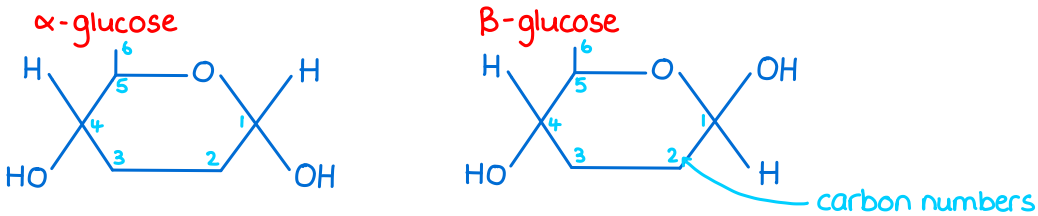
CARBOHYDRATES

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

Glucose

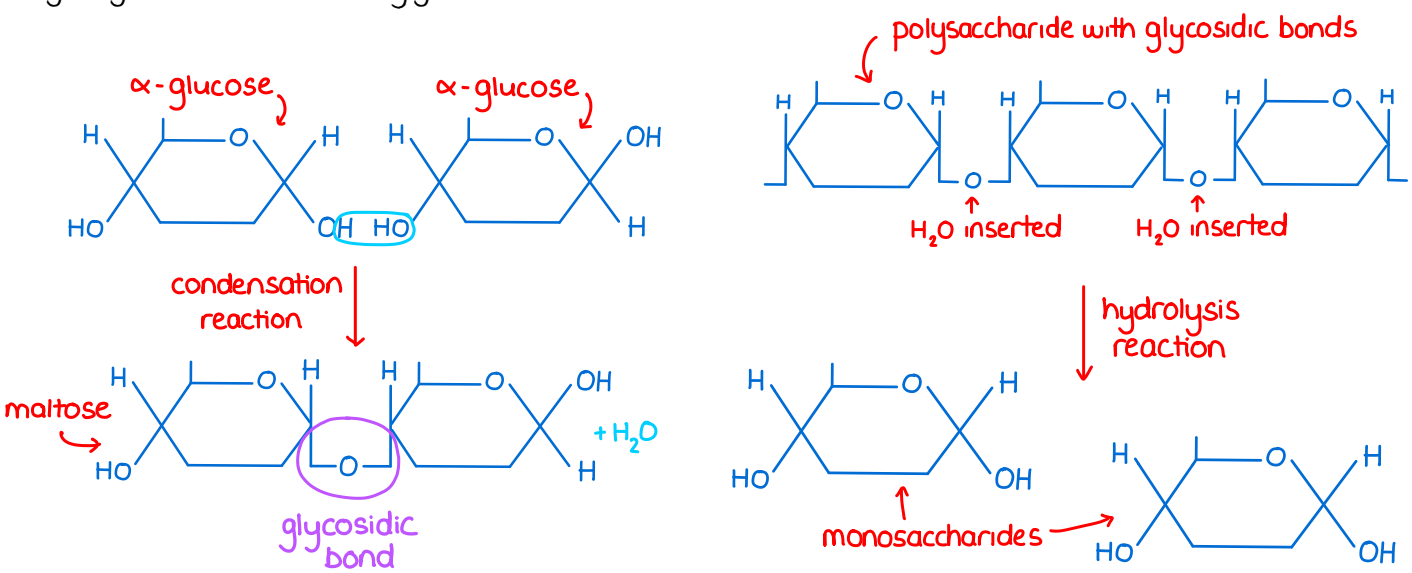
- A monosaccharide with the formula $C_6H_{12}O_6$
- A hexose monosaccharide (six carbon atoms) in a ring structure
- Soluble in water → easily transported
- Main energy source for animals and plants
  - → chemical bonds store lots of energy
  - → glucose is a substrate for respiration
- Two isomers: 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, condensation and hydrolysis reactions

- Condensation reaction → two molecules join to form a new chemical bond and a water molecule is eliminated
- Hydrolysis reaction → a water molecule is used and the chemical bond is broken
- Condensation reactions form glycosidic bonds between monosaccharides to create disaccharides and polysaccharides
- Hydrolysis reactions break glycosidic bonds between monosaccharides



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

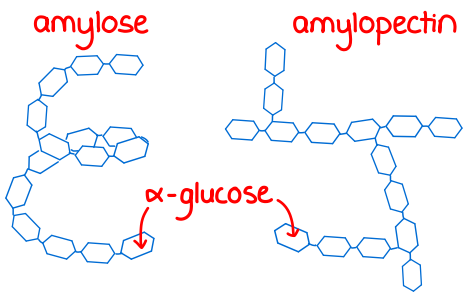
- Large polymers of monosaccharides joined with glycosidic bonds
- Starch and glycogen are large energy storage molecules which cannot leave cells

Starch

- Glucose storage in plant cells
  → hydrolysed when glucose is needed for respiration
- 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 provide more ends so glucose can be released quickly by enzymes, and it is compact

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.

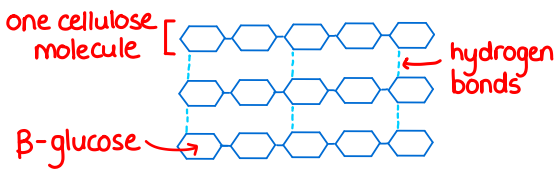


Glycogen

- Glucose storage in animal cells
  → easily hydrolysed when glucose is needed for respiration
- 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

- Gives strength to plant and algal cell walls
- Unbranched long and straight β-glucose polysaccharide (1,4 glycosidic bonds)
  → alternate β-glucose monomers are inverted
- Cellulose molecules linked with many hydrogen bonds to form strong rigid microfibrils

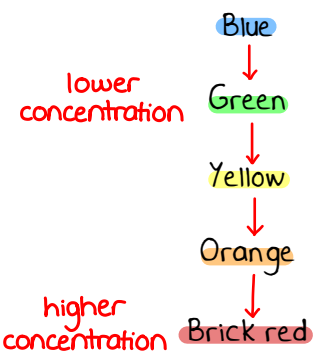


Benedict's test for sugars

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

1) Add an excess of blue Benedict's reagent to liquid sample in a 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) Hydrolyse non-reducing sugars to monosaccharides by adding dilute HCl to new tube of sample and heating in a water bath set to boil.
6) Neutralise by adding sodium hydrogencarbonate.
7) Repeat steps 1 and 2.
8) If coloured precipitate now forms, non-reducing sugars are present.
9) If the solution is still blue, neither type of sugars are present.

