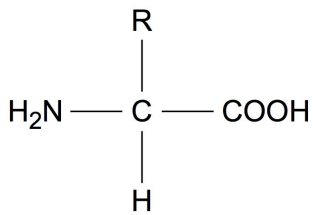
3.1.4 Proteins

3.1.4.1 General properties of protein

SPECIFICATION

- Amino acids are the monomers from which proteins are made. The general structure of an amino acid as:



- where NH_2 represents an amine group, COOH represents a carboxyl group and R represents a side chain. The twenty amino acids that are common in all organisms differ only in their side group.
- A condensation reaction between two amino acids forms a peptide bond.
  - Dipeptides are formed by the condensation of two amino acids.
  - Polypeptides are formed by the condensation of many amino acids.
- A functional protein may contain one or more polypeptides.
- The role of hydrogen bonds, ionic bonds and disulfide bridges in the structure of proteins.
- Proteins have a variety of functions within all living organisms. The relationship between primary, secondary, tertiary and quaternary structure, and protein function.
- The biuret test for proteins.
- Students **should be able to** relate the structure of proteins to properties of proteins named throughout the specification.

Source: AQA Spec

Proteins

Proteins are one of the most abundant organic molecules in living systems. Amino acids are the monomers that make up proteins.

Amino acids are so-called because they have both amino groups and acid groups, which have opposite charges.

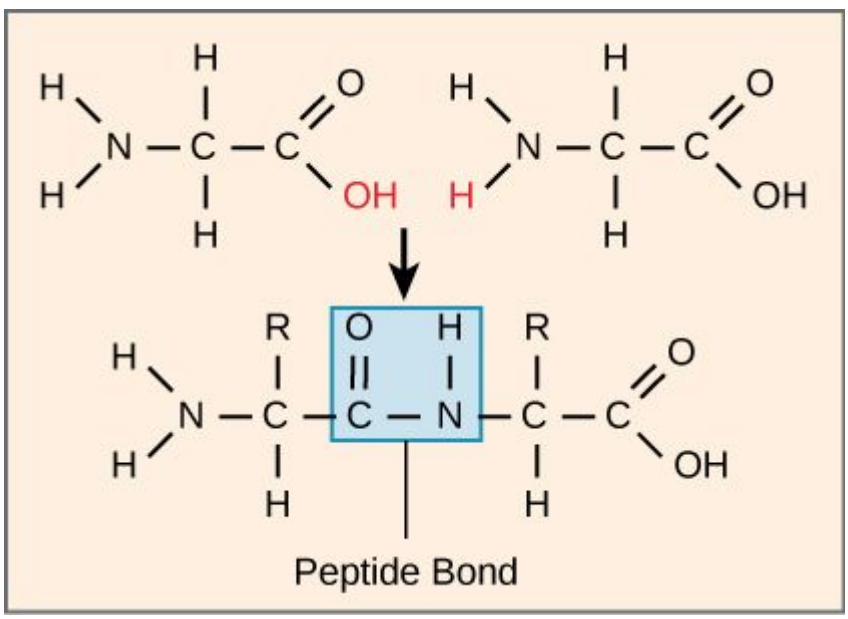
All living things share a bank of only 20 amino acids. There are 20 different R groups, and so 20 different amino acids. Since each R group is slightly different, each amino acid has different properties, and this in turn means that proteins can have a wide range of properties.

Peptide formation between two amino acids

Polypeptides are formed via condensation reactions between the amine group of one amino acid and the carboxyl group of another.

A molecule of water is released during reaction. The bonds formed between amino acids are called peptide bonds. The reverse reaction happens during digestion.

The sequence of amino acids in a polypeptide is determined by the sequence of the genetic code on mRNA being translated in the ribosomes.

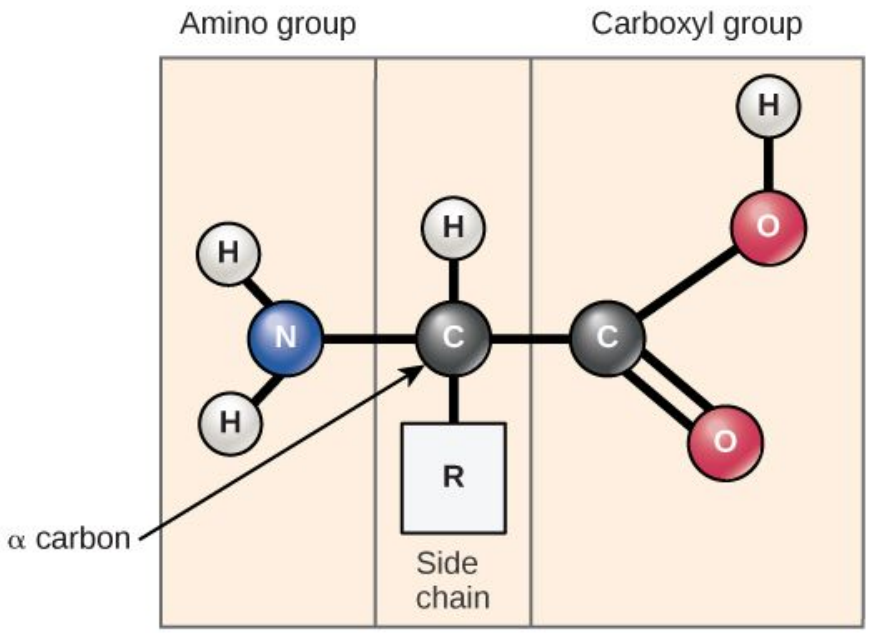


The 20 Amino Acids

Abbreviation	Amino Acid	Abbreviation	Amino Acid
Ala	Alanine	Leu	Leucine
Arg	Arginine	Lys	Lysine
Asp	Aspartic acid	Met	Methionine
Asn	Asparagine	Phe	Phenylalanine
Cys	Cysteine	Pro	Proline
Gln	Glutamine	Ser	Serine
Glu	Glutamic acid	Thr	Threonine
Gly	Glycine	Trp	Tryptophan
His	Histidine	Tyr	Tyrosine
Ile	Isoleucine	Val	Valine

