

قسم الكيمياء الحيوية  
Biochemistry Department  
College of Science - King Saud University

# BCH 322

## [Experimental Enzymology]

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# Course Description

- A set of special experiments designed to study parameters of enzyme activity, activation & inhibition, and characterization of enzymes.

	Title of the Experiments
1	Some Factors Affecting Polyphenol Oxidase Activity
2	Methods of Enzyme Assay
3	The Effect of Incubation Time on the Rate of an Enzyme Catalyzed Reaction
4	The Effects of Temperature on the Rate of an Enzyme Catalyzed Reaction
5	The Effects of Enzyme Concentration on the Rate of an Enzyme Catalyzed Reaction
6	The Effects of pH on the Rate of an Enzyme Catalyzed Reaction
7	The Effects of Substrate Concentration on the Rate of an Enzyme Catalyzed Reaction
8	The Inhibition of Acid Phosphatase by Inorganic Phosphate

# Mark Distribution

	Marks	
Conducting the experiment	5 Marks	
Report	15 Marks	
Quiz	12 Marks	
Research	3 Marks	
Midterm (25 Marks)	Practical	15 Marks
	Theoretical	10 Marks
Final (40 Marks)	Practical	25 Marks
	Theoretical	15 Marks
<b>Total</b>	<b>100 Marks</b>	

# How to write a scientific report?

- The laboratory reports should contain the following sections:

- Cover page: Title, course number and student name
- Objectives
- Brief Introduction [ **Theoretical background information** ]
- Materials and Methods [ **Rewrite the method -as the researcher you are- as paragraphs in the past passive tense** ]

*An Example* → Three tubes were labeled as (A,B,and C).To each tube, 5ml of dis. water,0.5ml of pNPP ,0.5ml of MgCl<sub>2</sub>, and 0.5 ml of pH5.7 buffer were added.....

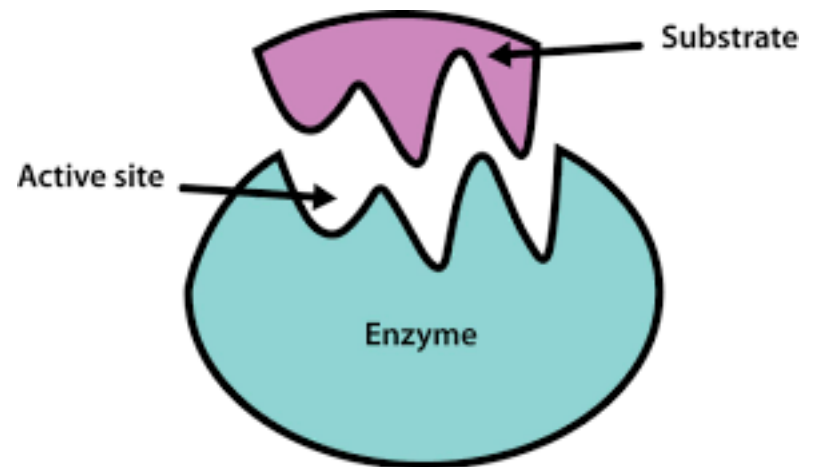
- Results [ **Tables, Graphs and/or Calculations** ]
- Discussion
  - In this section you are required to describe of what happened in the experiment **(Principle)**, explain your results and make conclusions by comparing your results to expected values (calculated or from the literature).
  - Even if you obtained unexpected results, the discussion section is the section to justify or explain the reasons why you have obtained such results.

