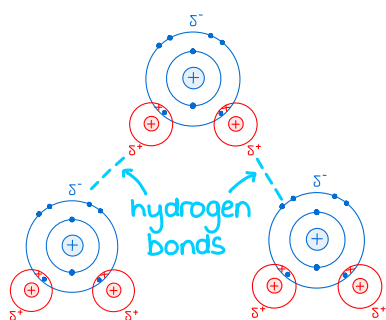
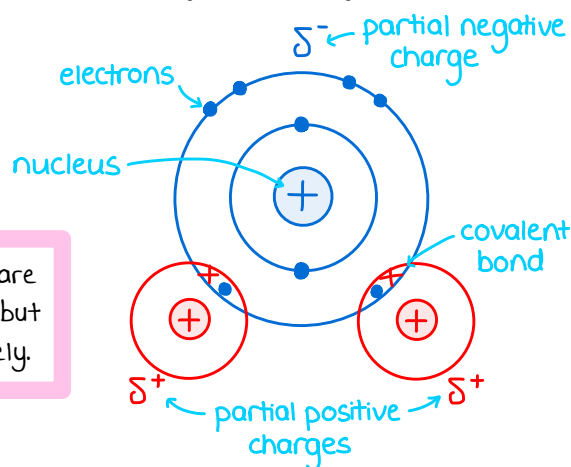


## Structure

- 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
- A polar molecule
- Partial charges attract other water molecules → this forms hydrogen bonds

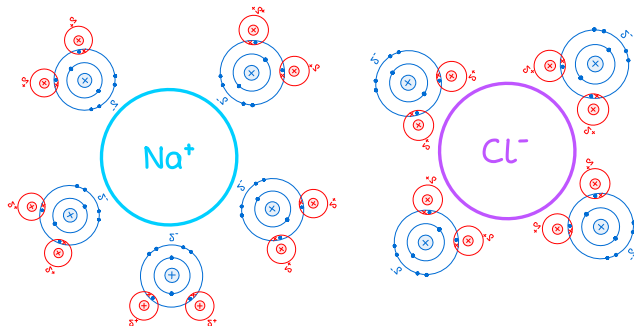


Hydrogen bonds are weak individually but strong collectively.



## Properties

- Good solvent → polar water molecules surround and are attracted to ions or other polar molecules, so they can dissolve and be transported e.g. glucose dissolves in blood plasma



If a molecule has polar OH groups it will dissolve in water. More free OH groups = more soluble.

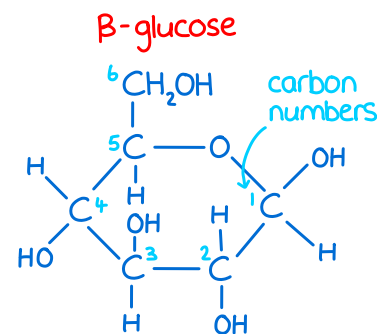
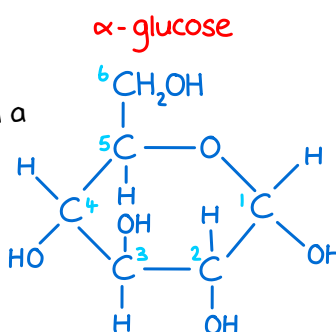
- Strong cohesion → polar water molecules attracted to each other with hydrogen bonds
  - water flows well e.g. it forms an unbroken chain in the xylem vessels
  - has a high surface tension so forms droplets, and can support small organisms
- Adhesion → water molecules can stick to other surfaces e.g. the walls of the xylem vessels
- Useful metabolite → used in metabolic reactions e.g. condensation and hydrolysis reactions
- High 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)
- Ice is less dense than liquid water → ice floats and forms an insulating layer on top of water so aquatic organisms can survive
- Transparent → allows light to pass through to reach aquatic plants
- Density allows organisms to float → gives buoyancy
- Water is a good habitat because of its properties

See Module 3 for more about water transport in plants.

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

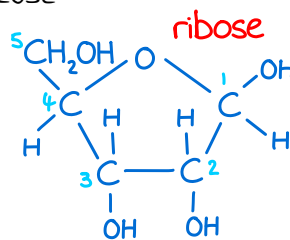
## Glucose

- 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:  $\alpha$ -glucose and  $\beta$ -glucose → H and OH groups on carbon 1 inverted in  $\beta$ -glucose



## Ribose

- A monosaccharide with the formula  $C_5H_{10}O_5$
- A pentose monosaccharide (five carbon atoms) in a ring structure
- A component of RNA nucleotides and ATP

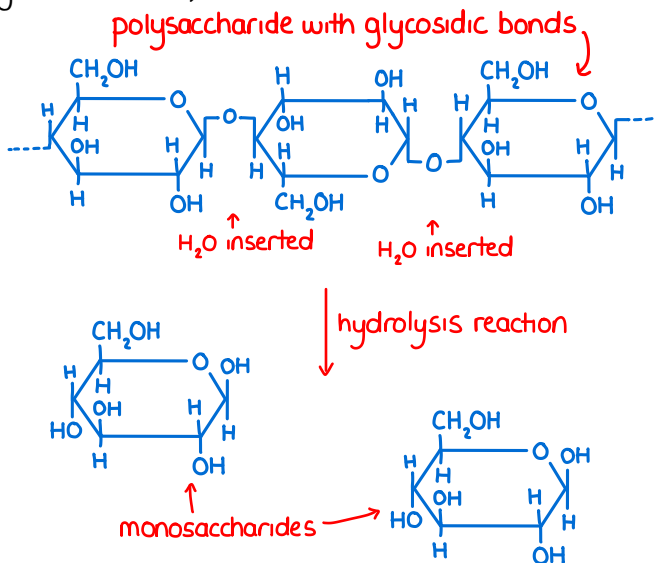
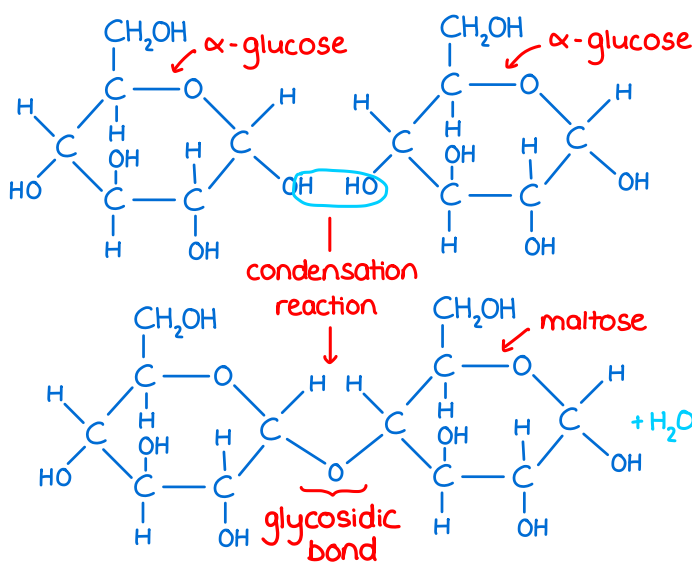


Monosaccharides are small soluble carbohydrate monomers. They also include fructose and galactose.