Colour of precipitate depends on the sugar concentration:



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

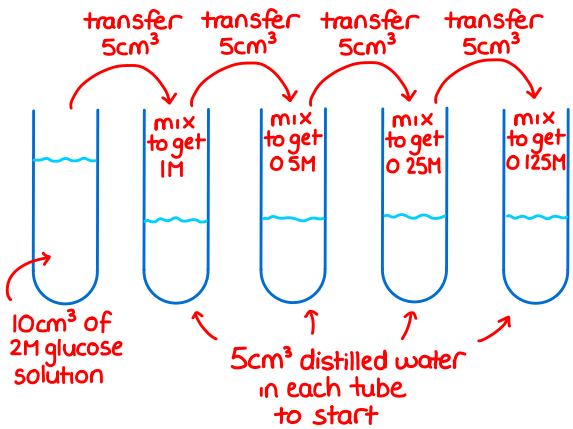
CARBOHYDRATES

Using a colorimeter

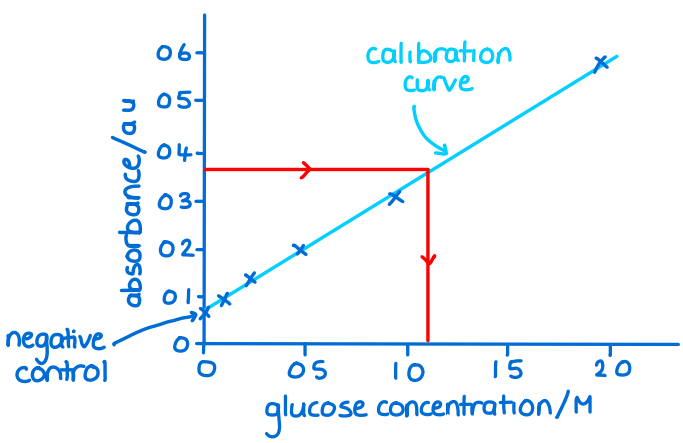
- A more accurate and quantitative way to measure glucose concentration after the Benedict's test
  - → judging colour is subjective and more affected by human error
- A colorimeter measures absorbance of light
  - → a lower absorbance reading means more light passes through the sample
- Higher glucose concentration in a sample results in more precipitate bring formed in the Benedict's test
  - → the absorbance of the solution containing the precipitate is measured in a colorimeter
  - → the precipitate blocks the light and results in a higher absorbance reading
  - → the higher the absorbance, the higher the glucose concentration
- Can quantify the concentration of glucose by producing a calibration curve:

1) Start with a solution of known glucose concentration (e.g. 2M) and create a serial dilution. In this case we are diluting the glucose solution by a factor of two each time.

Make sure to mix well at each stage.



2) Set up the colorimeter by selecting the red filter and zeroing to distilled water. This ensures the absorbance values are comparable.
3) Carry out the Benedict's test (detailed on previous page) on all serial dilution tubes, samples, and a negative control (distilled water). Control the volume and concentration of Benedict's solution used and control the time in the boiling water bath.
4) Measure the absorbance of each using the colorimeter. Produce a calibration curve as below from the serial dilution.
5) For the samples with unknown glucose concentration, use the calibration curve to read across from the absorbance value and down to find the concentration. This is called interpolation.

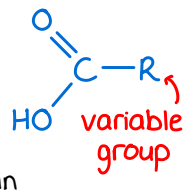


This could be done for other reducing sugars as well as glucose.

There is a different type of Benedict's reagent that works differently so produces a different calibration curve. You will come across this in a Topic 6 practical.

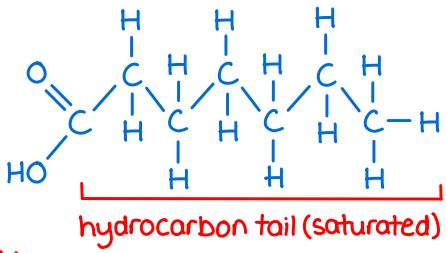
LIPIDS

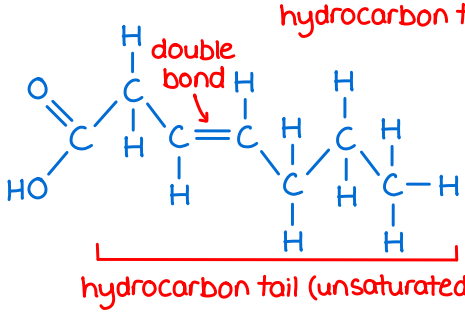
- Lipids contain carbon, hydrogen and oxygen



