
ENZYMES

FUNCTION

Enzymes catalyse biochemical reactions; they are biological catalysts

- control metabolic reactions in living cells
- convert substrate to product
- synthesis reactions join substrates

  e.g. protease combines amino acids to form protein

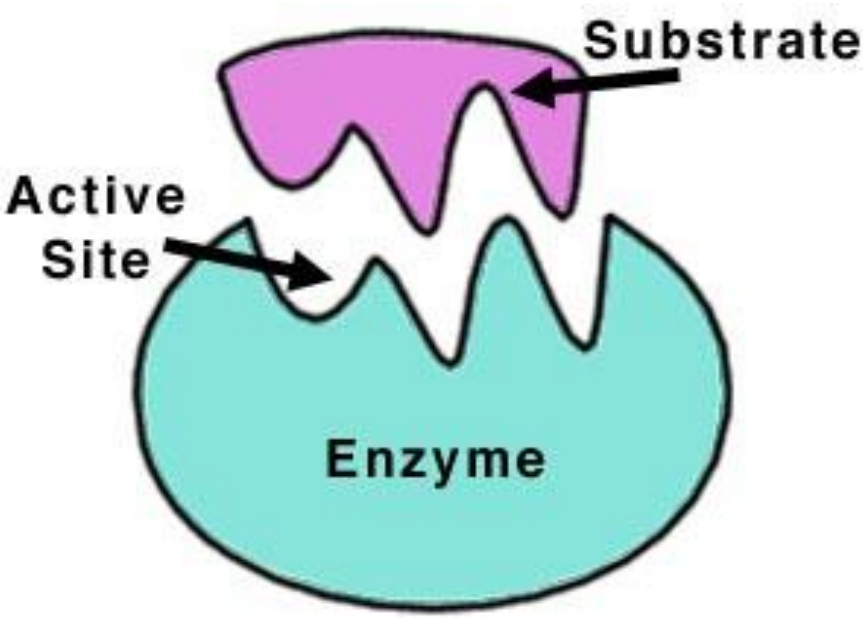
- breakdown reactions breakdown a substrate

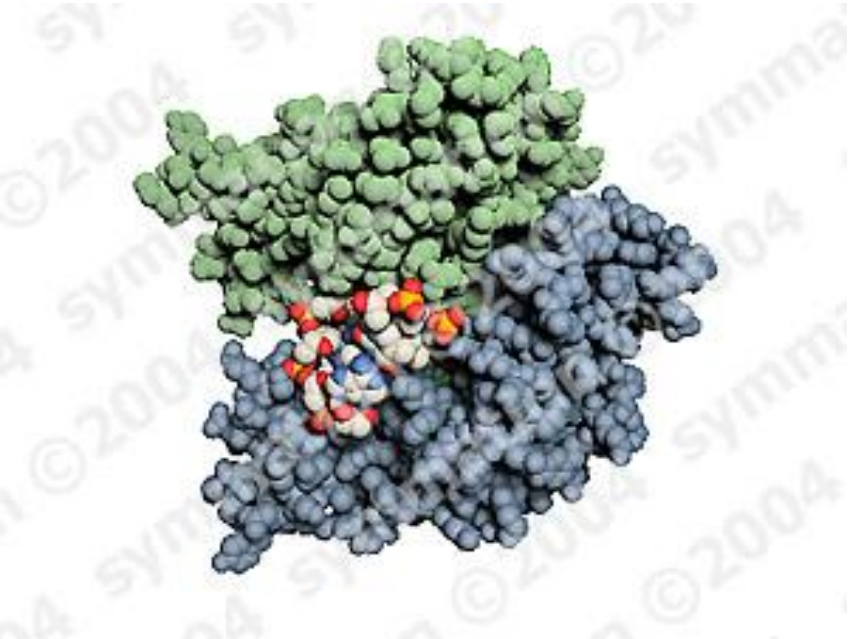
  e.g. lipase breaks down lipids to fatty acids and glycerol

- reactions are reversible

STRUCTURE

- globular proteins with a complex 3D tertiary structure
- the polypeptide chain is folded and twisted into shape
- each enzyme has an indent called the ACTIVE SITE which the substrate binds to

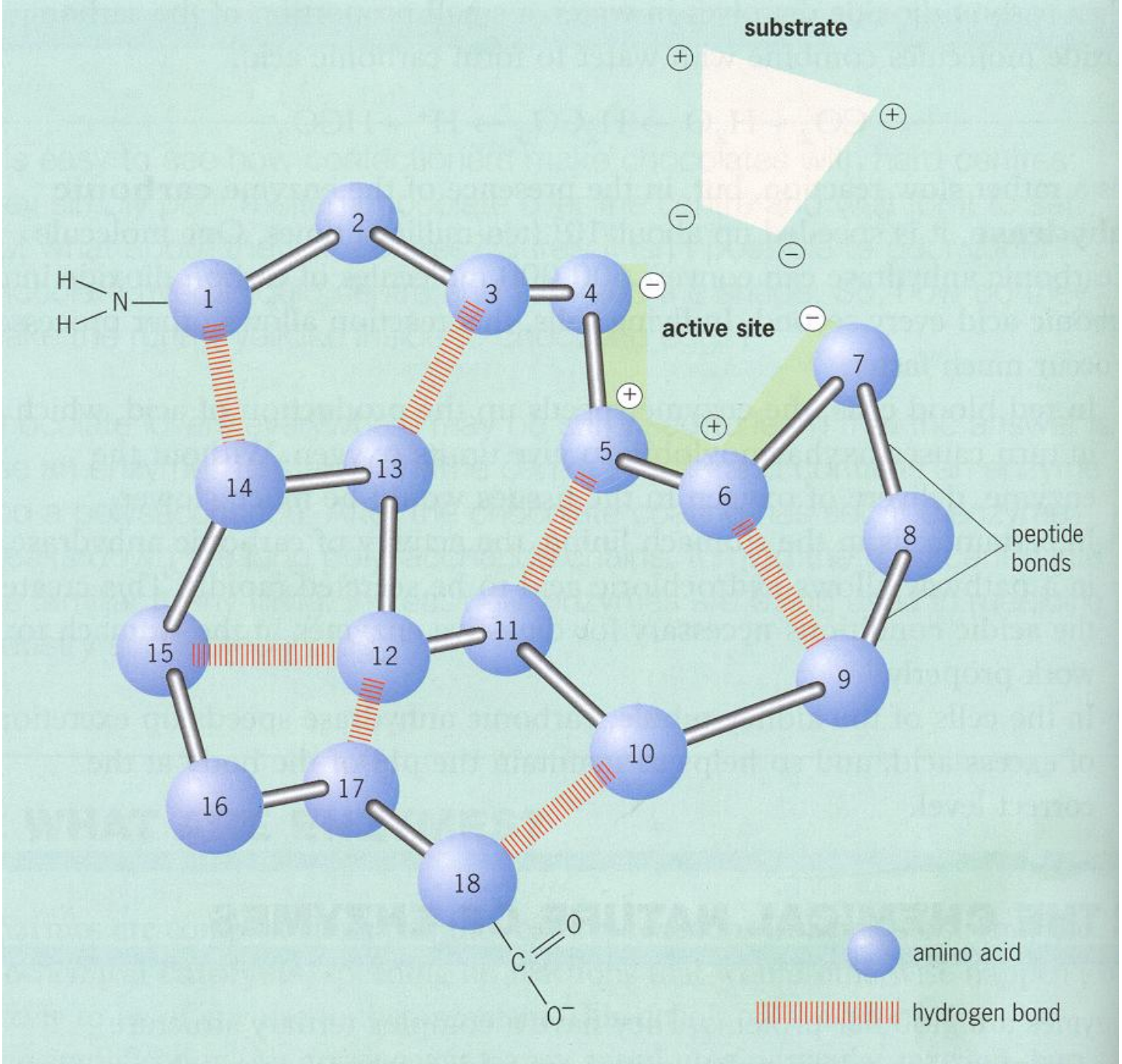




REMEMBER substrate = molecule the enzyme acts on

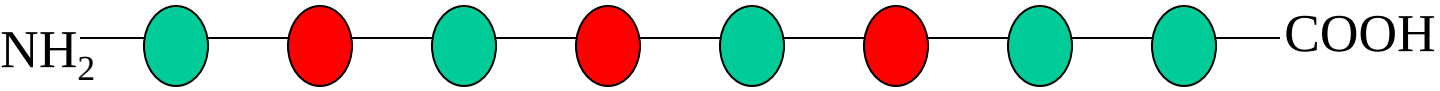
SPECIFICITY

- each enzyme catalyses only one kind of reaction
- only one substrate will fit the active site
- specific amino acids from different parts of the polypeptide chain are found within the active site and are responsible for binding to the substrate
- most other amino acids are involved in maintaining the correct globular shape of the molecule
- So enzyme specificity is due to the highly specific 3D shape


substrate
active site
peptide bonds
amino acid
hydrogen bond
H
N
H
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
O
C
O⁻

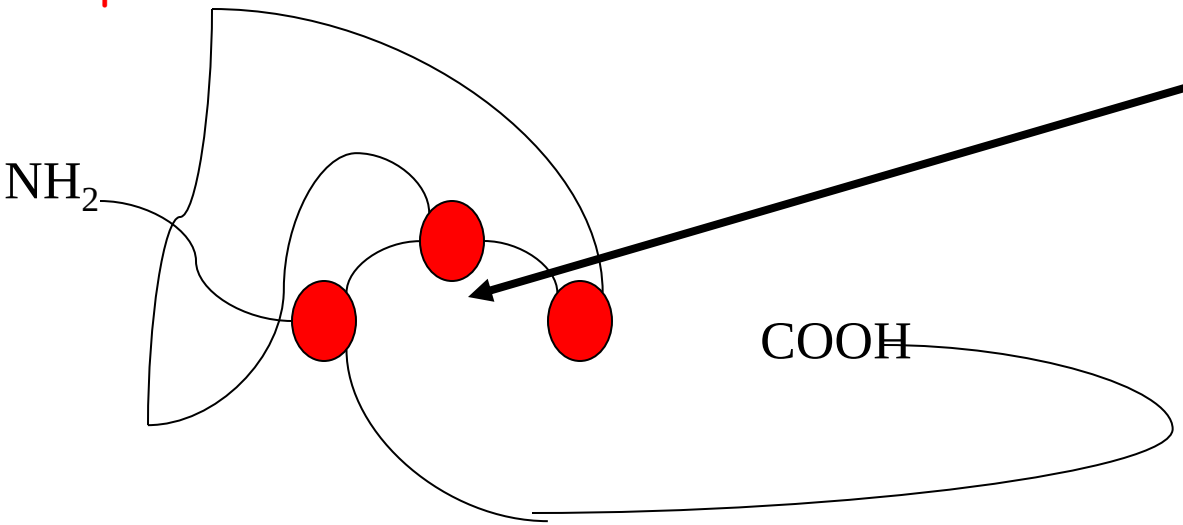
Enzymes

Primary structure of protein



Folds into functional shape inside cell

Tertiary structure of protein



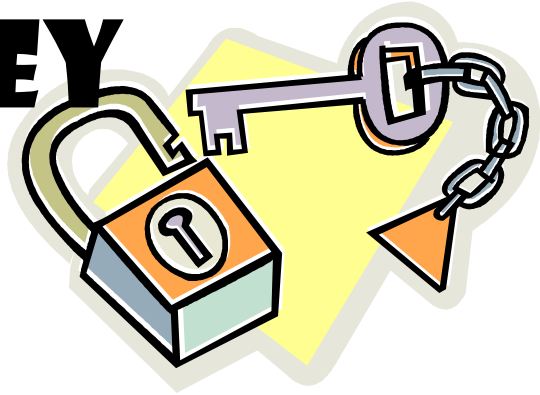
Active site

Red amino acids close together to form active site.Rest of amino acids make up bulk of enzyme

How Enzymes Work

2 theories:

