

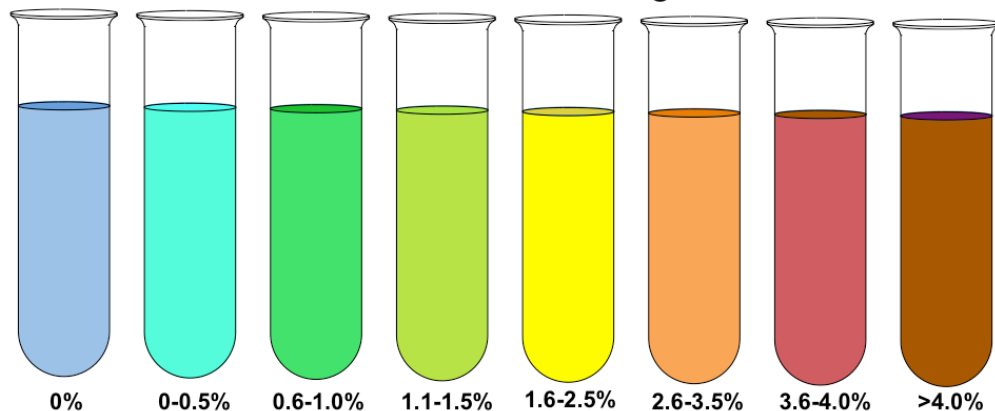
Biological molecules

A-Level Biology

Testing for biological molecules

Living things are built from four main kinds of biological **molecule** : **carbohydrates** , **lipids** , **proteins** and **nucleic acids** . You can use simple chemical tests to find out which kinds are present in a sample.

Benedict's test for sugars



Benedict's test: blue turns green, then orange, then brick-red as more reducing sugar is present

Test	What it finds	Method	Positive result
Benedict's test	reducing sugar	add Benedict's solution and heat in a water bath	a green, yellow, orange or brick-red precipitate
iodine test	starch	add orange-brown iodine solution	colour changes to blue-black
emulsion test	lipid	mix sample with ethanol, then pour into water	a white, cloudy emulsion forms
biuret test	protein	add biuret solution at room temperature	blue changes to purple

■ Semi-quantitative Benedict's test

The normal Benedict's test only tells you "yes or no". A **semi-quantitative** test gives a rough amount. First you **standardise** the test: you run it on solutions of known **concentration** and record the result for each. Then you can estimate an unknown by either:

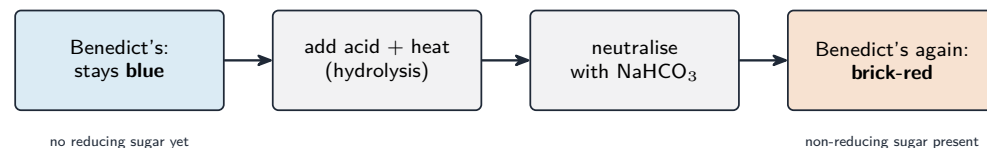
- the **time to the first colour change** (more sugar changes colour faster), or
- comparing the final colour to your set of colour standards.

Keep sample and reagent volumes, water-bath temperature and the colour-change endpoint the same for standards and the unknown. Use a water blank, repeat readings and use closer standards around the estimate; do not assume the time is proportional to concentration.

■ Testing for non-reducing sugars

Some sugars, such as sucrose, are **non-reducing sugar** : they give a negative Benedict's test. To detect them:

1. Do a normal Benedict's test first. It stays blue (reducing sugar is not detected under these conditions).
2. Take a fresh sample and add dilute **hydrochloric acid** , then heat. This **acid hydrolysis** breaks the sugar into smaller reducing sugars.
3. Cool, then **neutralise** the acid with sodium hydrogencarbonate.
4. Add Benedict's solution and heat in a water bath. A coloured precipitate after an initially negative test supports a non-reducing sugar in a sample known to contain sugars; it does not identify sucrose specifically.



Testing for a non-reducing sugar: hydrolyse it with acid first, then the second Benedict's test turns red

Worked example. A solution is known to contain one dissolved sugar and no starch. One portion gives a negative Benedict's test. A fresh portion is heated with dilute hydrochloric acid, cooled, neutralised and re-tested with Benedict's in a water bath; it gives an orange precipitate. This supports a non-reducing sugar: hydrolysis releases reducing monosaccharides, and neutralisation restores the alkaline conditions needed for the test. The sugar is not identified as sucrose by this result alone. Without the sample context, other carbohydrates that release reducing sugars on hydrolysis must also be considered. A positive initial test followed by another positive test alone would not establish an additional non-reducing sugar.

