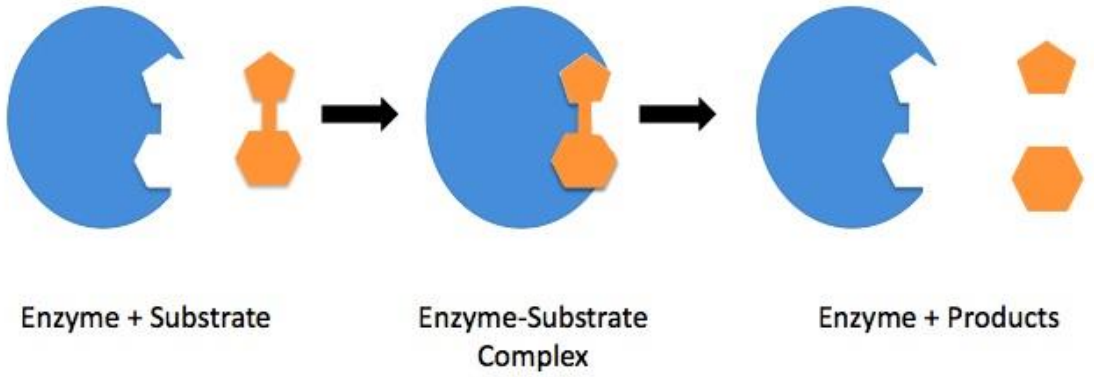
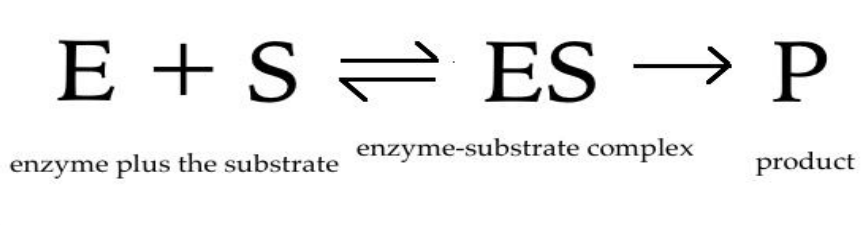
Effect of various factors on polyphenol oxidase activity



Enzymes:

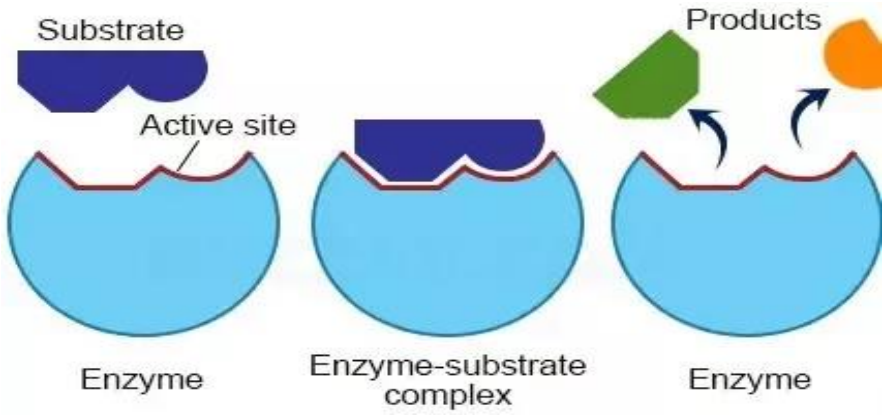
- A substance that speeds up a chemical reaction—without being consumed—is called a catalyst. The catalysts for biochemical reactions that occur in the living organisms are called **enzymes**.
- Enzymes are highly specialized proteins.
- Enzymes have a high degree of **specificity** for their substrates.
- They function under very mild conditions of temperature and pH.

