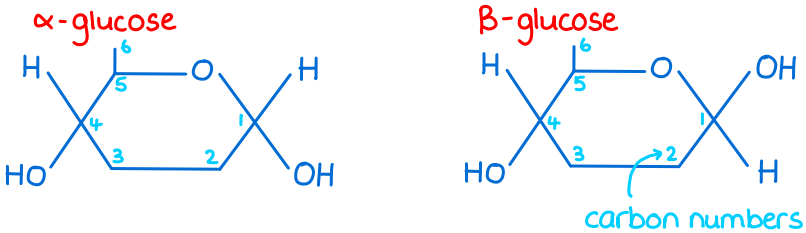
# CARBOHYDRATES

- Carbohydrates contain carbon, hydrogen and oxygen, usually with the general formula $C_nH_{2n}O_n$

## Glucose

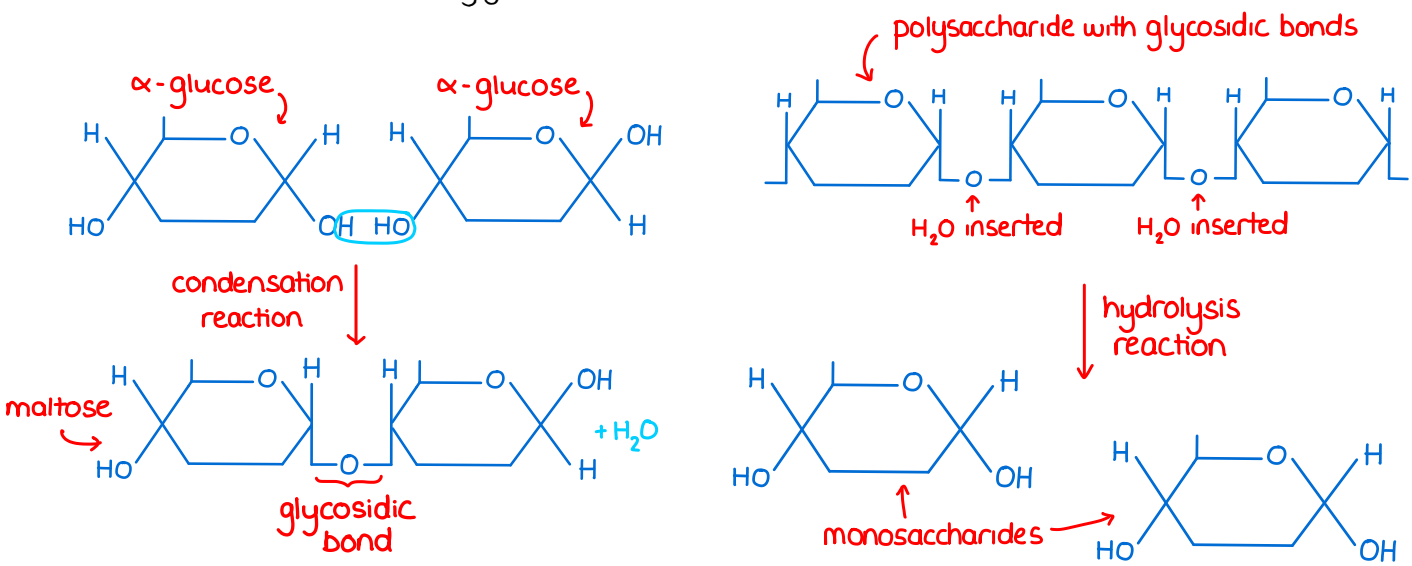
- A monosaccharide with the formula $C_6H_{12}O_6$
- A hexose monosaccharide (six carbon atoms) in a ring structure
- Soluble in water → easily transported
- Main energy source for animals and plants
  → chemical bonds store lots of energy
- Two isomers: α-glucose and β-glucose → H and OH groups on carbon 1 inverted in β-glucose

Monosaccharides are small soluble carbohydrate monomers. They also include fructose and galactose.




## Glycosidic bonds and condensation/hydrolysis reactions

- Condensation reaction: two molecules join to form a new chemical bond and a water molecule is eliminated
- Condensation reactions form glycosidic bonds between monosaccharides to create disaccharides and polysaccharides
- Hydrolysis reaction: a water molecule is used and the chemical bond is broken (reverse of a condensation reaction → breaks glycosidic bonds)




## Disaccharides

- Two monosaccharides joined together with a glycosidic bond in a condensation reaction
- Soluble in water
- Maltose = glucose + glucose
- Sucrose = glucose + fructose
- Lactose = glucose + galactose

Monosaccharides and disaccharides are sugars.

# CARBOHYDRATES

## Polysaccharides

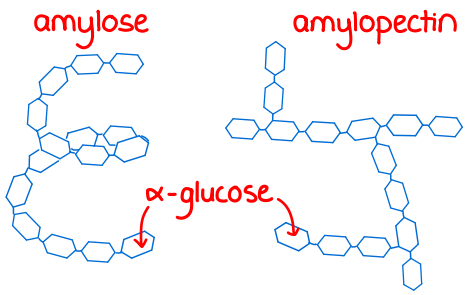
- Large polymers of monosaccharides joined with glycosidic bonds
- Starch and glycogen are large energy storage molecules which cannot leave cells

## Starch

- Glucose storage in plants → hydrolysed when glucose is needed
- Insoluble in water → does not affect the water potential of cells so water is not drawn in by osmosis
- Amylose → unbranched α-glucose polysaccharide (1,4 glycosidic bonds)
  → helical structure so is compact
- Amylopectin → branched α-glucose polysaccharide (1,4 and 1,6 glycosidic bonds)
  → branches mean enzymes can easily access more glycosidic bonds = faster glucose release

## Iodine test for starch

1) Add iodine in potassium iodide solution to sample.
2) If starch is present: goes from browny-orange to blue-black.

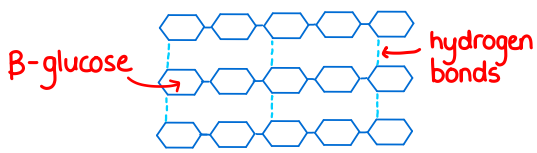



## Glycogen

- Excess glucose storage in animals
  → easily hydrolysed when glucose is needed
- Insoluble in water → does not affect the water potential of cells so water is not drawn in by osmosis
- Highly branched α-glucose polysaccharide (1,4 and 1,6 glycosidic bonds) → more ends so glucose can be released quickly by enzymes, and it is compact

## Cellulose

- Found in plant cell walls to give strength
- Unbranched long and straight β-glucose polymers (1,4 glycosidic bonds)
- Chains linked with many hydrogen bonds to form strong rigid microfibrils

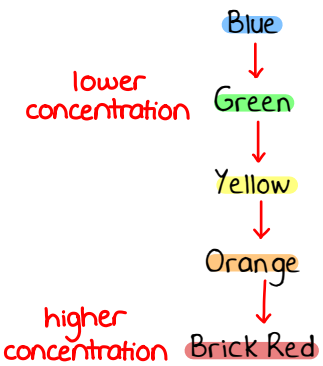



## Benedict's test for sugars

- Monosaccharides, maltose, and lactose are reducing sugars
- Sucrose is a non-reducing sugar

1) Add an excess of blue Benedict's reagent to liquid food sample in a test tube.
2) Heat the tube in a water bath set to boil.
3) If reducing sugars are present: coloured precipitate forms. End test here.
4) If no reducing sugars are present: solution stays blue. Go to step 5.
5) Break down non-reducing sugars to monosaccharides: add dilute HCl to new sample and heat in a water bath set to boil.
6) Neutralise with sodium hydrogencarbonate, then repeat steps 1) and 2).
7) If coloured precipitate now forms, non-reducing sugars are present in the sample.
8) If the solution is still blue, neither type of sugars are present.