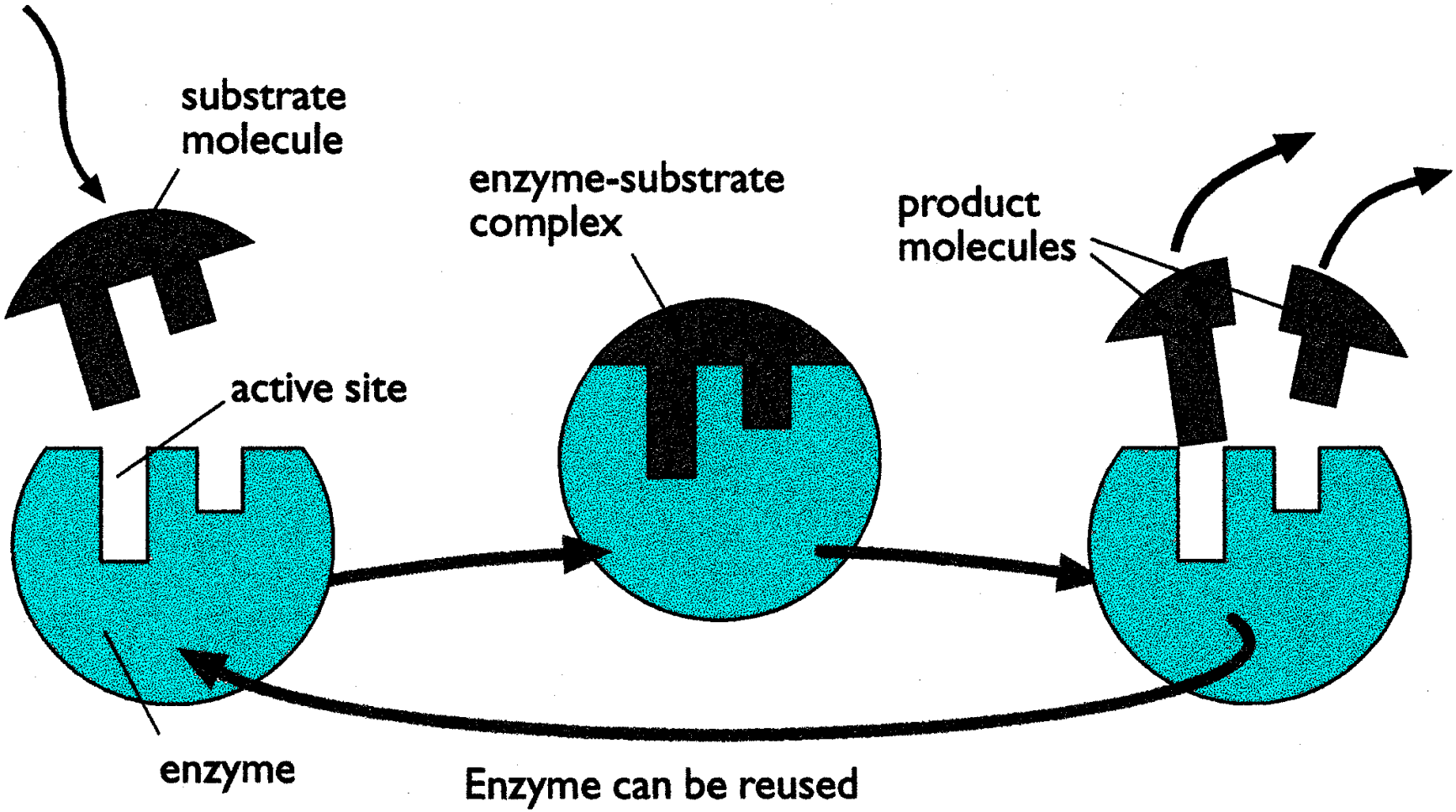
The Lock and Key Hypothesis



The Induced Fit Theory

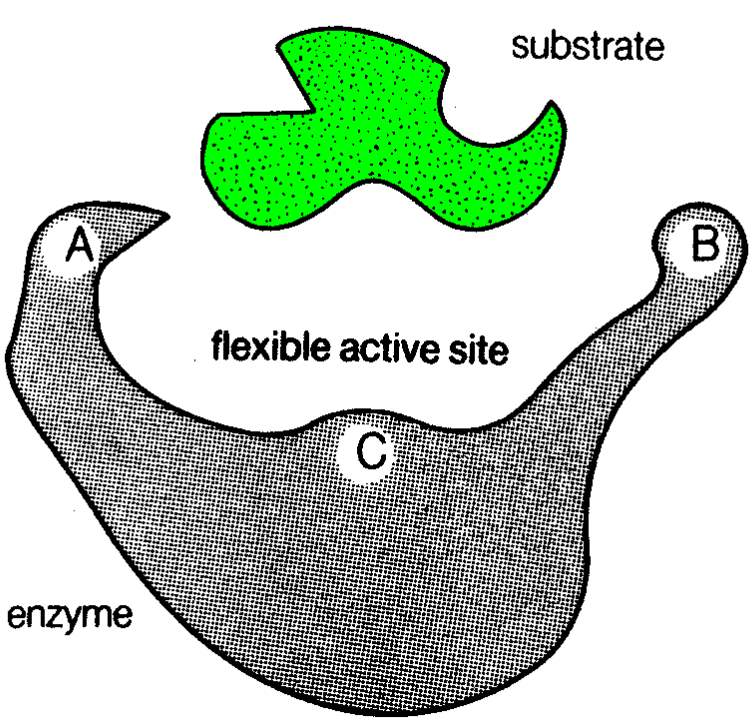
The Lock and Key Hypothesis

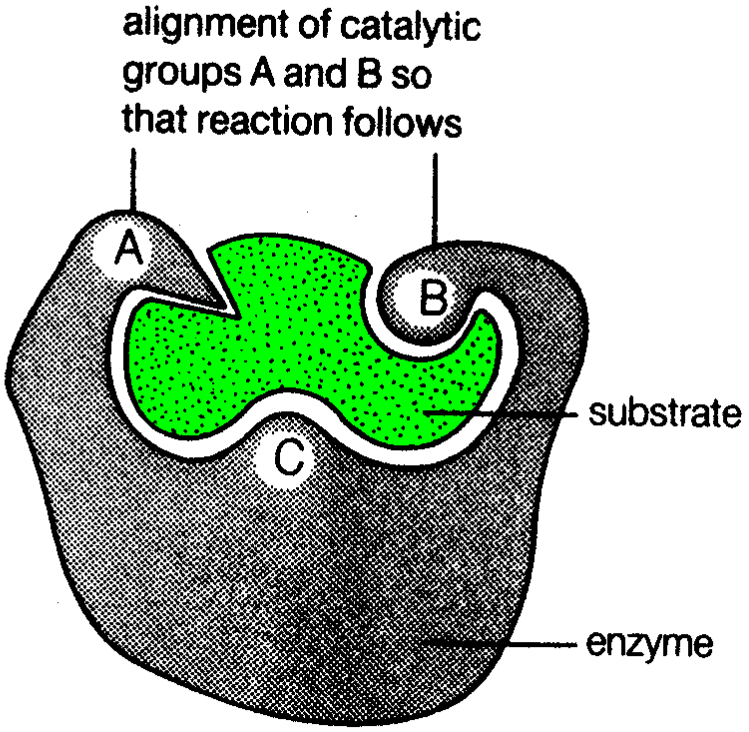
- The shape of the active site is **complementary** to part of the substrate
- The **substrate** molecule is like the **key** that fits into the **enzymes lock**, active site
- The 2 molecules form a temporary structure called an **enzyme-substrate complex**
- The reaction takes place at the **active site** where products are formed
- **Products** have a different shape to the substrate so no longer fit the active site and are **repelled**
- The active site is free to react with more substrate


substrate molecule
enzyme-substrate complex
product molecules
active site
enzyme
Enzyme can be reused

The Induced Fit Theory

- The active site is **not exactly complementary** to the substrate
- The active site is **flexible**
- As the substrate binds it causes the active site to **mould** itself into a tighter fit around the substrate
- The enzyme puts **strain** on the bonds in the substrate
- The shape of the substrate in the enzyme-substrate complex **distorts**, causing the reaction to occur rapidly
- As the product is a different shape to the substrate it diffuses out of the active site, which **returns to its original shape**, ready to bind with the next substrate molecule

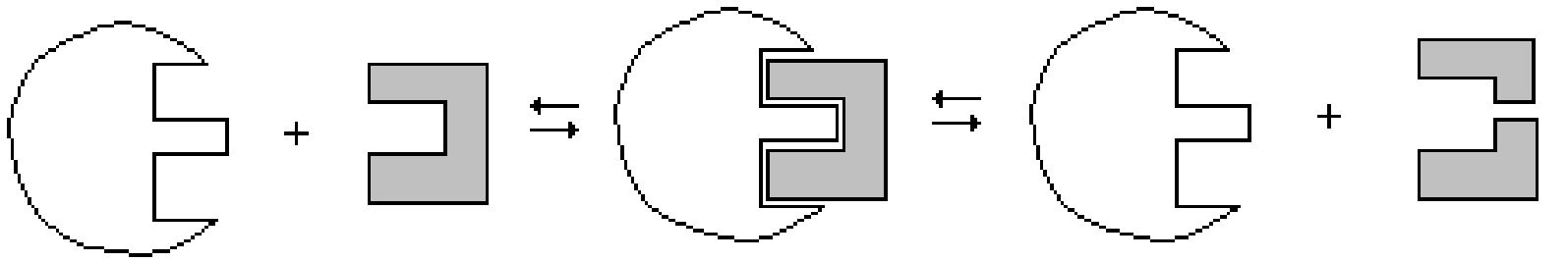

substrate
A
B
flexible active site
C
enzyme


alignment of catalytic groups A and B so that reaction follows
A
B
C
substrate
enzyme

BOTH THEORIES ARE SUBSTRATE SPECIFIC

ENZYMES AND ENERGY

If substrates and enzyme collide with sufficient energy they will form a high-energy, unstable intermediate called an **enzyme-substrate complex** that quickly changes into product .



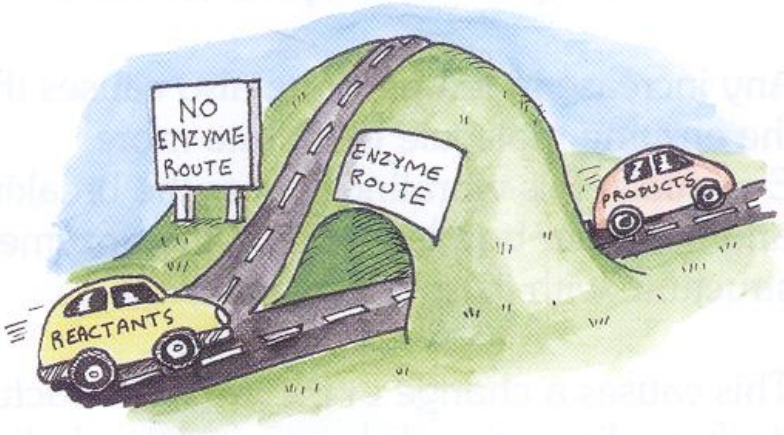
This energy is called **ACTIVATION ENERGY**

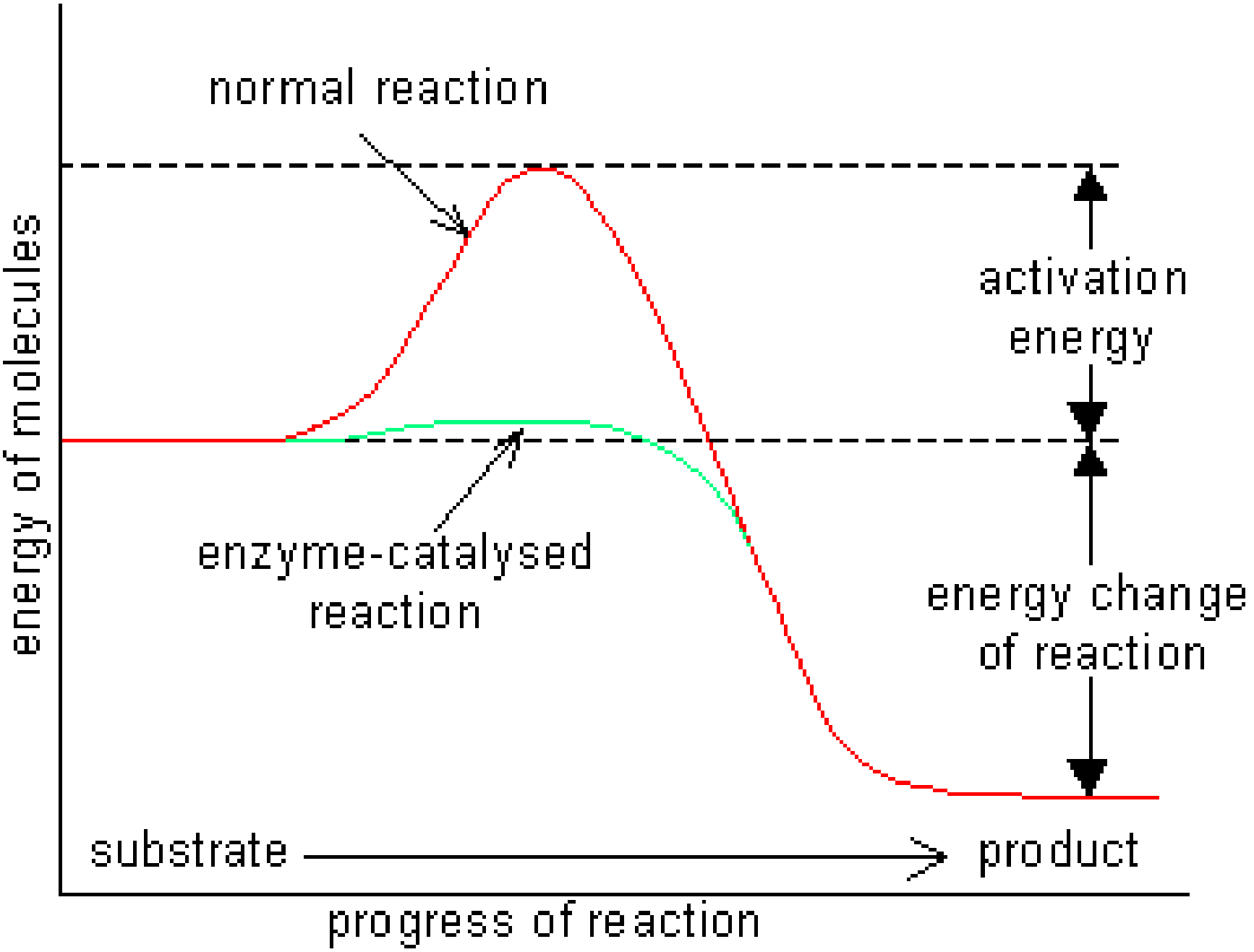
ACTIVATION ENERGY IS DEFINED AS THE MINIMUM AMOUNT OF ENERGY REQUIRED TO FORM AN ENZYME-SUBSTRATE COMPLEX.

Enzymes help to
reduce the activation energy
for a reaction.

This allows reactions to take place at the lower temperatures found in cells.

The enzyme provides a different pathway for the reaction to follow

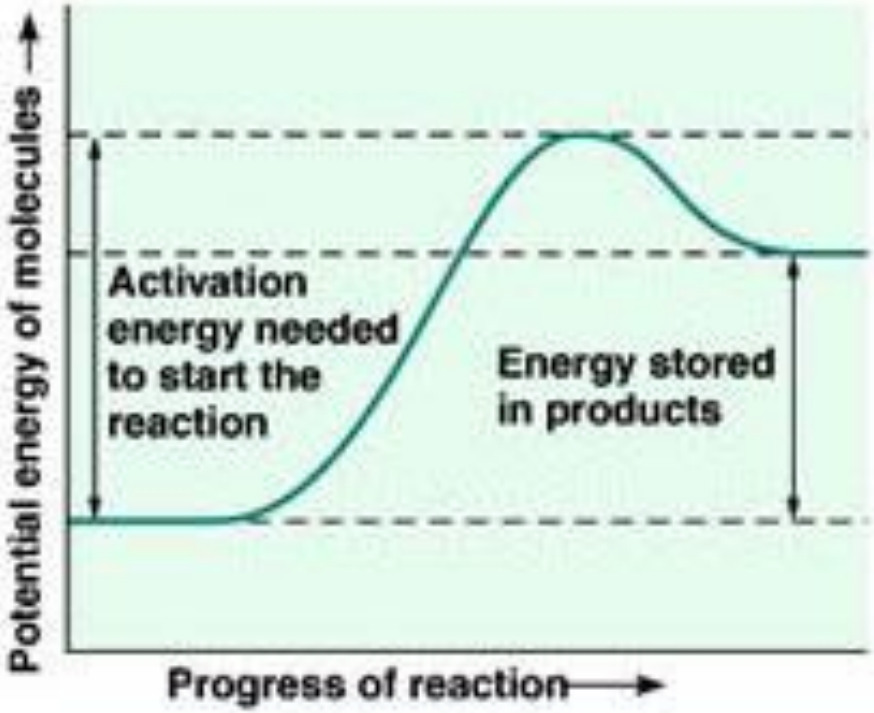



normal reaction
activation energy
energy change of reaction
enzyme-catalysed reaction
energy of molecules
substrate
product
progress of reaction

The reaction in this graph is exothermic/exogonic as the substrate energy level is more than product energy level