# Safety in the Lab



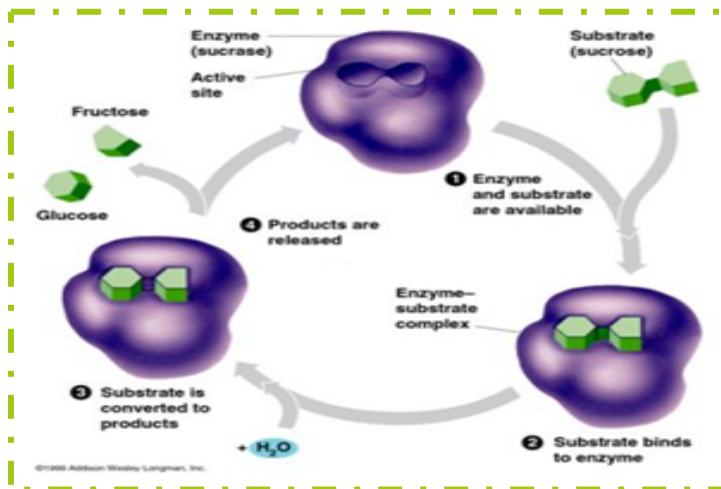
- You must wear a lab coat and hand gloves and a mask.
- Keep a safe distance.
- Open toed shoes must not be worn because they cannot protect you against chemical spills.
- Long hair should be tied back to avoid any interference with the experiment.
- In case of acid or base contact with your skin, wash it with large amount of clean, cold water and inform the instructor immediately.
- Do not handle broken glassware with your bare hands.
- **Do not eat, drink, or chew gum in the laboratory.**
- Do not depart from the lab leaving an experiment unattended. If you need to leave the lab you must **inform your instructor before leaving the lab.**
- You must wash your hands with soap after finishing the experiment.

# Introduction to enzymology



# What are Enzymes and its function?

- Enzymes (E) are biological molecules that increase the rates [catalyze] of biochemical reactions *without being consumed*.
- Most catalyzed reactions are **reversible**
- Nearly all known enzymes are **proteins** in nature with the exception of certain RNA molecules called *ribozymes*.

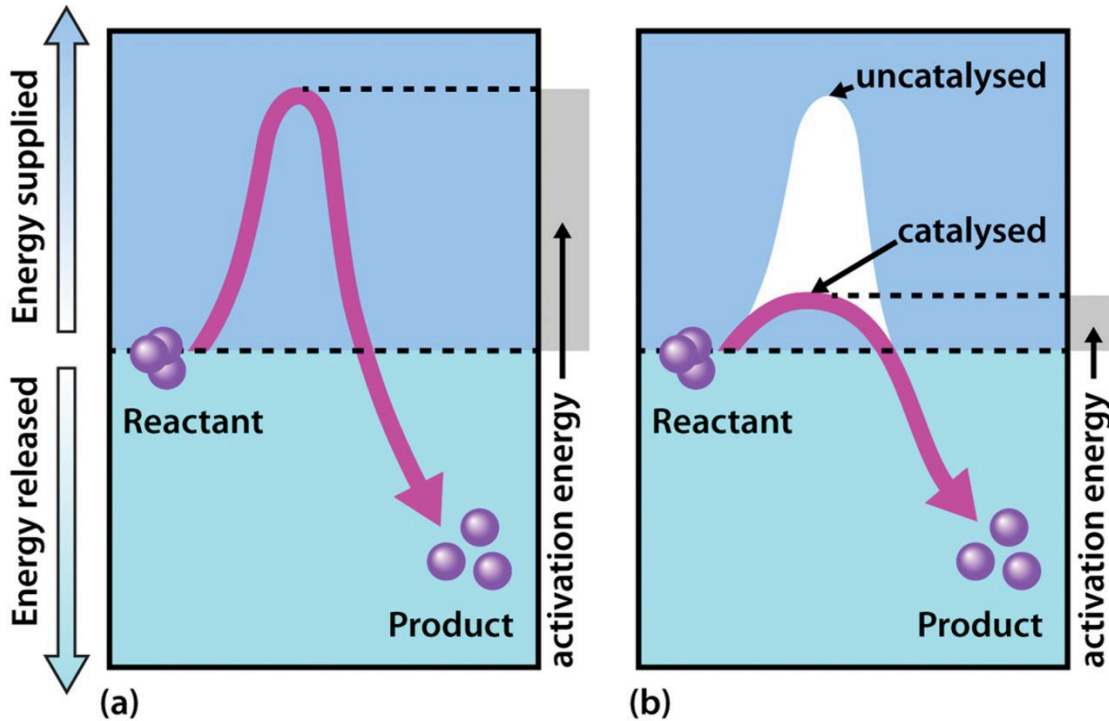


Copy this link to watch

[http://highered.mheducation.com/sites/0072495855/student\\_view0/chapter2/animation\\_how\\_enzymes\\_work.html](http://highered.mheducation.com/sites/0072495855/student_view0/chapter2/animation_how_enzymes_work.html)