Colour of precipitate depends on the concentration of reducing sugars:



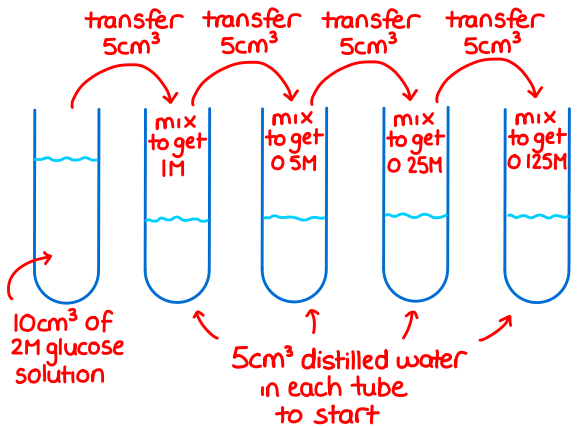

You could filter, dry, and weigh the precipitate to make more accurate comparisons.

# CARBOHYDRATES

## Using a colorimeter

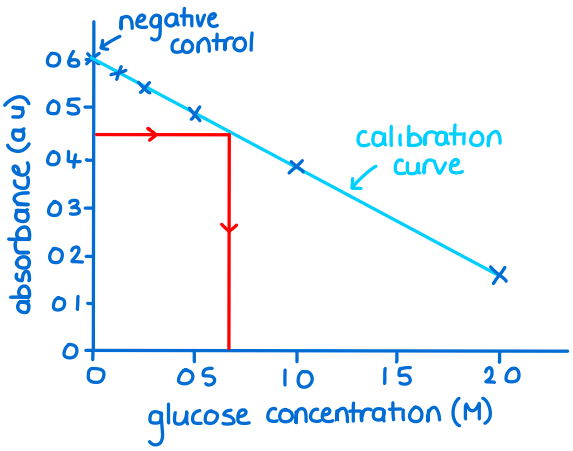
- A more accurate and quantitative way to measure glucose concentration after the Benedict's test (judging colour is subjective and more affected by human error)
- When the precipitate is filtered out of the solution, the solution left is the Benedict's reagent → more glucose = more precipitate = the less blue the remaining solution will be
- A colourimeter measures absorbance of light → lower absorbance = more blue colour lost = more glucose
- Zero the colorimeter to distilled water to make sure values are comparable
- Control the volume and concentration of Benedict's solution used
- Control the duration of time in the boiling water bath
- Can use a serial dilution of a known concentration of glucose to produce a calibration curve

In this case we are diluting the glucose solution by a factor of two each time.




Make sure to mix well at each stage.

Carrying out the Benedict's test on all tubes and a negative control (distilled water) will produce a calibration curve.

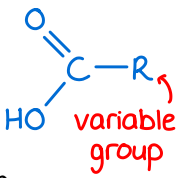



Now we can measure the absorbance of a glucose solution with an unknown concentration and use the calibration curve to read across from the absorbance value and down to find the concentration. This is called interpolation.

If the precipitate was not filtered out of the solution, absorbance would increase with increasing glucose concentration instead.

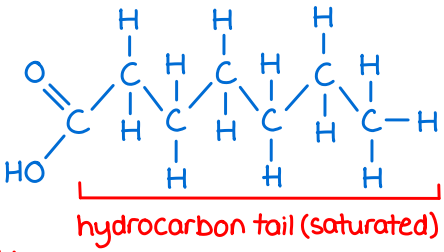
# LIPIDS

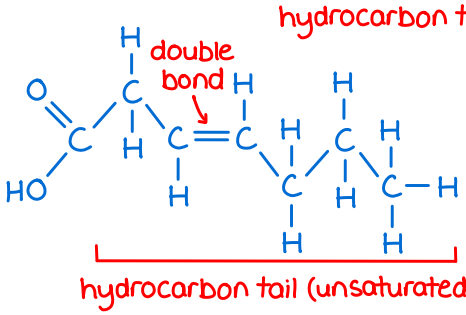
- Lipids contain carbon, hydrogen and oxygen




## Fatty acids

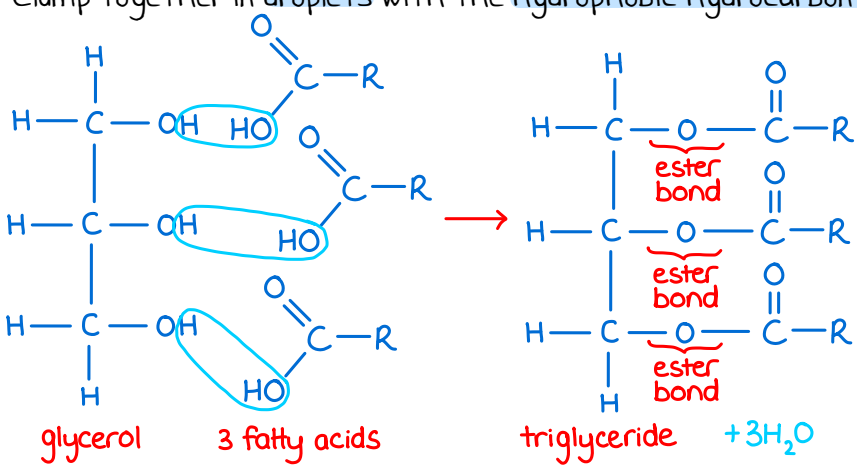
- Have a variable R group → the hydrocarbon tail
- Saturated fatty acids have no double C=C bonds in the hydrocarbon tail
- Unsaturated fatty acids have one or more double C=C bonds in the hydrocarbon tail so the chain kinks
- Hydrocarbon tails are hydrophobic (insoluble in water)



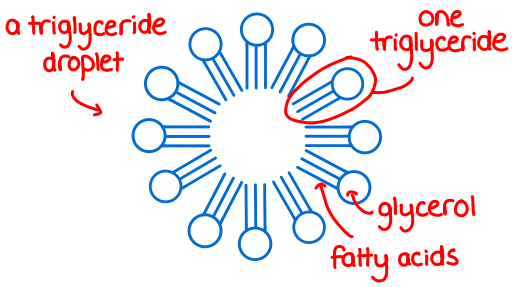




## Triglycerides

- One molecule of glycerol bound to three fatty acids
- Fatty acids join to glycerol in a condensation reaction → an ester bond is formed and a water molecule is released
- Three water molecules released and three ester bonds formed for each triglyceride
- Ester bonds are broken by a hydrolysis reaction
- Energy store → hydrocarbon tails release a lot of energy when broken down
- Insoluble in water → do not affect the water potential of cells so water is not drawn in by osmosis
- Clump together in droplets with the hydrophobic hydrocarbon tails facing inwards

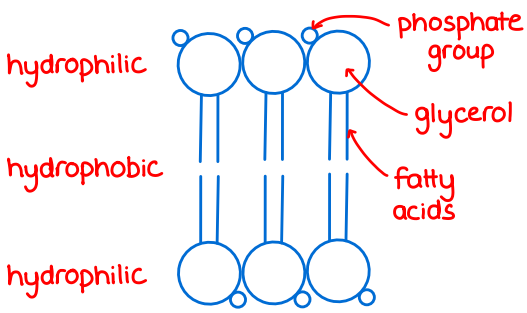



Know how to relate structure to function.