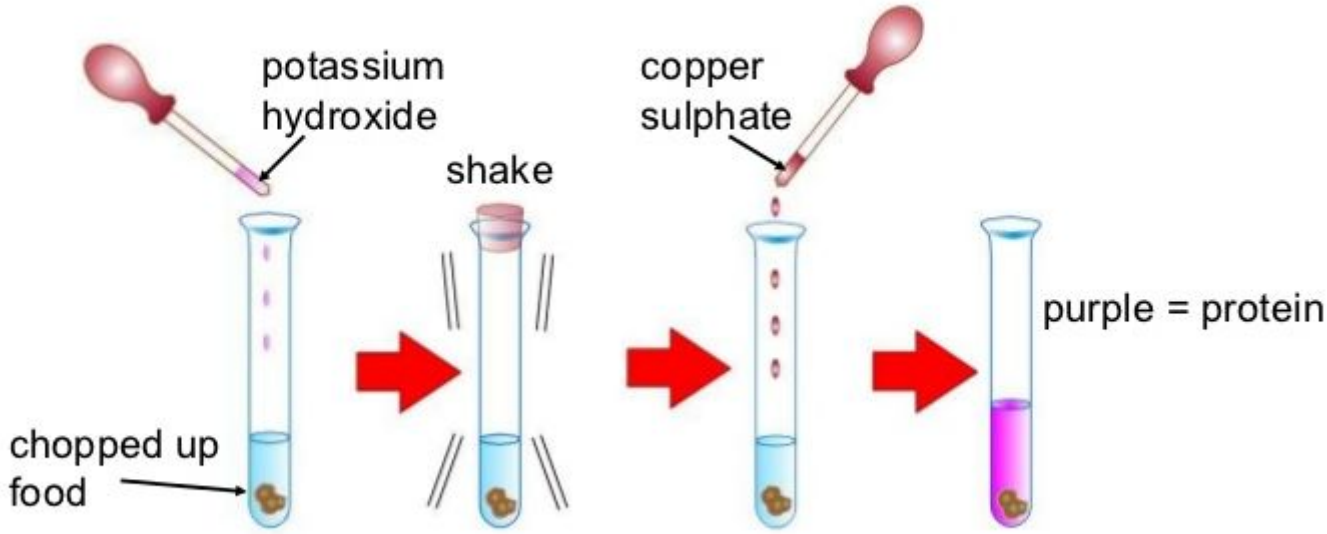
Amino Acid General Structure

Amino acids have a central asymmetric carbon to which an amino group, a carboxyl group, a hydrogen atom, and a side chain (R group) are attached.



Testing for Proteins

If you need to find out if a substance, like a food sample, contains protein, you would use the **biuret test**.



- The test solution needs to be alkaline, so you first need to add sodium or potassium hydroxide solution.
- Next you add a few drops of copper sulfate solution.
- If protein is present, the solution turns purple. If there is no protein, it will remain blue.

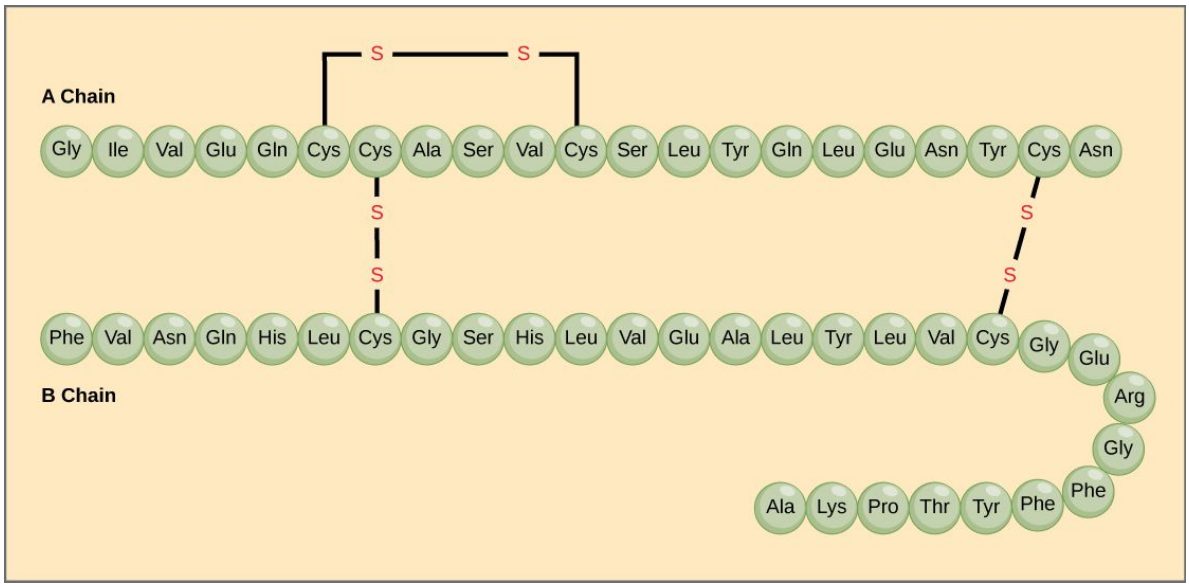
Forming Proteins

The shape of a protein is critical to its function. An enzyme, for example, can bind to a specific substrate at site known as the active site. If that active site is altered because of changes to the protein structure, the enzyme may be unable to bind to the substrate.

To understand how the protein gets its final shape, you need to know the four levels of protein structure: primary, secondary, tertiary and quaternary.