Carbohydrates

■ Monomers, polymers and macromolecules

- a **monomer** is a small molecule that is a single unit.
- a **polymer** is a long molecule made of many monomers joined together.
- a **macromolecule** is any very large molecule.

Sugars come in three sizes:

- a **monosaccharide** is a single sugar unit, such as **glucose** and fructose.
- a **disaccharide** is two units joined, such as maltose and sucrose.
- a **polysaccharide** is many units joined into a polymer.

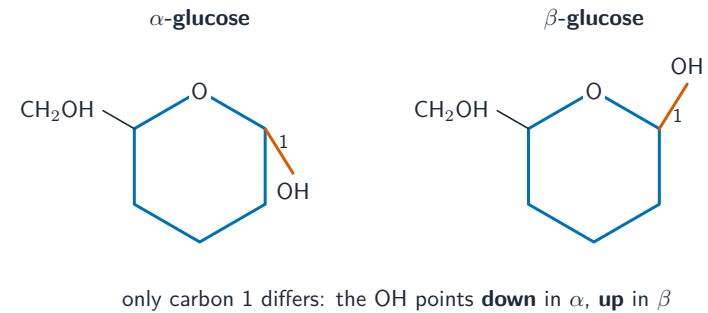
Glucose, **fructose** and **maltose** are reducing sugars. **Sucrose** is a non-reducing sugar.

■ Two ring forms of glucose

Glucose has six carbon atoms and forms a ring. There are two ring forms. They differ only at carbon 1:

- in **α -glucose**, the -OH group on carbon 1 points **down**, below the ring.
- in **β -glucose**, the -OH group on carbon 1 points **up**, above the ring.

This small difference decides which polysaccharide the glucose can build.

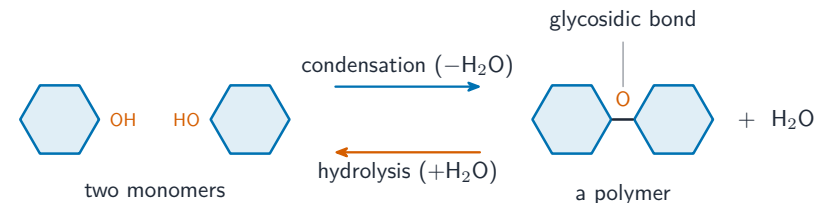


The two ring forms differ only at carbon 1: the -OH points down in α , up in β

■ Joining and breaking sugars

Monomers are joined by strong **covalent bonds**. When two sugars join, a **glycosidic bond** forms between them. This happens by **condensation**: a molecule of water is removed each time a bond forms.

The reverse is **hydrolysis**: a water molecule is added to break a glycosidic bond. This is why the non-reducing sugar test needs acid and heat – they hydrolyse sucrose into glucose and fructose.



Condensation removes water to join monomers; hydrolysis adds water to split them

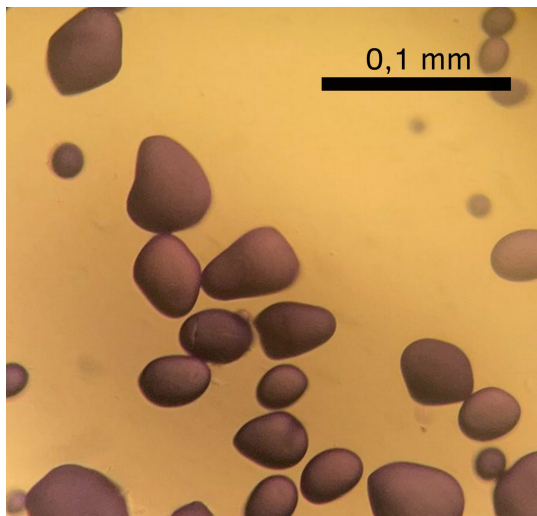
■ Storage polysaccharides: starch and glycogen

Starch is the energy store in plants. It is made of two polymers of α -glucose:

- **amylose** – a long, unbranched chain that coils into a spiral.
- **amylopectin** – a chain with many side branches.