## Phospholipids

- One molecule of glycerol bound to two fatty acids and a phosphate group
- Phosphate groups are hydrophilic, fatty acids are hydrophobic
- Form a bilayer in cell membranes → water soluble (polar) substances cannot pass through the hydrophobic centre of the bilayer




## Emulsion test for lipids

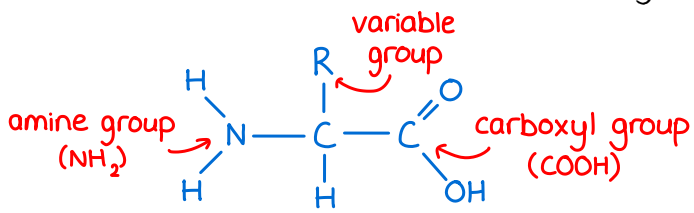
1) Mix food sample with ethanol and shake until dissolved.
2) Pour mixture into water.
3) If lipid is present → milky emulsion forms (the more obvious it is, the more lipid there is).
4) If no lipid is present → stays clear.

# PROTEINS

## Amino acids

- The monomers of proteins
- Contain carbon, hydrogen, nitrogen, oxygen and sometimes sulfur
- Have a carboxyl group, an amine group, and a variable R group
- There are 20 different amino acids, each with a different R group

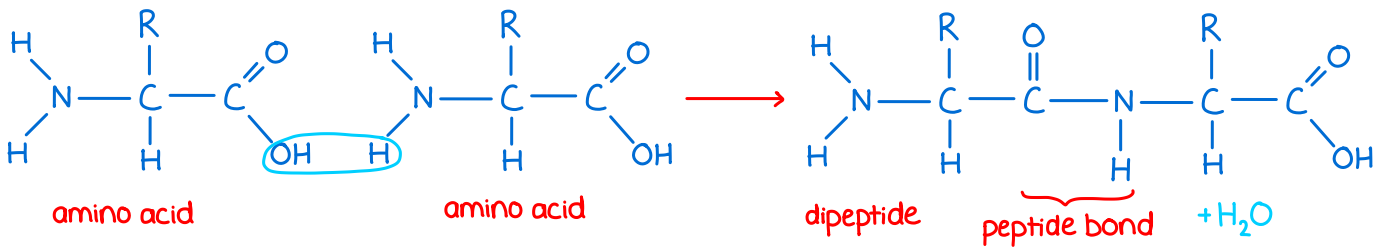
A monomer is a smaller unit from which larger polymers are made.




Amino acids can be joined in any order and length.

## Peptide bonds and dipeptides

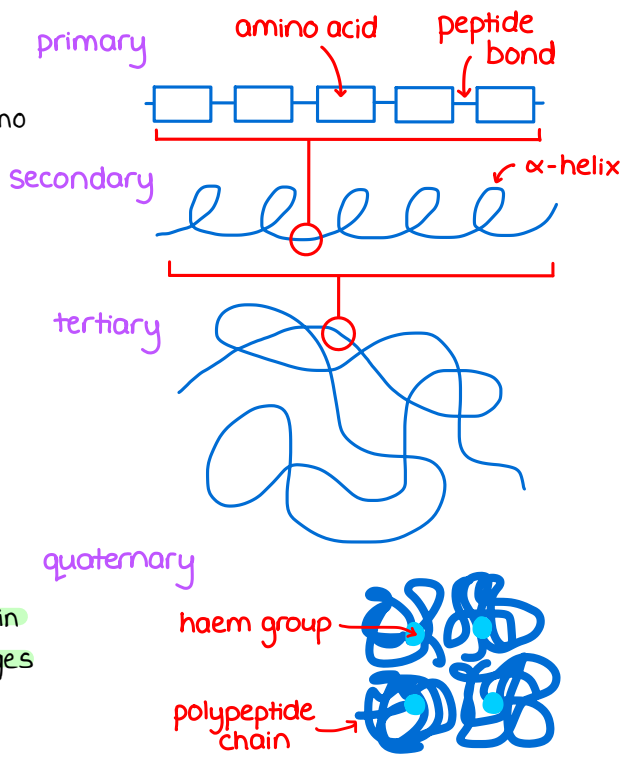
- A condensation reaction joins two amino acids with a peptide bond
  - → produces a dipeptide and a molecule of water
- A hydrolysis reaction breaks a peptide bond by adding a molecule of water
- A polypeptide is a polymer of amino acids (a long chain of amino acids joined with peptide bonds)




## Protein structure

- Primary structure
  - → order of amino acids in the polypeptide chain
  - → determines the overall protein structure
- Secondary structure
  - → alpha helix (coiled) or beta pleated sheet (folded)
  - → hydrogen bonds between N-H and C=O parts of amino acids in the polypeptide
- Tertiary structure
  - → further folding (bonds form between R groups of amino acids in the polypeptide)
  - → more hydrogen bonds
  - → ionic bonds between positively and negatively charged R groups
  - → disulfide bridges between cysteine amino acids
  - → final structure for proteins made from one polypeptide
- Quaternary structure
  - → some proteins have more than one polypeptide chain
  - → chains held together with bonds e.g. disulfide bridges
  - → example opposite: haemoglobin has four polypeptide chains

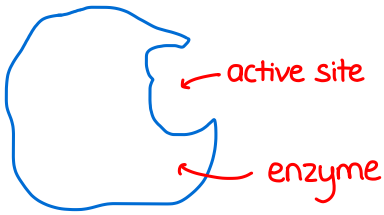
Cysteine contains a sulfur atom in the R group.



