase reaction rate. If there is no substrate present, there will be no reactions. The more substrates there are, the better the reaction rate – but only up to a certain 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

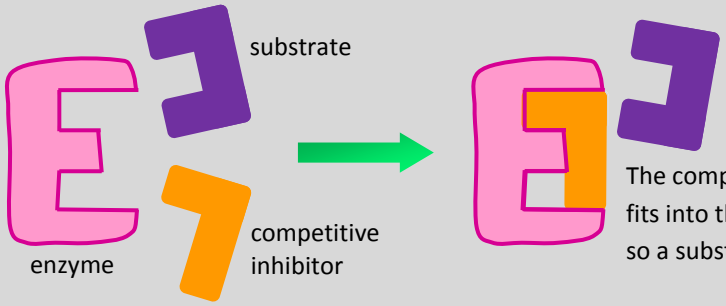
Enzymes and enzyme concentration: When there is a fixed concentration of substrate molecules, again the relationship is immediately obvious: more enzymes mean more reactions are possible and more likely as collisions will be more frequent

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

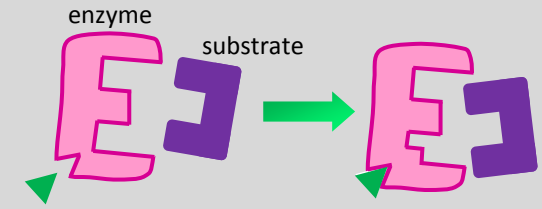


The competitive inhibitor fits into the active site and so a substrate cannot enter

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*

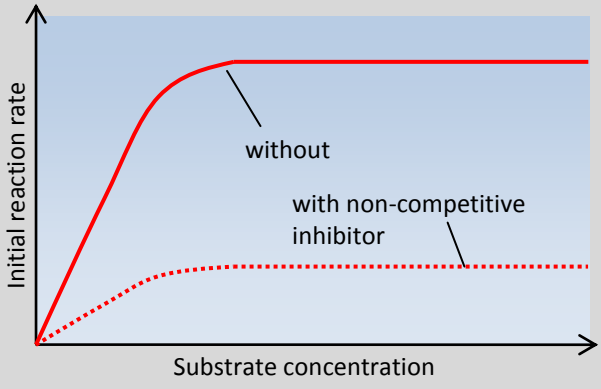
A **non-competitive inhibitor** does not compete with substrate for the active site of an enzyme. Instead, it attaches itself to another region of the enzyme

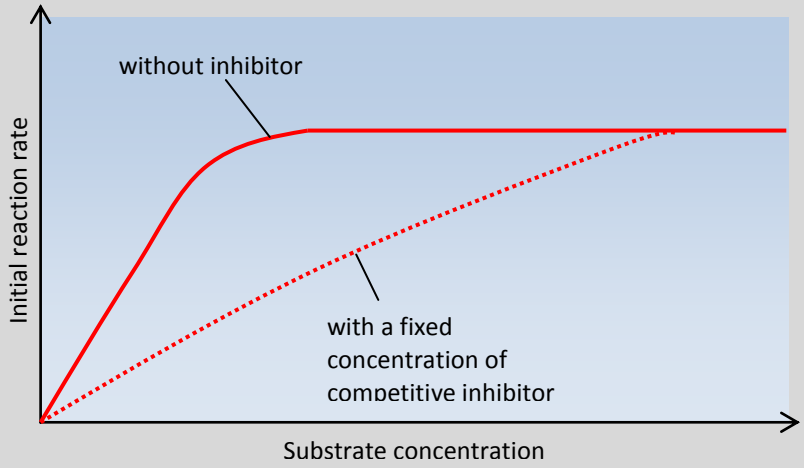


▲ *Non-competitive inhibition*

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