# Recap

We learnt about lipids and how different types have different functions within the body.You now understand triglycerides and their role as energy stores, as well as phospholipid's role as a structural component of cell membranes.

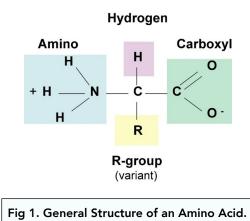


- 1. Different structures of proteins and amino acids.
- 2. Test used to detect presence of proteins.
- 3. Biological roles of proteins in the body.

## 1.4.1 Proteins

## Structure of Amino Acids

- Amino acids are the monomers of proteins.
- There are 20 amino acids and they all have very similar structures. On one end, they contain an amine group (-NH2). This is known as the N-terminus. On the opposite end they contain a carboxyl group (-COOH) which makes them an acid. This is known as the C-terminus.
- The amine group and carboxyl group are covalently bonded to a central carbon atom. The central carbon atom is bonded to one hydrogen (H) atom, and a variable "R" group.
- The variable "R" group is different for each amino acid. This variable group is what gives each amino acid its unique chemical and physical properties. This group is also commonly known as the side chain.
- Different amino acids can be grouped together based on their "R" groups.





Amino acids are the monomers from which proteins are made. Thee general structure of an amino acid involves an amine group, carboxyl group and side chain (R).

## **Peptide Bonding**

- Condensation reactions join amino acids. Condensation reactions lead to formation of strong covalent bonds, called **peptide bonds**, which hold the amino acids together. Water is also released during the reaction.
- Condensation reactions can form dipeptides or polypeptides.



A condensation reaction between two amino acids forms a peptide bond. Condensation of: two amino acids forms a dipeptide; many amino acids forms a polypeptide. A functional protein may contain one or more polypeptides.

- Two amino acids linked to each other are known as a dipeptide.
- Several amino acids linked together are known as a polypeptide.
- Proteins consist of one or more polypeptides.
- Peptide bonds can be broken down by hydrolysis reactions. This can break proteins and peptides down to their constituent amino acids.
- There are 20 common amino acids present in living organisms. The only difference between them is the R side group.
- Glycine is the smallest amino acid. In glycine the R group is just a hydrogen atom.

There is no need to memorise the amino acids below, but use Figure 2 to understand the ways in which amino acids can differ.

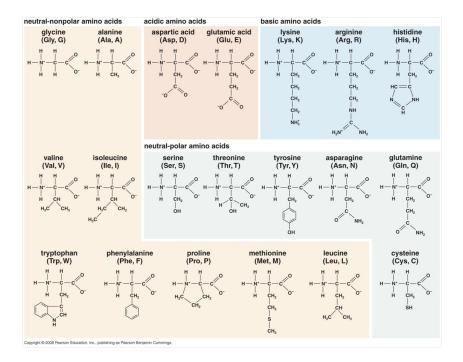


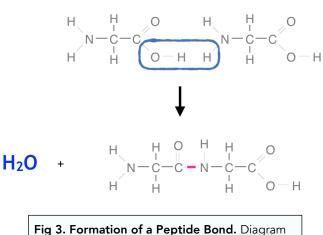
Fig 2. List of 20 Amino Acids.



The twenty amino acids that are common in all organisms differ in just their side group.

# AQA Specification

Proteins have a variety of functions within all living organisms. Understand the relationship between primary, secondary, tertiary and quaternary structure, and protein function. Recognise the role of hydrogen bonds, ionic bonds and disulphide bridges in the structure of proteins.



shows the condensation reaction of two animo acids to from a dipeptide molecule and a water molecule. The peptide bond is shown in pink.

## **Structure of Proteins**

Proteins are fairly large molecules and are extremely complex. To make things simpler, we can describe protein structure in terms of four levels, with each new level building upon the previous level.

## **Primary Structure**

The **primary structure** of a protein is the sequence of amino acids that make up its polypeptide chains.

## Secondary Structure

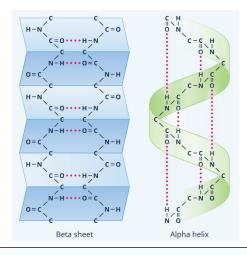
The primary structure is flat and 2D. Proteins, like other molecules, are 3D objects. Hydrogen bonds between different amino acids in the chain are responsible for the **secondary structure**.