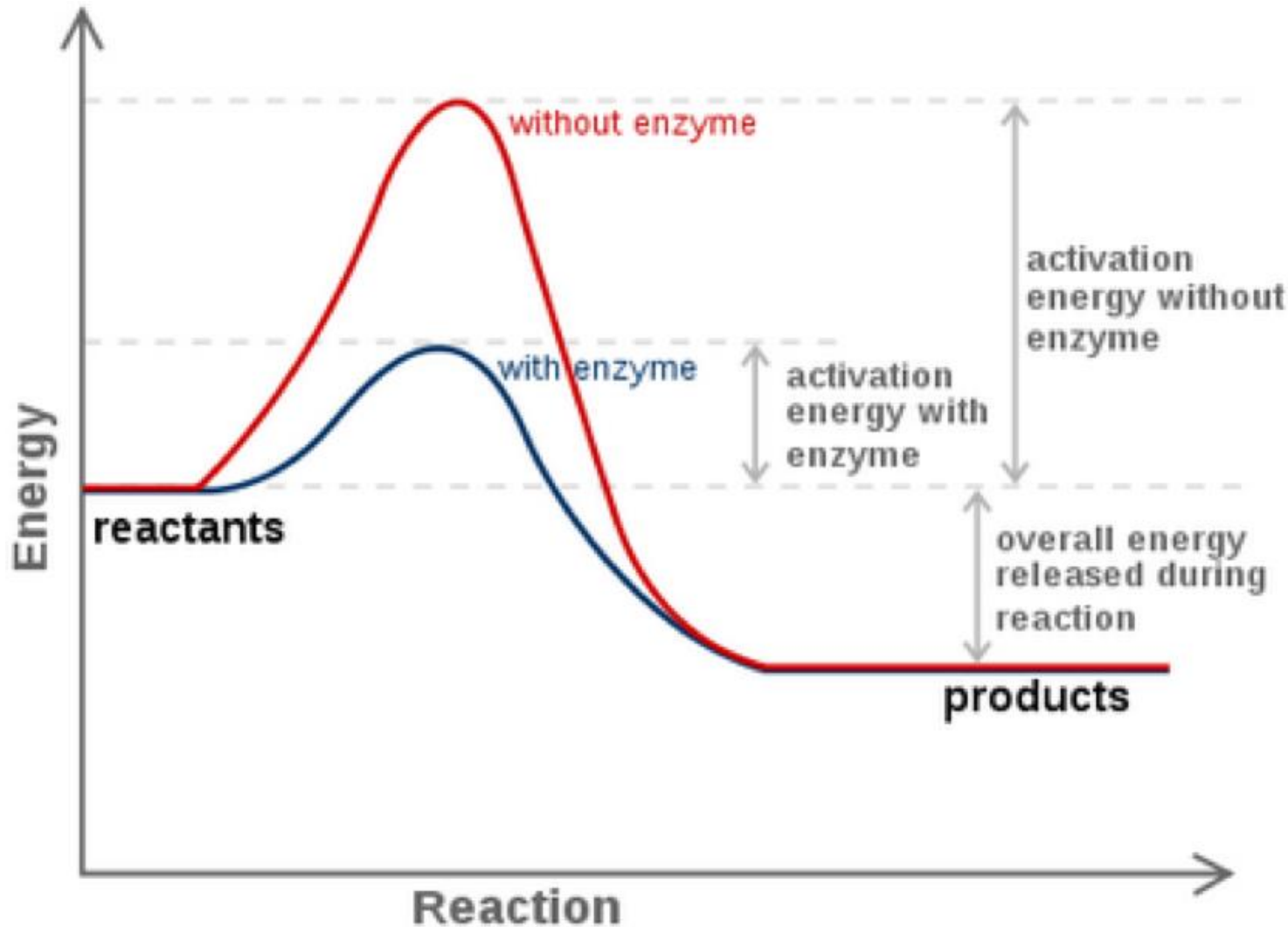


- Enzymes perform the critical task of lowering a reaction's activation energy—that is, the amount of energy that must be supplied for a reaction to begin.

- **Enzyme-catalyzed reaction** takes place within a pocket on the enzyme called the active site.

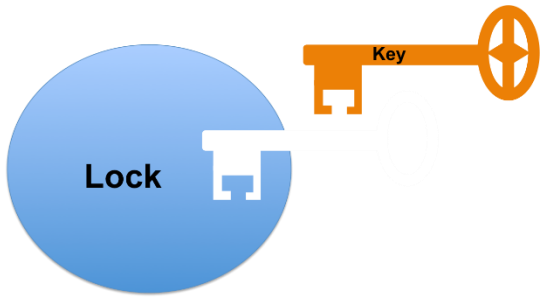
- To catalyse a reaction, an enzyme will bind to one or more reactant molecules, these molecules are the enzyme's **substrates**, to give the **product**.