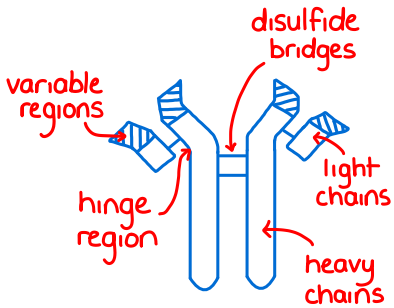
# PROTEINS

## Functions of proteins

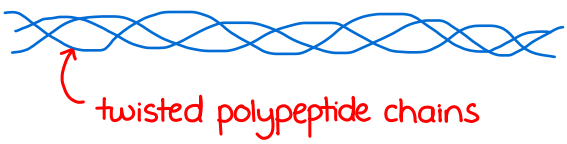
- Enzymes
  - → soluble and almost spherical
  - → tightly folded polypeptides
  - → catalyse metabolic reactions
  - → examples: amylase digests starch, lipase digests lipids



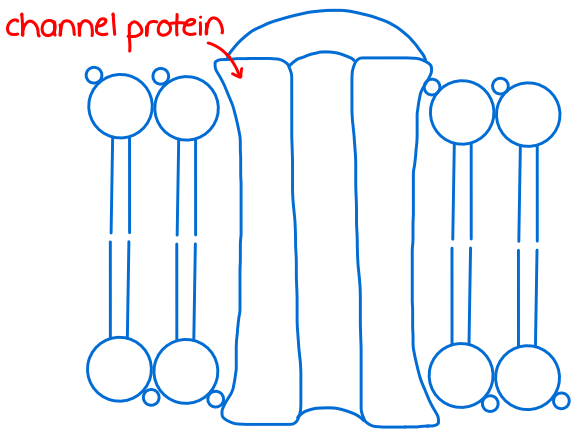

- Antibodies
  - → made by plasma cells in the immune response
  - → bind to one specific antigen




- Structural proteins
  - → provide strength and support
  - → long polypeptide chains parallel to each other or twisted round each other into a rope shape
  - → chains held together with cross-links e.g. disulfide bonds.
  - → examples: collagen in connective tissue, and keratin in hair and nails




- Transport proteins
  - → found in cell membranes
  - → channel proteins and carrier proteins
  - → hydrophobic and hydrophilic regions of the protein help it to form its shape




## Biuret test for proteins

1) Add a few drops of sodium hydroxide solution to the sample to make it alkaline.
2) Add copper (II) sulfate solution.
3) If protein is present: solution turns purple.
4) If no protein is present: solution stays blue.

The colour change is subtle in this test.

# PROTEINS

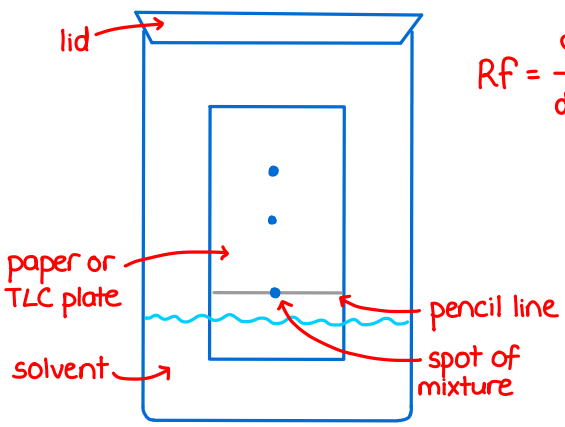
## Separating and identifying amino acids using chromatography

- Chromatography separates particles based on their different affinities for the stationary phase vs the mobile phase
  - → particles can adsorb to stationary phase
  - → particles are soluble in the mobile phase
- Paper chromatography → stationary phase is paper, mobile phase is normally water
- Thin layer chromatography (TLC) → stationary phase is silica gel, mobile phase is normally an organic solvent
- Only R groups differ in amino acids → R group determines the interaction with the stationary and mobile phases
- A more soluble amino acid spends more time in the mobile phase

Molecules adsorb to the stationary phase, not absorb.

Method:

1) Draw a pencil line near the bottom of the paper or TLC plate
   - → do not use pen because it will dissolve in the solvent
2) Put concentrated spots of your mixtures onto the pencil line
   - → make sure the spots are spread out so they do not merge
3) Place the paper or TLC plate into the solvent
   - → make sure the pencil line is above the solvent so the spots do not dissolve into the solvent
   - → make sure the paper or TLC plate is supported to be kept level and at a constant height
4) Put a lid on the container to prevent the solvent from evaporating
5) Allow the solvent to move up until it is nearly at the top, then remove paper or TLC plate
   - → mark the solvent front with pencil to avoid confusion when it dries out
   - → stain the paper or TLC plate with ninhydrin to visualise the amino acids
6) Calculate the Rf values
   - → repeat the experiment and find the average Rf value to increase accuracy
7) Look up Rf values in standard reference tables to find out which amino acids you have in the mixture




$$Rf = \frac{\text{distance moved by solute}}{\text{distance moved by solvent}}$$

You will come across chromatography again in Topic 5.

Chromatography can also be used to separate carbohydrates, vitamins and nucleic acids.

- Safety considerations
  - → wear gloves to protect skin and to prevent contamination (your hands might have amino acids on them!)
  - → work in a fume cupboard if using organic solvents or ninhydrin

# ENZYMES

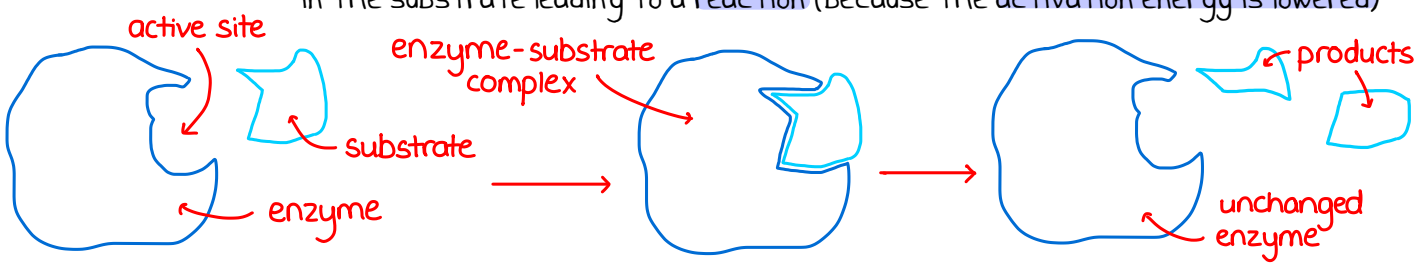
## Function of enzymes

- Enzymes are proteins with a specific tertiary structure
- The active site is complementary to a specific substrate
- Biological catalysts → speed up a reaction by lowering the activation energy
- Can be intracellular (act inside cells) or extracellular (act outside cells)
- Determine structure and function of cells and whole organisms

Very few substrates would have enough energy to react at body temperature without an enzyme.