**Hydrogen bonds** form between the R groups of amino acids. Hydrogen bonding between the variable "R" groups of amino acid side-chains causes coiling (**alpha helices**) or folding (**beta sheets**).

• Alpha (a) helices form due to coiling. Hydrogen bonding leads to coiling, forming a spiral, cylindrical (helical) shape.



Make sure you remember what hydrogen bonds are and how they work. A quick Google search for "hydrogen bonding" and "intermolecular forces" should will do the trick!  Beta (β) pleated sheets form due to folding. Hydrogen bonding leads to folding, forming an almost flat, but kinked sheet of amino acids. They are formed by two amino acids chains running anti-parallel to each other (see Figure 4).



**Fig 4. Secondary Structure:** Notice how the beta sheet has two amino acid chains running side by side, whilst the alpha helix has coiling. The red dots are hydrogen bonds which hold together both the beta pleated sheets and the alpha helix.

## **Tertiary Structure**

- The secondary structure of a protein can be further folded or coiled into a **tertiary structure**.
- Tertiary structure can involve further coiling and folding. The tertiary structure is made up by different combinations of alpha helices and beta pleated sheets.
- The tertiary structure involves four types of bonds:
  - Ionic bonds
  - Disulfide bridges
  - Hydrophobic forces
  - Hydrogen bonds
- Ionic bonds contribute to folding. Ionic bonds result from the electrostatic interactions between electrochemically charged sidechains of different amino acids. These bonds contribute to the folding process of the tertiary structure.



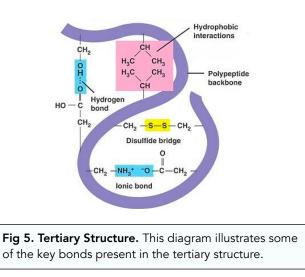
## 1. Define the primary structure of a protein?

- 2. What causes alpha helices to form?
- What is the main type of bonding involved in forming the secondary structure?
- 4. What are the 4 types of bonds involved in forming the tertiary structure?

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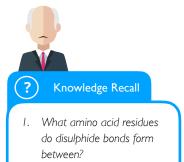


- Disulfide bonds are covalent bonds which form between cysteine residues. Cysteine is the only amino acid that contains sulfur containing sulfhydryl groups (-SH). When in close proximity to another cysteine, the sulfur atom of one cysteine can covalently bond with a sulfur atom of the neighbouring cysteine to produce a disulfide bond, which is a covalent bond and is very important for the structure of proteins.
- Hydrophobic forces are also key. Hydrophobic (non-polar) amino acids, such as valine and proline, are 'repelled' from water. Therefore they get pushed "inside" the protein due to water molecules in their environment. Hydrophobic Forces occur between non-polar amino acids.
- The tertiary structure may be the final structure. Proteins consisting of only one polypeptide chain have their tertiary structure as their final structure.



### **Quaternary Structure**

The **quaternary structure** refers to the way in which different polypeptide chains come together to form the final protein structure.



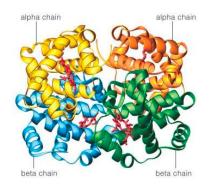
- 2. What type of amino acids do hydrophobic forces form between?
- What type of amino acids fo ionic bonds form between?



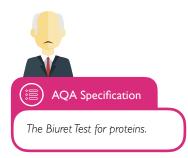
- The quaternary is the final 3D conformation. Some proteins only have one polypeptide chain, but those with multiple chains undergo additional folding to form the quaternary structure.
- Various bonds join together the polypeptide chains. Different polypeptide chains coming together to form the final protein are typically bonded together either via hydrogen bonds, covalent bonds, or ionic bonds.
- Proteins can be dimers, trimers, tetramers and so on. Proteins made up of two polypeptide chains are called dimers. Proteins made of three polypeptide chains are called trimers, four chains tetramers, and so on.
- Proteins can either be homomers or heteromers.
  - In homomers, the polypeptide chains are all identical
  - In heteromers, the polypeptide chains are non-identical.
- Haemoglobin is a tetrameric heteromer. Haemoglobin consists of four polypeptide chains (tetrameric) which are non-identical (heteromer) (See Figure 6).