Draw a graph to show an endothermic/ endogonic reaction

Endergonic reactions



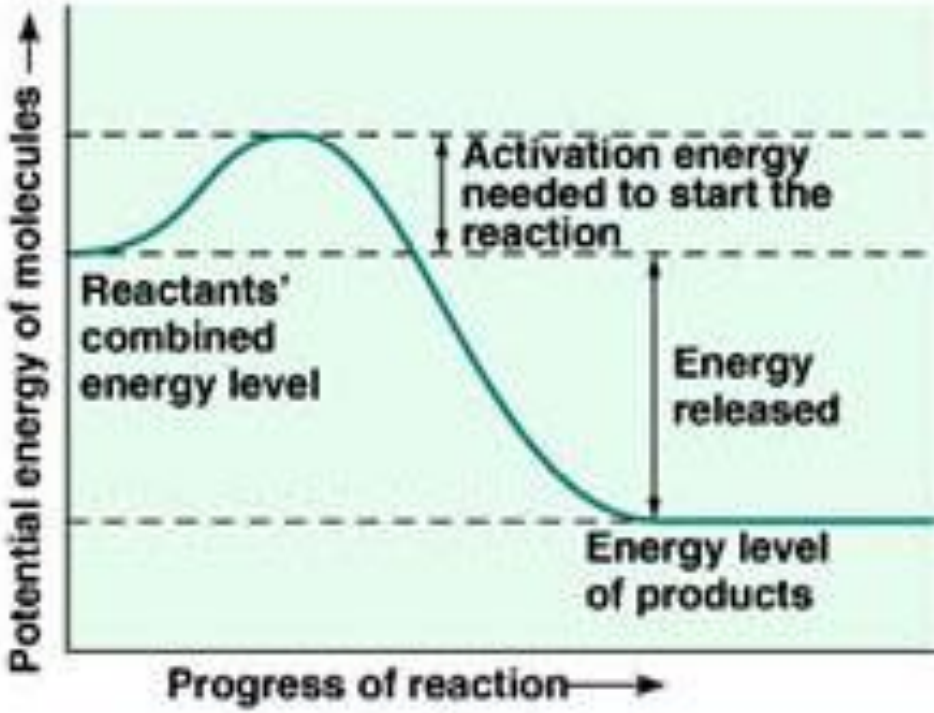
Energy required

$CO_2 + H_2O \rightarrow C_6H_{12}O_6 + O_2$

Carbon dioxide — water — glucose — oxygen

photosynthesis

Exergonic reactions



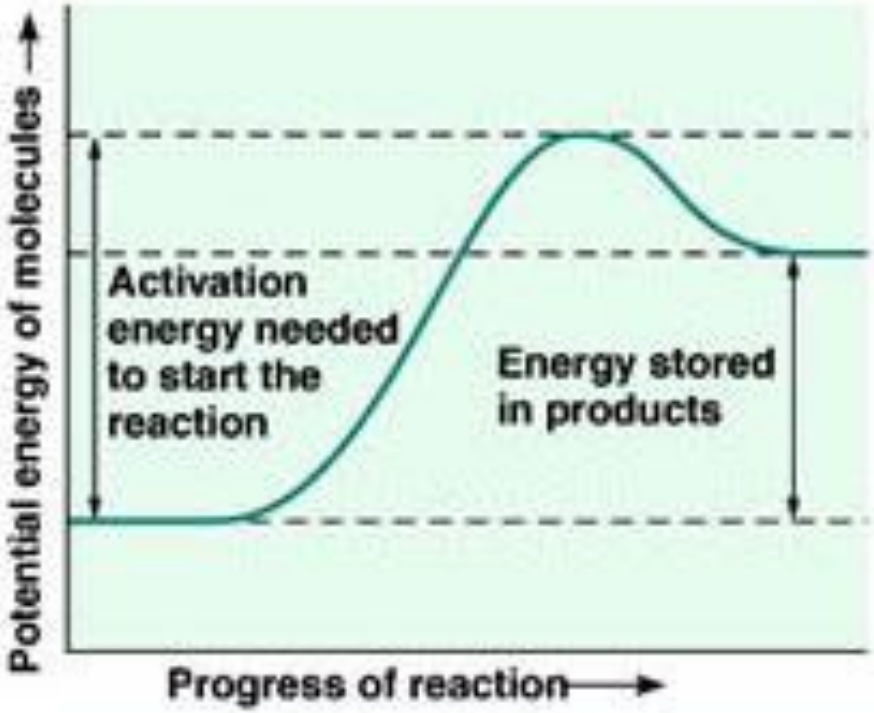
Energy released

$6O_2 + C_6H_{12}O_6 \rightarrow 6CO_2 + 6H_2O$

Oxygen — Glucose — Carbon dioxide — Water

respiration

Endergonic reactions



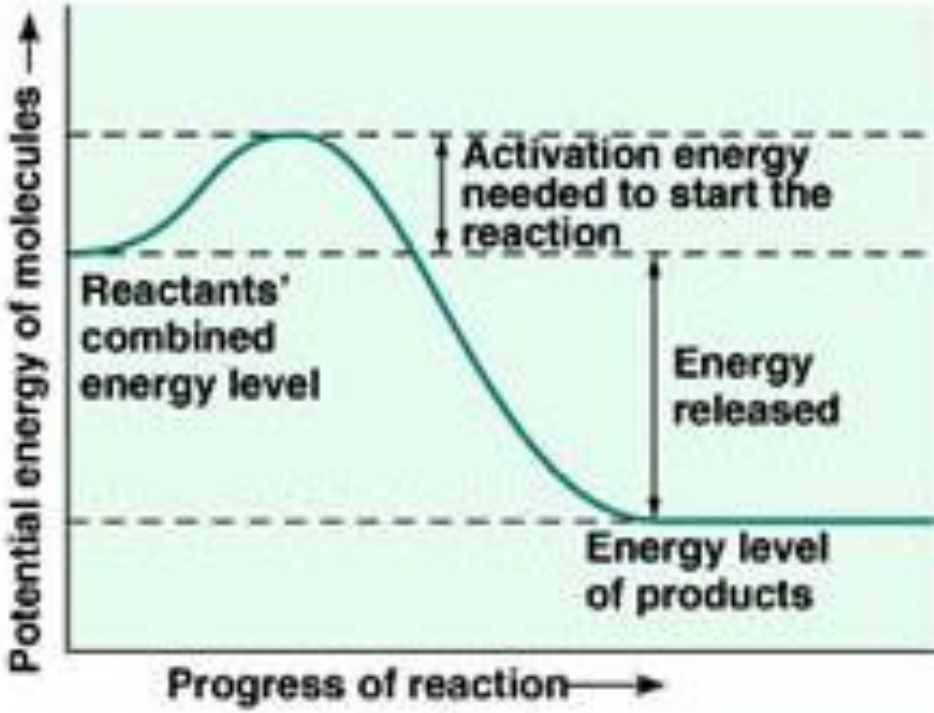
Energy required

$CO_2 + H_2O \rightarrow C_6H_{12}O_6 + O_2$

Carbon dioxide — water — glucose — oxygen

photosynthesis

Exergonic reactions



Energy released

$6O_2 + C_6H_{12}O_6 \rightarrow 6CO_2 + 6H_2O$

Oxygen — Glucose — Carbon dioxide — Water

respiration

A NUMBER OF FACTORS AFFECT ENZYME ACTIVITY

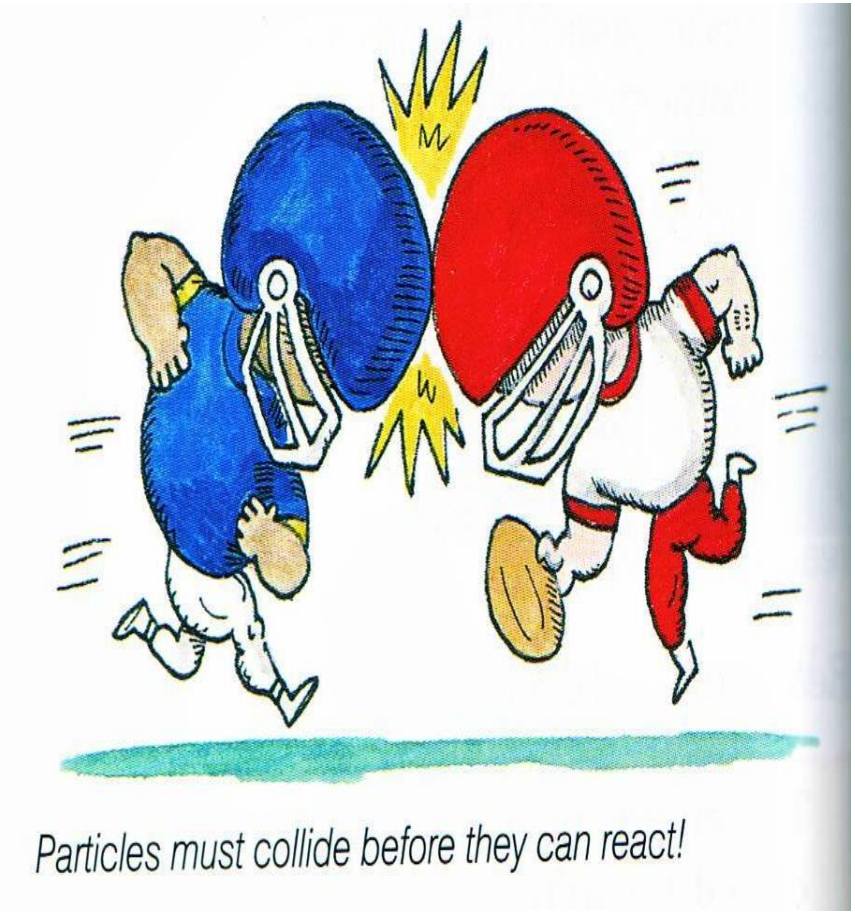
temperature

pH

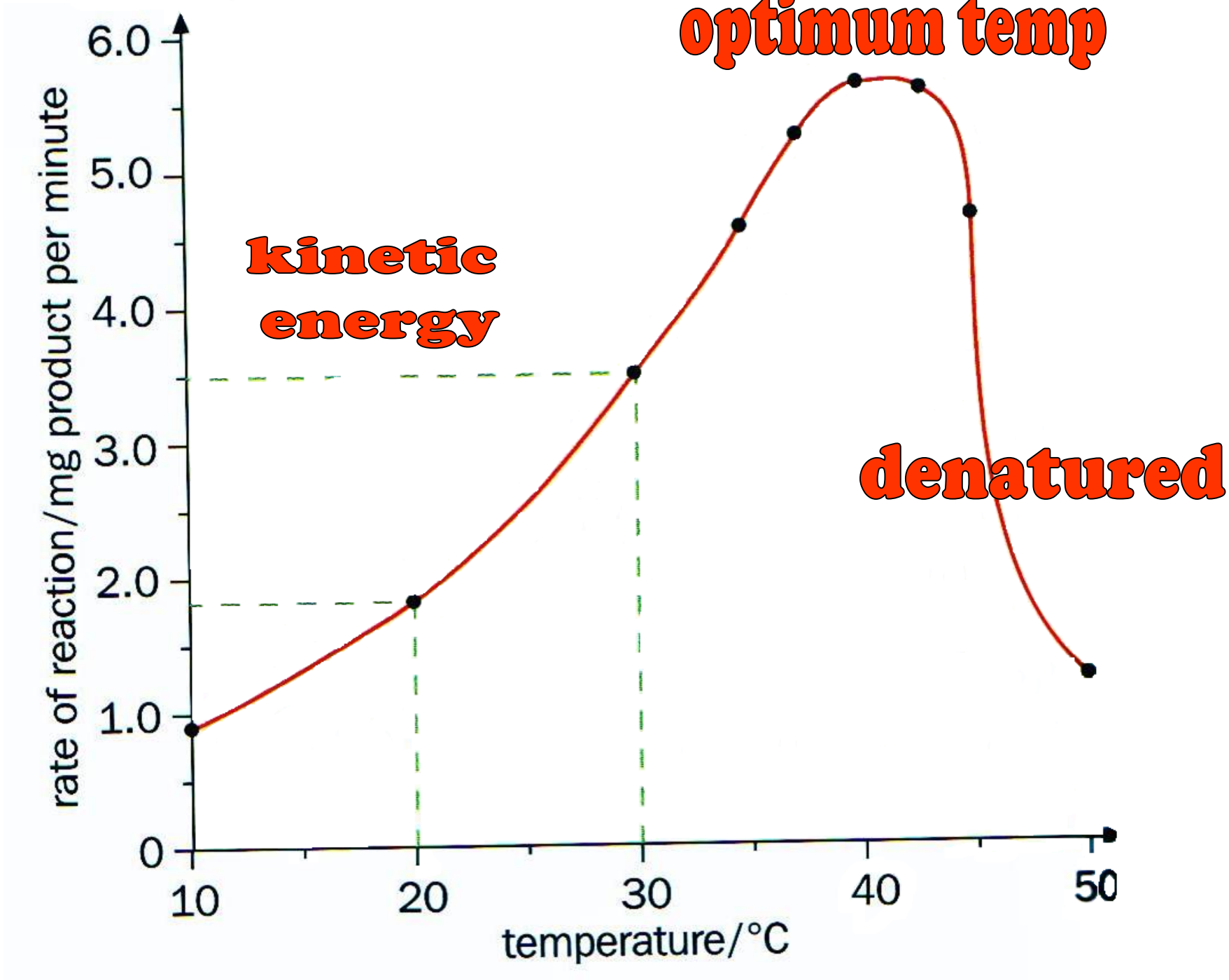
substrate concentration

enzyme concentration

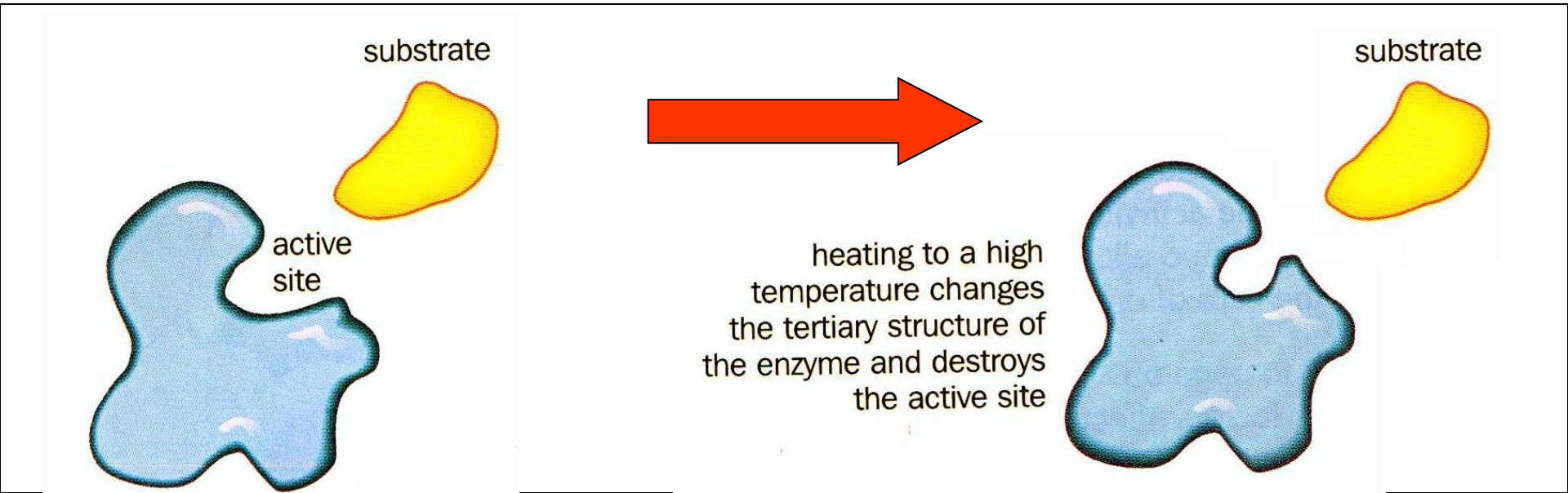
temperature



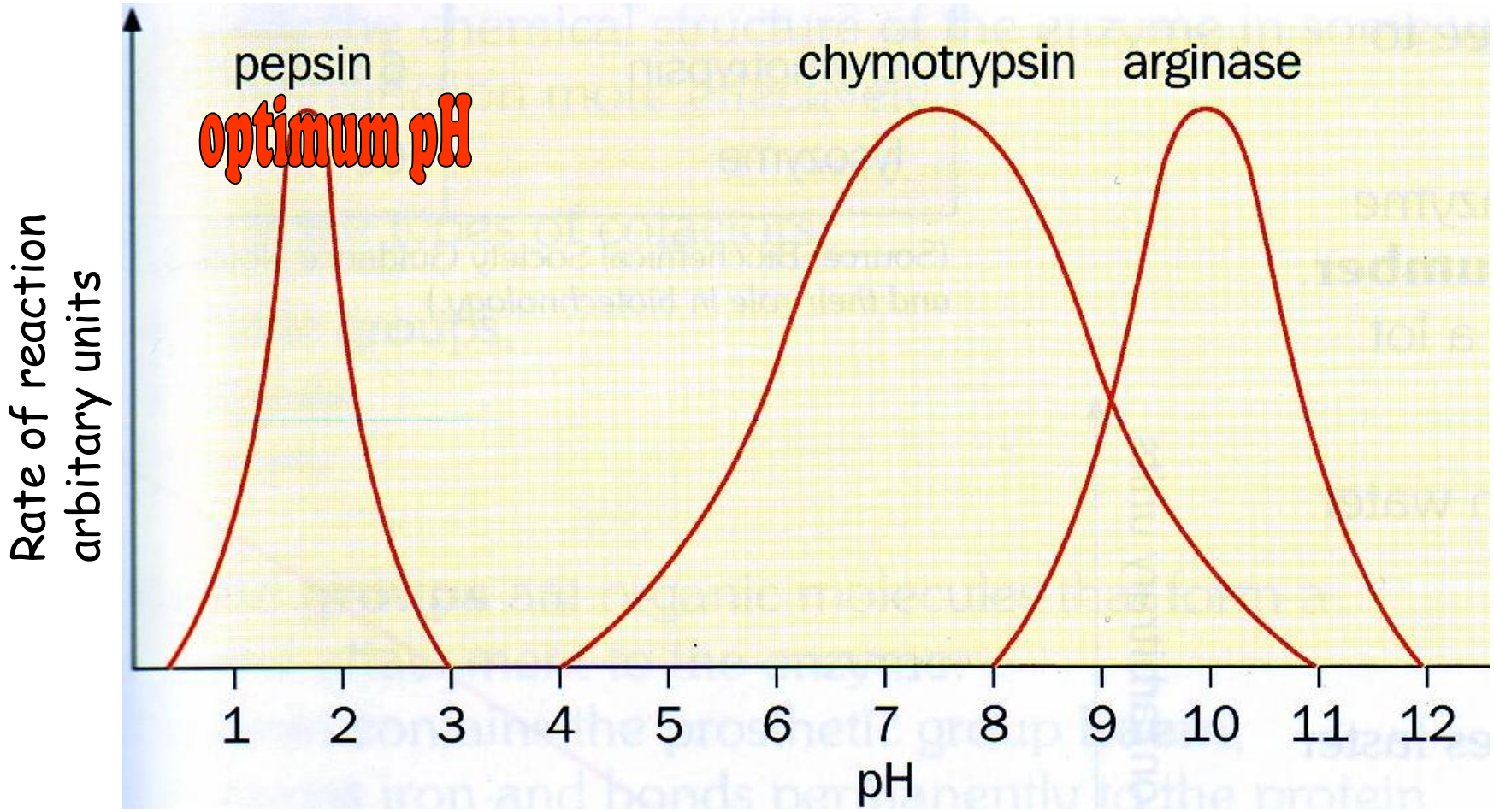
Raising the temp gives molecules more **kinetic energy** therefore there is a greater chance of collisions occurring. More enzyme-substrate complexes are formed making reaction more likely.


optimum temp
kinetic energy
denatured
rate of reaction/mg product per minute
6.0
5.0
4.0
3.0
2.0
1.0
0
10
20
30
40
50
temperature/°C

- An increase of 10°C causes the rate of enzyme controlled reactions to double (exponential) up to an **optimum temperature.**
- This is because increasing temp causes particles to vibrate more which eventually results in H bonds and other bonds breaking. This changes the shape of the tertiary structure of the enzyme which includes its active site which will no longer fit the substrate.