## 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 inserted and the chemical bond is broken (reverse of a condensation reaction → breaks glycosidic bonds)

Monosaccharides and disaccharides are sugars.



## Disaccharides

- Two monosaccharides joined together with a glycosidic bond in a condensation reaction
- Soluble in water
- Maltose =  $\alpha$ -glucose +  $\alpha$ -glucose
- Sucrose =  $\alpha$ -glucose + fructose
- Lactose =  $\beta$ -glucose + galactose → this disaccharide has a  $\beta$ -glycosidic bond

## Polysaccharides

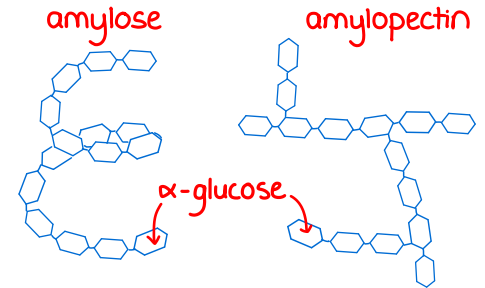
- Large insoluble polymers of monosaccharides joined with glycosidic bonds
- Starch and glycogen are large metabolically inactive energy storage molecules which cannot leave cells

## 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  $\alpha$ -glucose polysaccharide ( $\alpha$ ,4 glycosidic bonds)
  - coiled structure so is compact
- Amylopectin → branched  $\alpha$ -glucose polysaccharide ( $\alpha$ ,4 and  $\alpha$ ,6 glycosidic bonds) so is compact
  - 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 brownly-orange to blue-black.

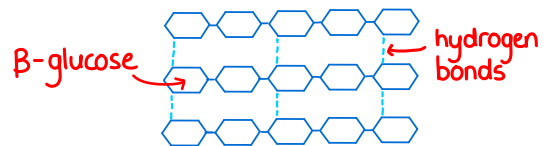


## Glycogen

- 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  $\alpha$ -glucose polysaccharide ( $\alpha$ ,4 and  $\alpha$ ,6 glycosidic bonds) → more ends so glucose can be released quickly by enzymes, and more energy can be stored in a small space
- More branched and compact than amylopectin

## Cellulose

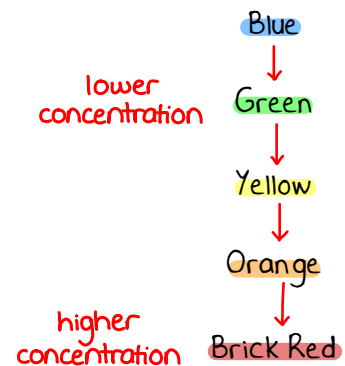
- Found in plant cell walls to give strength
- Unbranched long and straight  $\beta$ -glucose polymers ( $\beta$ ,4 glycosidic bonds)
- Chains linked with many hydrogen bonds to form inflexible microfibrils with high tensile strength



## 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:

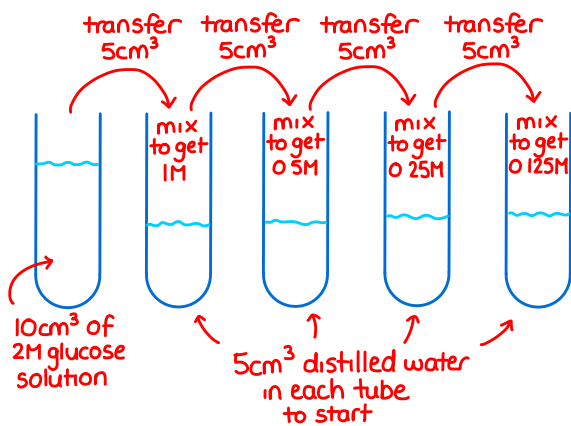


You could filter, dry, and weigh the precipitate to make more accurate comparisons.

### Using a colorimeter

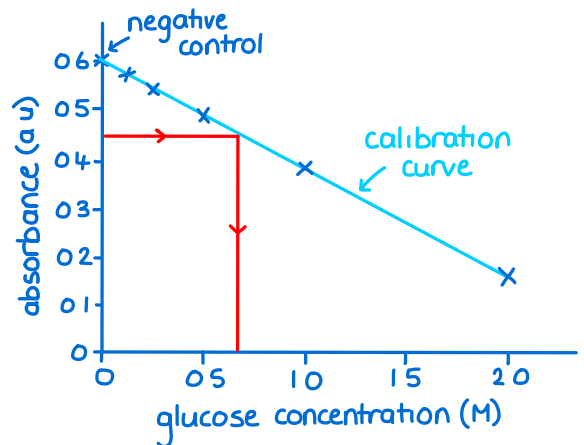
- A more accurate and quantitative way to measure glucose concentration after the Benedict's test (judging colour is subjective and more affected by human error)
- When the precipitate is filtered out of the solution, the solution left is the Benedict's reagent → more glucose = more precipitate = the 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
- 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. Use the red filter on the colorimeter to measure absorbance.



Now we can measure the absorbance of a glucose solution with unknown concentration and use the calibration curve to predict the concentration. This is called interpolation.

### Reagent test strips and biosensors

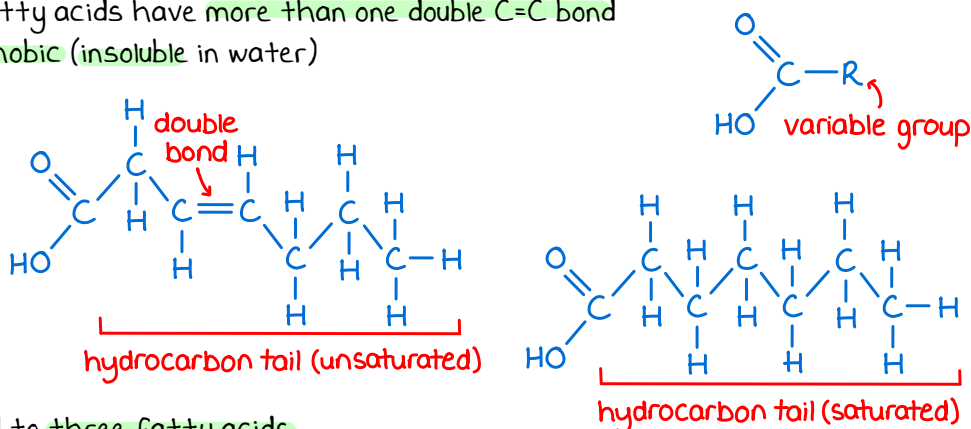
- Glucose test strips have enzymes which will test for the presence of glucose → insert the test strip into the liquid sample, then compare the colour to a colour chart to find the concentration of glucose
- Biosensors are electrical devices which can measure concentration of glucose using an enzyme and electrodes
- Useful for testing glucose concentration in urine and blood
- Other molecules can be tested for using these methods