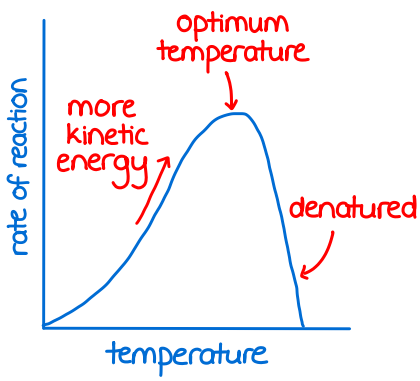
## Induced fit model

- Replaced the lock and key model
  - → lock and key model suggested enzyme and substrate are exactly complementary shapes
  - → substrate binds to active site to form an enzyme-substrate complex (active site does not change shape)
- Induced fit model → active site is not fully complementary to the substrate before the reaction
  - → shape of the active site changes slightly as the substrate binds to become complementary and form the enzyme-substrate complex
  - → formation of an enzyme-substrate complex puts stress on bonds or forms bonds in the substrate leading to a reaction (because the activation energy is lowered)



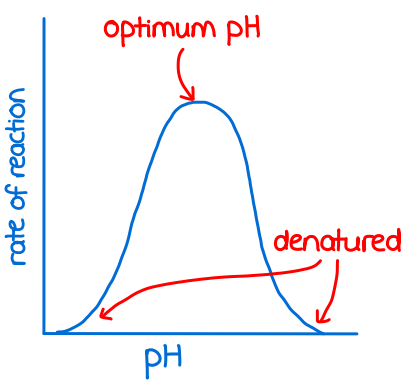

## Temperature

- As temperature increases, enzyme and substrate have more kinetic energy so there are more frequent successful collisions and more enzyme-substrate complexes form
- After the optimum temperature, the enzyme becomes denatured
  - → too much kinetic energy breaks the hydrogen bonds and ionic bonds between amino acid R groups
  - → the shape of the active site changes so it is no longer complementary to the substrate
  - → enzyme-substrate complexes cannot form
- Effects of low temperature are reversible, high temperature are not




## pH

- Enzymes are denatured above and below the optimum pH
  - → $H^+$ (acidic) or $OH^-$ (alkali) ions interfere with the hydrogen bonds and ionic bonds between amino acid R groups, so the active site changes shape

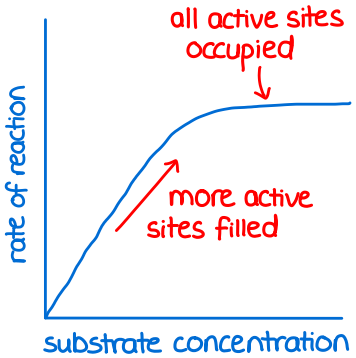


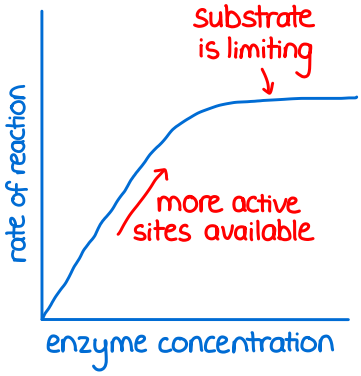

Remember that a change in the primary structure often results in a change in the tertiary structure.

# ENZYMES

## Enzyme or substrate concentration

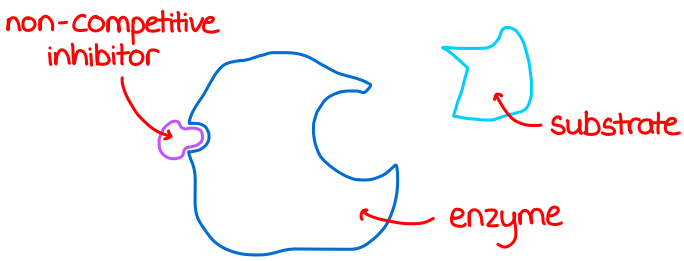
- Increasing enzyme or substrate concentration increases the frequency of collisions between enzyme and substrate, so more enzyme-substrate complexes form
- Eventually all available active sites are filled, or substrate concentration becomes limiting

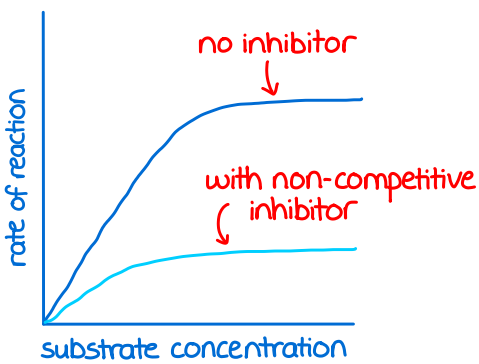


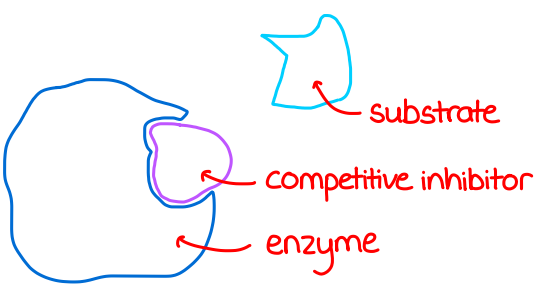

## Inhibitors

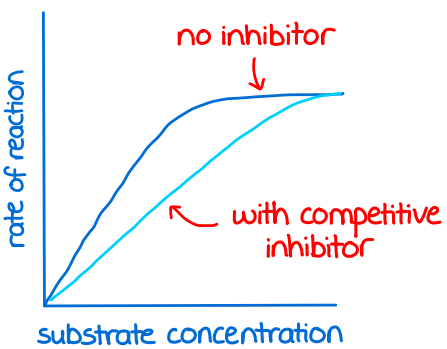
- Non-competitive inhibitors
  - → bind to an allosteric site on the enzyme (not the active site)
  - → alter the tertiary structure so the active site changes shape
  - → active site no longer complementary to substrate, so less enzyme-substrate complexes form
  - → increasing substrate concentration does not increase the rate of reaction




- Competitive inhibitors
  - → have a very similar tertiary structure to the substrate
  - → bind to the active site in place of the substrate so less enzyme-substrate complexes form
  - → increasing substrate concentration will increase the rate of reaction (because the substrate "wins" the competition more often)



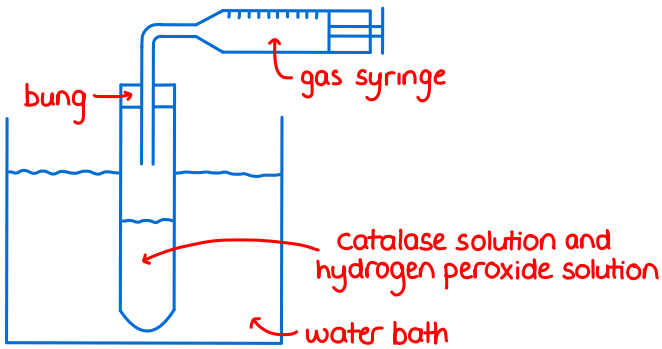



## Investigating enzyme-controlled reactions

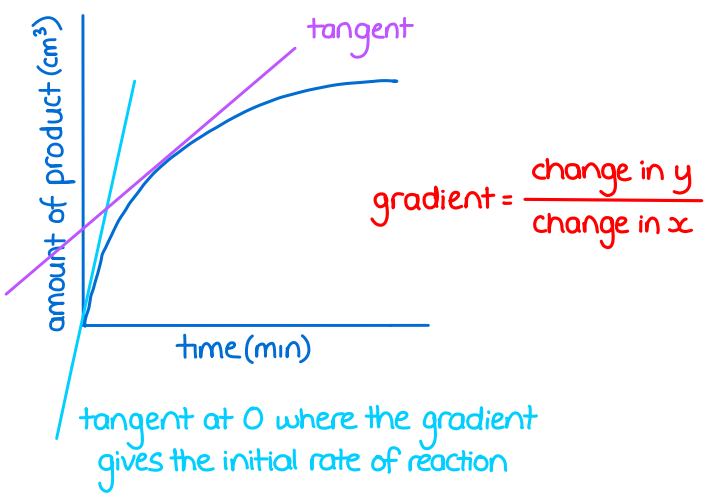
- Many different possibilities for what to investigate and which reaction to use
- Only change one variable at a time and control all other variables e.g. temperature, pH
- Example: the enzyme catalase converts hydrogen peroxide to oxygen and water
  - → could use a gas syringe to record the volume of oxygen gas produced over time and repeat at different temperatures
  - → have a negative control experiment with the same volume and concentration of denatured catalase to show no oxygen is produced without the active enzyme
- Possible control variables
  - → volume of the substrate solution
  - → concentration of the substrate solution
  - → volume of the enzyme solution
  - → concentration of the enzyme solution
  - → temperature of the solutions (use a water bath)
  - → pH of the solutions (use a buffer solution)

Control variables depend on the investigation - be specific to the question.