- The enzyme is **denatured**. This is a permanent change that can't be reversed by cooling. The enzyme has lost its activity as it can no longer form enzyme/substrate complexes



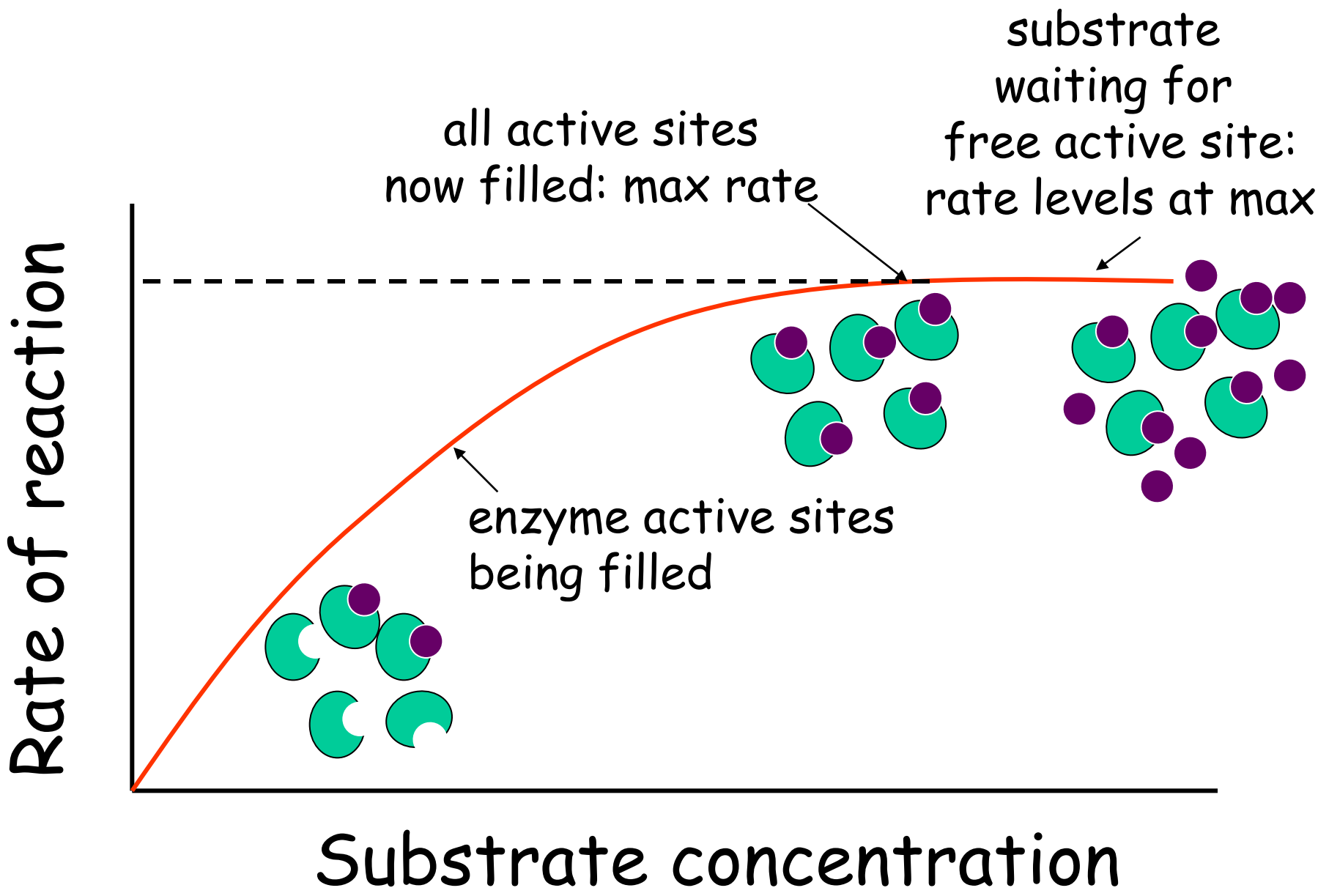
pH



Graph showing the optimum pH for 3 different enzymes

- Small changes in pH can affect the rate of reaction without denaturation occurring but at the extreme pH the enzyme can become unstable and denature.
- Many of the bonds holding the enzyme's 3D shape together are **ionic** bonds.
- Free H^+ and OH^- ions can affect the charges on the amino acid side chains of the enzyme's active site. This will affect the ionic bonding and so change the 3D shape of the enzyme and its active site. The substrate will no longer fit the active site the enzyme loses it activity and the rate of reaction falls.

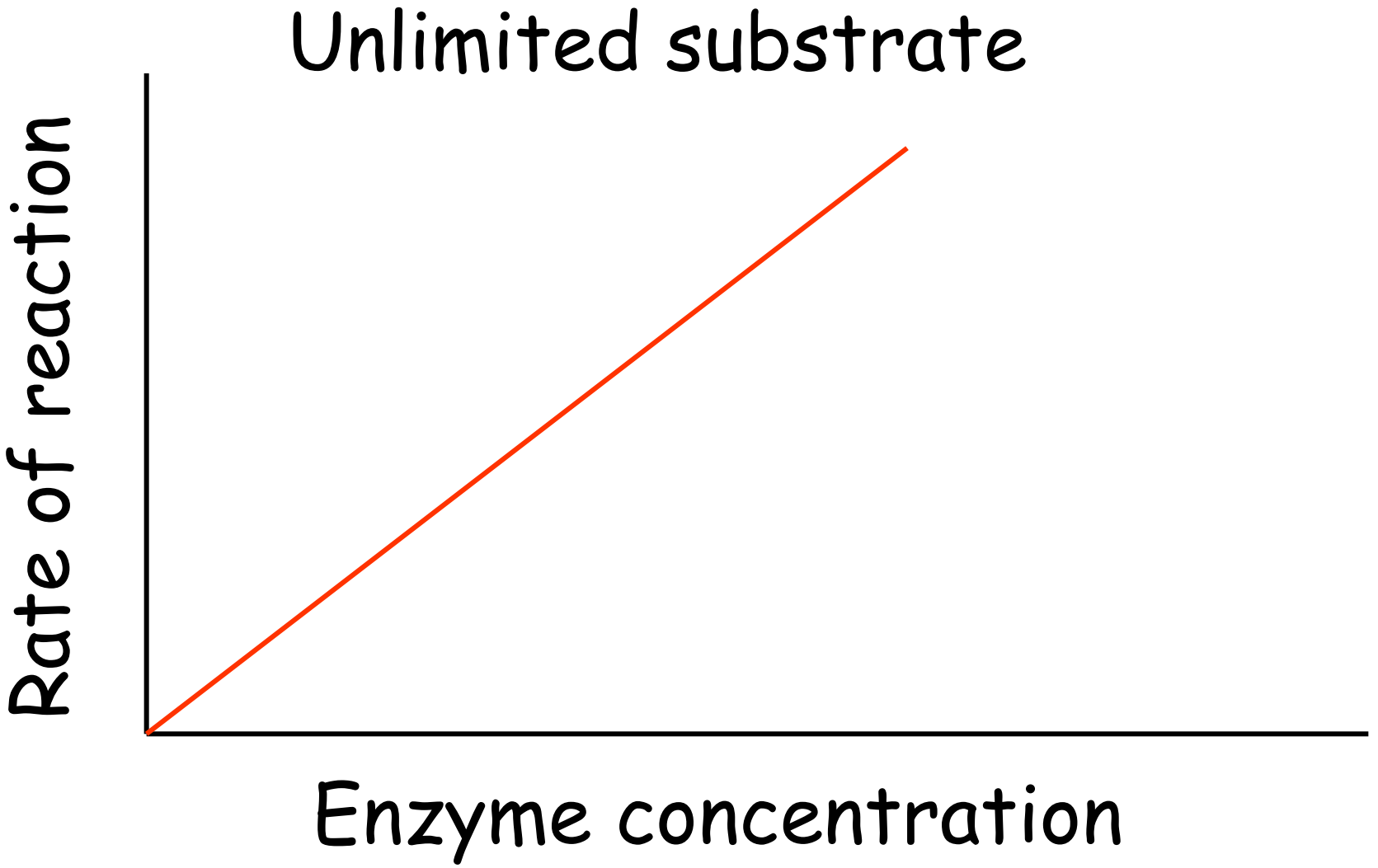
substrate concentration



- If the amount of enzyme remains constant and you add more substrate the rate of reaction will increase as the enzymes active sites are being filled until all the active sites are filled.
- When all the active sites are filled the rate of reaction cannot further increase when more substrate is added as it can't bind with the enzymes active site as they are all filled.

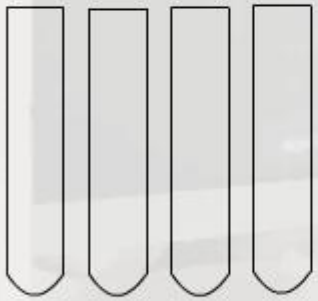
enzyme concentration

As long as there is excess substrate any increase in the number of enzyme molecules will result in an increase in the number of enzyme-substrate complexes and therefore an increase in the rate of reaction.


Unlimited substrate
Rate of reaction
Enzyme concentration

Effect of temperature on catalase activity

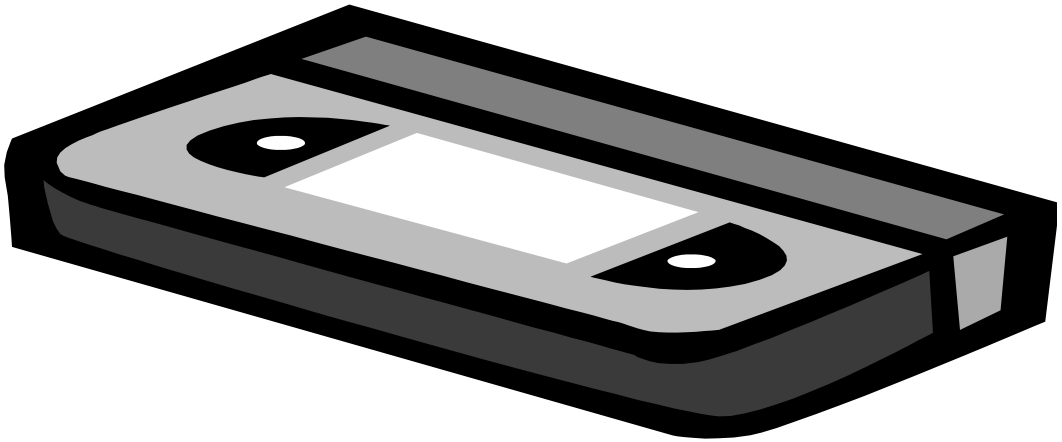
Obtain four clean tubes.
Add 3 ml. of Hydrogen Peroxide (H_2O_2) to each



H_2O_2

Click on the Graduated cylinder.