Glycogen is the energy store in animals. It is like amylopectin but has even more branches, so it can be broken down quickly when energy is needed.

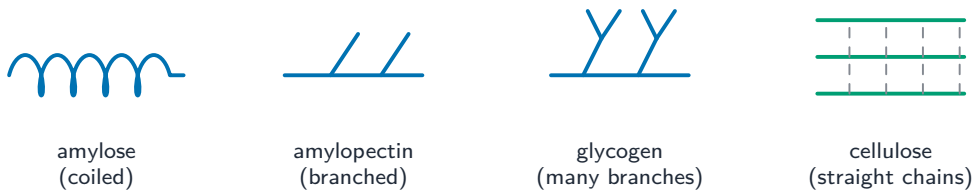
These stores suit their job well: they are compact, they are **insoluble** (so they do not leave the cell), and they do not change the **water potential** of the cell (so they do not pull water in by **osmosis**). The many branches give many ends, so glucose can be added or removed fast.



Real starch grains inside a potato, stained by iodine. Each grain is a dense, insoluble package of amylose and amylopectin – note the scale: the largest are only about 0.1 mm across

■ Cellulose

Cellulose is made of β -glucose. Because of the β form, every other glucose is flipped over, so the chains are long and straight. Many straight chains lie side by side and are held together by **hydrogen bonds** into strong bundles called **microfibrils**. These give the plant **cell wall** its strength and stop the cell bursting.



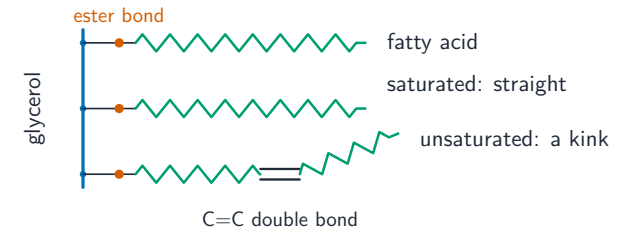
The shape fits the job: amylose coils and amylopectin/glycogen branch (for compact stores), while straight cellulose chains pack into strong fibres

Lipids

■ Triglycerides

A **triglyceride** is the main fat or oil. It is **non-polar** and **hydrophobic** (it does not mix with water). It is made from one **glycerol** molecule joined to three **fatty acids** by **ester bonds**. Each ester bond

forms by condensation, so three water molecules are removed.



3 ester bonds form $\rightarrow$ 3 H_2O removed (condensation)

One glycerol plus three fatty acid tails; a straight tail is saturated, a kinked one unsaturated

Fatty acids are of two kinds:

- **saturated** – no carbon-carbon **double bonds**; these fats are usually solid.
- **unsaturated** – one or more double bonds; these oils are usually liquid.

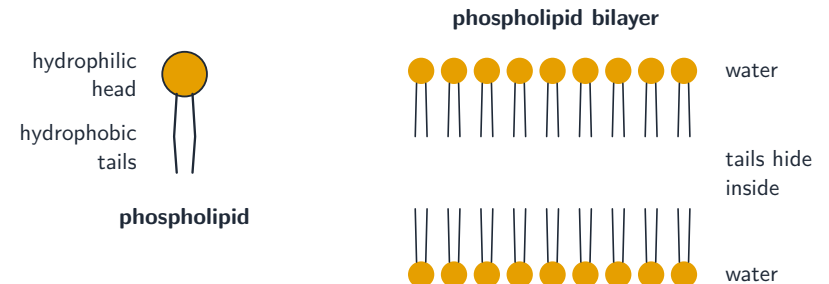
Triglycerides make a good long-term energy store: they release about twice as much energy per gram as carbohydrates, they are insoluble, and they store little extra mass because they hold no water. Under the skin they also give **insulation** and protect the organs.

■ Phospholipids

A **phospholipid** is like a triglyceride, but one fatty acid is replaced by a **phosphate** group. This gives the molecule two ends with different behaviour:

- a **hydrophilic** (“water-loving”) **polar** phosphate head.
- two hydrophobic (“water-fearing”) fatty acid tails.

This split personality is why phospholipids form the membranes around cells.