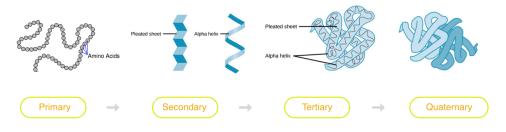
## ?) Knowledge Recall

- 1. Define the quaternary structure of a protein?
- 2. Name a protein that has a quaternary structure?
- 3. How many polypeptide chains does this protein have?



**Fig 6. Quaternary Structure.** The quaternary structure consists multiple polypeptide chains coming together.





**Fig 7. The Different Progressive Structures During Protein Formation.** Primary structure made from the joining together of amino acids. Secondary structure could be a beta pleated sheet or an alpha helix. Tertiary structure might be the final protein structure. Quaternary structure is formed by different polypeptides chain come together.

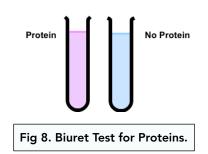
## **Biuret Test:** Proteins

We can use the biuret test to test for the presence of proteins. To perform the test, follow these steps:

- 1. **Make the solution alkaline**. In order to properly test for the presence of proteins, your test solution needs to be alkaline. To make it alkaline, you need to add a few drops of sodium hydroxide (which is a base) to your solution.
- 2. Add copper (II) sulphate. Next, you need to add a few drops of aqueous copper(II) sulphate solution to your test solution.
- 3. Observe the colour change of your test solution.

If the solution remains **blue**, this means there is **no protein** present in your sample.

If the solution turns **purple**, this is a positive result which means there is **protein** present in your sample.





- For the biuret test for proteins, it is important that the solution is alkaline or acid?
- 2. What needs to be added to make the solution like this?
- What colour change indicates the presence of a protein?







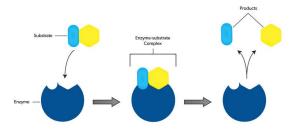
Relate the structure of proteins to properties of proteins mentioned in the specification.

## Protein Function is Related to Protein Shape

In this section, we will discuss four different classes of proteins, and focus on understanding how their structure determines their biological function. We will learn more about each of these molecules in later sections of the AQA specification.

### Enzymes

- Enzymes usually have an almost spherical shape. Enzymes can be large, or they can be compact, depending on the substrates to which they bind.
- Enzymes are usually soluble in water. This means that they have a large number of polar (hydrophilic) amino acids in their polypeptide chains.
- Enzymes reduce the activation energy of a reaction. Enzymes work by reducing the activation energy of a chemical reaction by binding directly to one or more substrates.
- Most enzymes are capable of binding only a very unique substrate (target). Therefore, the shape of an enzyme directly impacts and determines its biological function.



**Fig 9. How an Enzyme Generally Works.** Substrate binds to the enzyme's substrate binding site. This form an enzyme-substrate complex, allowing the chemical reaction to take place. The products are then released.

## Antibodies

• Antibodies play a critical role in the immune response. Antibodies directly recognise and bind to unique antigens on the surfaces of foreign pathogens such as bacteria and viruses.



Students should be able to relate the structure of proteins to properties of proteins named throughout the specification.

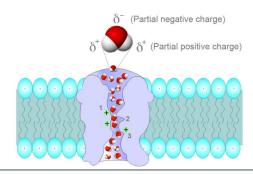
# ? Knowledge Recall

- 1. Name three properties enzymes have?
- What is the general name given to the target of an enzyme?
- 3. What is the main role of an antibody?
- 4. What does the variable region of an antibody determine?

• Each antibody is complementary to a specific antigen. Each antibody has a unique shape which allows it to only bind the antigen which it is designed to bind to. While the majority of the structure of an antibody is common across most antibodies, the structural specificity of an antibody to its complementary antigen is determined by a section called the "variable region".