Video AS Guru enzymes



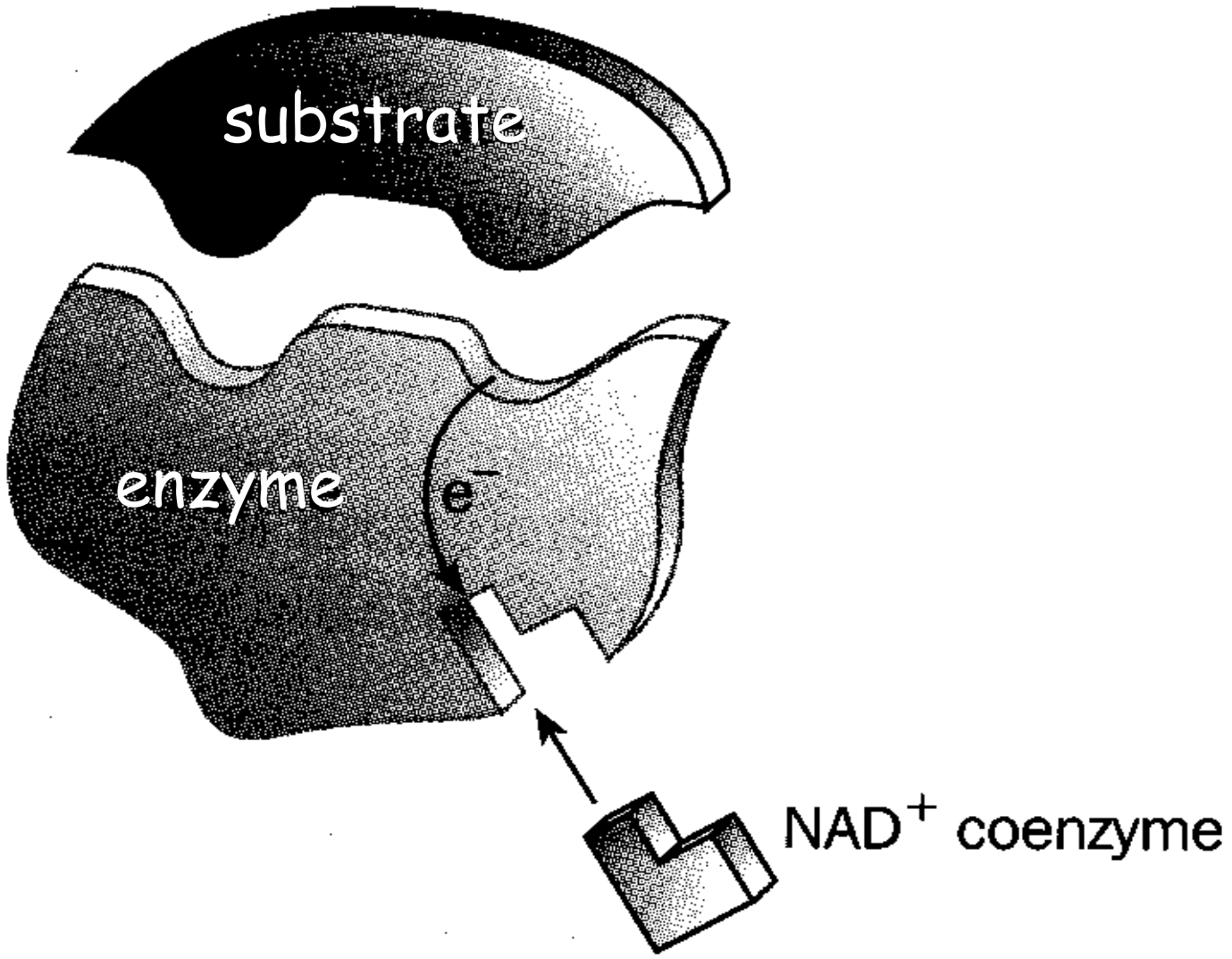
COFACTORS

- Some enzymes need the presence of **another molecule** if they are to work
- These cofactors are **non-organic** molecules
- They **modify** the chemical structure of enzymes in some way so that it can function more effectively

e.g. prosthetic groups, activators

COENZYMES

- Small non- protein **organic** molecules that are **not permanently attached** to an enzyme
- **Help** enzyme and substrate to **bond** with each other
- Enzyme only functions if the coenzyme is present
- Many derived from **vitamins**
- **NAD**, from vitamin nicotinic acid, acts as a coenzyme for a number of dehydrogenase enzymes, by acting as a hydrogen acceptor. Involved in respiration reactions.


substrate
enzyme
e⁻
NAD+ coenzyme

Enzyme activity

Lew-Port's Biology Place

enzyme inhibition

enzyme inhibitors

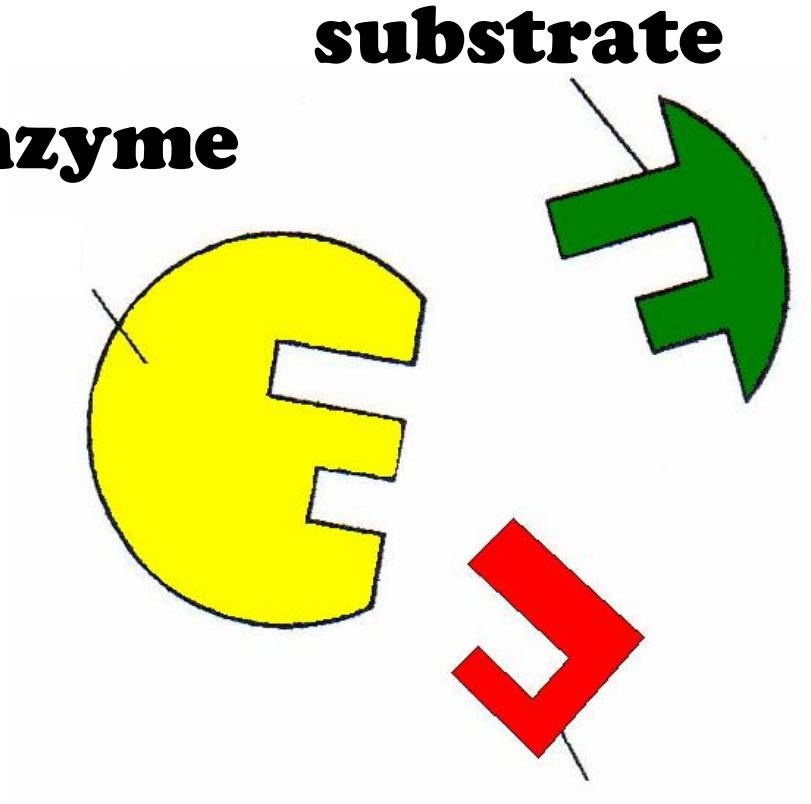
- Substances that prevent substrate attaching to the active site and forming an enzyme-substrate complex.
- 2 types of inhibitor:

Competitive

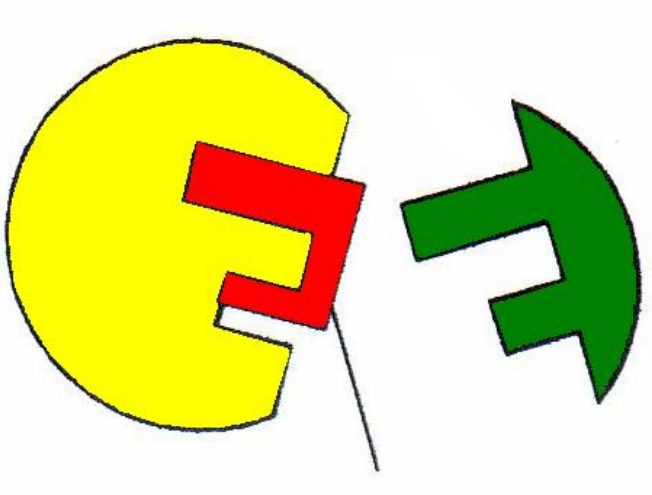
Non-competitive

competetive inhibitors

- have a molecular structure similar to the substrate
- attach to the active site forming inhibitor-enzyme complexes
- reaction slowed or stopped as substrate complexes cannot form



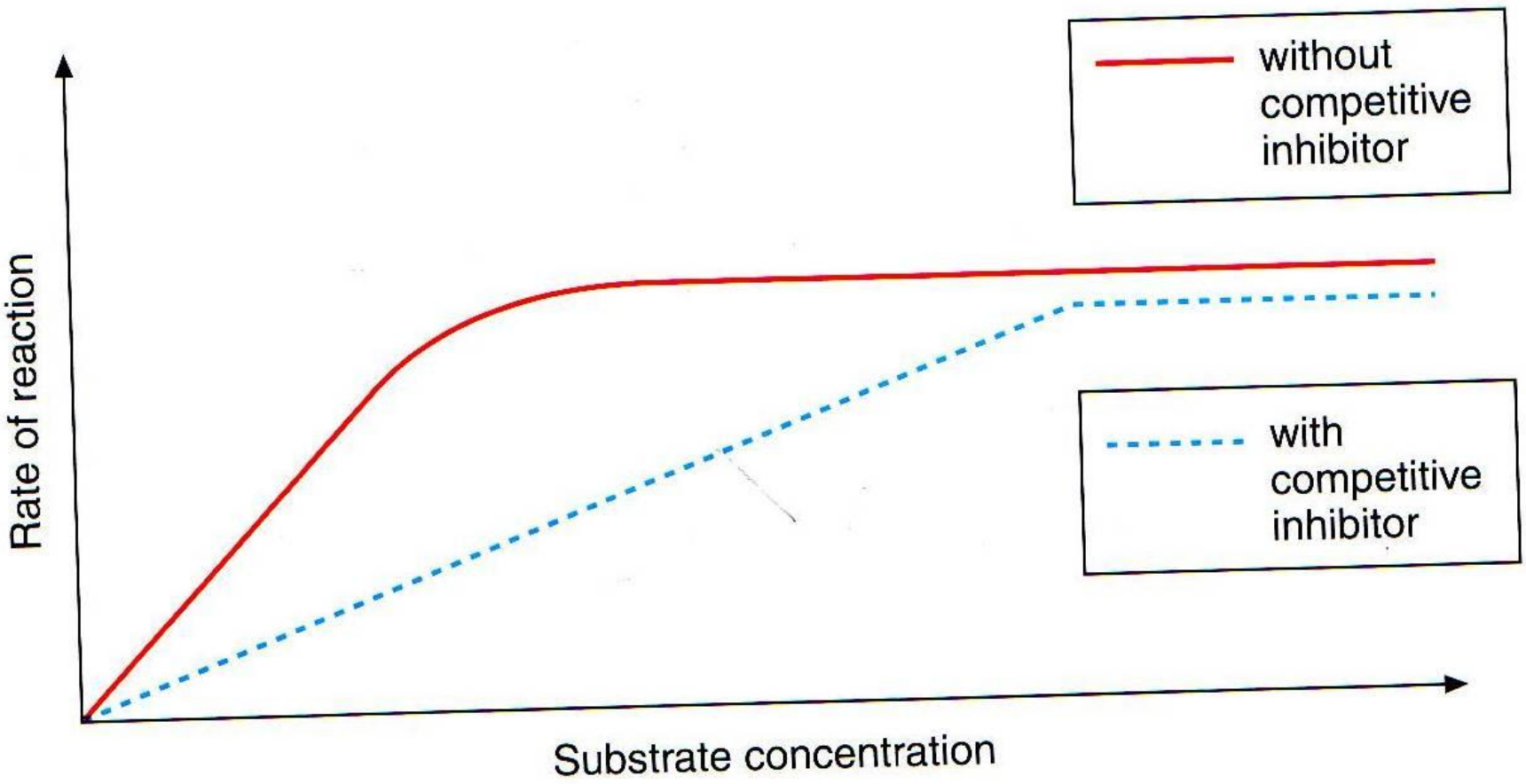
Inhibitor molecule competes for active site



Inhibitor occupies the active site preventing the formation of enzyme-substrate complexes

- Read page 72 Froggy
- Explain how malonate acts as a competitive inhibitor of the enzyme succinate dehydrogenase

The effect of competitive inhibitors depends on the relative concentration of the substrate and inhibitor

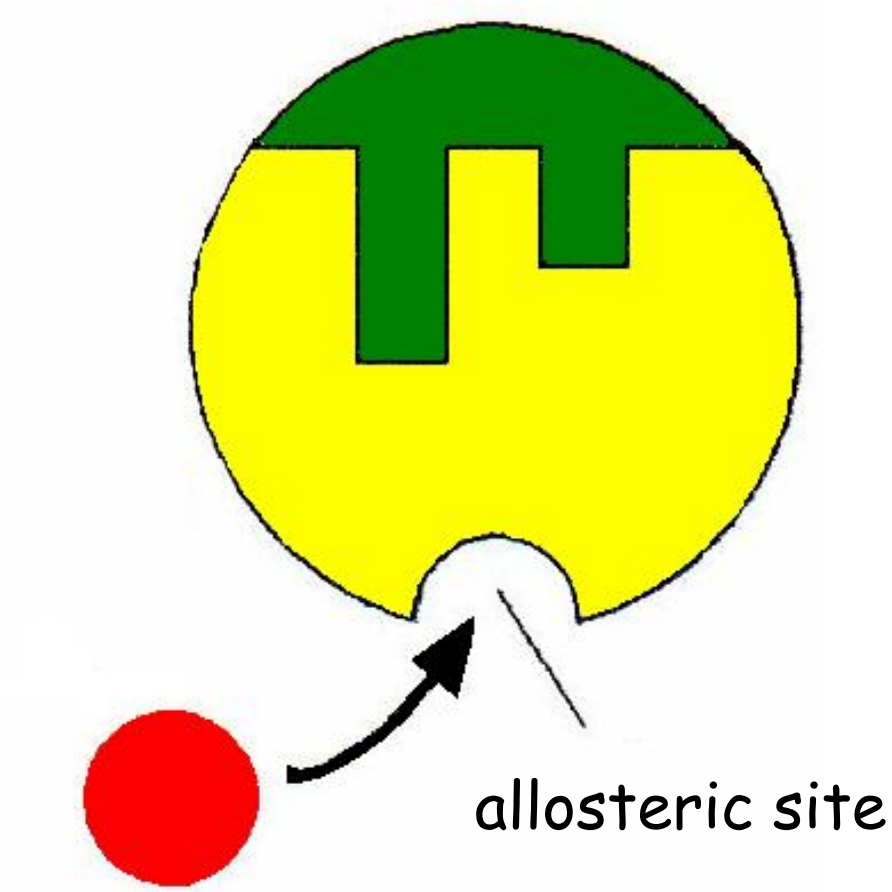


At **low substrate concentrations** there is a higher chance of the inhibitor colliding with the active site, so reaction rates are greatly reduced.