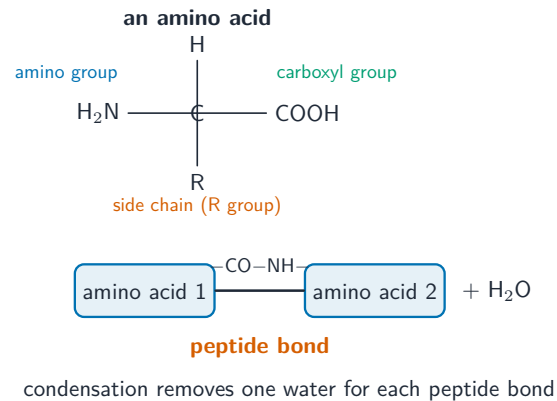
The hydrophilic heads face the water; the hydrophobic tails hide inside, forming a bilayer

Proteins

■ Amino acids and the peptide bond

Proteins are polymers of **amino acids**. Every amino acid has the same general structure around a central carbon atom: an **amino group** ($-\text{NH}_2$), a **carboxyl group** ($-\text{COOH}$), a hydrogen atom, and a variable **side chain** (the R group). The R group is different in each amino acid.

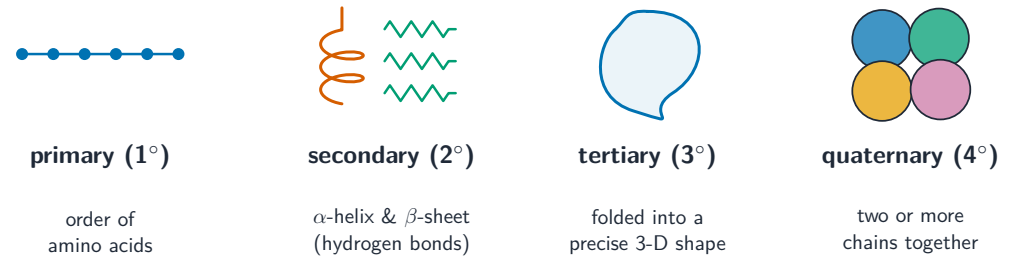
Two amino acids join by condensation. The bond formed between the amino group of one and the carboxyl group of the next is a **peptide bond**, and a water molecule is removed. Many amino acids joined this way make a **polypeptide**. Adding water (hydrolysis) breaks a peptide bond.



Every amino acid has an amino group, a carboxyl group and an R group; two join by a peptide bond

■ Four levels of protein structure

Level	What it means
primary structure	the order of amino acids in the chain
secondary structure	local shapes – the α - helix and the β - pleated sheet – held by hydrogen bonds between backbone groups
tertiary structure	the whole chain folded into a precise 3-D shape
quaternary structure	two or more polypeptide chains joined into one protein



The four levels: primary → secondary → tertiary → quaternary structure

The tertiary folding is stabilised by several kinds of interaction involving R groups:

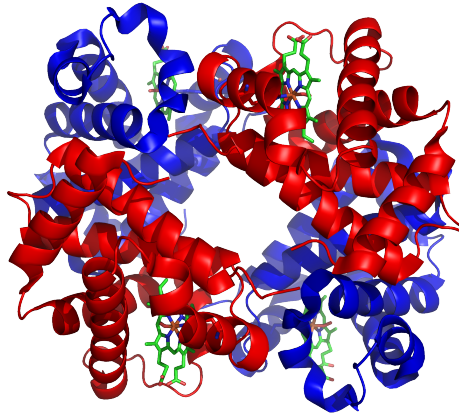
- **hydrophobic interactions** (non-polar R groups cluster away from water).
- hydrogen bonding.
- **ionic bonds** (between charged R groups).
- covalent bonding, including strong **disulfide bonds**.

■ Globular and fibrous proteins

- **globular proteins** fold into a rounded shape, are usually **soluble**, and do jobs in the body (for example enzymes and haemoglobin).
- **fibrous proteins** form long strands, are usually insoluble, and give structure and support (for example collagen).

■ Haemoglobin – a globular protein

Haemoglobin carries **oxygen** in red blood cells. It has a quaternary structure made of four polypeptide chains: two alpha (α -globin) chains and two beta (β -globin) chains. Each chain holds a **haem group**. At the centre of each haem group is an **iron** ion, and this is where one oxygen molecule binds. Four chains mean one haemoglobin molecule can carry four oxygen molecules.