- Lipids contain carbon, hydrogen and oxygen

## Fatty acids

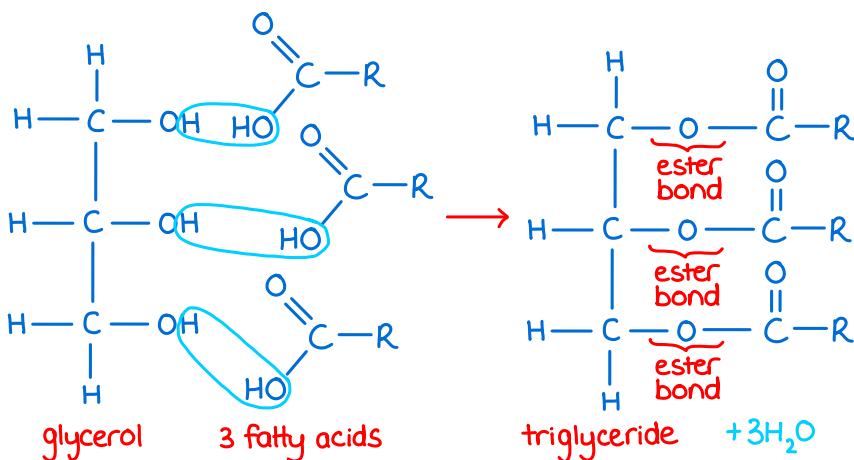
- Have a variable R group → the hydrocarbon tail
- Saturated fatty acids → no double C=C bonds in the hydrocarbon tail
  - more tightly packed together so higher melting point e.g. butter
- Unsaturated fatty acids → one or more double C=C bonds in the hydrocarbon tail so the chain kinks
  - kinks mean molecules are less tightly packed together so lower melting point e.g. vegetable oils
  - monounsaturated fatty acids have one double C=C bond, polyunsaturated fatty acids have more than one double C=C bond
- Hydrocarbon tails are hydrophobic (insoluble in water)

Saturated fatty acid  
general formula =  
 $C_nH_{(2n+1)}COOH$

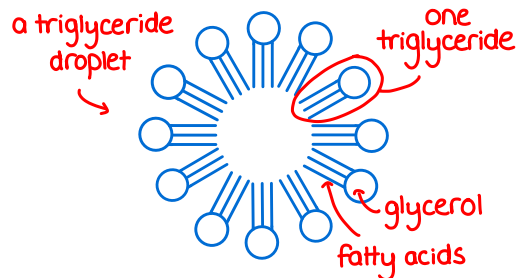


## Triglycerides

- 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 (this can also be called esterification)
- 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.



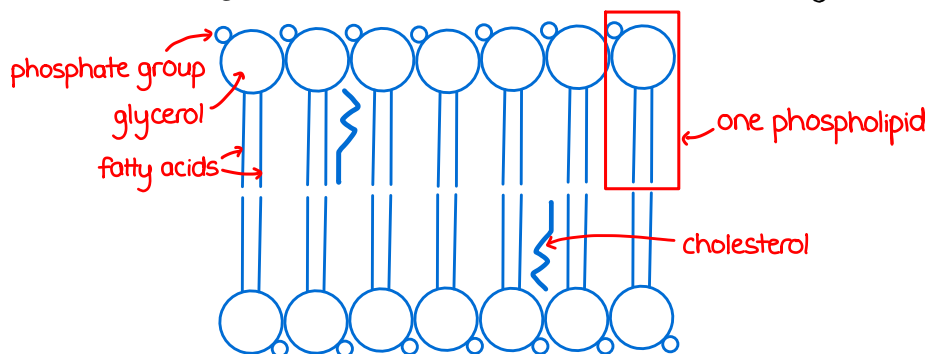
Fat stored as triglycerides also provides thermal insulation and protects organs.

**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

**Cholesterol**

- Hydrocarbon ring (with a polar hydroxyl group) and a hydrocarbon tail
- Small and flat shape → fits between phospholipids and binds to hydrophobic tails
- Reduces fluidity of eukaryotic cell membranes → phospholipids are more tightly packed so the membrane is more rigid

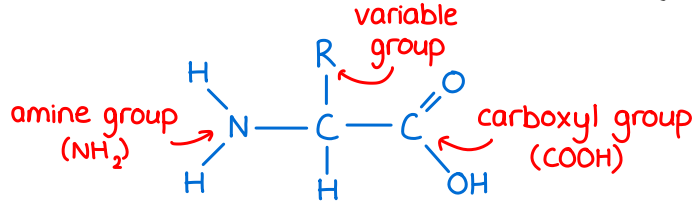
**Emulsion test for lipids**

- 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

## 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

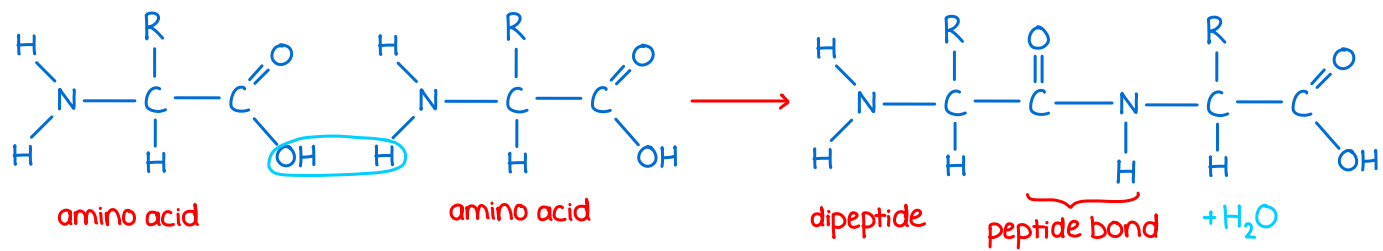
A monomer is a smaller unit from which larger polymers are made.



Amino acids can be joined in any order and length.

## Peptide bonds and dipeptides

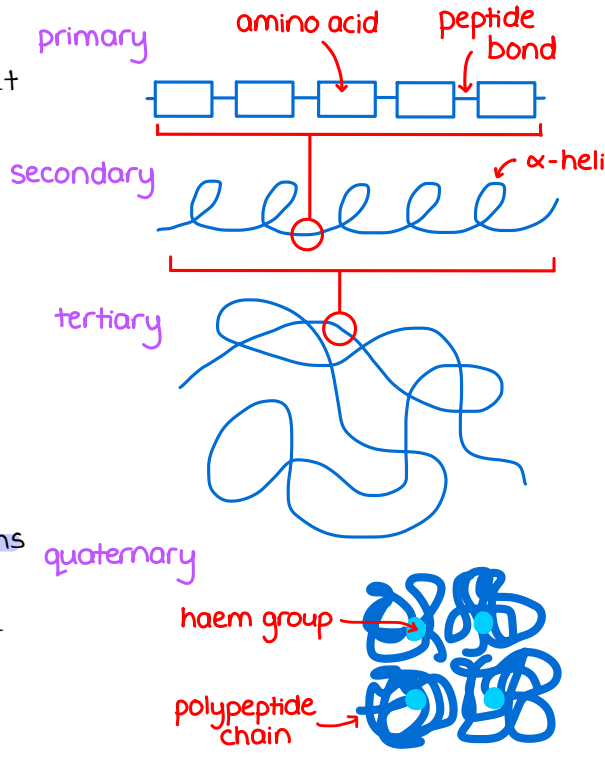
- A condensation reaction joins two amino acids with a peptide bond → produces a dipeptide and a molecule of water
- A hydrolysis reaction breaks a peptide bond by adding a molecule of water
- A polypeptide is a polymer of amino acids (a long chain of amino acids joined with peptide bonds)



## Protein structure

- Primary structure** → order of amino acids in the polypeptide chain → amino acids are joined with peptide bonds
- Secondary structure**
  - alpha helix (coiled) or beta pleated sheet (folded)
  - can be areas of both alpha helix and beta pleated sheet within the same polypeptide
  - hydrogen bonds between partial charges on parts of amino acids in the polypeptide
- Tertiary structure**
  - further folding and coiling (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
  - hydrophobic regions clump together, hydrophilic regions turn outwards
  - 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

Cysteine contains a sulfur atom in the R group.