## **Transport Proteins**

- Transport proteins regulate what enters and exits a cell. They help facilitate the movement of molecules such as ions, water, and glucose into and out of cells.
- The structure of a transport protein determines its transport properties. For example, aquaporin is a transport protein responsible for moving water molecules into and out of cell, and its positively charged interior helps it do this job (See Figure 10).



**Fig 10. Aquaporin.** Aquaporin is a transport protein that helps facilitate the movement of water into and out of cells. Water molecules are polar molecules and are not able to freely cross the non-polar, hydrophobic cell membrane. Aquaporin has a cylindrical shape (due to its many alpha helices) which allows the water to tunnel through the membrane. It is made up of many polar amino acids carrying a positive charge which electrostatically interact with the partially negatively charged water molecules and help them to enter into the cell.



- 1. Give an example of a transport protein? What
- does this transport?What type of bond do most structural proteins contain?
- 3. Give an example of structural protein?

## **Structural Proteins**

- Structural proteins provide structure to a cell or organism. This means that they have to have flawless structural stability.
  - To support **cells**, structural proteins make up the cytoskeleton.
  - To support whole **organisms**, structural proteins can be found in various locations, such as hair and nails (keratin) or connective tissue such as cartilage (collagen).



• Most structural proteins contain covalent bonds. The proteins are made up of extremely long peptide chains that run parallel to each other and are almost directly opposed onto one another. Covalent bonds between these chains results in extremely strong structures.







Each enzyme lowers the activation energy of the reaction it catalyses.

## 1.4.2 Enzymes

## **Enzymes Speed Up Biochemical Reactions by Reducing Activation Energy**

- Enzymes reduce the activation energy (Ea) of a chemical reaction. Activation energy is the minimum amount of energy required for a collision between two particles to result in a reaction.
- Reducing the Ea of a reaction increases the rate at which the reaction occurs. In many instances, chemical reactions in the body require a higher temperature than normal body temperature. Reduction of Ea by enzymes allows for these reactions to occur at lower temperatures and at a greater rate.

## The Structure of an Enzyme Is Crucial to its Function

Remember, the functionality of a protein is directly determined by its biochemical structure.

- All enzymes have an active site with a specific shape. The active site of an enzyme binds to a substrate (the target). The structure of an enzyme's active site determines which substrates it is capable of binding to.
- Enzymes are substrate specific. Because of the unique structure of each enzyme's active site, most enzymes can only readily bind to two substrates which "fit" into its active site.

## **Enzyme Substrate Complexes**

• Enzymes optimise the position of reactants. In order for chemical reactions to happen, reactants need to be in the right place at the right time, and they need to be very close to each other.



## AQA Specification

The properties of an enzyme relates to the tertiary structure of its active site and its ability to combine with complementary substrate(s) to form an enzymesubstrate complex.

## • Enzyme-substrate complexes form. When enzymes bind to their substrates, they form an "enzyme-substrate complex". These complexes reduce activation energy in two ways:

- **Bringing substrates close together**. By bringing two substrates together, the enzyme puts them in very close proximity to each other thereby allowing them to readily bond with each other.
- Putting chemical strain. In catalysis (breakdown) reactions, the active site of an enzyme can put chemical strains on the bonds of a molecule causing them to break easily.

## **Changes in Tertiary Structure**

- Enzymes are similar to most proteins. Enzymes are proteins and therefore their chemical properties are more or less similar to most proteins. The majority of the properties that will be discussed in this section can be readily applied to proteins as well.
- Like proteins, enzymes derive their properties from their tertiary structure. Changes to their tertiary structure will lead to changes in their functionality. The tertiary structure of an enzyme determines the structure of its active site, and therefore its substrate binding ability.

We will explore how factors such as pH and temperature can affect the tertiary structure of enzymes, and therefore impact rate of reaction.

## **Measuring Rate of Reaction**

- Product formation or reactant consumption is used to measure rate. Rates of chemical reactions are measured by how quickly a reactant disappears or by how quickly a product appears.
- We can measure changes in concentrations during a reaction. Because enzymes take a substrate (reactant) and turn it into a new product, we can measure how enzymes affect reaction rates by taking samples from a chemical reaction at different time points and measuring the change in concentrations of our starting substrates and the final product.