When there is a **high concentration of substrate** there is less chance of inhibitor colliding and more chance of enzyme substrate complexes forming; therefore inhibitors have less effect at high substrate concentrations

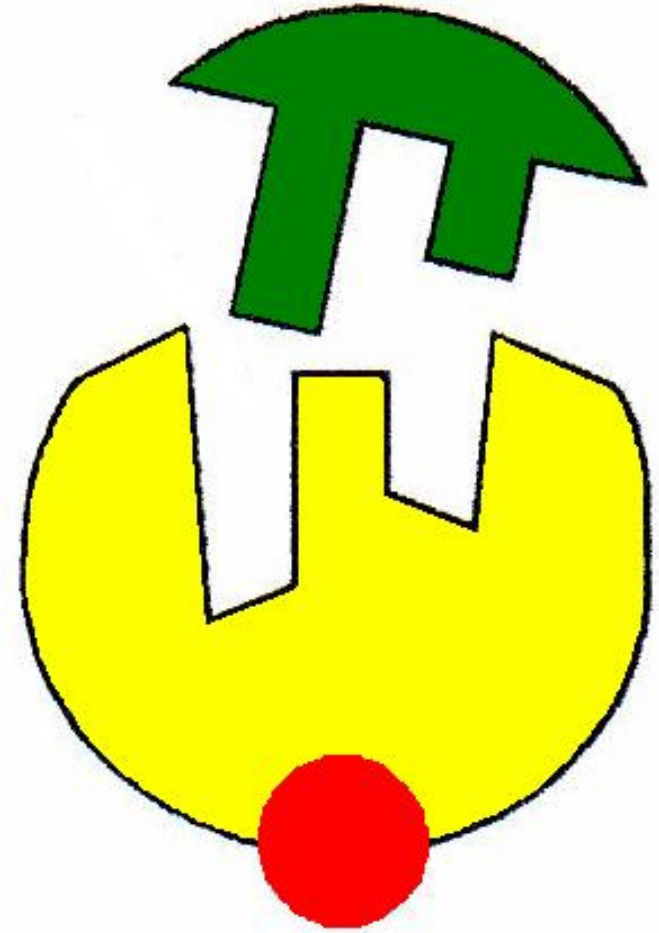
non competetive inhibitors

- Different shape to the substrate molecule
- Do not bind with the active site
- Bind at another area away from the AS called an **allosteric site**
- Causes the active site to change shape preventing the formation of substrate-enzyme complexes.

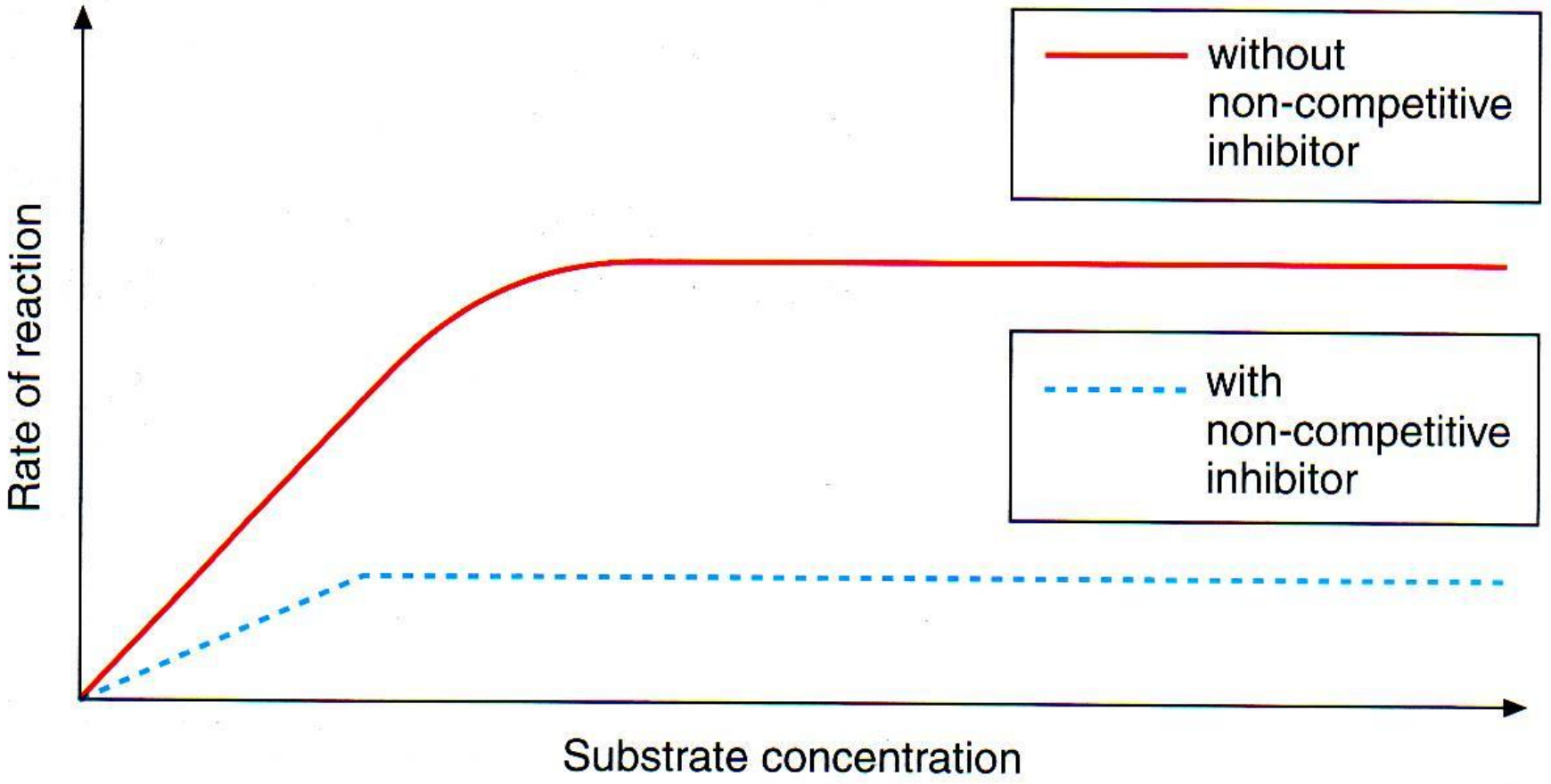


1. inhibitor attaches to an allosteric site on the enzyme

2. inhibitor-enzyme complex alters the shape of the active site preventing substrate binding



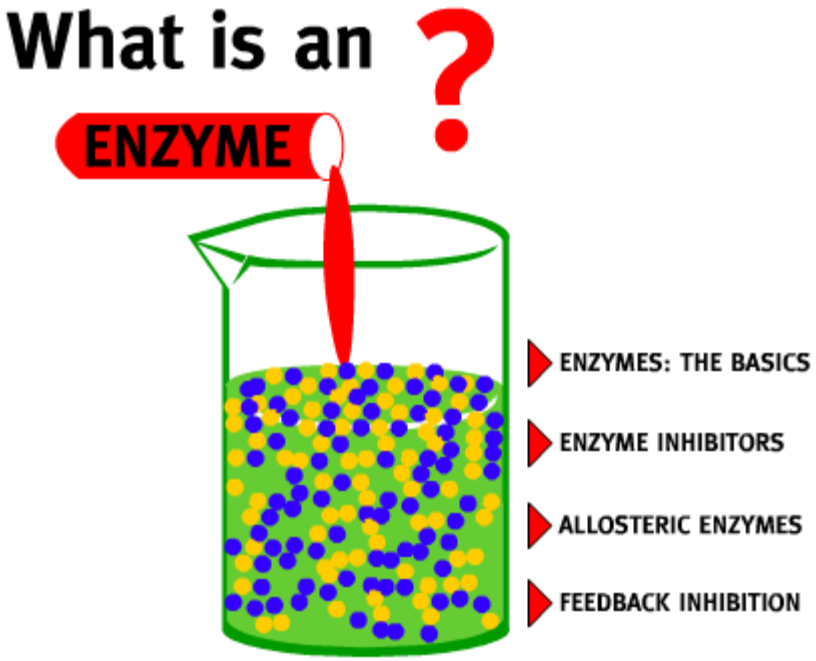
- Non-competitive inhibitors are not affected by substrate concentration.



- The ions of some heavy metals such as **arsenic** and **cyanide** may bind onto allosteric sites and act as poisons, stopping important enzyme controlled reactions.
- End point inhibition is an example of non-competitive inhibition and is an important method of regulating the activity of enzymes.

- Read pages 72-73 Froggy
- Explain how end point inhibition can be used as an example of negative feedback in controlling enzyme activity.

enzyme inhibition



IMMOBILISED ENZYMES

- As enzymes are catalytic molecules they are not directly used up in reactions but due to denaturation, they lose activity with time. **Therefore they should be stabilised against denaturation**.

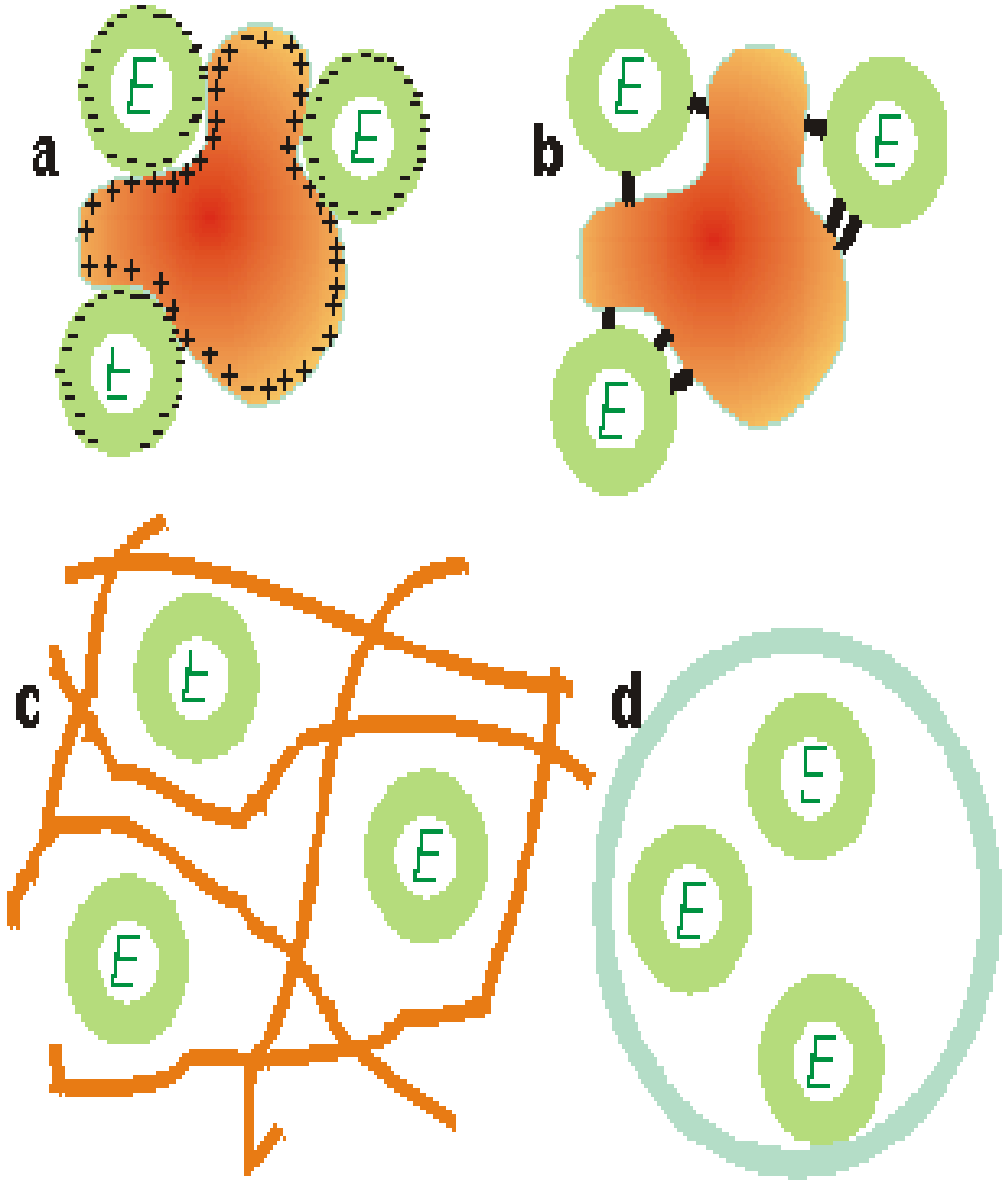
- When the enzymes are used in a soluble form they can contaminate the product, and its removal may involve extra **purification costs**.
- To eliminate wastage and improve productivity the enzyme and product can be separated during the reaction.
- The enzyme can be imprisoned allowing it to be reused but also preventing contamination of the product – this is known as **immobilisation**.

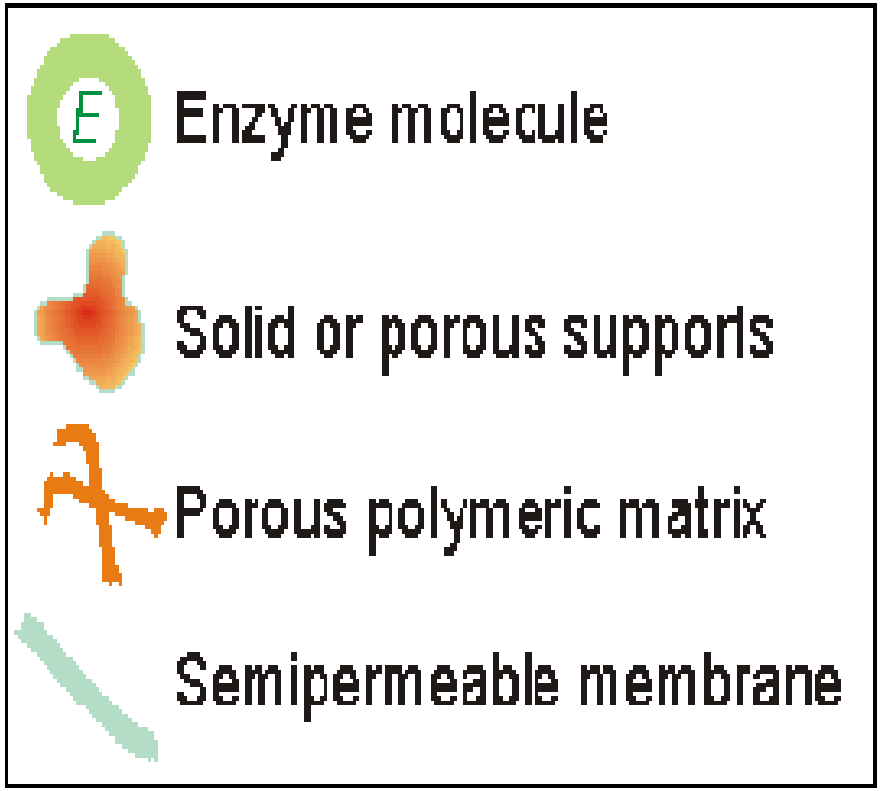
- Unstable enzymes may be **immobilised** by being attached to or located within an insoluble support, therefore the **enzyme is not free in solution**. Once attached, an **enzyme's stability is increased**, possibly because its ability to change shape is reduced.