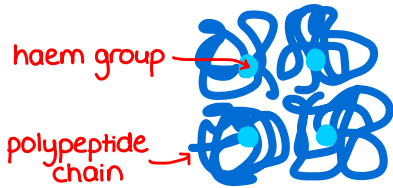


## Globular and fibrous proteins

- **Globular** → soluble, spherical, often complementary to another molecule
  - hydrophilic regions face outwards and hydrophobic regions face inwards which makes them soluble
  - easily transported

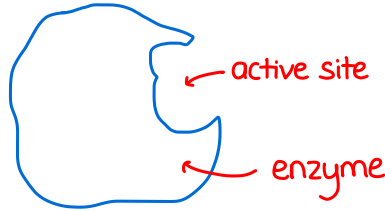
### Haemoglobin

carries oxygen in red blood cells



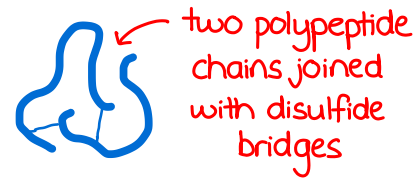
### Enzymes

e.g. amylase



### Hormones

e.g. insulin



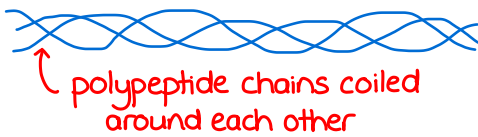
Haemoglobin is a conjugated protein: a globular protein with a non-protein prosthetic group (haem) attached with covalent, ionic, or hydrogen bonds.

Primary structure determines the tertiary structure and function.

- **Fibrous** → long chains of amino acids, insoluble (many hydrophobic R groups), flexible, strong
  - little tertiary structure and mostly unreactive
  - chains parallel to each other or twisted round each other into a rope shape
  - chains held together with many crosslinks e.g. disulfide bridges
  - provide strength, support, and flexibility (structural functions)

### Collagen

in connective tissue for strength



### Elastin

in elastic connective tissue to allow stretch and recoil  
e.g. in arteries

### Keratin

in skin and other external structures for strength  
e.g. hair, nails, horns

## 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.



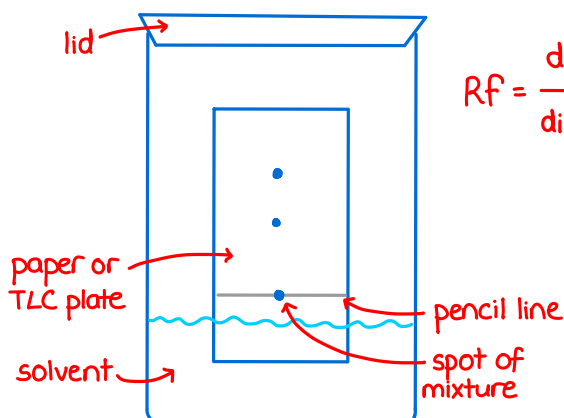
## Separating and identifying amino acids using chromatography

- 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 e.g. butanol (water cannot be used if the amino acids are hydrophobic)
- 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 amino acid mixtures onto the pencil line
  - make sure the spots are spread out so they do not merge
  - let the spot dry and then repeat to build up a concentrated spot
- 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 R<sub>f</sub> values
  - repeat the experiment and find the average R<sub>f</sub> value to increase accuracy
- 7) Look of R<sub>f</sub> values in standard reference tables to find out which amino acids you have in the mixture



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

This process cannot be automated.

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

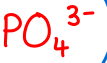
### Safety considerations

- 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

- 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

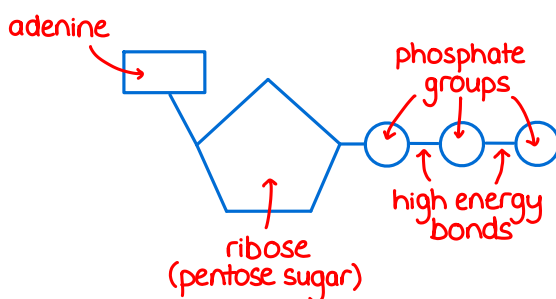
- **Phosphate ions** → attached to other molecules to become a phosphate group (phosphorylation)  
→ found in ATP, DNA, RNA, phospholipids and calcium phosphate (in bones)  
→ give DNA and RNA a negative charge  
→ used in photosynthesis and respiration
- **Nitrate ions** → source of nitrogen for plants (absorbed from the soil)
- **Hydrogencarbonate ions** → help to maintain pH of the blood by acting as a buffer
- **Chloride ions** → a cofactor for amylase  
→ help to maintain pH of the blood (chloride shift)
- **Hydroxide ions** → more OH<sup>-</sup> ions means a high (more alkaline) pH
- **Hydrogen ions** → more H<sup>+</sup> ions present means a lower (more acidic) pH  
→ enzyme activity is affected by pH (and therefore H<sup>+</sup> concentration)
- **Sodium ions** → used in co-transport to help molecules cross membranes e.g. glucose absorption in the small intestine  
→ needed to create an action potential in neurones  
→ involved in regulation of fluid balance
- **Potassium ions** → needed to create an action potential in neurones  
→ involved in muscle contraction and regulation of fluid balance  
→ activate some enzymes in photosynthesis
- **Ammonium ions** → source of nitrogen for plants (absorbed from the soil)
- **Calcium ions** → important role at synapses between neurones and in muscle contraction  
→ a cofactor for some enzymes involved in coagulation (blood clotting)  
→ important for the secretion of insulin from the pancreas
- **Zinc ions** → a prosthetic group of carbonic anhydrase



Inorganic normally means no carbon, but  $\text{HCO}_3^-$  is an exception.