Technically speaking, enzymes provide an alternative reaction pathway with a lower activation energy, so they technically don't lower the activation energy of the original reaction path. Imagine you had to climb a hill to cross a city; enzymes would provide an alternative route via a tunnel through the hill - they don't lower the original path! But this distinction is more important in chemistry, so in biology you should be fine with saying that "enzymes lower the activation energy".

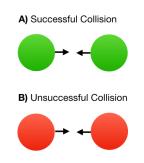




Understand the effects of the following factors on the rate of enzyme-controlled reactions - enzyme concentration, substrate concentration, pH and temperature.

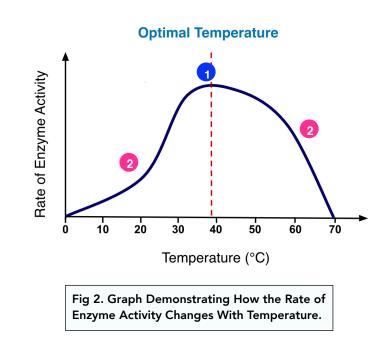
## **Optimum Conditions for Enzymes**

- Enzyme tertiary structures are sensitive to changes. Protein and enzyme tertiary structures are very sensitive to environmental changes and require **optimal** conditions to keep functioning.
- Changes in environmental conditions can cause a protein to denature. Denaturation means a protein loses its shape. The normal shape of a protein or enzyme is known as its native conformation. The reversal of denaturation is known as renaturation.



**Fig 1. Collision Theory.** (A) A collision between two particles has enough energy to cause a reaction. (B) A collision between two particles does not have enough energy so the particles bounce off each other. Enzymes increase the number of successful collisions.

## **Changes in Temperature**





There are some exceptions to this diagram (figure 2), and every enzyme has its own optimum temperature. Even in humans there are some proteins that can function at higher and lower temperatures. Certain organism known as extremophiles, which live in harsh environments, have proteins and enzymes that can tolerate a wide range of temperatures.



#### Knowledge Recall

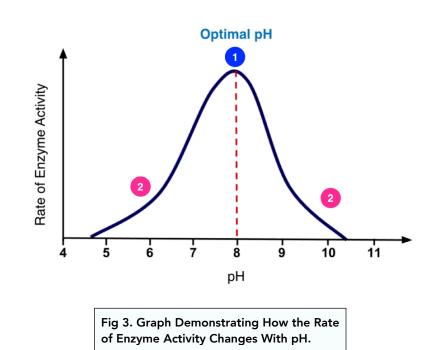
- How does an enzymesubstrate complex reduce the activation energy?
- 2. Explain a possible way to measure the rate of a reaction?
- 3. Why does increasing the temperature (before the optimal temperature) increase the rate of the reaction?

**Optimal temperature**. As you increase temperature, temperature rises because the kinetic energy of reactants increases. This increases the rate in two ways:

- More frequent collisions the reactant particles move faster, collide more often with each other and with enzymes, so there are more successful collisions (leading to a reaction)
- More successful collisions the reactant particles have higher energy, so any given collision is more likely to be successful and result in a reaction.
- Denaturation. As you increase temperature further, bonds in the active site begin to break, and the tertiary structure is disrupted.
  This alters the specific shape of the active site, so it may no longer be complementary to the substrate. This can happen at low or high temperatures.

There are some exceptions to the above diagram, and every enzyme has its own optimum temperature. Even in humans there are some proteins that can function at higher and lower temperatures. Certain organism known as extremophiles, which live in harsh environments, have proteins and enzymes that can tolerate a wide range of temperatures.

#### Changes in pH





- works best at what pH? 3. How can mutations affect
- enzyme function?

- **Optimal pH**. Most proteins and enzymes optimally function at a natural pH of 7.4. However, certain proteins and enzymes can tolerate higher or lower pH levels. For example, the human protein, pepsin, which is found in the stomach, works best at a pH of 2, which is highly acidic.
- **Denaturation**. Denaturation can occur at low or high pH. The enzyme is affected due to disruption of the ionic and hydrogen bonds in the tertiary structure, which leads to an alteration in the specific shape of the active site.