Primary Structure

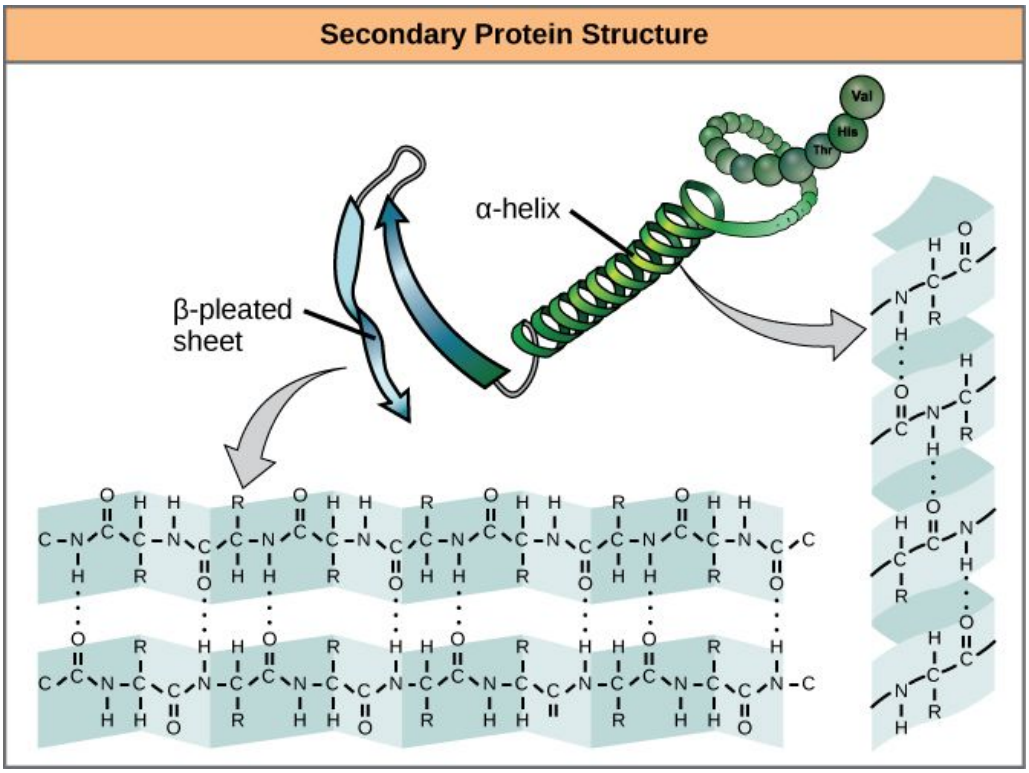
The unique sequence of amino acids in the polypeptide chain is its **primary structure**.



Secondary Structure

This is a result of protein folding, and consists of a few basic motifs that are found in all proteins. The secondary structure is held together by hydrogen bonds between the carboxyl groups and the amino groups in the polypeptide backbone. The two most common secondary structure motifs are the α-helix and the β-pleated sheet, but it does not have to be either of these

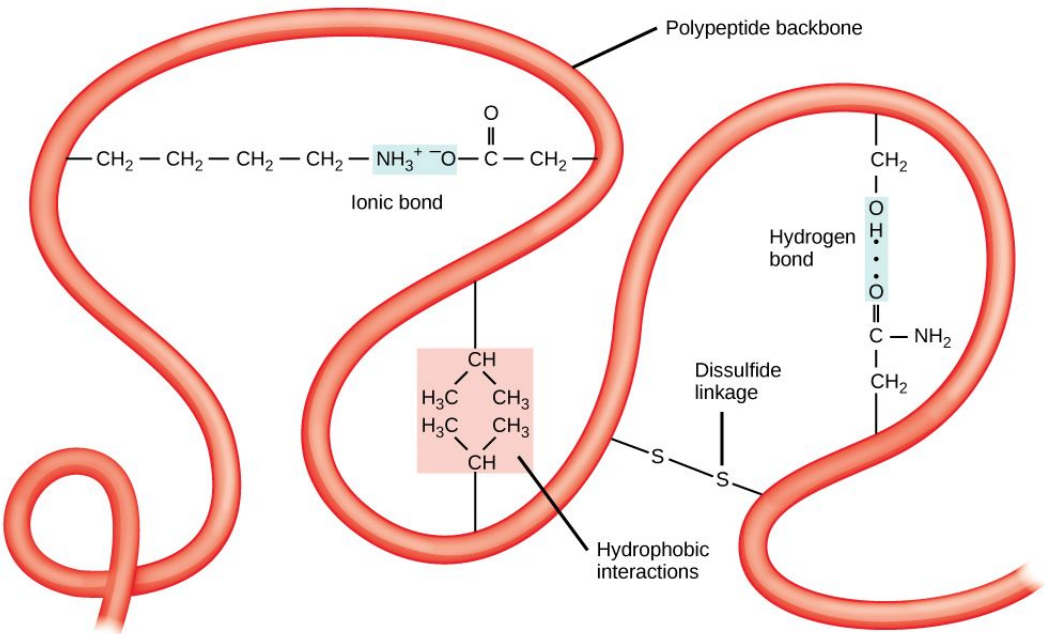
The α-helix is held together by hydrogen bonds running parallel with the long helical axis. There are so many hydrogen bonds that this is a very stable and strong structure.