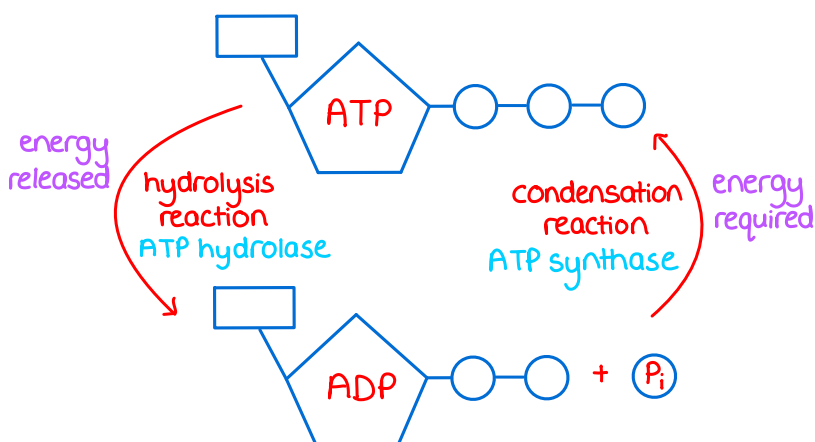
## Structure

- ATP = **adenosine triphosphate**
- Contains **carbon, hydrogen, oxygen, phosphorous and nitrogen**
- A **phosphorylated nucleotide**
- A **ribose sugar** bound to **adenine** (a purine base) and **three phosphate groups**
- Energy stored in **high energy bonds** between phosphate groups
- **Phosphodiester bond** between the phosphate and ribose



## Function

- **Diffuses** to areas of cells where energy is needed and **does not leave the cell**
- **Hydrolysed** to ADP (adenosine diphosphate) and  $P_i$  (inorganic phosphate) → **energy is released** for use in metabolic reactions
- **Immediate energy supply** → not stored long term
- **Rapidly re-synthesised** by phosphorylating ADP with  $P_i$  → **requires energy** released from **glucose** in respiration



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

- DNA = deoxyribonucleic acid, RNA = ribonucleic acid
- Contain carbon, hydrogen, phosphorous, oxygen and nitrogen

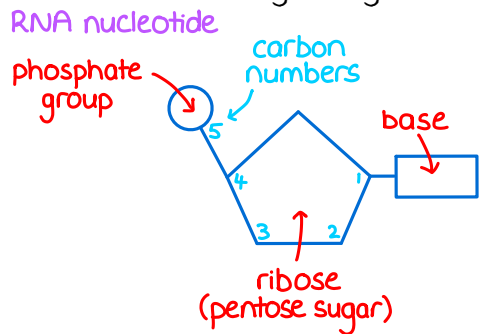
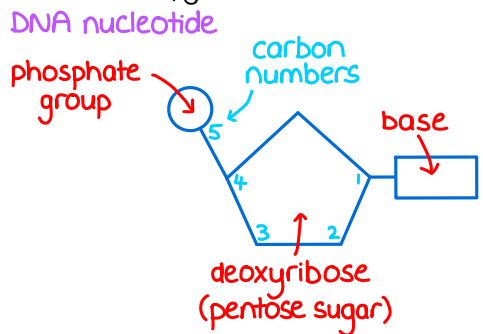
## DNA nucleotides

- The monomers of DNA
- Consist of a pentose sugar (deoxyribose), a nitrogenous 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 nitrogenous base, and a phosphate group
- The base can be adenine (A), uracil (U), guanine (G) or cytosine (C)

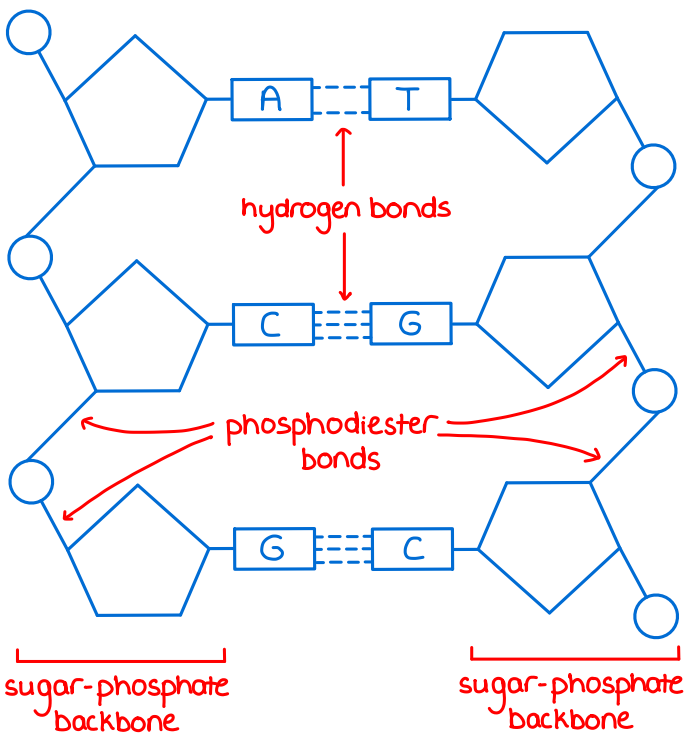
- Both DNA and RNA nucleotides form polynucleotides → phosphodiester bonds form between the phosphate group of one and the deoxyribose of the next in a condensation reaction
- A and G are purine bases → contain two carbon-nitrogen rings
- T, U and C are pyrimidine bases → contain one carbon-nitrogen ring (smaller than purines)



A pentose sugar has five carbon atoms.

## DNA

- Stores genetic information
- 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



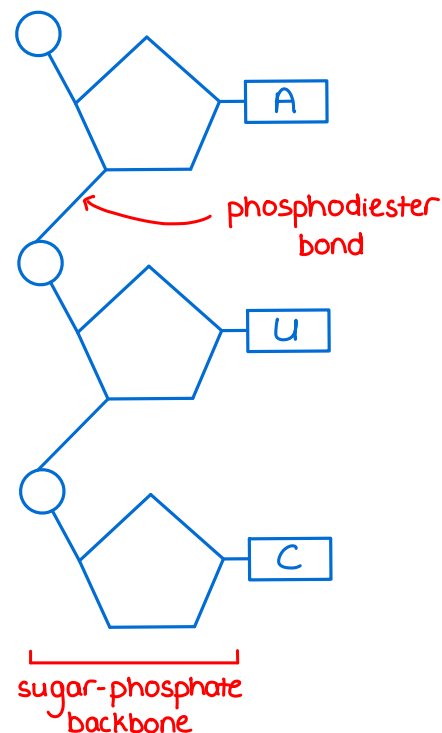
Antiparallel means the strands run in opposite directions: one is 5' to 3', the other is 3' to 5'.

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.

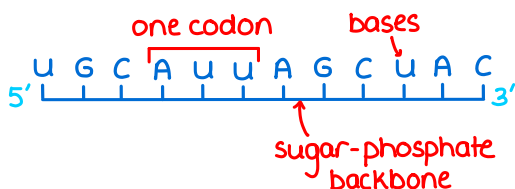
## RNA

- A single polynucleotide chain with different functions
- Single-stranded polymer of RNA nucleotides
- 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



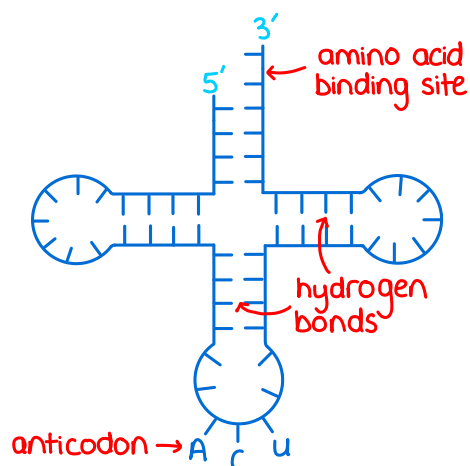
## Messenger RNA

- A single linear polynucleotide strand made during transcription in the nucleus
- 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 clover leaf shape
- Hydrogen bonds between complementary bases hold the shape
- Contains an amino acid binding site and an anticodon
- Found in the cytoplasm