## **Mutations**

• Mutations can disrupt enzymes. Mutations in the DNA of an organism can lead to the development of proteins and enzymes with mutations. These mutations can cause a protein or enzyme to lose its intended function.

## **Natural Degradation**

• Unlike most proteins, enzymes are reusable. Once they bind to a substrate and catalyse a reaction, enzymes will release the substrate and the active site will regain its shape, ready to bind to another set of substrates. However, over time, enzymes can degrade and be replaced by new enzymes.

## **Substrate Concentration**

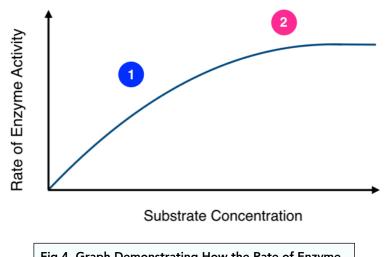


Fig 4. Graph Demonstrating How the Rate of Enzyme Activity Changes With Substrate Concentration.



During the course of a reaction, the substrate concentration inevitably falls as substrate is used up and converted to product. Hence rate of reaction falls during any reaction unless the substrate is replaced.



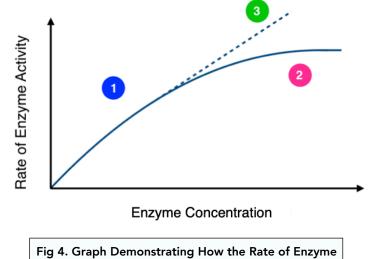


In the exam, when describing changes in enzyme concentration, it is useful to write "assuming a constant substrate concentration". And the reverse for when you describe substrate concentration. **Increasing [substrate] initially increases rate.** Increasing the concentration of substrate will increase the rate of collisions, so there will be more successful collisions per second. This is assuming a constant enzyme concentration.

2

After a while the enzyme active sites are saturated. After a certain point, increasing the concentration of a substrate, while keeping the enzyme concentration constant, no longer increases the rate of reaction. We call this point the saturation point. The enzyme concentration is now the rate-limiting factor.

## **Enzyme Concentration**



Activity Changes With Enzyme Concentration.

 Explain the two phases of how increasing [substrate] affects the rate of enzyme activity?

Knowledge Recall

- 2. What is the saturation point?
- What is meant by a "ratelimiting factor"?

**Increasing [enzyme] initially increases rate**. Similar to increasing the concentration of substrate in a reaction, increasing the number of enzymes increases the rate by increasing the amount of collisions between enzymes and substrates.

After a while there is a lack of substrate. After a certain point, if the amount of substrate is kept constant, the rate of the reaction will not increase with increasing enzyme concentration.

1

2



## ? Knowledge Recall

- How does increasing [enzyme] initially affect the rate of enzyme activity?
- 2. How does increasing [enzyme] affect the rate of enzyme activity if the reaction is with an unlimited substrate?



Understand the effects of the concentration of competitive and of non-competitive inhibitors.

The dotted line represents a reaction with unlimited substrate. If the supply of substrate is unlimited, addition of enzymes will continue to result in increased reaction rates.

## **Inhibition of Enzymes**

3

Often, it is necessary to inhibit the activity of an enzyme. In many biological processes, enzymes function in a negative feedback loop. In this loop, the product of the enzyme will inhibit the enzyme once enough product has been made in order to stop the enzyme from producing more product than what is needed. Additionally, many pharmaceutical drugs are designed to inhibit enzymes for therapeutic purposes. In this section, we will discuss the two major mechanisms by which enzymatic activity can be inhibited.

## **Competitive Inhibition**

- Competitive inhibitors compete with the substrate for the active site. The inhibitor molecule, which has a similar shape to the substrate molecule, competes with the substrate to bind to the active site of an enzyme. When the inhibitor binds, it occupies the active site without causing a reaction.
- Increasing substrate concentration can reduce inhibition. If there is a greater concentration of substrate, the substrate will outcompete the inhibitor for the active site, and vice versa. Therefore increasing the substrate concentration will increase the rate of reaction (up to a certain point, after which the inhibitor is outnumbered and has a negligible effect).