Always repeat the experiment and calculate a mean.




- Rate of reaction can be calculated by finding the gradient of a line
  - → draw a tangent at zero to find the initial rate of reaction
  - → draw a tangent at any part of the curve to find the rate at that specific point
- Initial rate of reaction is highest because plenty of substrate is available
  - → initially very frequent collisions and many enzyme-substrate complexes form
  - → rate slows as the substrate is used up and there are less frequent collisions
  - → the reaction stops when there is no substrate left




In this example the units of rate would be $cm^3\ min^{-1}$ ($cm^3$ per min).

# DNA AND RNA

- DNA = deoxyribonucleic acid, RNA = ribonucleic acid

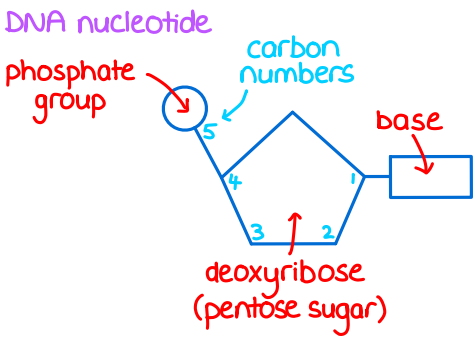
## DNA nucleotides

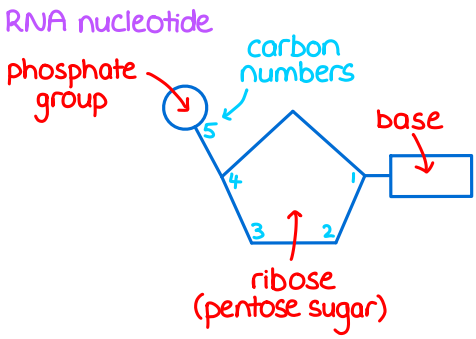
- The monomers of DNA
- Consist of a pentose sugar (deoxyribose), a nitrogen-containing base, and a phosphate group
- The base can be adenine (A), thymine (T), guanine (G) or cytosine (C)

## RNA nucleotides

- The monomers of RNA
- Consist of a pentose sugar (ribose), a nitrogen-containing base, and a phosphate group
- The base can be adenine (A), uracil (U), guanine (G) or cytosine (C)

- Both DNA and RNA nucleotides form polynucleotides → phosphodiester bonds form between the phosphate group of one and the deoxyribose of the next in a condensation reaction



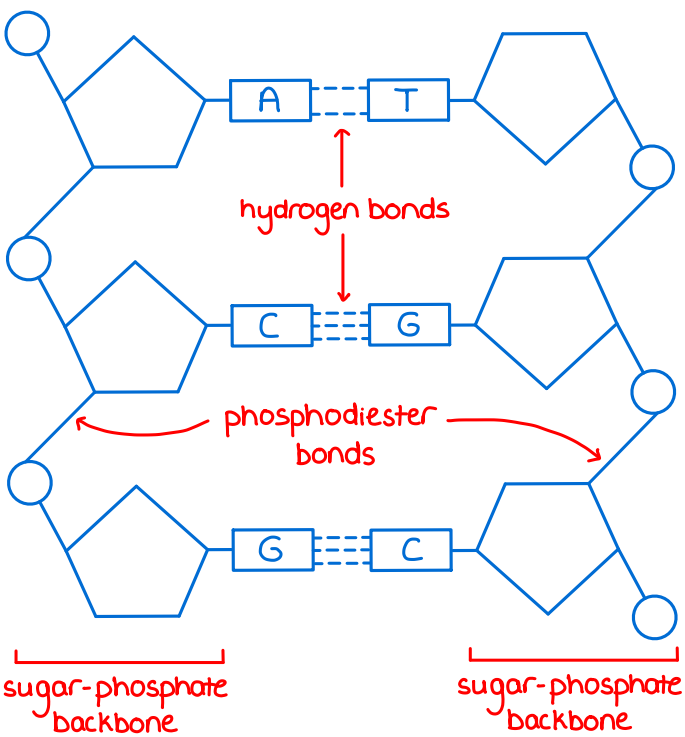




A pentose sugar has five carbon atoms.

Bases are organic (contain carbon).

## DNA

- Stores genetic information
- A long double-stranded polymer of DNA nucleotides
- Two antiparallel polynucleotide chains twisted into a double-helix structure
- The phosphate groups and pentose sugars form the sugar-phosphate backbone
- The bases join by complementary base pairing
  - → A pairs to T with two hydrogen bonds
  - → C pairs to G with three hydrogen bonds
  - → holds the two strands together
  - → there is always the same amount of A and T and the same amount of C and G in DNA



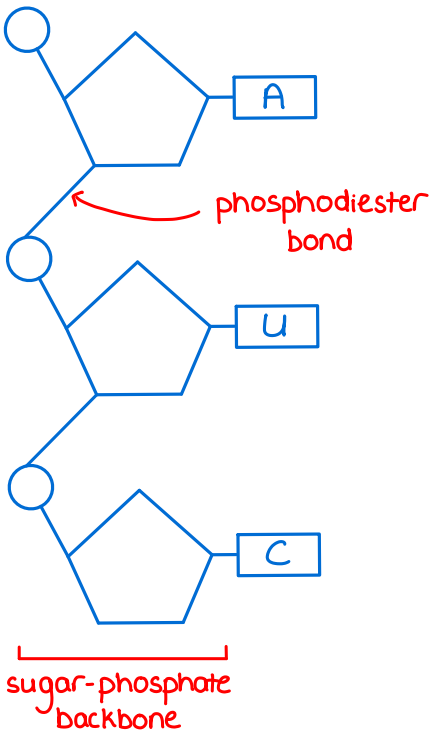

The double-helix was discovered by Watson and Crick in 1953. Scientists first doubted that DNA stored complex genetic information because of its simple structure.

Antiparallel means the strands run in opposite directions: one is 5' to 3', the other is 3' to 5'.