A real haemoglobin molecule, worked out from X-ray data. Count them: two alpha chains (red), two beta chains (blue), and one green haem group held in each – so four oxygen molecules in total

■ Collagen – a fibrous protein

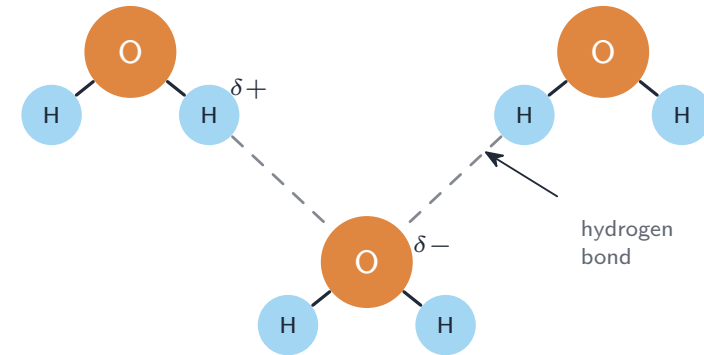
Collagen gives strength to skin, **tendons**, bone and blood vessel walls. One collagen molecule is three helical polypeptide chains wound tightly into a triple helix, stabilised by hydrogen bonds between chains. Glycine occurs at about every third position; its small side chain allows close packing. Many of these molecules lie side by side, slightly staggered, and have covalent cross-links between molecules, forming thick **fibres**. The staggered, cross-linked arrangement makes collagen very strong when pulled.

Water

Water is a small molecule, but its two O–H bonds are **polar**: the oxygen end is slightly negative and the hydrogen ends are slightly positive. So one water molecule attracts its neighbours, forming weak hydrogen bonds between them. These hydrogen bonds explain water's useful properties:

- **solvent action** – water is a good **solvent**, so many substances dissolve in it. This lets reactions happen and lets substances be carried around the body.

- **high specific heat capacity** – water needs a lot of energy to warm up, so its temperature stays steady. This protects living things from quick temperature changes.
- **latent heat of vaporisation** – water needs a lot of energy to **evaporate**. So when water evaporates (for example as sweat dries), it carries away a lot of heat and cools the body.



Water is polar ($\delta-$ oxygen, $\delta+$ hydrogens), so its molecules attract each other by hydrogen bonds – the reason for all the properties above

Common mistakes.

- Writing that condensation “adds water” or that hydrolysis “removes water”. **Condensation** removes a water molecule as a bond forms; **hydrolysis** uses a water molecule to break one.
- Saying cellulose is made of α -glucose, or that starch has hydrogen-bonded microfibrils. Cellulose is **β -glucose**: alternate units are flipped, so the chains are straight and lie side by side, held by hydrogen bonds.
- Saying glycogen is a good store “because it is soluble”. It is **insoluble**, so it does not change the water potential of the cell; its many branches give many ends for fast release of glucose.
- Saying a triglyceride is a polymer. Three fatty acids on one glycerol is not a chain of repeating monomers, so lipids are **not polymers**.
- Saying the secondary structure is held by bonds between R groups. The α -helix and β -pleated sheet are held by hydrogen bonds between **backbone** groups; the R groups hold the tertiary structure.
- Saying a disulfide bond forms between any two amino acids. It forms only between two **cysteine** R groups, which contain sulfur.
- Calling hydrogen bonds strong. Each hydrogen bond is **weak**; there are so many of them that together they give strength (cellulose) or a high specific heat capacity (water).

Exam tips

- Learn each **food test** as reagent + positive result + colour change (Benedict's → coloured precipitate, not necessarily brick-red; iodine → blue-black; biuret → purple; emulsion → white).
- Benedict's detects **reducing** sugars; for a non-reducing sugar you must heat a fresh portion with acid, cool and neutralise it, then re-test in a water bath.
- Name the bond precisely: **glycosidic** (carbohydrates), **ester** (lipids), **peptide** (proteins) – all made by **condensation** (water removed).
- Link structure to function: cellulose (straight chains, H-bonds → strong), glycogen/starch (branched/coiled → compact store).
- For proteins, name the bond at each level: primary (peptide), secondary (hydrogen), tertiary (R-group interactions), quaternary.