5methods used to immobilise enzymes

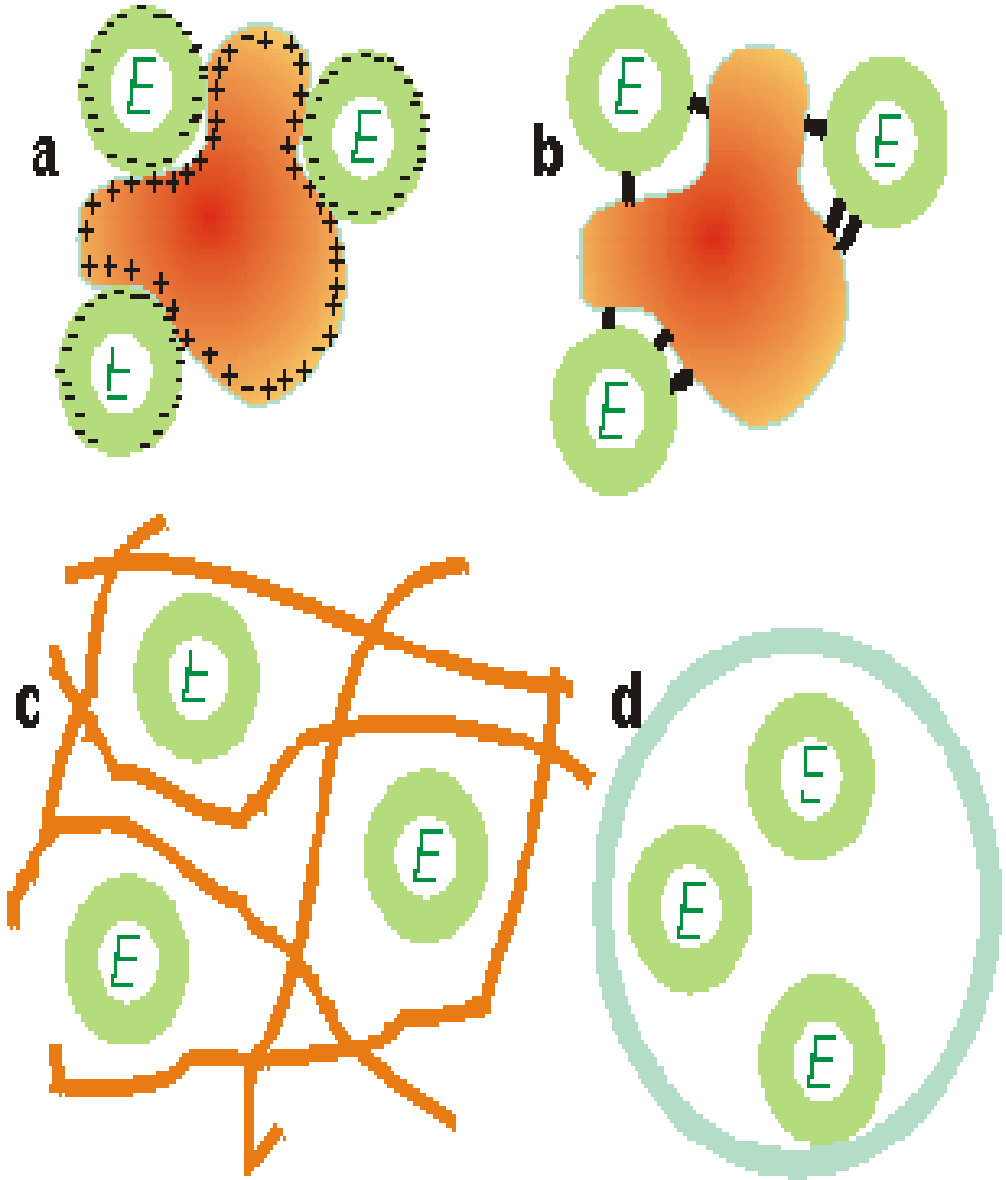
a. **Adsorption** in glass or alginate beads – enzyme is attached to the outside of an inert material.
b. **Cross-linkage** to another chemical e.g. cellulose or glyceraldehydes.
c. **Entrapment** in a silica gel – enzyme is held in a mesh or capsule of an inert material.
d. **Membrane confinement**
e. **Covalent bonding-** A non essential part of the enzyme binds to the support.

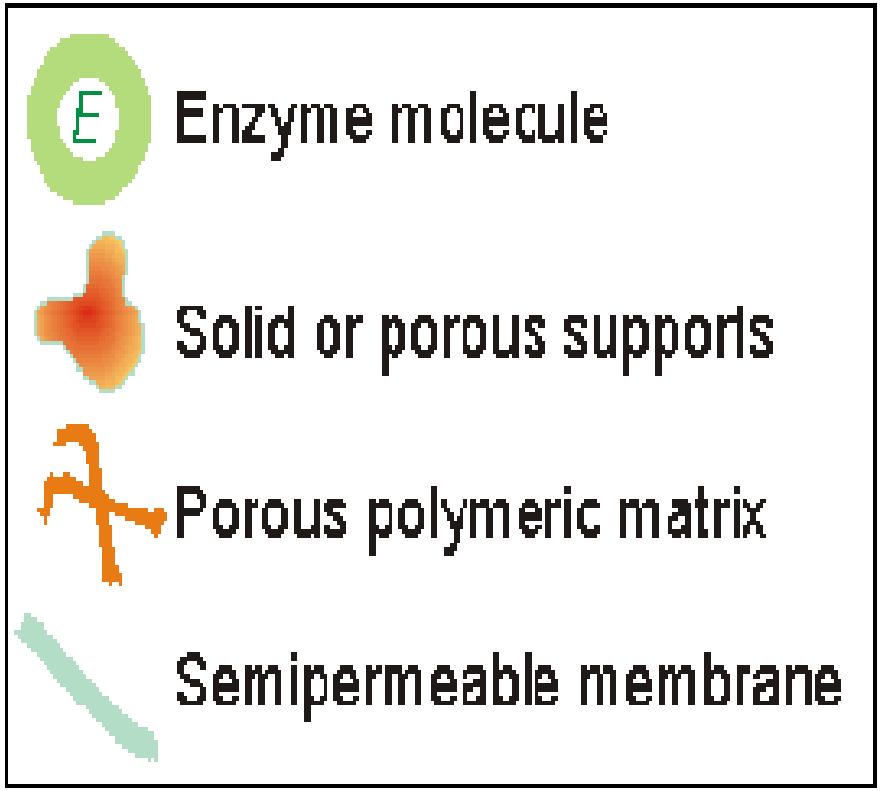
4 methods
(covalent bonding not shown)





4 methods
(covalent bonding not shown)

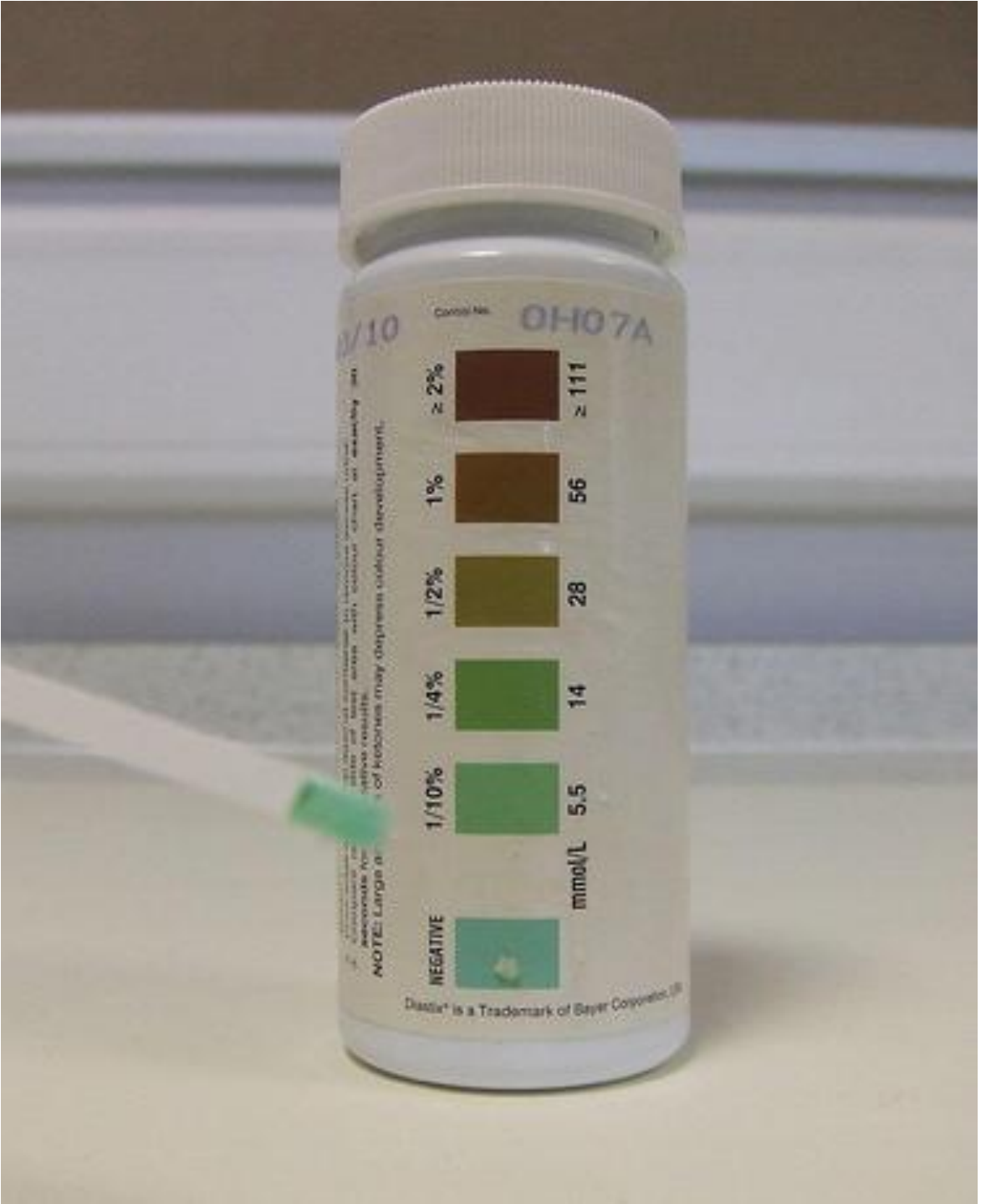




DIAGNOSTIC REAGENT STRIPS

- Immobilised enzymes are used in diagnostic reagent strips as **biosensors** which can detect trace amounts of biologically important molecules e.g.
- **CLINISTIX** -detects glucose (Red- Dark blue)
- **ALBUSTIX** detects protein (yellow green to blue green)

diastix



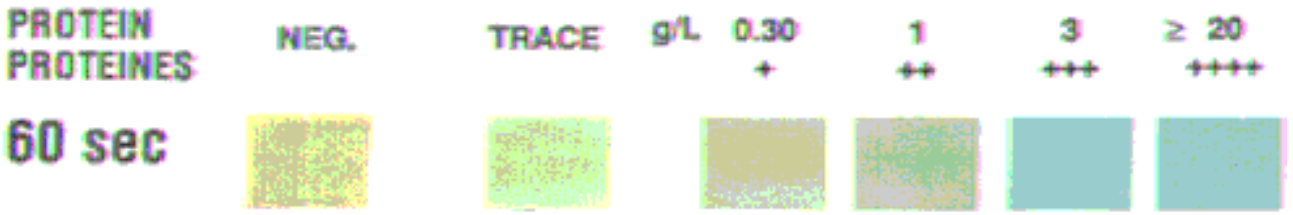
Clinistix

Red (negative) **(light) Purple** **medium (maroon)** **(Dark)Dark blue**


6B05A
BAYER
Albus
Reagent Strips
Teststrips
Teststreifen
Bandelettes
analyse d'urine
Protein
For Self-Testing
Zur Selbstkontrolle
Pour l'auto
IVD
50 Strips
NEG.
Spur/TRACE
mg/dl
g/L
0.30
30
100
1
300
3

Albustix

ALBUSTIX



Yellow/green **Blue green**

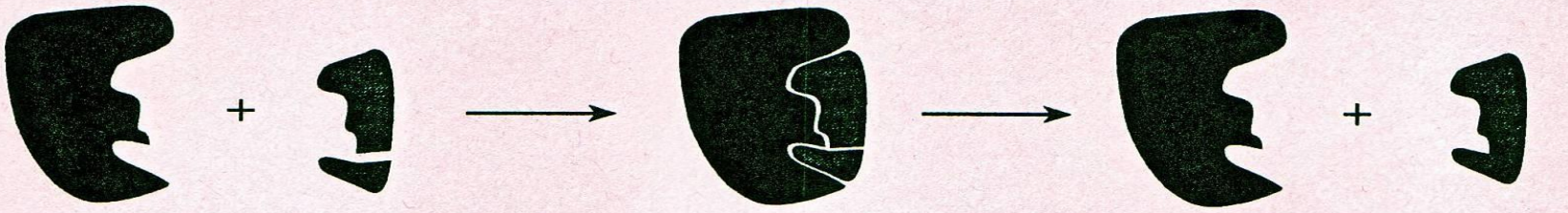
Advantages of immobilisation	Disadvantages of immobilisation
1. Easier to separate enzyme and products	1. Immobilisation may alter shape of enzyme
2. Allows catalysis in unfavourable media	2. May alter catalytic ability
3. Increases stability and can be manipulated easily	3. Enzyme may become detached
4. Allows continuous production/enzyme used for longer	4. Expensive
5. Enzyme can be recovered and reused	
6. Enzyme does not contaminate product/no purification required	

Immobilised enzymes other advantages

- More stable at high temperatures and are resistant to changes in pH.
- Less likely to be degraded by organic solvents
- Use of columns of immobilised enzyme allows automation of the industrial process.

- These advantages are very important for industrial processes. These processes often use extremes of pH and organic solvents.