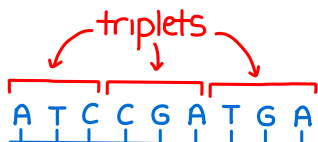


## DNA purification

- A method to extract pure DNA from cells
- 1) If using plant cells, crush and grind the piece of plant using a pestle and mortar or blender  
→ this breaks the cell walls
  - 2) Mix and incubate in a 60°C water bath with a solution of detergent and salt for about 15 mins  
→ the detergent breaks cell membranes to release the contents of the cells and nuclei, the salt binds to the DNA to help it precipitate
  - 3) Transfer to an ice bath to cool then filter the solution  
→ low temperature reduces the activity of enzymes which could break down DNA
  - 4) Add protease and RNAse enzymes to the filtered solution, then add ethanol  
→ protease hydrolyses the histone proteins associated with DNA  
→ RNAse hydrolyses any RNA present  
→ ethanol precipitates the DNA from solution and it will be visible as a white layer which can be removed carefully

## The genetic code

- A triplet of bases codes for one amino acid → sequence of bases determines primary protein structure
- Non-overlapping → each nucleotide is only part of one triplet of bases, there is never overlap
- Degenerate → more than one triplet codes for each specific amino acid
- Universal → the genetic code is the same in all species
- A gene is a sequence of DNA that codes for a polypeptide

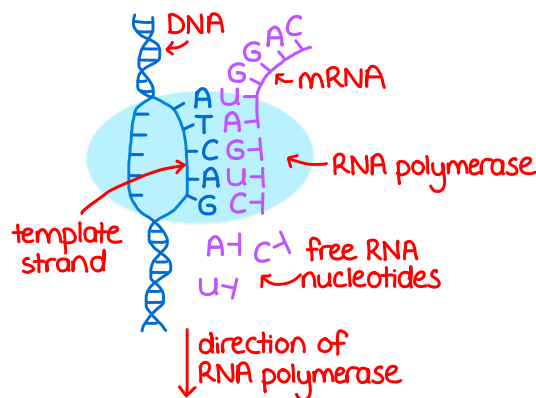


{	CTT	CTG	}	all code for leucine (an amino acid)
	CTC	TTA		
	CTA	TTG		

## Transcription

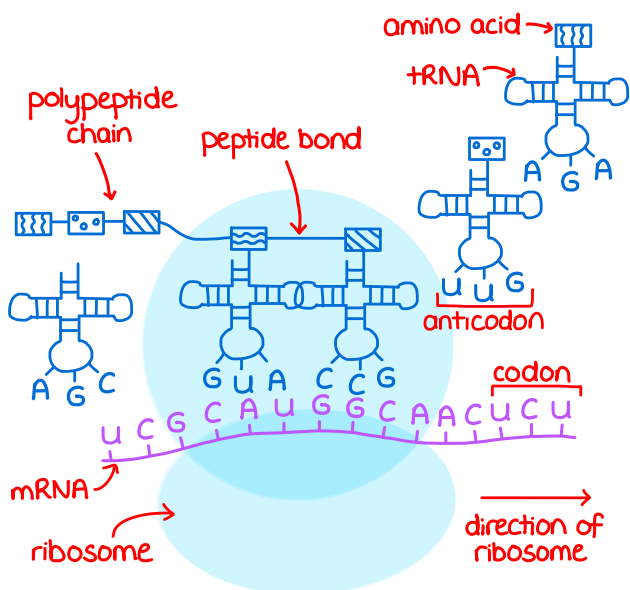
- Transcribing DNA to mRNA
- Happens in the nucleus in eukaryotic cells and the cytoplasm in prokaryotic cells
- DNA is too large to fit through the nuclear pores → mRNA is shorter so can travel to the ribosomes

- 1) RNA polymerase attaches to DNA at the start of a gene (the start codon).
- 2) Hydrogen bonds between the DNA strands break leaving exposed bases on the template strand.
- 3) Free RNA nucleotides complementary base pair with the exposed bases.
- 4) RNA polymerase joins the RNA nucleotides together with phosphodiester bonds in condensation reactions.
- 5) RNA polymerase moves along the DNA until it reaches the end of the gene (the stop codon), then detaches.



## Translation

- Translating mRNA into a polypeptide
- Happens at the ribosomes



- 1) The mRNA from the nucleus travels to the ribosomes and the ribosome attaches at the start codon.
- 2) tRNA molecules bring specific amino acids to the ribosomes.
- 3) The anticodon on the tRNA complementary base pairs with the codon on the mRNA.
- 4) A second tRNA molecule lines up next to the first and a peptide bond forms between the amino acids. (in a condensation reaction catalysed by rRNA)
- 5) The ribosome moves along until it reaches a stop codon then detaches.

mRNA codons are complementary to DNA triplets:

DNA triplet ATC  
mRNA codon UAG

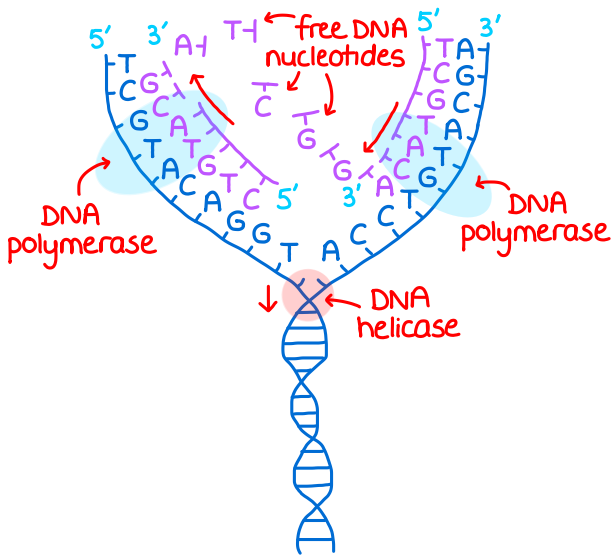
tRNA anticodons are complementary to mRNA codons:

mRNA codon UAG  
tRNA anticodon AUC

Ribosomes are made of ribosomal RNA (rRNA) and protein.

## 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
  - a purine must pair with a pyrimidine because they are different sizes
  - the right number of hydrogen bonds must be able to form, so each base only has one option

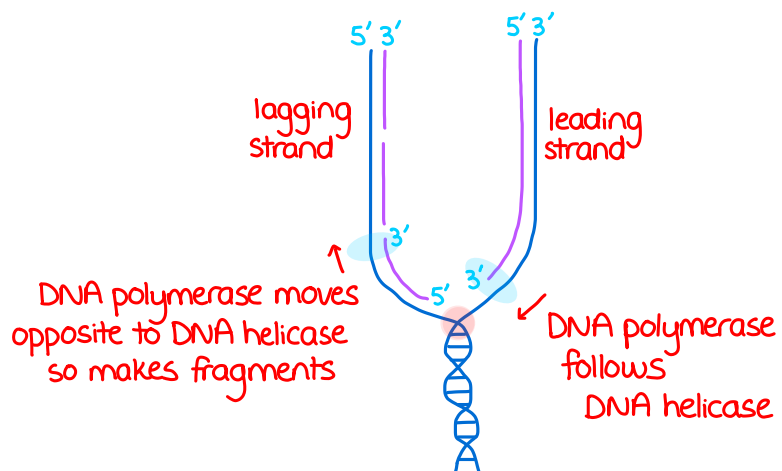


- DNA helicase breaks weak hydrogen bonds between complementary bases so the double helix unwinds and the two strands separate.
- Free DNA nucleotides are attracted to exposed bases on the two template strands and pair up by complementary base pairing.
- DNA polymerase joins the adjacent nucleotides with phosphodiester bonds in condensation reactions to form the sugar-phosphate backbone.
- The DNA winds into the double-helix shape.