## Non-competitive Inhibition

- Non-competitive inhibitors do not bind to the active site. The inhibitor molecule can bind to a site on an enzyme that is not the active site.
- Non-competitive inhibitors change the shape of the active site. Binding of the inhibitor to the alternative site results in a conformational change (change in shape) of the active site. Because of

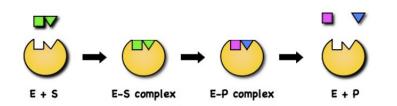


Knowledge Recall

- What part of the enzyme does a competitive inhibitor bind?
- What part of the enzyme does a non-competitive inhibitor bind?
- What effect does increasing [competitive inhibitor] have on rate of enzyme activity?
- What effect does increasing [non-competitive inhibitor] have on rate of enzyme activity?

this conformational change, the enzyme is no longer able to bind to its substrate.

• Increasing substrate concentration has no effect. The inhibitor is binding to an alternative site, so adding more substrate is useless.



**Fig 5**. Lock and Key Model for Enzyme-Substrate Binding. A substrate with the correct structure can bind to an enzyme with a similar active site. Once the enzyme's active site binds to the substrate, it leafs to the formation of the enzyme-substrate complex (E-S) complex. Once the enzyme begins its chemical activity, it forms an enzyme-product (E-P) complex. Upon completion of the reaction, the enzyme releases the products from its active site.

• Non-competitive inhibition is common. Non-competitive inhibition is a common mechanism by which negative feedback loops work in many biochemical processes.

## **Models of Enzymatic Function**

Substrate binding to an enzyme's active site is a tightly regulated biochemical process. It is crucial that the right substrate binds to the right enzyme. There are two main models that explain enzyme and substrate binding.

## Lock and Key Model

- The lock and key model is simple. Every lock can only be opened by a particular key which matches the lock. In order for the lock to open, the key has to fit into the lock perfectly.
- The enzyme is the lock, and substrate the key. In order for a substrate to bind to the active site of an enzyme, it must have the correct shape which allows it to "fit" into the active site.



Be able to appreciate how models of enzyme action have changed over time. Understand the induced fit model of enzyme action.

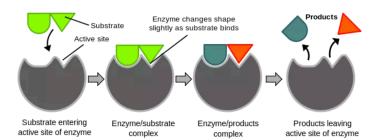


- How does the induce fit model differ from the lock and key model?
- 2. What are the limitations of the lock and fit model?

## **Induced Fit Model**

While the lock and key model is an excellent starting point for understanding how enzymes work, it's not entirely complete.

- The lock and key model has limitations. Enzymes are very selective in substrate binding, but many substrates can have similar shapes. With the lock and key theory, this should lead to inappropriate binding. However, enzymes still manage to have a surprisingly high level of specificity, and this is due to the induced fit model.
- The induced fit model involves a <u>two-step verification</u> method. The induced fit model suggest that when a substrate binds to an enzyme's active site, it causes the active site to change shape as well. For the reaction to happen, the substrate has to change the active site's shape in the right way. This two-step verification method is widely accepted to be the reason for the high specificity with which enzymes function.



**Fig 6. Induced Fit Model.** Substrate binds to the enzyme's active site. As substrate binds, the shape of the active site changes slightly. If the substrate enables the active site's shape to change in the right way then the reaction takes place and an enzyme-product complex is formed. The products are then released from the active site.

We have already discussed much of the content for this specification point. Enzymes are crucial in catalysing both intracellular and extracellular reactions. Here are a few examples:

• **Respiration (intracellular)**. Most steps of aerobic respiration are catalysed by enzymes, as you will learn in later chapters

AQA Specification

Be able to appreciate that enzymes catalyse a wide range of intracellular and extracellular reactions that determine structures and functions from cellular to whole-organism level.



- 2. What is the function of RNA?
- 3. What is the function of ribosomes?

- **Digestion (whole body)**. The digestive process requires many enzymes including protease, carbohydrase and lipase enzymes.
- Liver. Many enzymes in the liver are important in breaking down substances.
- Photosynthesis. In plants, enzymes drive photosynthesis.