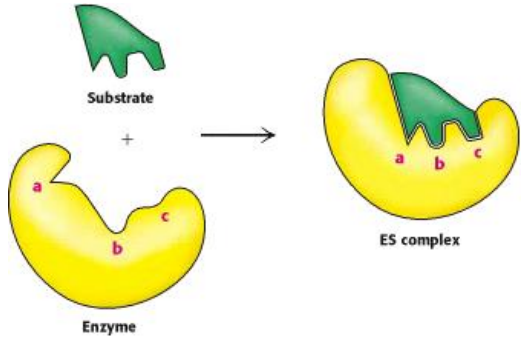



without enzyme
with enzyme
activation energy without enzyme
activation energy with enzyme
overall energy released during reaction
reactants
products
Energy
Reaction

- It was thought that enzyme-substrate binding took place in a simple "**lock-and-key" fashion,** this model asserted that the enzyme and substrate fit together perfectly in one instantaneous step.

- Current research supports an **induced fit model**. 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 substrate.





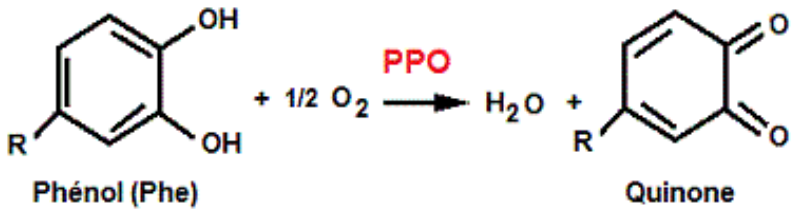
- Many enzymes have been named by adding the suffix **“-ase”.**

- In some diseases, there may be a deficiency or even a total absence of one or more enzymes. For other disease conditions, excessive activity of an enzyme may be the cause.

- Measurements of the activities of enzymes in blood plasma, erythrocytes, or tissue samples are important in **diagnosing** certain illnesses.

polyphenol oxidase:

- browning is a usual phenomenon that can be observed commonly in fruits and vegetables, This occurs when the phenolic compounds present in them react with polyphenol oxidase.
- Polyphenol oxidase (PPO) is an enzyme that catalyse the **oxidation** of dihydroxy-and trihydroxy phenol to corresponding quinone which has a brown color.
- In this lab, activity of polyphenol oxidase extracted from potato will be examined qualitatively.



Phénol (Phe) Quinone

Practical Part

Experiment (1). Examine the protein nature of polyphenol oxidase:

Objective:

Examine the protein nature of polyphenol oxidase by biuret test.

Principle:

- Majority of enzymes are proteins. Detection of protein nature will be done using **biuret reagent**, where the peptide bonds in the proteins (enzymes) treated with an alkaline solution of dilute copper sulphate CuSO4 (biuret reagent) forming a purple colored complex.

Protocol:

1. Label a test tube and add 1ml of enzyme crude extract.
2. Add 2 ml of biuret reagent.

Result

Tube	Observation
Enzyme + Biuret reagent	

Experiment (2). Test the activity of polyphenol oxidase:

Objective:

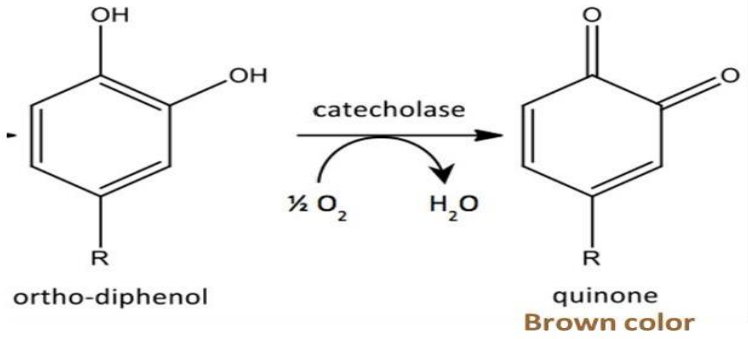
To demonstrate activity of the enzyme.