Fatty acids

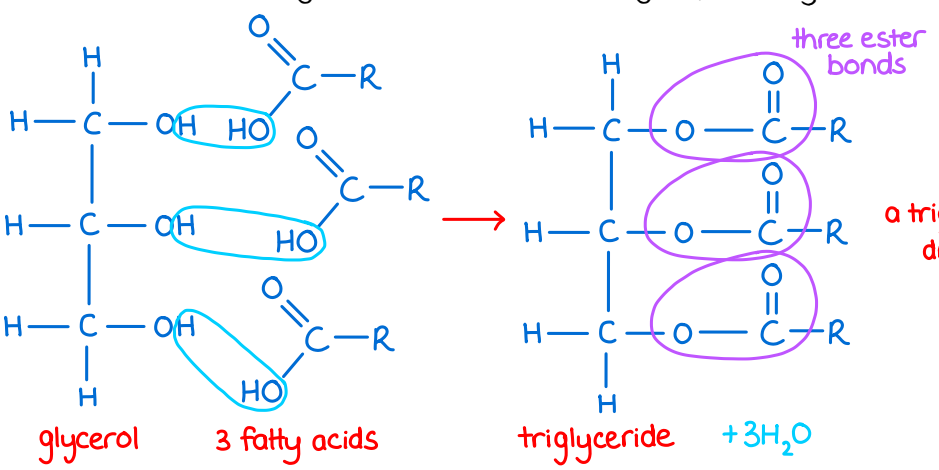
- Have a variable R group → the hydrocarbon tail
- Saturated fatty acids have no double C=C bonds in the hydrocarbon tail
- Unsaturated fatty acids have one or more double C=C bonds in the hydrocarbon tail so the chain kinks
- Hydrocarbon tails are hydrophobic (insoluble in water)



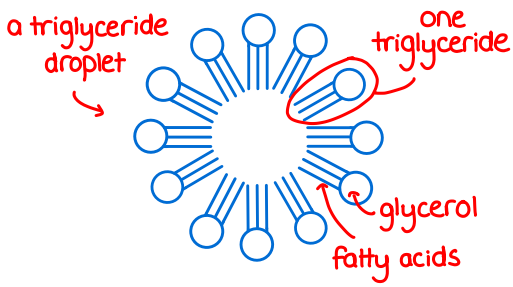


Triglycerides

- One molecule of glycerol bonded 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 storage molecules → 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
  → do not form a bilayer because there is no hydrophilic region

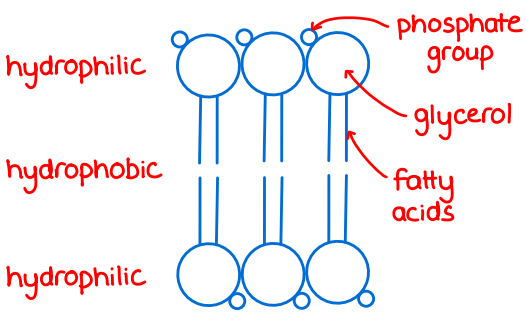


Know how to relate structure to function.



Phospholipids

- One molecule of glycerol bonded to two fatty acids and a phosphate group
- Phosphate groups are hydrophilic, fatty acids are hydrophobic
- Form a bilayer in cell membranes → hydrophilic phosphate groups attract water either side of the bilayer
- See Topic 2 for more on the phospholipid bilayer



Emulsion test for lipids

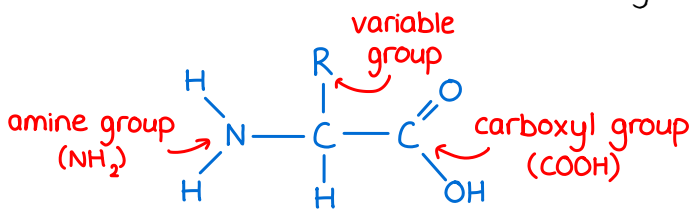
1) Mix food sample with ethanol and shake.
2) Pour mixture into water.
3) If lipid is present → milky emulsion forms.
4) If no lipid is present → stays clear.

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

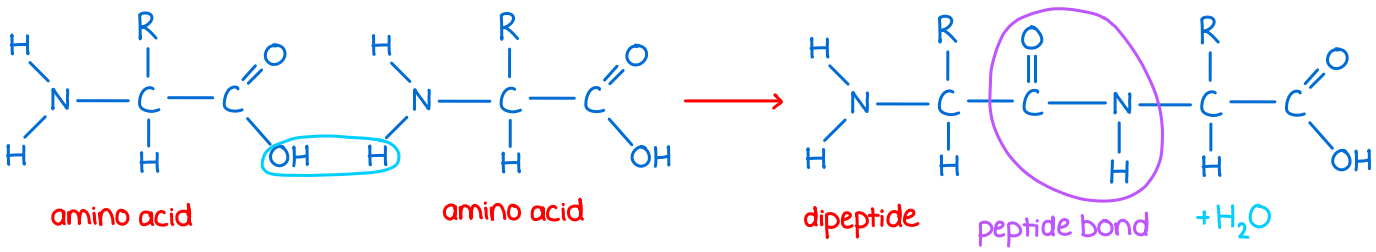
A monomer is a smaller unit from which larger polymers are made.



Amino acids can be joined in any order and length.

Peptide bonds and dipeptides

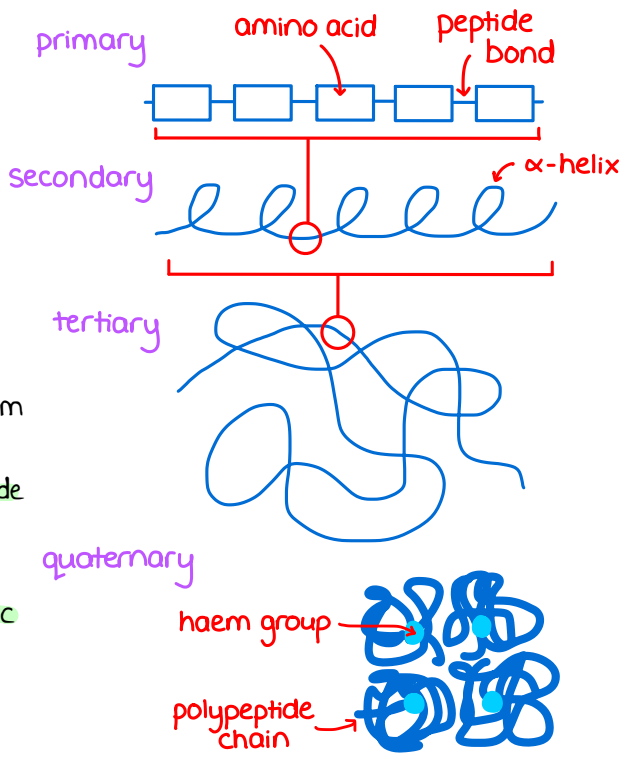
- A condensation reaction joins two amino acids with a peptide bond
  - → produces a dipeptide and a molecule of water
- A hydrolysis reaction breaks a peptide bond by adding a molecule of water
- A polypeptide is a polymer of amino acids (a long chain of amino acids joined with peptide bonds)