# DNA AND RNA

## RNA

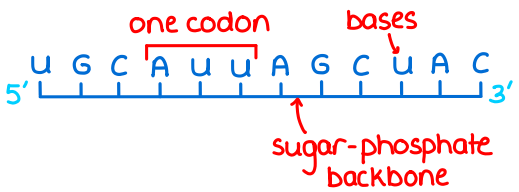
- A single polynucleotide chain with different functions
- Single-stranded polymer of RNA nucleotides
  → shorter polymer than DNA
- The phosphate groups and pentose sugars form the sugar-phosphate backbone
- Complementary base pairing happens in transcription and translation → A pairs with U, C pairs with G
- Messenger RNA (mRNA) transfers genetic information from DNA to the ribosomes
- Transfer RNA (tRNA) brings amino acids to the ribosomes
- Ribosomal RNA (rRNA) is part of the structure of ribosomes along with proteins




Transcription and translation are covered in Topic 4.

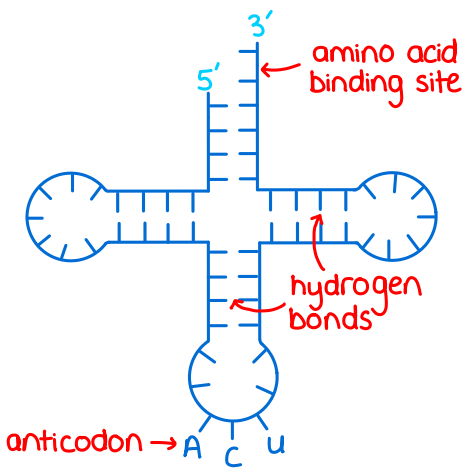
## Messenger RNA

- A single linear polynucleotide strand made during transcription
- Can be different lengths
- Much shorter than DNA → can fit through the nuclear pores
- A three base sequence is a codon




## Transfer RNA

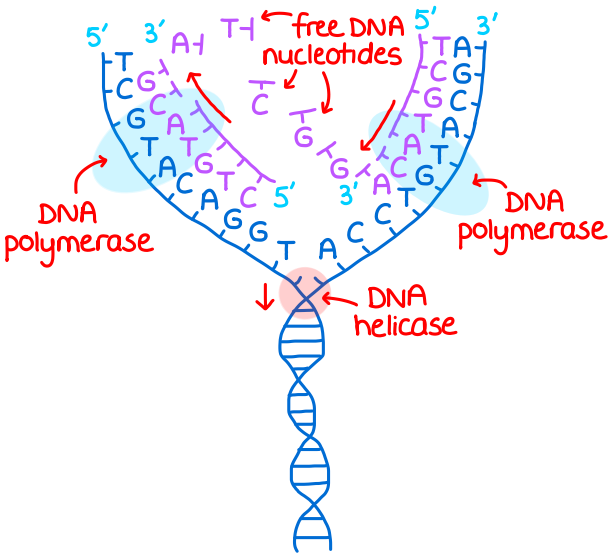
- A single polynucleotide strand folded into a cloverleaf shape
- Hydrogen bonds between complementary bases hold the shape
- Contains an amino acid binding site and an anticodon
- Found in the cytoplasm



# DNA REPLICATION

## Semi-conservative replication

- The Watson-Crick model shows that DNA replication is semi-conservative
  - → each new DNA molecule has one strand from the original DNA molecule and one newly synthesised strand
- Both strands of the DNA act as template strands and determine the order of bases
- Complementary base pairing makes sure that DNA replication is accurate

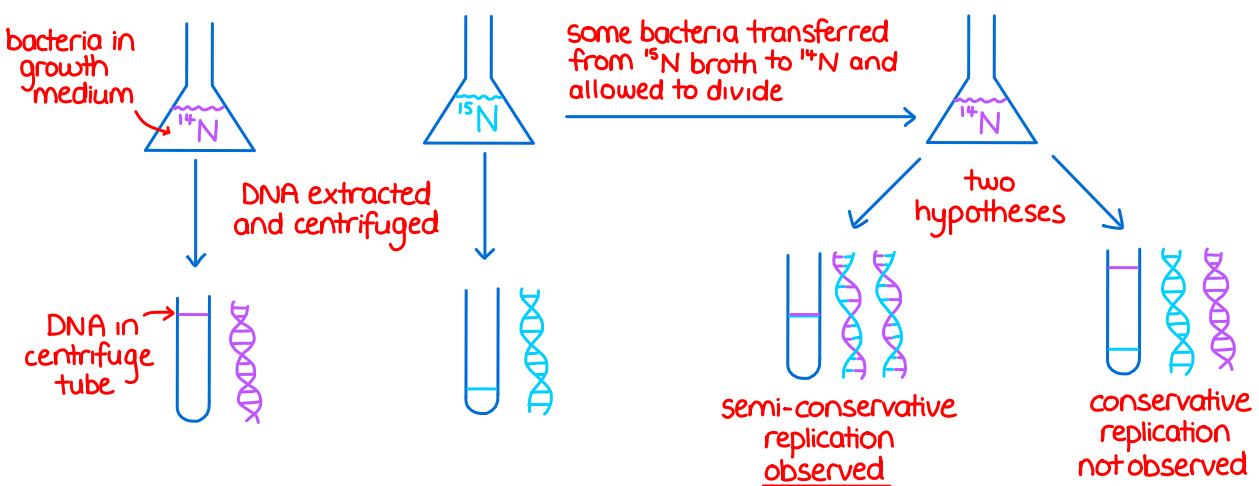



1) DNA helicase breaks weak hydrogen bonds between complementary bases so the double helix unwinds and the two strands separate.
2) Free DNA nucleotides are attracted to exposed bases on the two template strands and pair up by complementary base pairing (A with T, C with G).
3) DNA polymerase joins the adjacent nucleotides with phosphodiester bonds in condensation reactions to form the sugar-phosphate backbone.

DNA polymerase can only move in the 5' to 3' direction because it can only add nucleotides at the 3' end of a strand due to enzyme specificity.

## Evidence from Meselson and Stahl

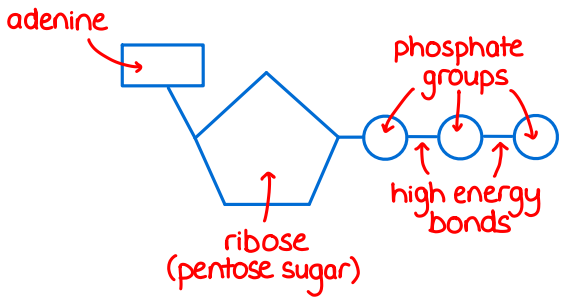
- Used bacteria grown in growth medium with either heavy nitrogen ($^{15}N$) or light nitrogen ($^{14}N$)
  - → bacteria use the nitrogen to make bases for DNA
  - → DNA with only $^{15}N$ settles out near the bottom of a centrifuge tube, DNA with only $^{14}N$ settles out near the top
  - → if DNA has one $^{15}N$ strand and one $^{14}N$ strand it settles out in the middle
- Experiment showed that DNA replication is semi-conservative, not conservative