- To investigate the effect of incubation time on enzyme activity.

Principle:

- Polyphenol oxidase activity will be examined **qualitatively** by following the change in the color. The oxidation-reduction reaction that catalysed by this enzyme is accompanied by a color change i.e **browning** (the product quinone).

- The intensity of the brown color is proportional to the enzyme's activity.



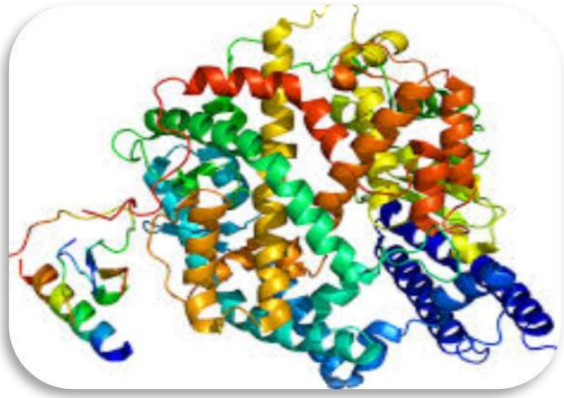
Protocol:

1. Label 3 test tube as A, B and C.
2. **In tube A** (control): add 15 drops of the enzyme and 15 drops of catechol.
3. **In tube B**: add 15 drops of the enzyme and 15 drops of distilled water.
4. **In tube C**: add 15 drops of distilled water and 15 drops of catechol.
5. Place all the tubes in the water bath at 37 °C.
6. Shake each tube every 5 minutes to aerate, thereby adding oxygen to the solution.

Result

Incubation Time (min)	Degree of color intensity (-, +, ++, +++)		
	A	B	C
0			
5			
10			
15			
20			

Experiment (3). Demonstrate the chemical nature of polyphenol oxidase:



Objective:

To examine the chemical nature of polyphenol oxidase.

Principle:

- Polyphenol oxidase **is a protein** in nature, and thus effected by various factors that affect proteins. Applying an external denaturation factors or compound such as a strong acid or base, an organic solvent, or heat on the enzyme leading to lose its structure and so its function.

- In addition, enzyme activity is compromised by sequestering its cofactor. Polyphenol oxidase is a **copper** enzyme, Adding of a chemical like phenylthiourea inhibit PPO by interact with copper ions at its active site.

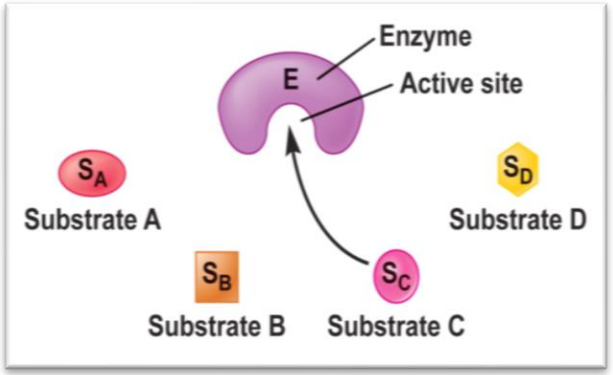
Protocol:

1. Label 3 test tube as A, B and C.
2. **In tube A** (control): add 15 drops of the enzyme and 15 drops of catechol. Shake it.
3. **In tube B**: add 10 drops of the enzyme and 10 drops of TCA. Shake the tube thoroughly and after 5 minutes, add 10 drops of catechol.
4. **In tube C**: add 10 drops of the enzyme and few crystals of phenylthiourea. Shake the tube continually for 5 min, then add 10 drops of catechol.
5. Place all the tubes in the water bath at 37 °C for 10 minutes.
6. Compare the results obtained from B and C to the control (A).

Result

Tube	Degree of color intensity (-, +, ++, +++)
A (Control)	
B	
C	

Experiment (4). Investigating the substrate specificity of polyphenol oxidase:



Objective:

To investigate the substrate specificity of the enzyme using structurally related chemicals.