Tertiary Structure

Further folding of the secondary structure. The tertiary structure is held together by bonds between the R groups of the amino acids in the protein, and so depends on what the sequence of amino acids is. There are three kinds of bonds involved:

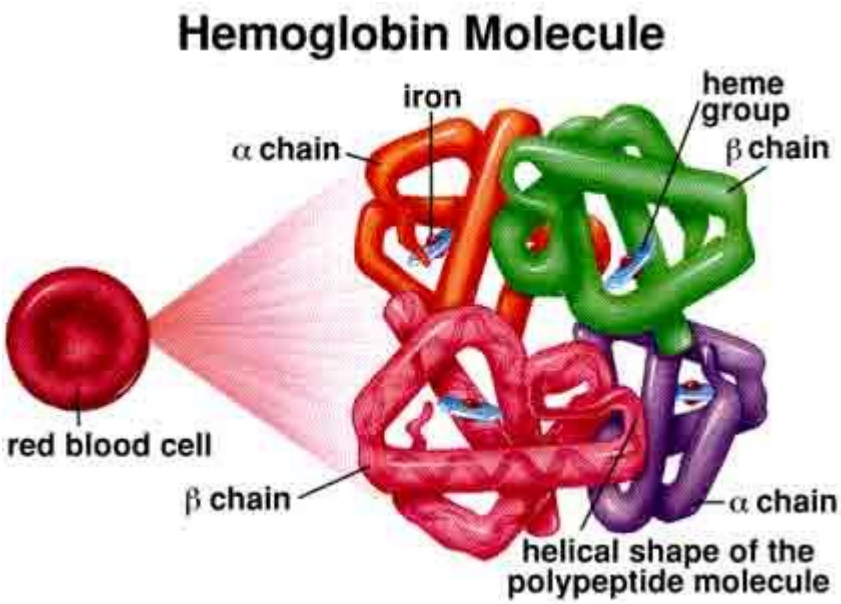
1. Hydrogen bonds, which are weak.
2. Ionic bonds between R-groups with positive or negative charges, which are quite strong.
3. sulphur bridges - covalent S-S bonds between two cysteine amino acids, which are strong.



Quaternary Structure

In nature, some proteins are formed from several polypeptides, also known as subunits, and the interaction of these subunits forms the quaternary structure. Examples of this association of several polypeptides include:

- Collagen - a fibrous protein of three polypeptides (trimetric) that are supercoiled like a rope)
- Hemoglobin - a globular protein with four polypeptide chains (tetrameric)
- Insulin - two polypeptide chains (dimeric) held together by disulfide bond



Thin Layer Chromatography to Separate/Identify Amino Acids

Amino acids have different R groups which means they will have different solubilities in different solvents and can be separated by thin layer paper chromatography.

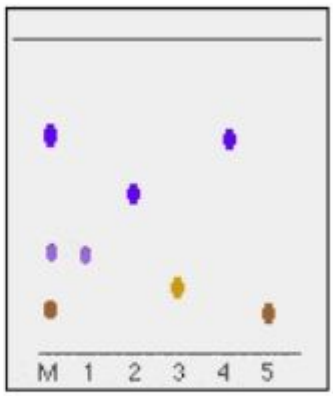
- A container is filled to a depth of 2 cm with a suitable chromatography solvent. The container is sealed and left to allow the solvent to **saturate the atmosphere**. This is important because solvent **must not evaporate** from the chromatogram as it is running.
- A strip of chromatography paper has a pencil line drawn on it slightly more than 2 cm from the bottom and small crosses are drawn on it to indicate where to add the amino acids.

  It is important not to touch the paper as there are amino acids on your fingers.
- A spot of amino acid is applied to a cross on the line using a capillary tube; the spot must not exceed 2mm in diameter.
- The spot is dried with a hair drier and another is applied over the top. This is done until a small but concentrated dot of amino acid has been built up on the cross.
- The nature of the amino acid is indicated by writing in pencil below the spot.
- This is repeated until each amino acid dot has been added.
- The strip of chromatography paper is placed in the container, making sure that the solvent doesn't splash above the line.
- The solvent is drawn up the paper by capillary action but does not evaporate from it due to the saturated air around it. The amino acid was be carried by the solvent and each will travel at different speeds.
- When the solvent has nearly reached the top the paper is removed and a line is drawn to show where the solvent has reached, this is the **solvent front**.
- The chromatogram is dried in a **fumes cupboard** and then sprayed with **ninhydrin spray**. When the chromatogram dries, the amino acids will appear as purple spots at different distances up the chromatogram.

Thin Layer Chromatography to Separate/Identify Amino Acids

- The distance each spot has traveled from the center of the cross to the center of the spot is measured, call this D_1.
- The distance the solvent front has traveled from the cross is measured, call this S.
- The R_f value can then be calculated as **$R_f = D_1 / S$**
- Each amino acid will have a unique R_f value and as the R_f values for all amino acids are known they can be identified knowing the R_f value

before spraying with ninhydrin



after spraying with ninhydrin

3.1.4 Proteins

3.1.4.2 Many proteins are enzymes

SPECIFICATION

- Each enzyme lowers the activation energy of the reaction it catalyses.
- The induced-fit model of enzyme action.
- The properties of an enzyme relate to the tertiary structure of its active site and its ability to combine with complementary substrate(s) to form an enzyme-substrate complex.
    - The specificity of enzymes
    - The effects of the following factors on the rate of enzyme-controlled reactions – enzyme concentration, substrate concentration, concentration of competitive and of noncompetitive inhibitors, pH and temperature.
- Students should be able to:
    - appreciate how models of enzyme action have changed over time
    - appreciate that enzymes catalyse a wide range of intracellular and extracellular reactions that determine structures and functions from cellular to whole-organism level.

Required practical 1: Investigation into the effect of a named variable on the rate of an enzyme-controlled reaction.

Source: AQA Spec

Enzymes are Biological Catalysts

Enzymes come up a lot in biology because they make reactions work quickly. They increase the rate of reactions by lowering the activation energy.

A **catalyst** is a substance which speeds up a chemical reaction without actually being used up in the reaction itself.

Enzymes catalyze metabolic reactions at both a **cellular** level (for example, respiration) and for the **organism** as a **whole** (for example, digestion in mammals.