# How can enzyme increase the rate of a biochemical reactions?

1. Lowering the activation energy
2. Reducing the chance in the collisions of molecules or ions

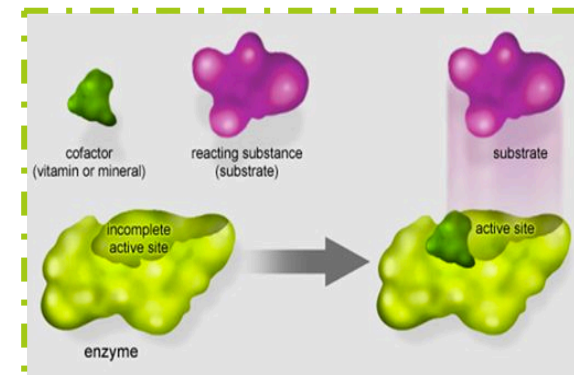


**Activation energy** : Is the energy that must be overcome in order for a chemical reaction to occur, given in units of **kilojoules/mole**.



# Enzyme Terminology

- Substrate (S): a molecule upon which an enzyme acts on.
- Active site: The specific site on an enzyme where the S binds and catalysis occur.
- E-S complex: an intermediate formed when the substrate molecule binds to the active site of the enzyme.
- Product (P): a substance produced as a result of the reactions.
- Cofactors: Small molecules that binds with some E and are necessary for their function. Could be either:
  - Inorganic** - metal ions such as  $Zn^{+2}$ ,  $Cu^{+2}$ , or  $Mg^{+2}$
  - Organic (Coenzymes)** – Mostly vitamins and their derivatives such as NAD [derived from niacin (vit B3)]

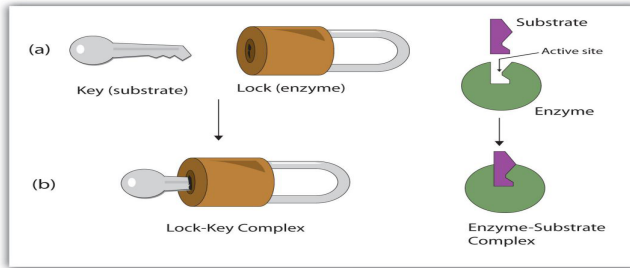


# Turnover number

- It is the total number of substrate molecules that an enzyme can convert to product per minute, when the enzyme is fully saturated with substrate.
- It varies from enzyme to another.
- Many enzymes have a high turnover number. For example, **catalase has a turnover number of 5 million per minute.**
- Thus enzymes are generally effective in relatively minute concentrations in the living cell.

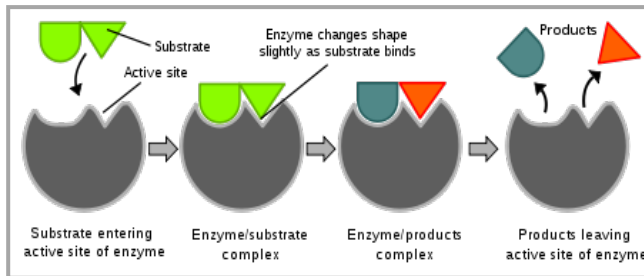
# How can a substrate bind to the Enzyme?

## The Key and lock hypothesis



Suggests that both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another

## Induced fit model



Suggests that enzymes are flexible structures, where the the amino acid side chains which make up the active site are continually reshaped by interactions with the substrate.



# Notes in practical work

In this course enzymes will be extracted from cells and the enzyme catalyzed reactions will be studied in a test tube.

In any experiment you should know:

- The aim and what you are doing while working
- You must know why and what are the importance of the chemicals that you are adding during the experiment
- You should be accurate

Instruments used in this course are:



Spectrophotometer



Waterbath



Pipette



Micropipette