Random spontaneous mutations can occur during DNA replication, which can alter protein structure and function.

- DNA polymerase can only form phosphodiester bonds in the 5' to 3' direction of the new strands
  - it can only add nucleotides at the 3' end due to enzyme specificity
  - the leading strand is made in one piece
  - the lagging strand is made in fragments which are joined by DNA ligase

The fragments are called Okazaki fragments.





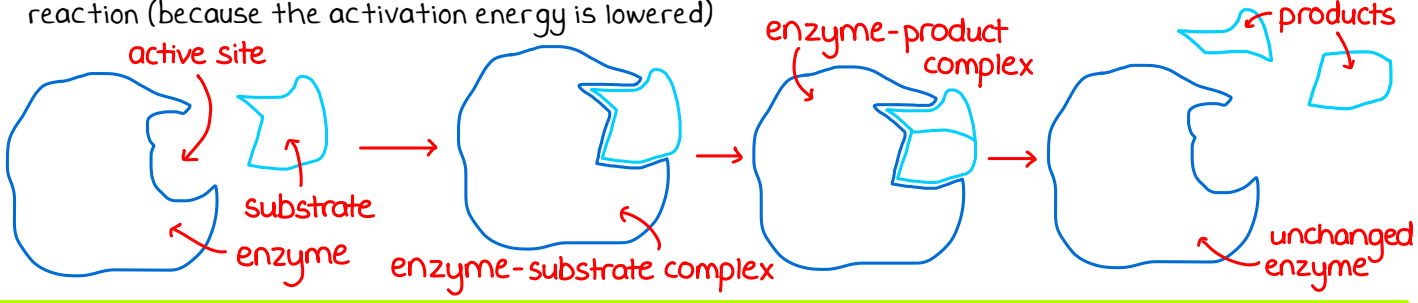
## Function of enzymes

- Enzymes are globular proteins with a specific tertiary structure
- The active site is complementary to one 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 without an enzyme.

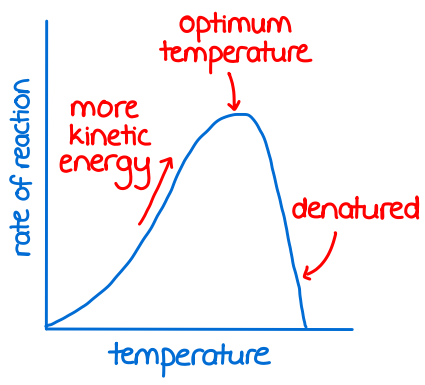
## Induced fit model

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



## Temperature

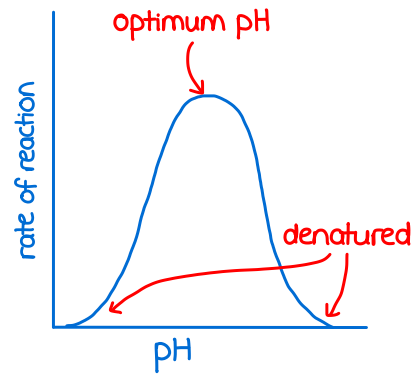
- As temperature increases, enzyme and substrate have more kinetic energy so there are more frequent successful collisions and more enzyme-substrate complexes form
- After the optimum temperature, the enzyme becomes denatured
  - too much kinetic energy breaks the hydrogen bonds and ionic bonds between 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
- The temperature coefficient ( $Q_{10}$ ) tells you by what factor the rate of reaction changes when you increase the temperature by 10°C



$$Q_{10} = \frac{R_2 \leftarrow \text{rate at temperature} + 10^\circ\text{C}}{R_1 \leftarrow \text{rate at temperature}}$$

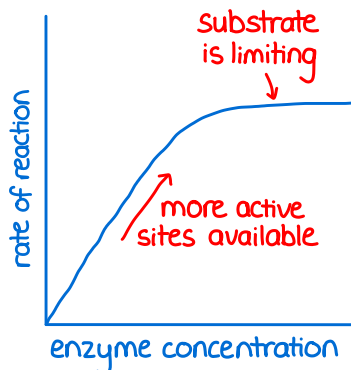
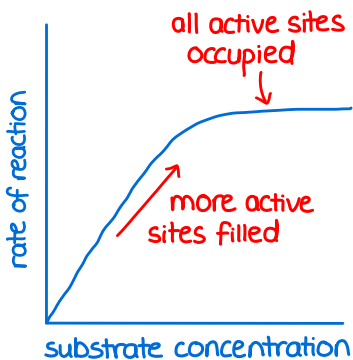
## pH

- Enzymes are denatured above and below the optimum pH
  - $\text{H}^+$  (acidic) or  $\text{OH}^-$  (alkali) ions interfere with the hydrogen bonds and ionic bonds between amino acid R groups, so the tertiary structure is altered and the active site is no longer complementary to the substrate



## Enzyme or substrate concentration

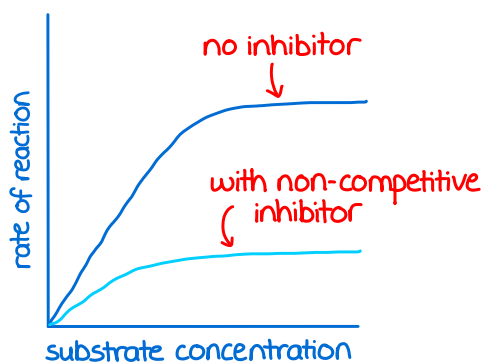
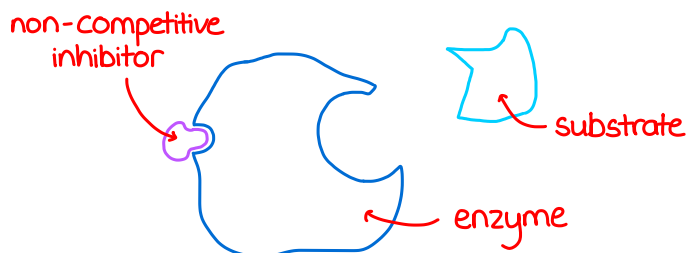
- Increasing enzyme or substrate concentration increases the frequency of collisions between enzyme and substrate, so more enzyme-substrate complexes form
- Eventually all available active sites are filled, or substrate concentration becomes limiting