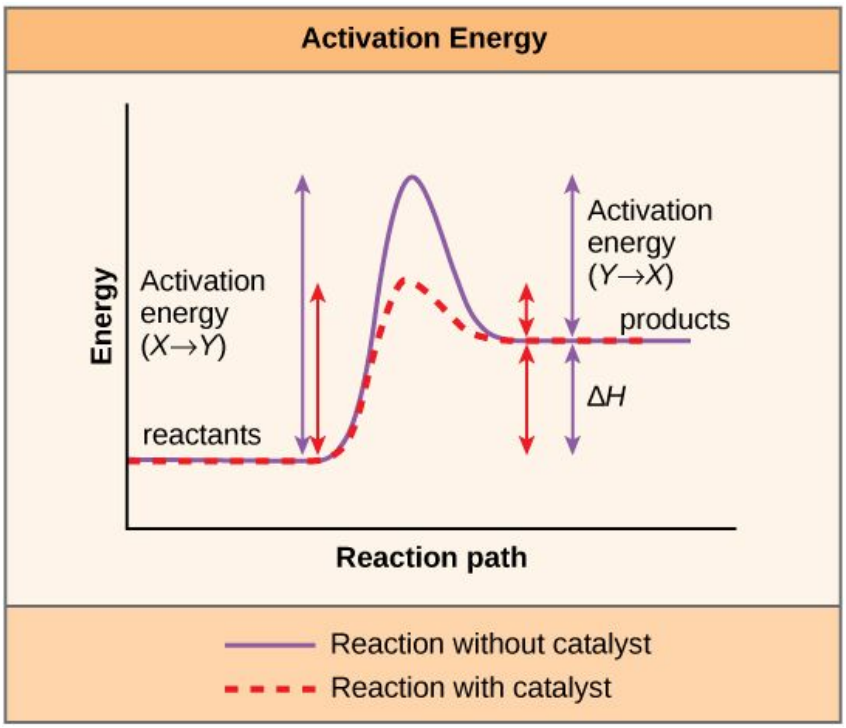
They can affect **structures** in an organism. For example, enzymes are involved in the production of collagen - an important protein in the connective tissue of animals.

Enzymes can also affect organism **functions**, like **respiration**.

An enzyme action can be **intracellular** (within the cells) or **extracellular** (outside the cells).

Enzymes have an **active** site, which is a specific shape. It's part of the enzyme where the substrate molecules bind to.

Enzymes Effect on Activation Energy of a Reaction

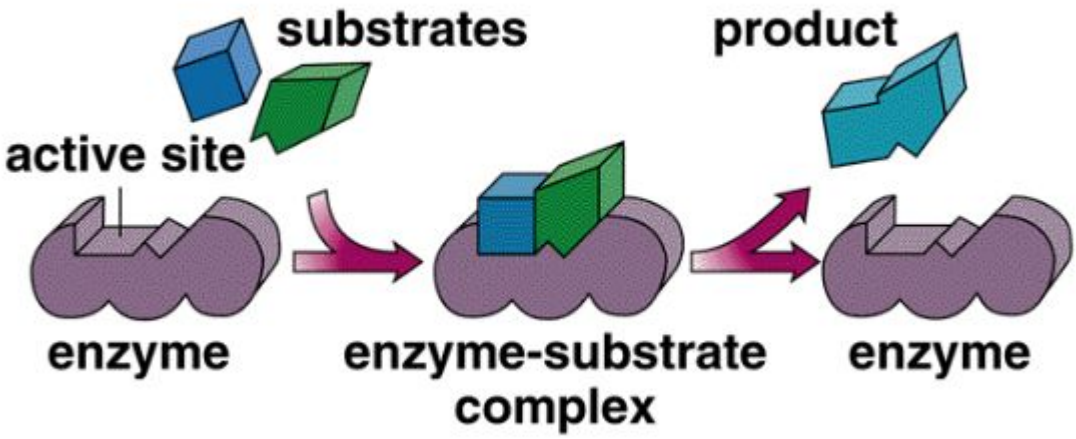


Enzymes lower the activation energy of a reaction but do not change the free energy of the reaction

Lock and Key Model

For many years, scientists thought that enzyme-substrate binding took place in a simple "**lock-and-key**" fashion.

This model suggested that the active site has a rigid shape and that only a substrate with the correct complementary shape can bind to the active site. However, this has its limitations.



Scientists soon realised that this lock and key had limitations. In particular:

- It does not easily explain how activation energy is lowered
- It does not easily explain the role of competitive inhibitors
- It does not easily explain the role of non-competitive inhibitors

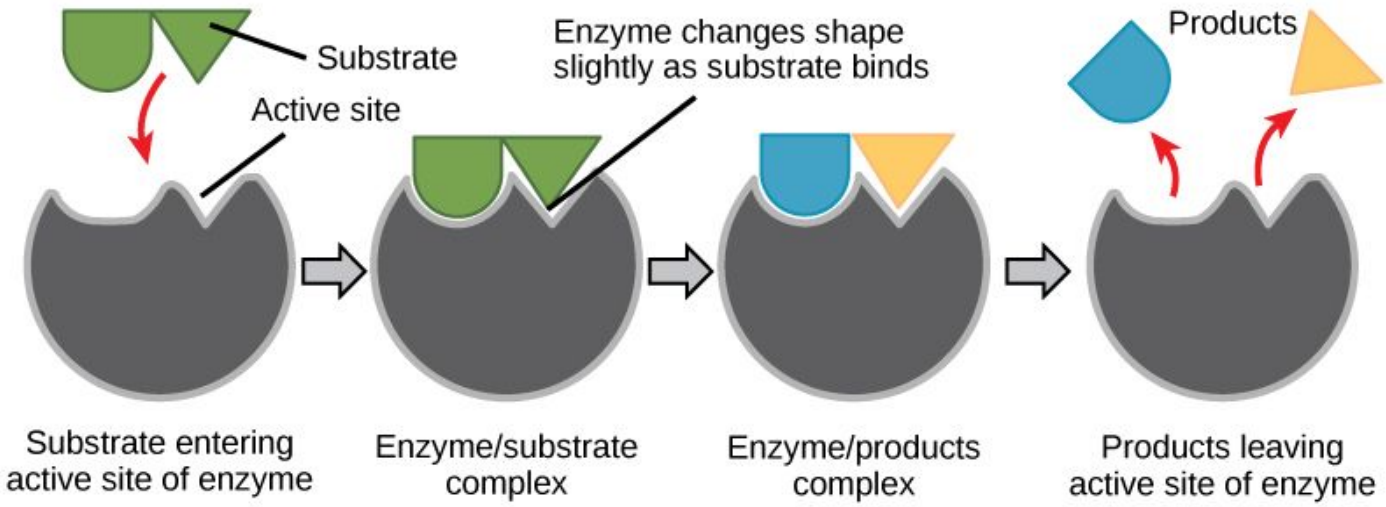
Current research supports a more refined view called induced fit. The induced fit model is a better mechanism to explain enzyme activity.