Protein structure

- Primary structure
  - → order of amino acids in the polypeptide chain joined with peptide bonds
  - → determines the secondary, tertiary and quaternary structures
- Secondary structure
  - → hydrogen bonds form between N-H and C=O parts of different amino acids in the polypeptide
  - → alpha helix (coiled structure) or beta pleated sheet (folded structure) forms due to hydrogen bonding
- Tertiary structure
  - → further folding
  - → hydrogen bonds, ionic bonds, and disulfide bridges form between R groups of amino acids in the polypeptide
  - → final structure for proteins made from one polypeptide
- Quaternary structure
  - → some proteins have more than one polypeptide chain
  - → polypeptides held together with hydrogen bonds, ionic bonds, and disulfide bridges
  - → example opposite: haemoglobin has four polypeptide chains

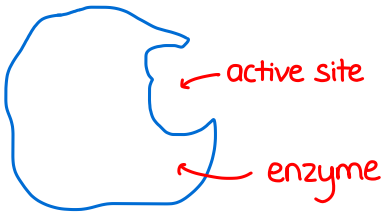
Cysteine contains sulfur in the R group.



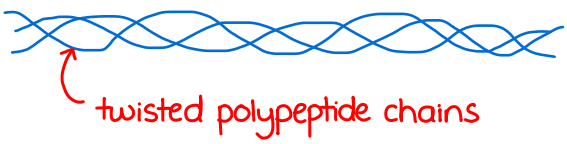
PROTEINS

Functions of proteins

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

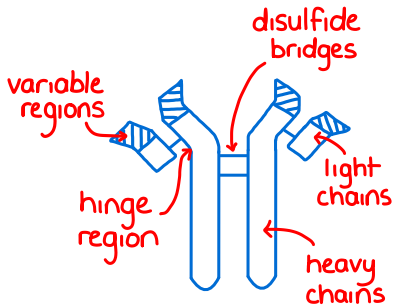


- Structural proteins
  - → provide strength and support
  - → long polypeptide chains parallel to each other or twisted round each other into a rope shape
  - → chains held together with cross-links e.g. disulfide bridges
  - → examples: collagen in connective tissue, and keratin in hair and nails

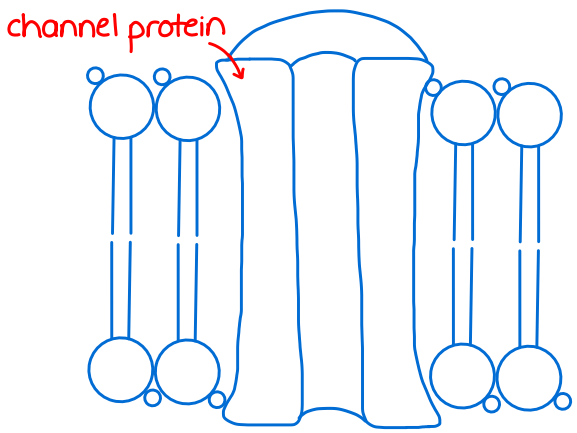


The different R groups make some protein regions hydrophilic and some hydrophobic.

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



- Transport proteins
  - → found in cell membranes
  - → channel proteins and carrier proteins
  - → hydrophobic and hydrophilic regions of the protein help it to form its shape
  - → e.g. in a channel protein, hydrophobic regions turn towards hydrophobic hydrocarbon tails but hydrophilic regions form a channel through the membrane



Biuret test for proteins

1) Add Biuret reagent.
2) If protein is present: solution turns purple.
3) If no protein is present: solution stays blue.

ENZYMES

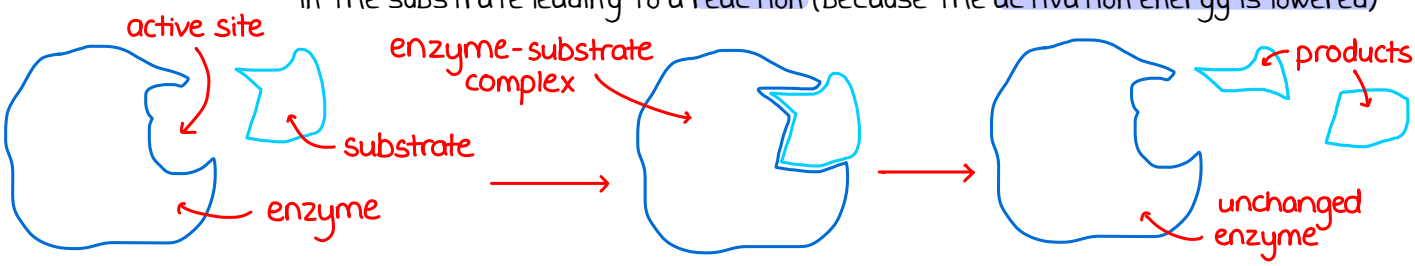
Function of enzymes

- 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
  → are not used up during the reaction
- 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.