2 The diagram below shows the action of an enzyme on substrate molecules according to the "lock and key" hypothesis.



(a) Use the information in the diagram above to explain the specificity of enzymes.

[3]

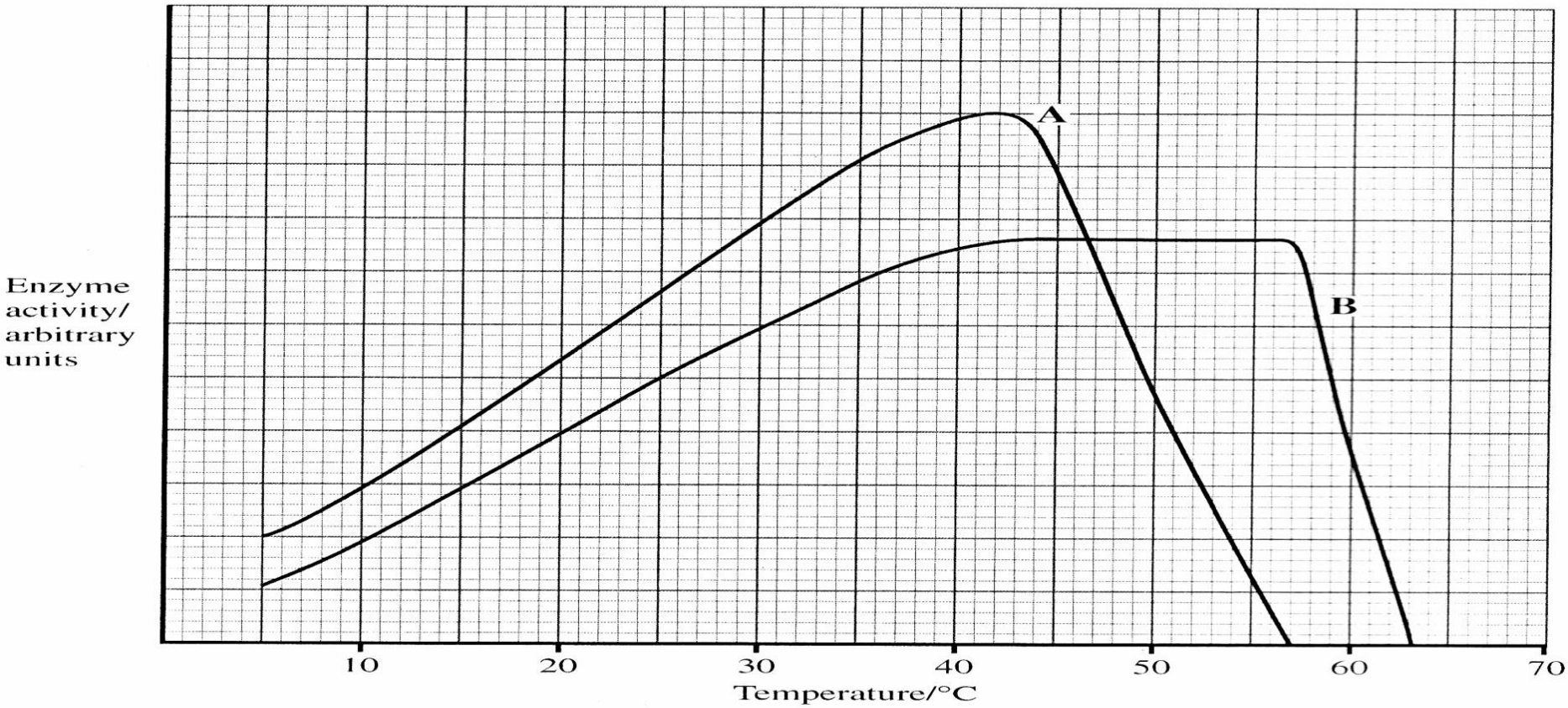
(b) Explain how an enzyme affects the activation energy of a reaction such as the one shown above.

[2]

(c) Explain how the "induced fit" hypothesis of enzyme action differs from the "lock and key" hypothesis.

[2]

5 The graphs below illustrate the results of two experiments in which the activity of a single enzyme was investigated over a range of temperatures. In one experiment, the enzyme was in its soluble form. In the other experiment, an identical concentration of the same enzyme was in an immobilised form. Study the graphs and use them to help you answer the questions that follow.



(a) In graph **A**, at which temperature does this enzyme achieve its maximum activity?

_____ [1]

(b) At which temperature are both forms of this enzyme equally active?

(c) **(i)** Which of the two graphs, **A** or **B**, represents the results for the enzyme in its immobilised form?

_____ [1]

(ii) Identify, and explain, **two** pieces of evidence in the graph that justify your decision in part **(i)**.

1. _____

_____

2. _____

_____ [2]

(d) Explain the rapid decline in activity of both forms of the enzyme at the higher temperatures investigated.

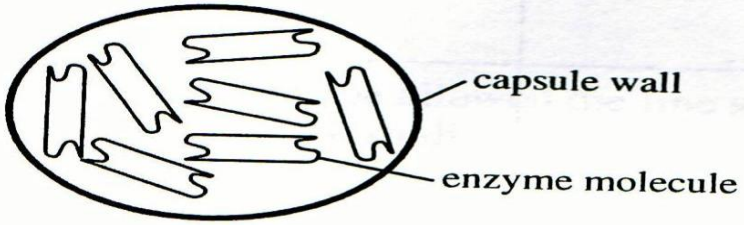
_____

_____ [1]

5 **(a)** In terms of molecular structure, explain fully the changes which occur in most enzymes when the temperature exceeds 45 °C.

[3]

(b) **(i)** Entrapment of enzyme molecules in microcapsules is a common method of enzyme immobilisation.



Suggest **two** properties of the capsule wall essential for optimal functioning of the entrapped enzyme.

1.

2.

[2]

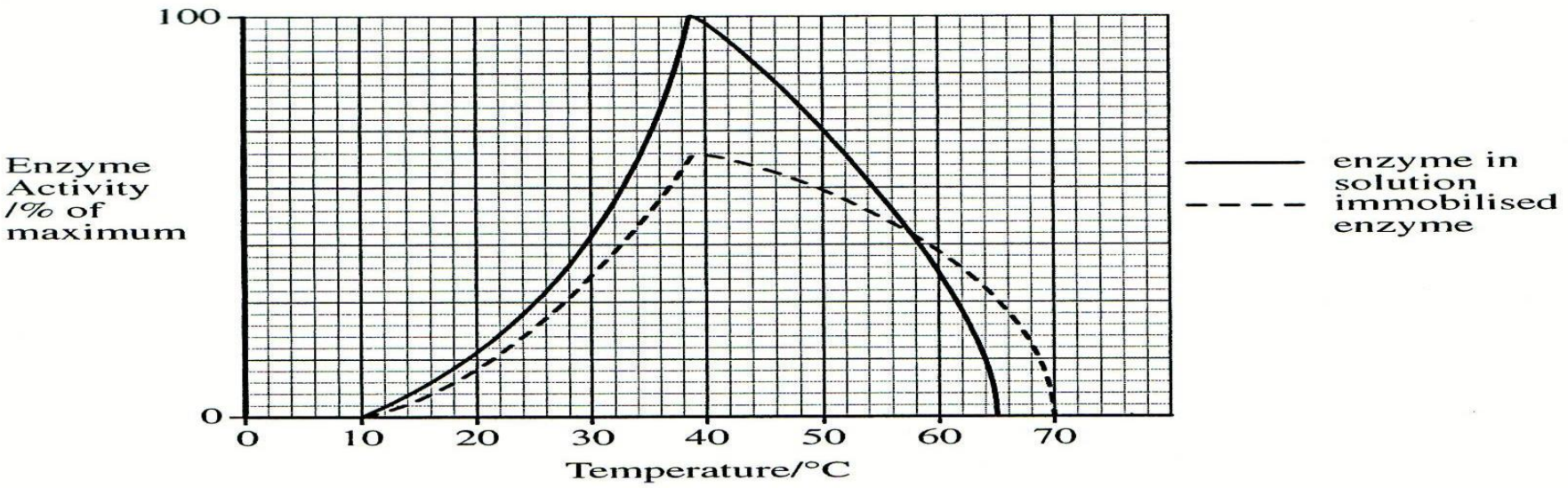
(ii) Diagnostic reagent strips contain immobilised enzymes. Give **one** example of such a reagent strip and describe how the enzyme(s) involved are immobilised in this example.

_____

_____

_____ [2]

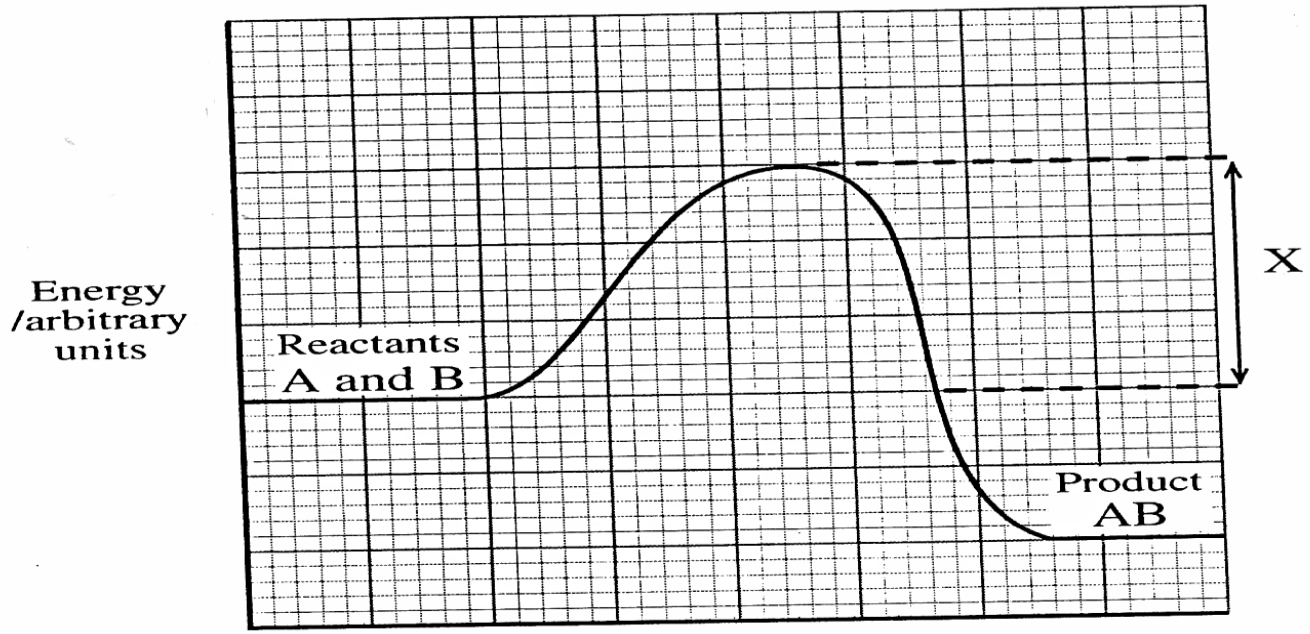
(c) The graph below shows the effect of temperature on the activity of an enzyme when free in solution and when immobilised.



Compare and contrast the enzyme's activity when free in solution and when immobilised.

_____

4 The graph below shows the energy profile for an exergonic reaction between two molecules, A and B.



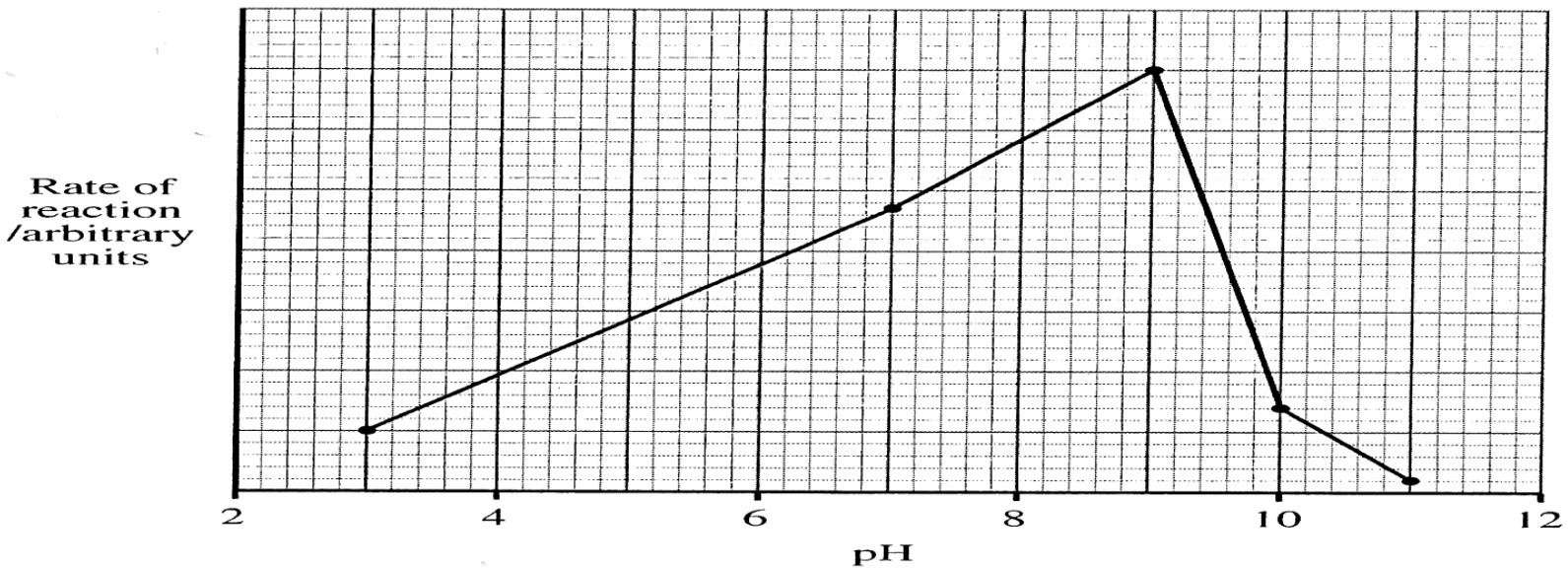
(a) (i) What does the value X on the graph represent?

_____ [1]

(ii) **On the graph above**, draw the alternative energy profile if an appropriate enzyme had been used to catalyse the reaction between molecules A and B. [1]

(b) Explain, concisely, how an enzyme acts as a catalyst.

(c) An enzyme extract, made by crushing various types of fruit, was added to an appropriate substrate and mixed in solutions with different pH values. The results of this experiment were plotted in the graph below.



(i) Use the graph to determine the optimum pH for the action of this enzyme.

_____ [1]

(ii) Compare and contrast the effect of increasing acidity and alkalinity on the activity of this enzyme extract.

_____

_____

_____

_____ [2]

(d) When this experiment was repeated using another enzyme extract, the graph showed two optimum pH values. What would this suggest about the **purity** of this second enzyme extract?