Induced Fit and Enzyme Function

The induced-fit model expands upon the lock-and-key model by describing a more dynamic interaction between enzyme and substrate.

As the enzyme and substrate come together, their interaction causes a mild shift in the enzyme's structure that confirms an ideal binding arrangement between the enzyme and the transition state of the substrate.

This ideal binding maximizes the enzyme's ability to catalyze its reaction.



According to the induced-fit model, both enzyme and substrate undergo dynamic conformational changes upon binding. The enzyme contorts the substrate into its transition state, thereby increasing the rate of the reaction.

Advantages of the induced fit model

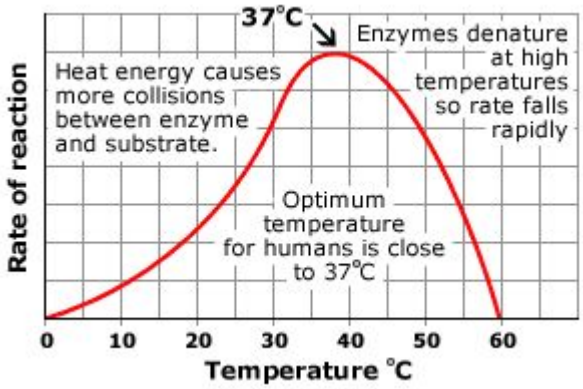
- Can explain how the activation energy is lowered, the stretching and distorting of bonds or causing the closer orientation of reactive groups.
- Explain how non-competitive inhibitors can bind to a region away from the active site and change its shape so that substrate can no longer bind to the active site.
- Explains how competitive inhibitors can bind to the active site or other molecules with similar shapes to the substrate.

Factors Affecting the Rate of Enzyme Activity

Now you know what enzymes are and how they work, you need to understand the factors that affect their activity.

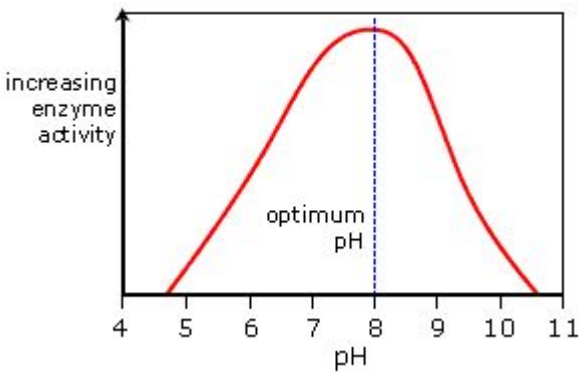
Temperature

- Increase in temperature increases kinetic energy
- so more enzyme substrate complexes form
- High temperatures cause denaturation, due to the breaking of breaking of bonds holding the tertiary structure together (H bonds/disulphide bridges/ionic bonds)
- Active site altered (changes shape) substrate cannot bind, no enzyme substrate complexes form



pH

- Deviations from the optimum pH cause a decrease in enzyme activity.
- Small deviations change the charge at the active site and affect the binding of the substrate
- Larger deviations can cause the hydrogen and ionic bonds holding the tertiary structure together to change and the enzyme denatures, meaning enzyme substrate complexes can no longer form

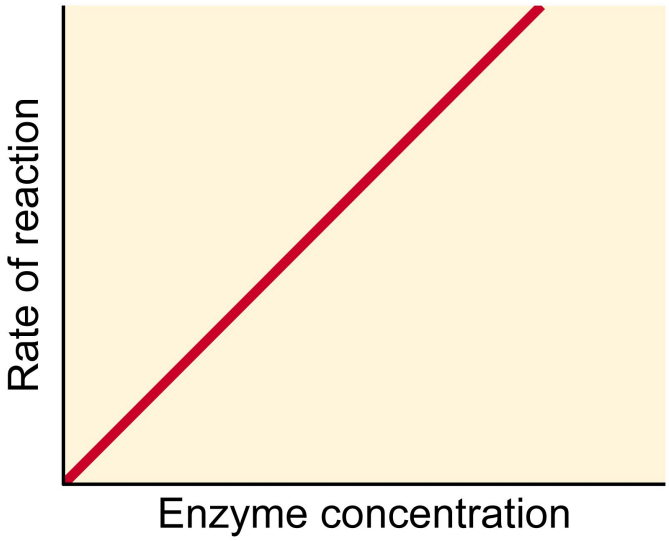


Images: BBC

Factors Affecting the Rate of Enzyme Activity

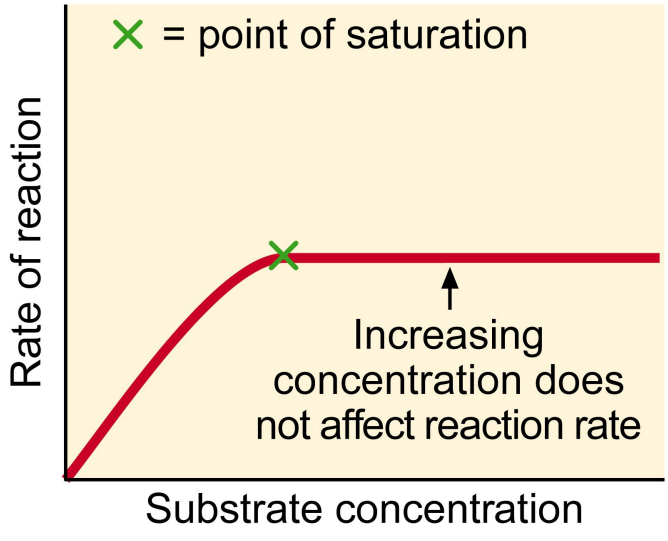
Enzyme Concentration

- As enzyme concentration increases so will the rate: as there are more enzyme substrate complexes forming.
- At very high concentrations of enzyme the rate remains constant as substrate becomes the limiting factor Add more enzyme will cause rate to increase again.