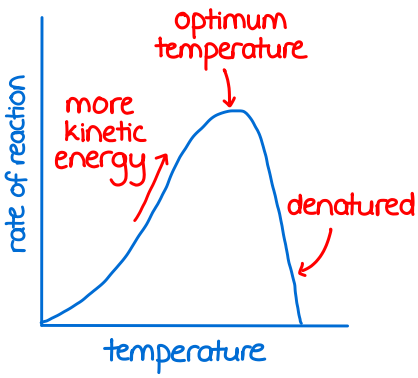
Induced fit model

- 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)



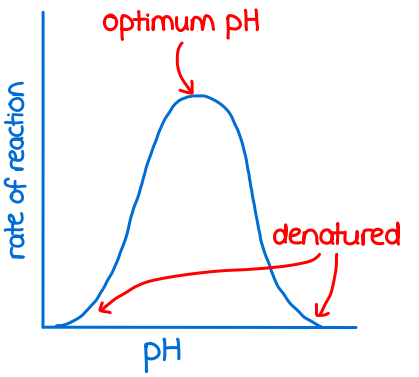
Temperature

- 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 tertiary structure of the enzyme changes, and the active site changes shape 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

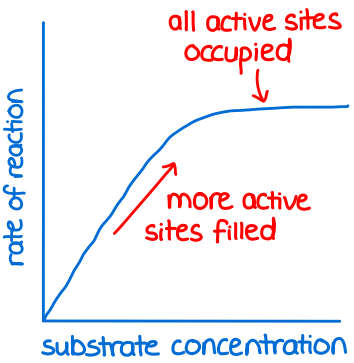
- Enzymes are denatured above and below the optimum pH
  → H^+ (acidic) or OH^- (alkali) ions interfere with the hydrogen bonds and ionic bonds between amino acid R groups
  → the tertiary structure of the enzyme changes, and the active site changes shape so it is no longer complementary to the substrate
  → enzyme-substrate complexes cannot form

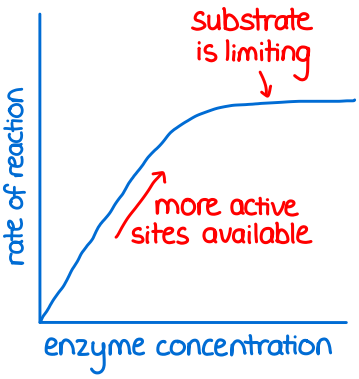


ENZYMES

Enzyme or substrate concentration

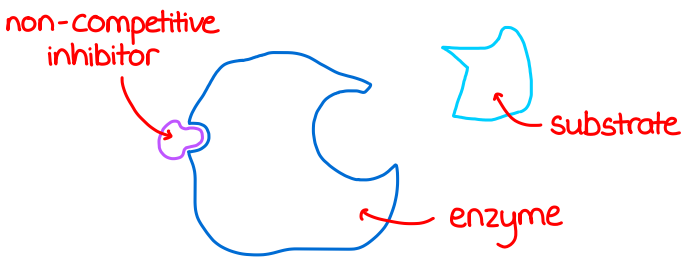
- Increasing enzyme or substrate concentration increases the frequency of collisions between enzyme and substrate, so more enzyme-substrate complexes form
- Eventually all available active sites are filled, or substrate concentration becomes limiting

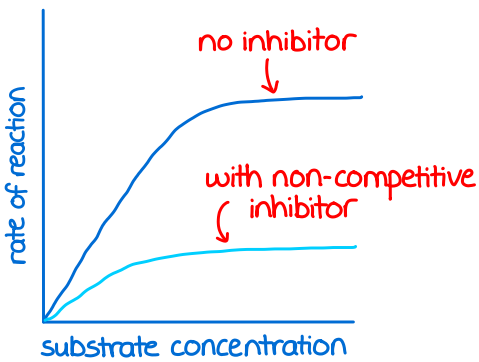




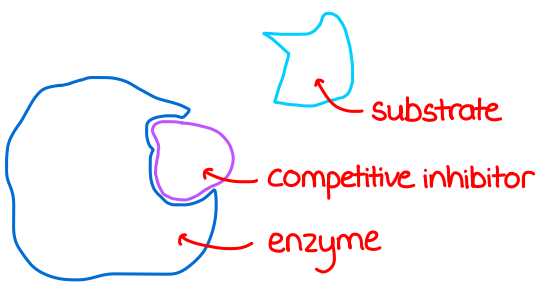
Inhibitors

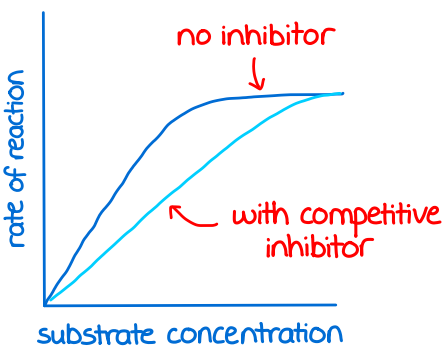
- Non-competitive inhibitors
  - → bind to an allosteric site on the enzyme (not the active site)
  - → alter the tertiary structure so the active site changes shape
  - → active site no longer complementary to substrate, so less enzyme-substrate complexes form
  - → increasing substrate concentration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