Principle:

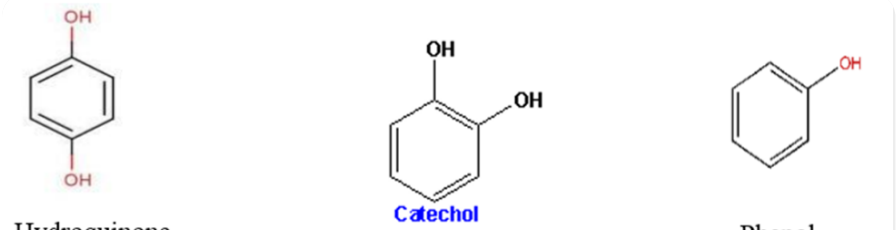
- There may be one or more substrates for each type of enzyme, depending on the particular chemical reaction.
- The enzyme's **active site** binds to the substrate, Since enzymes are proteins, this site is composed of a unique combination of **amino acid residues,** a specific chemical substrate matches this site like a puzzle piece and **makes the enzyme specific to its substrate**.
- Potato polyphenol oxidase catalyses the oxidation of dihydroxy-and trihydroxy phenol to the corresponding quinine

Protocol:

1. Label 3 test tube as A, B and C.
2. **In tube A (control):** add 15 drops of the enzyme and 15 drops of catechol.
3. **In tube B:** add 15 drops of the enzyme and 15 drops of phenol.
4. **In tube C:** add 15 drops of the enzyme and 15 drops of hydroquinone.
5. Place all the tubes in the water bath at 37 °C for 10 minutes.
6. Shake each tube every 5 minutes to aerate, thereby adding oxygen to the solution.

Result

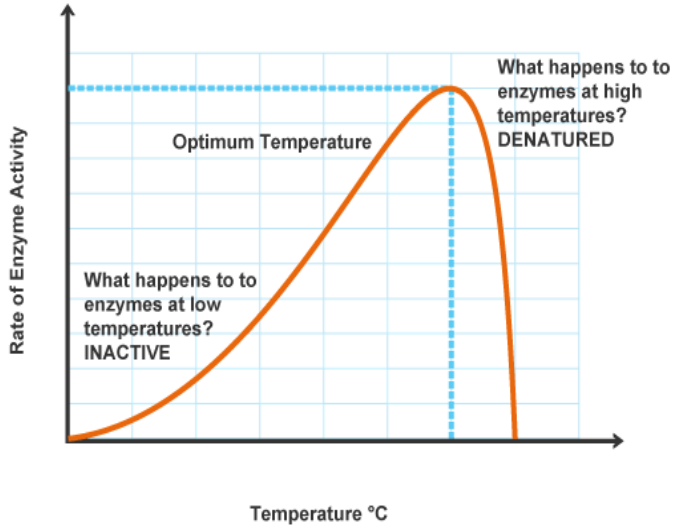
Tube	Degree of color intensity (-, +, ++, +++)
A (Control)	
B	
C	



Experiment (5). Investigating the effect of temperature on polyphenol oxidase activity:

Objective:

To investigate the effects of temperature on the enzyme activity.



Principle:

- **Each enzyme has a temperature that it works optimally**.
- Increasing temperature above 40 ℃ increases the rate of reaction, because it excites molecules and increases the rate at which enzymes/reactants collide and react to make product.
- However, increasing temperature too much may denature the enzyme and prevent it from working at all.
- Meanwhile, at 0°C enzyme action is low because the movement of molecules is low. This causes the collision frequency between enzyme and substrate to be low

Protocol:

1. Label 3 test tube as A, B and C.
2. **In tube A**: add 15 drops of the enzyme and incubate at 0 °C for 10 min.
3. **In tube B**: add 15 drops of the enzyme and incubate at 37 °C for 10 min.
4. **In tube C**: add 15 drops of the enzyme and incubate at 95 °C for 10 min.
5. Add 15 drops of catechol for all tubes.
6. Wait for 15 minutes. Then, examine each tube without removing it from its temperature condition

Result

Tube	Degree of color intensity (-, +, ++, +++)
0	
37	
95	