Substrate Concentration

- As the substrate concentration increases, the rate increases because more substrate molecules can collide with enzyme molecules, so more reactions will take place.
- At higher concentrations the enzyme molecules become saturated with substrate, so there are few free enzyme molecules, so adding more substrate doesn't make much difference.



Factors Affecting the Rate of Enzyme Activity

Enzyme Activity can be **Inhibited**

Enzyme activity can be prevented by **enzyme inhibitors**. These are molecules that bind to the enzyme that they inhibit. Inhibition can be either **competitive** or **non-competitive**.

Inhibitors prevent the binding of substrate to active site; therefore fewer enzyme-substrate complexes formed, reducing the rate of their reactions.

They are found naturally, but are also used artificially as drugs, pesticides and research tools.

COMPETITIVE INHIBITORS

- They have a similar shape to that of the substrate molecules.
- They compete with the substrate molecules to bind to the active site, but no reaction takes place.
- Instead of a reaction, they block the active site so no substrate molecules can fit in it.
- The level of activity inhibited depends on the relative concentrations of the inhibitor and the substrate.
- High concentration of the inhibitor means it will take up nearly all of the active sites and hardly any substrate will get to the enzyme.
- With a lower concentration, the chances of the substrate getting to an active site before the inhibitor increase.
- So, increasing the concentration of a substrate will increase the rate of reaction - up to a point.

NON-COMPETITIVE INHIBITORS

- They bind to the enzyme **away from its active site**.
- This causes the active site to change shape so the substrate molecules cannot bind to it.
- They don't "compete" with the substrate molecules to bind to the active site because they are different shapes.
- Increasing the concentration of substrate doesn't affect the reaction rate - enzyme activity is still inhibited.