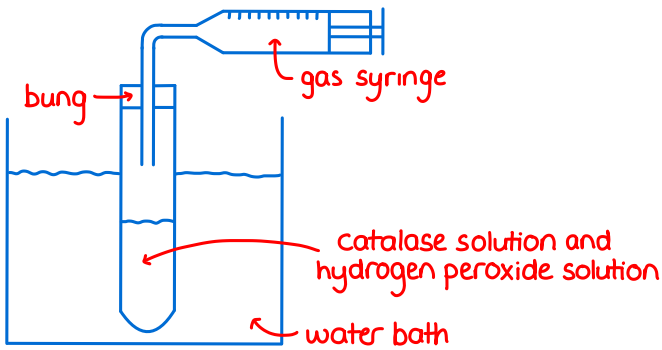
ENZYMES

Investigating enzyme-controlled reactions

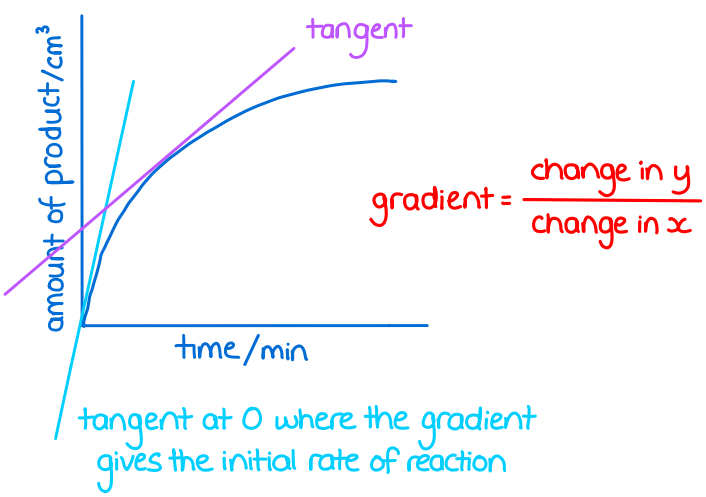
- Many different possibilities for what to investigate and which reaction to use
- Only change one variable at a time and control all other variables which could affect enzyme activity
- Example: the enzyme catalase converts hydrogen peroxide to oxygen and water
  - → could use a gas syringe to record the volume of oxygen gas produced over time and repeat at a range of 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 between enzyme and substrate and many enzyme-substrate complexes form
  - → rate decreases as the substrate concentration decreases and there are less frequent collisions
  - → the reaction stops when there is no substrate left



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

DNA AND RNA

- DNA = deoxyribonucleic acid, RNA = ribonucleic acid

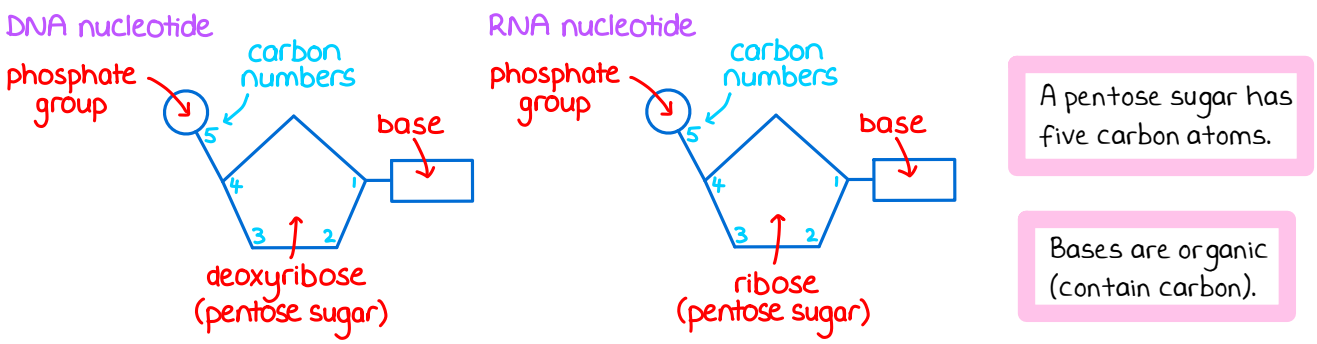
DNA nucleotides

- 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

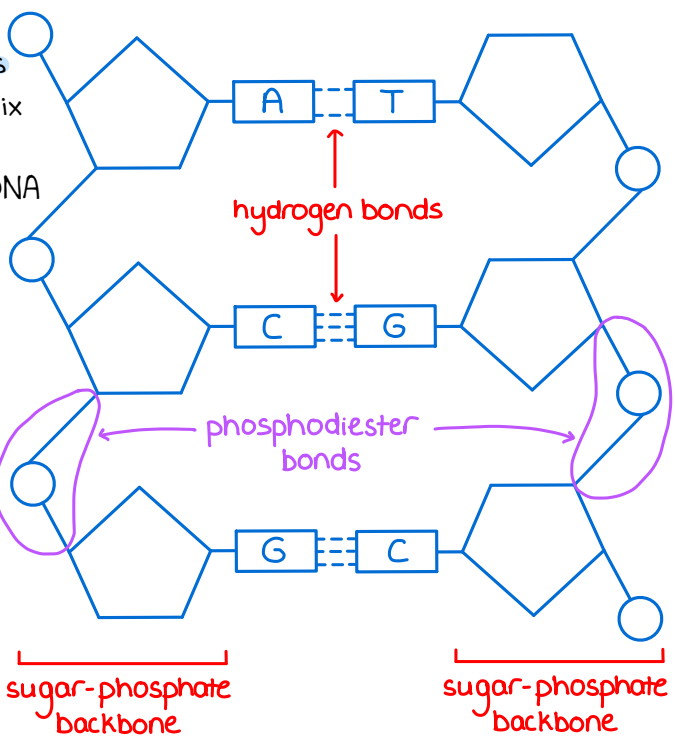


A pentose sugar has five carbon atoms.

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
  - → adenine pairs thymine with two hydrogen bonds
  - → cytosine pairs guanine with three hydrogen bonds
  - → holds the two strands together in the double-helix
  - → always the same amount of adenine and thymine and the same amount of cytosine and guanine in DNA



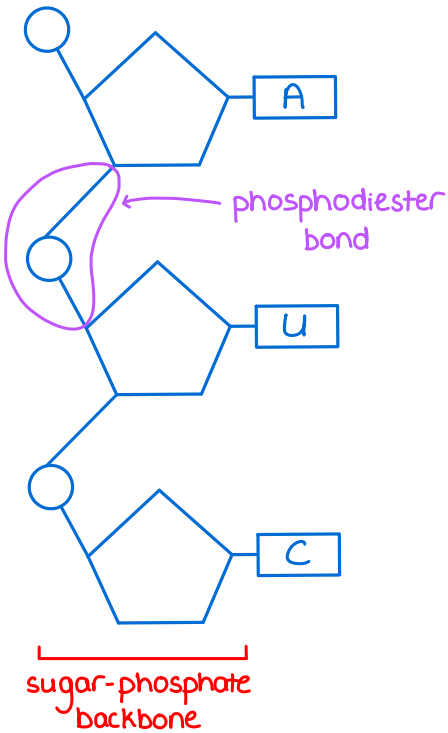
The double-helix was discovered by Watson and Crick in 1953. Scientists first doubted that DNA stored complex genetic information because of its simple structure.