## Inhibitors

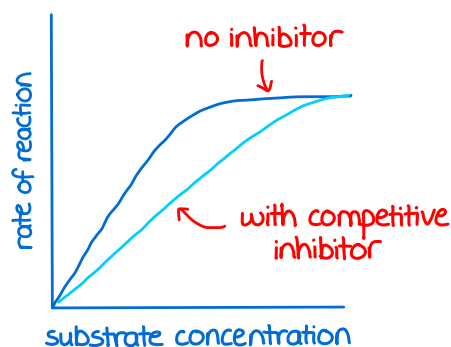
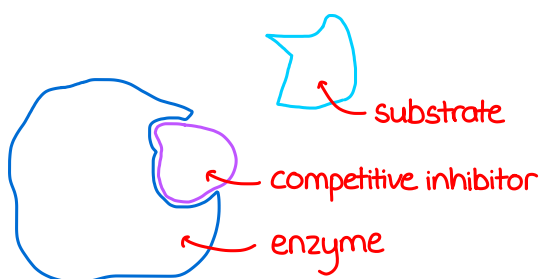
### Non-competitive inhibitors

- 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

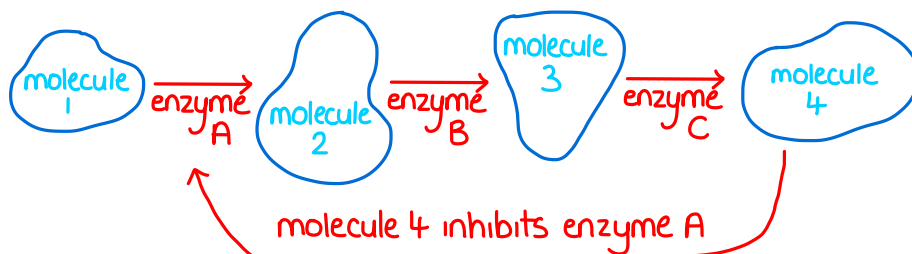
- have a very similar tertiary structure to the substrate
- bind to the active site in place of the substrate so less enzyme-substrate complexes form
- increasing substrate concentration will increase the rate of reaction (because the substrate "wins" the competition more often)



- Non-reversible inhibitors bind to the enzyme with strong covalent bonds
- Reversible inhibitors bind to the enzyme with weak hydrogen bonds

## End-product inhibition

- When the final product in a metabolic pathway inhibits an enzyme further up the pathway
- Helps to regulate the pathway and control the amount of products
- A type of reversible inhibition



A metabolic pathway is a linked series of reactions happening in a cell.

- Enzymes are sometimes synthesised as inactive precursors
  - enzyme is inactive until part of it is removed
  - allows enzymes to be activated under specific conditions
  - can prevent an enzyme damaging cells e.g. protease enzymes digesting intracellular proteins

## Drugs and metabolic poisons

- Some medicinal drugs or metabolic poisons are enzyme inhibitors
- **Statins** → a drug commonly taken to lower blood cholesterol
  - competitive reversible inhibitors of HMG-coA reductase which synthesises cholesterol in the liver
- **Cyanide** → a metabolic poison which irreversibly inhibits cytochrome c oxidase
  - cytochrome c oxidase is needed for oxidative phosphorylation in aerobic respiration

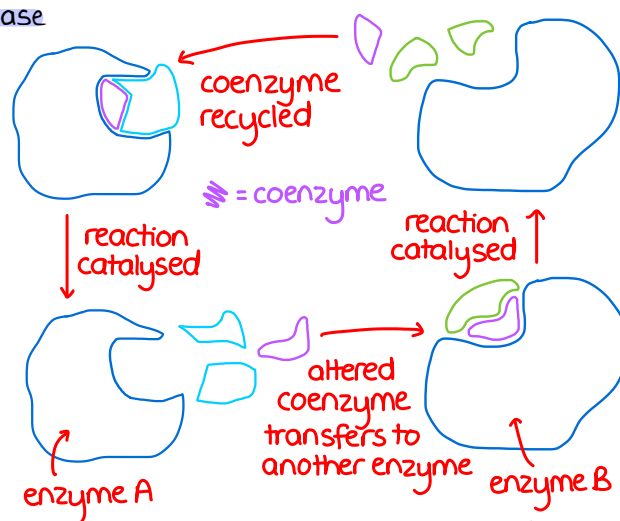
Inhibiting aerobic respiration means inhibiting ATP production so there is not enough energy for other processes.

## Cofactors

- Non-protein inorganic molecules or ions which help the substrate to bind to the enzyme
- Called a prosthetic group if tightly bound to the enzyme with bonds e.g. covalent bonds
- Not used up or changed in the reaction
- Zinc ions ( $Zn^{2+}$ ) → prosthetic group for carbonic anhydrase
- Chloride ions ( $Cl^-$ ) → cofactor for amylase

## Coenzymes

- Non-protein organic molecules not permanently attached to an enzyme
- Needed to allow an enzyme to function
- Transfer chemical groups between enzymes and are continually recycled
- Many vitamins are coenzymes

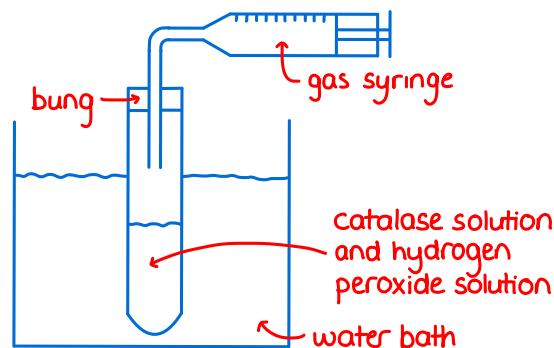


### Investigating enzyme-controlled reactions

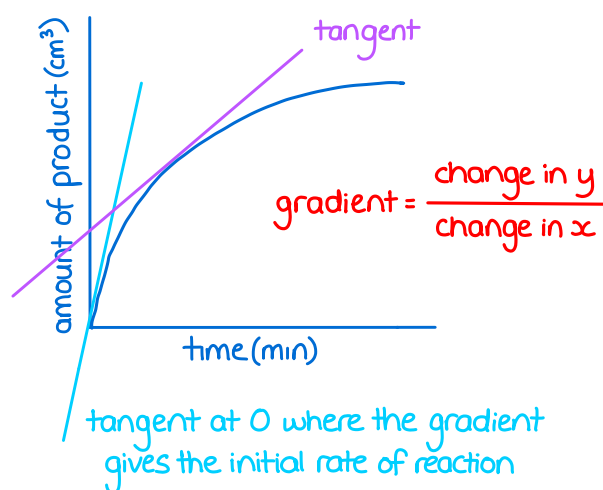
- Many different possibilities for what to investigate and which reaction to use
- Only change one variable at a time and control all other variables e.g. temperature, pH
- Example: the enzyme catalase converts hydrogen peroxide to oxygen and water
  - could use a gas syringe to record the volume of 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 enzymes
- 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<sup>3</sup> min<sup>-1</sup> (cm<sup>3</sup> per min).

### Specific enzyme examples

- Catalase → an intracellular enzyme
  - catalyses the conversion of toxic hydrogen peroxide to non-toxic oxygen and water
- Amylase → an extracellular enzyme secreted by the salivary glands and pancreas
  - hydrolyses starch to maltose in digestion (breaks glycosidic bonds)
- Trypsin → an extracellular enzyme secreted by the pancreas
  - hydrolyses peptide bonds in large polypeptides to create smaller polypeptides