Competitive and Non-Competitive Enzyme Inhibitors

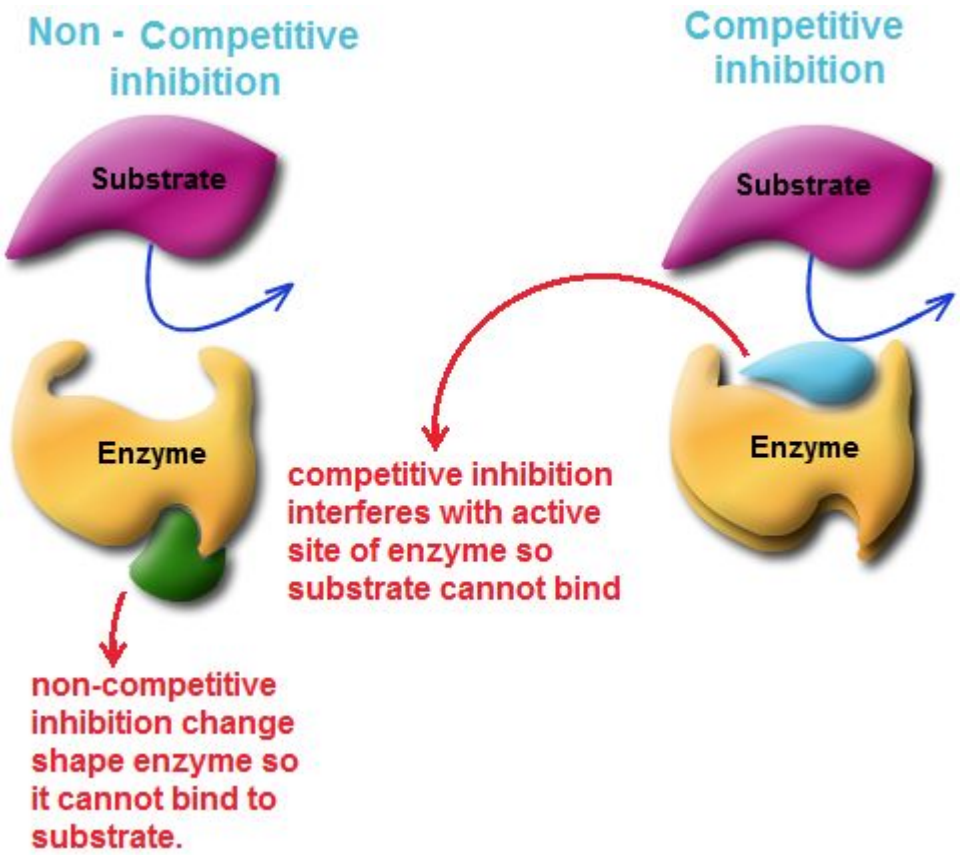
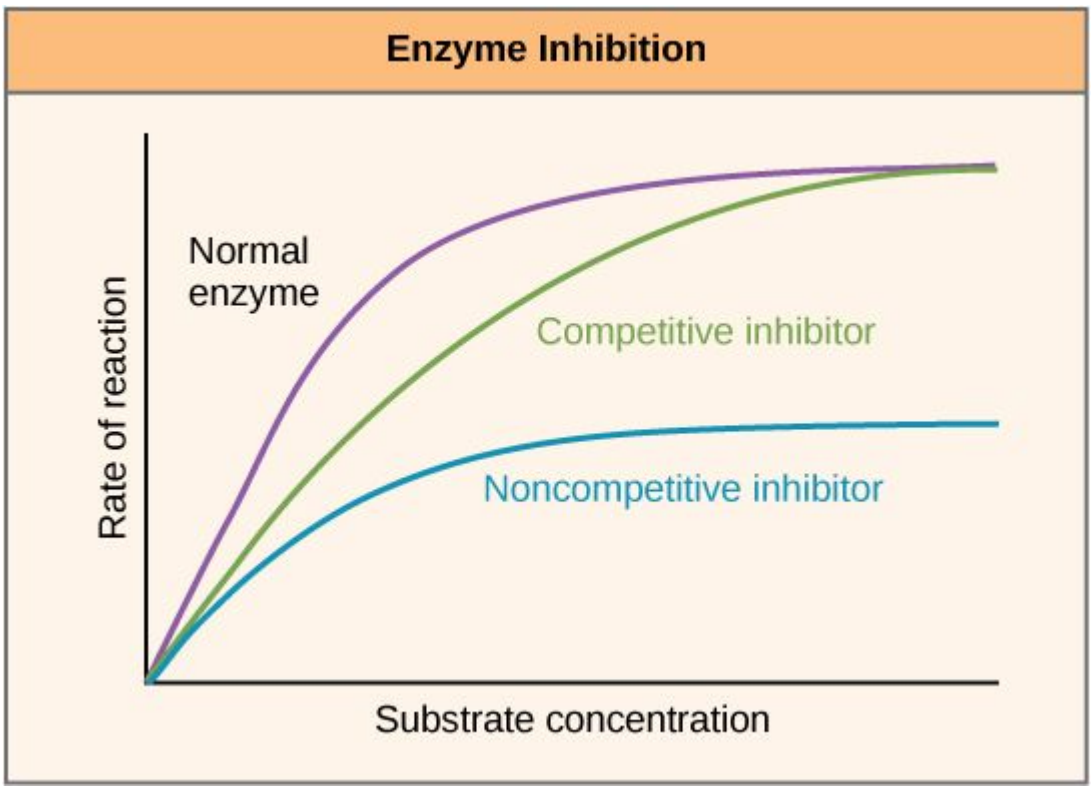
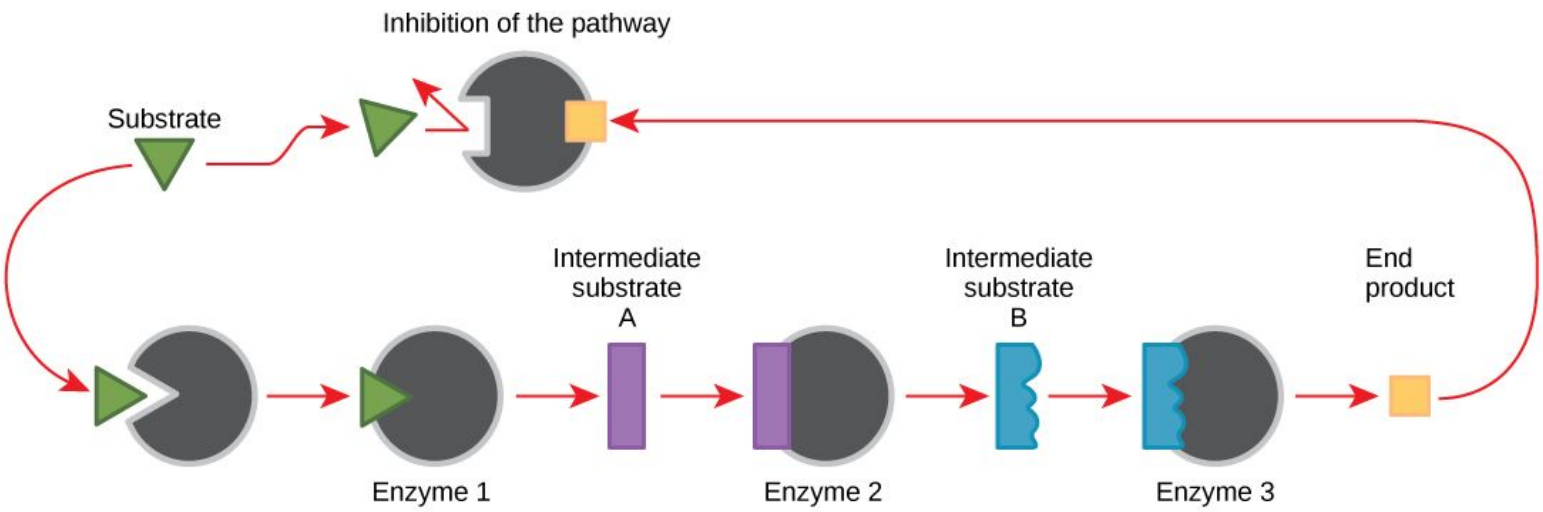


Diagram showing the difference between competitive and non-competitive enzyme inhibition



Competitive and noncompetitive inhibition affect the rate of reaction differently. Competitive inhibitors affect the initial rate but do not affect the maximal rate, whereas noncompetitive inhibitors affect the maximal rate.

Competitive and Non-Competitive Enzyme Inhibitors



Metabolic pathways are a series of reactions catalyzed by multiple enzymes. Feedback inhibition, where the end product of the pathway inhibits an upstream step, is an important regulatory mechanism in cells.

End product inhibition/Allosteric effectors

The activity of some enzymes is controlled by certain molecules binding to a specific regulatory (or allosteric) site on the enzyme, distinct from the active site.

Different molecules can inhibit or activate the enzyme, allowing sophisticated control of the rate. Only a few enzymes can do this, and they are often at the start of a long biochemical pathway.

They are generally activated by the substrate of the pathway and inhibited by the product of the pathway, thus only turning the pathway on when it is needed.