Antiparallel means the strands run in opposite directions: one is 5' to 3', the other is 3' to 5'. These numbers relate to the position of the carbon atoms in the pentose sugar.

DNA AND RNA

RNA

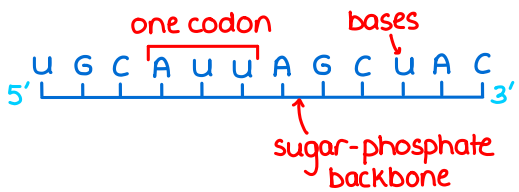
- A single polynucleotide chain with different functions
- Single-stranded polymer of RNA nucleotides
  → shorter polymer than DNA
- The phosphate groups and pentose sugars form the sugar-phosphate backbone
- Complementary base pairing happens in transcription and translation → adenine pairs with uracil,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



Transcription and translation are covered in Topic 4.

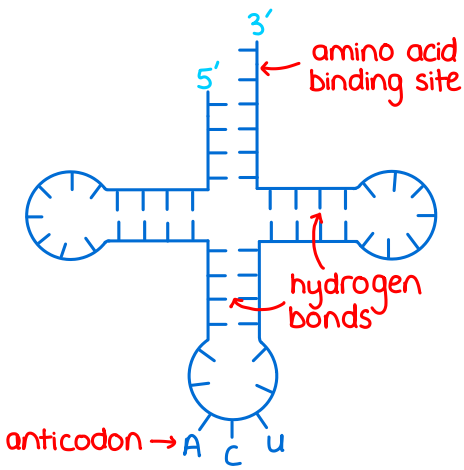
Messenger RNA

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



Transfer RNA

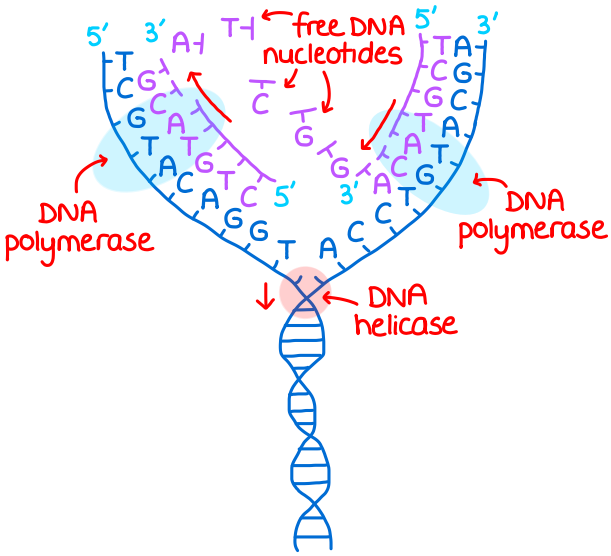
- A single polynucleotide strand folded into a cloverleaf shape
- Hydrogen bonds between complementary bases hold the shape
- Contains an amino acid binding site and an anticodon
- Found in the cytoplasm



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

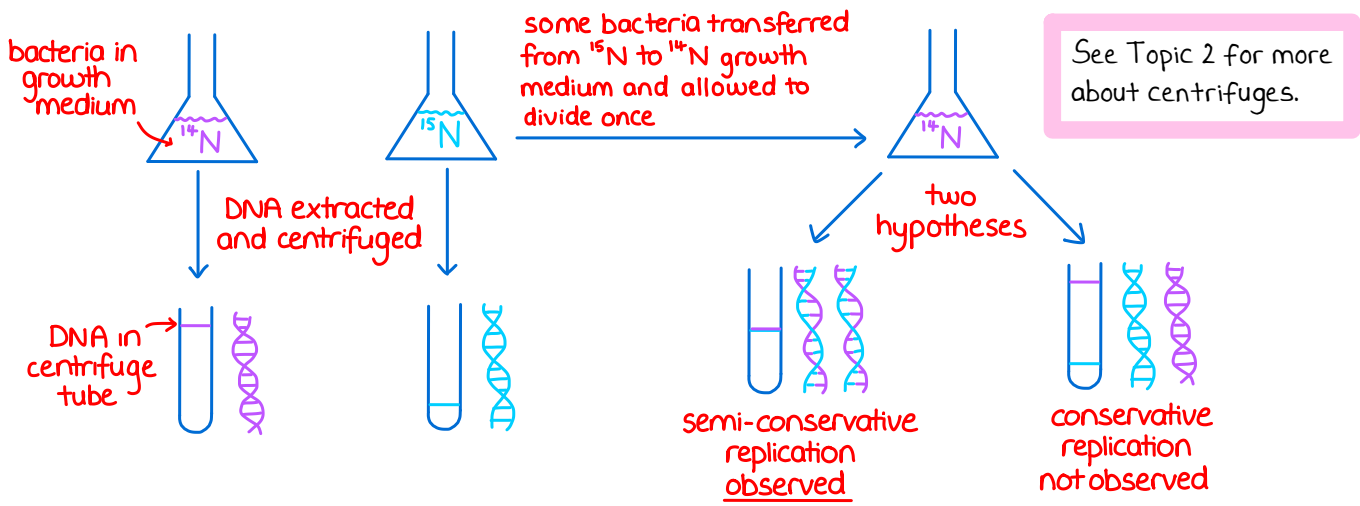


1) DNA helicase breaks weak hydrogen bonds between complementary bases so the double-helix unwinds and the two strands separate.
2) Free DNA nucleotides are attracted to exposed bases on the two template strands and pair up by complementary base pairing (adenine with thymine, cytosine with guanine).
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 containing either heavy nitrogen (^{15}N) or light nitrogen (^{14}N)
  - → bacteria use the available nitrogen to make bases for DNA
  - → DNA containing only ^{15}N settles out near the bottom of a centrifuge tube, DNA containing only ^{14}N settles out near the top because it is lighter
  - → if DNA contains one ^{15}N strand and one ^{14}N strand it settles out in the middle of the tube
- Experiment showed that DNA replication is semi-conservative, not conservative