# ATP

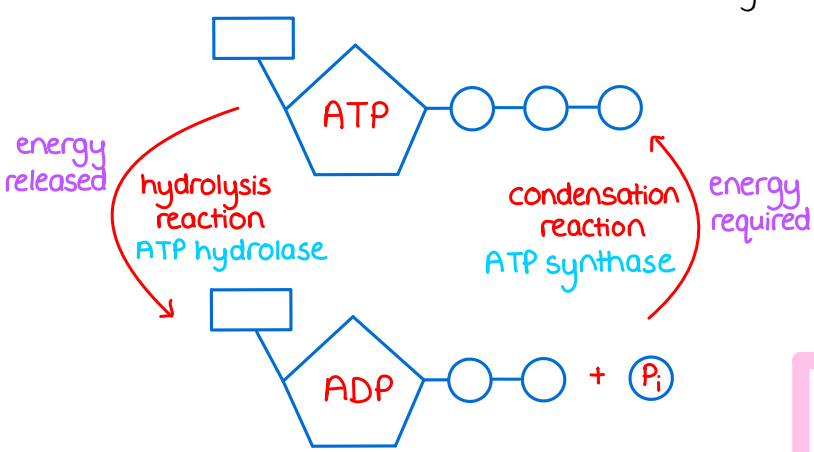
## Structure

- ATP = adenosine triphosphate
- A nucleotide derivative (a modified nucleotide)
- A ribose sugar bound to adenine (a base) and three phosphate groups
- Energy stored in high energy bonds between phosphate groups
- Made during respiration using energy released from glucose




## Function

- Provides energy for other reactions
- Diffuses to areas the cell where energy is needed and does not leave the cell
- Hydrolysed by ATP hydrolase to ADP (adenosine diphosphate) and $P_i$ (inorganic phosphate) in a hydrolysis reaction → energy is released
- Energy can be used straight away in a coupled reaction → a reaction requiring energy which is coupled to ATP hydrolysis
- Immediate energy supply → little energy is lost as heat
- Rapidly re-synthesised by ATP synthase in a condensation reaction → happens in respiration and photosynthesis
- $P_i$ can be used in a phosphorylation reaction → a phosphate group is added to another molecule
  → phosphorylation can make compounds more reactive or change their shape



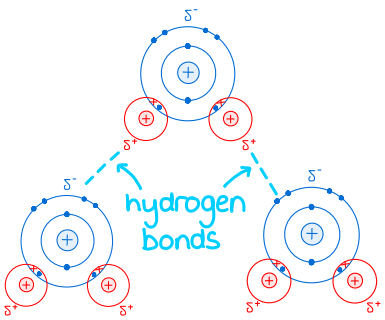

Remember that water is produced in condensation and used in hydrolysis.

An example of a reaction coupled to ATP hydrolysis is the synthesis of sucrose from glucose and fructose.

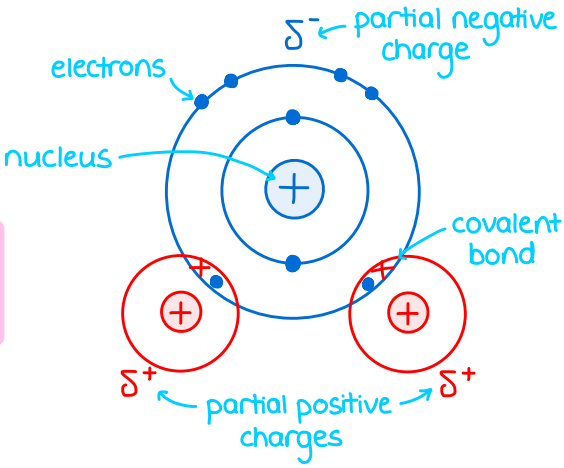
# WATER

## Structure

- Two hydrogen atoms share electrons with one oxygen atom (covalent bonds)
- Electrons pulled towards oxygen → hydrogens have a partial positive charge
- Oxygen has two lone (unshared) electron pairs → oxygen has a partial negative charge
- Partial charges attract other water molecules
  → this forms hydrogen bonds

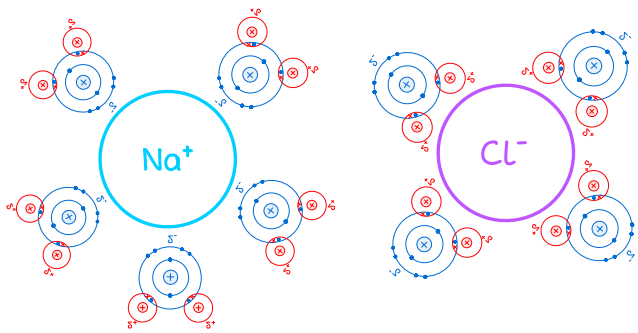



Water is a polar molecule.




## Properties

- Good solvent → polar water molecules surround and are attracted to ions or other polar molecules, so these substances can dissolve and be transported




This is important to enable metabolic reactions to occur in water.

- Strong cohesion → polar water molecules stick together with hydrogen bonds
  → water flows well e.g. it forms an unbroken column in the xylem vessels
  → has a high surface tension so forms droplets, and can support small organisms

- Useful metabolite → used in metabolic reactions e.g. condensation and hydrolysis reactions

- Large latent heat of vaporisation → lots of energy needed to break hydrogen bonds
  → uses lots of heat energy to evaporate so it has a cooling effect

- High specific heat capacity → hydrogen bonds can absorb lots of energy
  → can buffer changes in temperature because it can lose or gain a lot of energy without changing temperature (good for aquatic organisms)

Sweat forms droplets on the skin then uses heat energy from the skin to evaporate, cooling you down in the process.

Specific heat capacity = energy needed to raise temperature of 1g of a substance by 1°C.

# INORGANIC IONS

- Ions have electric charge → anions have negative charge
  → cations have positive charge
- Inorganic ions are soluble → dissolved in the fluids of an organism and in the cytoplasm

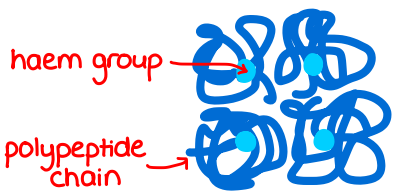
## Examples

- Phosphate ions → used to phosphorylate other molecules which often makes them more reactive
  → phosphorylation of enzymes could change the shape of the active site to make it complementary to the substrate
  → found in ATP, DNA, RNA and phospholipids
  → the hydrophilic part of the phospholipid bilayer
  → used in photosynthesis and respiration to phosphorylate ADP to ATP

$PO_4^{3-}$

- Iron ions → $Fe^{2+}$ is part of the haem group in haemoglobin that binds oxygen to form oxyhaemoglobin ($Fe^{2+}$ becomes $Fe^{3+}$ until oxygen is released again)

$Fe^{2+}$




- Sodium ions → used in co-transport to help glucose and amino acids cross cell membranes e.g. glucose absorption in the small intestine
  → needed to create an action potential in neurones
  → affect the water potential of cells

$Na^+$

- Hydrogen ions → more $H^+$ ions present means a lower (more acidic) pH
  → enzyme activity is affected by pH (and therefore $H^+$ ion concentration)

$H^+$

You will come across many other ions in biology e.g. $Ca^{2+}$ ions at synapses.