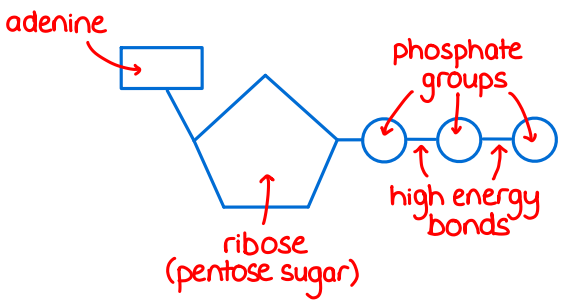


> See Topic 2 for more about centrifuges.

ATP

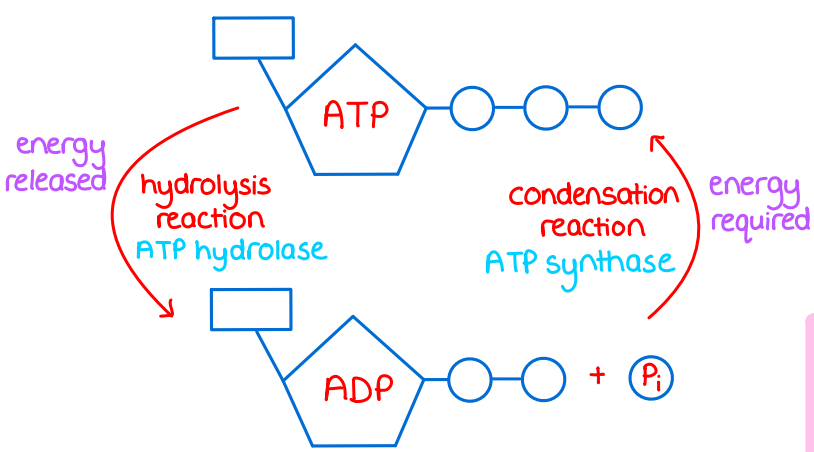
Structure

- 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
- Mainly made during respiration using energy released from glucose



Function

- Provides an immediate energy supply for other reactions and cellular processes
- Diffuses to areas of 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 immediately uses the energy released from ATP hydrolysis
  - → only a little energy is lost as heat
- Rapidly re-synthesised by ATP synthase in a condensation reaction → happens in respiration and photosynthesis
- Phosphate groups (P_i) released from ATP hydrolysis can be used in phosphorylation reactions
  - → a phosphate group is added to another molecule
  - → phosphorylation can make compounds more reactive or change their shape



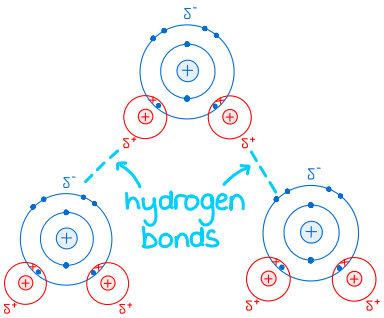
Remember that water is produced in condensation and used in hydrolysis.

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

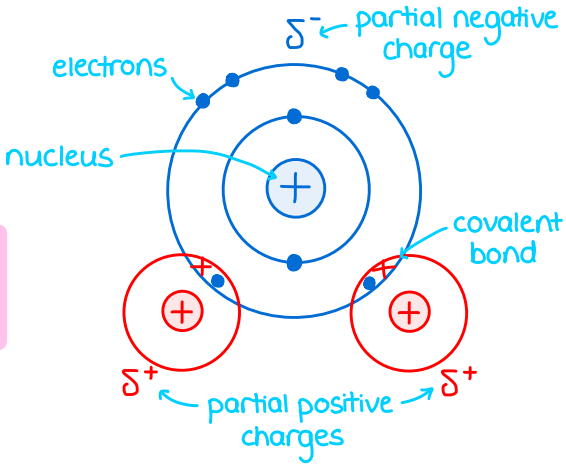
WATER

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

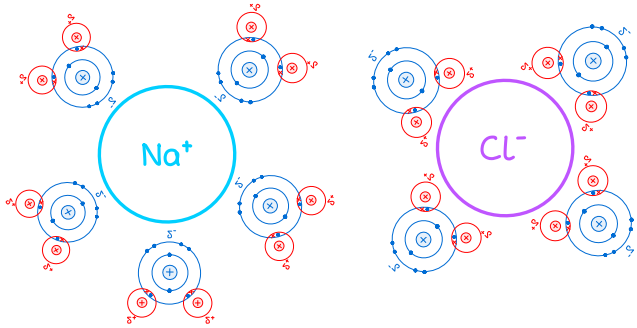


Water is a polar molecule.



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

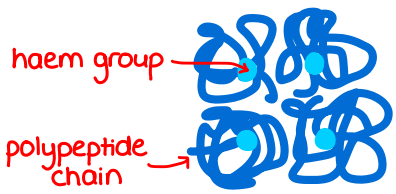
Examples

- Phosphate ions → used to phosphorylate other molecules which often makes them more reactive
  → phosphorylation of enzymes could change the tertiary structure and therefore 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-surface membranes e.g. glucose and amino acid absorption in the small intestine
  → needed to create an action potential in neurones
  → affect the water potential of cells

Na^+

- Hydrogen ions → a higher concentration of H^+ ions means a lower (more acidic) pH
  → enzyme activity is affected by pH (and therefore H^+ concentration)
  → a high H^+ concentration could denature proteins

H^+

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