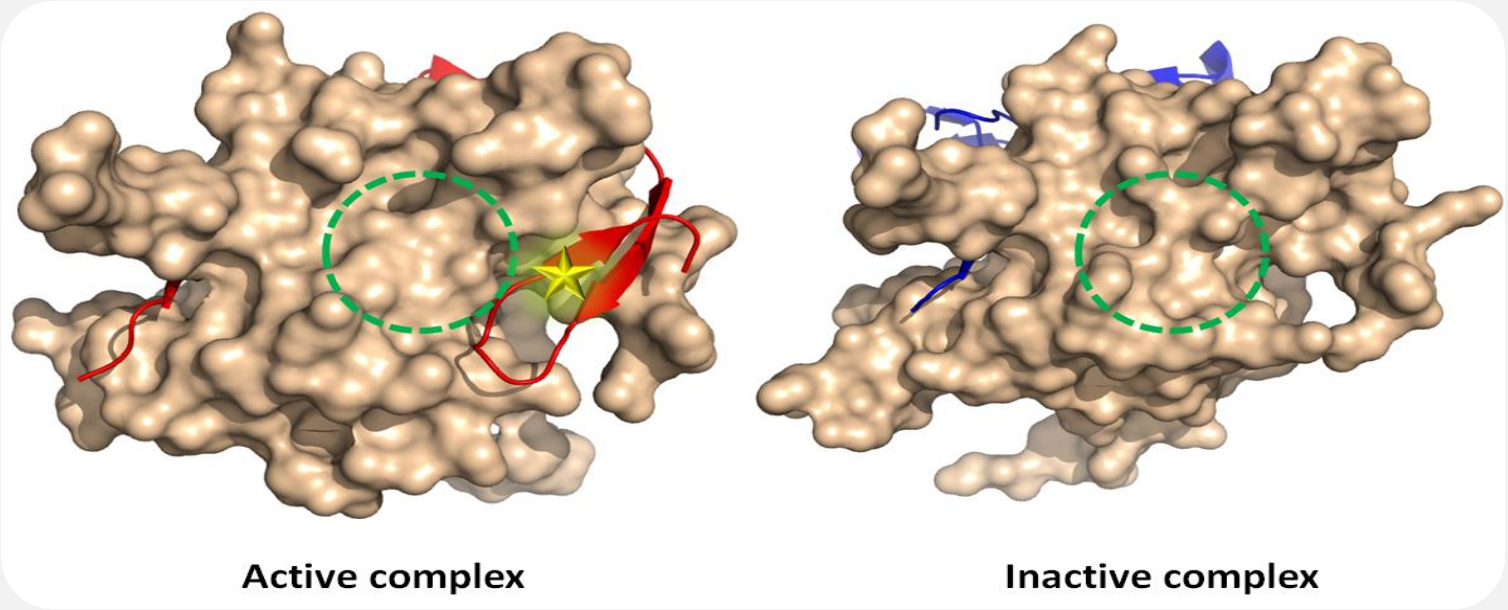
Some factors Affecting polyphenol Oxidase Activity

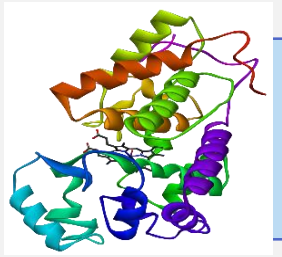


Studying Enzymes

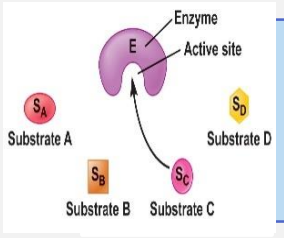
- To detect and follow the progress of the reaction in this experiment a simple, **qualitative** method will be used.
- More sophisticated, **quantitative methods** of following enzyme catalyzed reactions will be introduced later in the course.
- In this lab **Polyphenol Oxidase** will be studied as an example to understand factors that could affect enzymes.

Objectives:

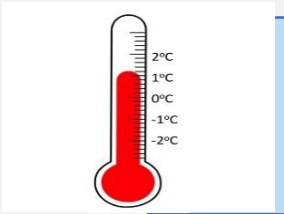
To demonstrate activity of the enzyme



To demonstrate the Chemical nature of the enzyme



To investigate the substrate specificity of the enzyme



To investigate the effects of temperatures on the enzyme activity

of polyphenol oxidase in crude extract from potato

** In this experiment you will notice the change qualitatively (change in the color only)*

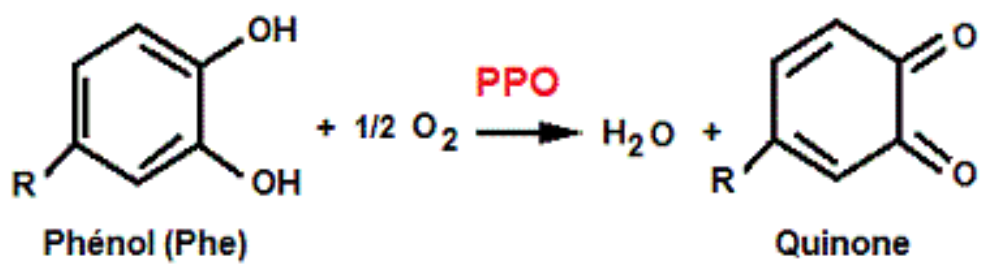
Polyphenol Oxidase:

• Copper-containing enzyme with an **optimum pH of 6.7**.

• It mainly catalyses the oxidation of **di-and tri-hyroxy phenol** to the corresponding quinine.

• This **oxidation-reduction reaction** is accompanied by a color change.

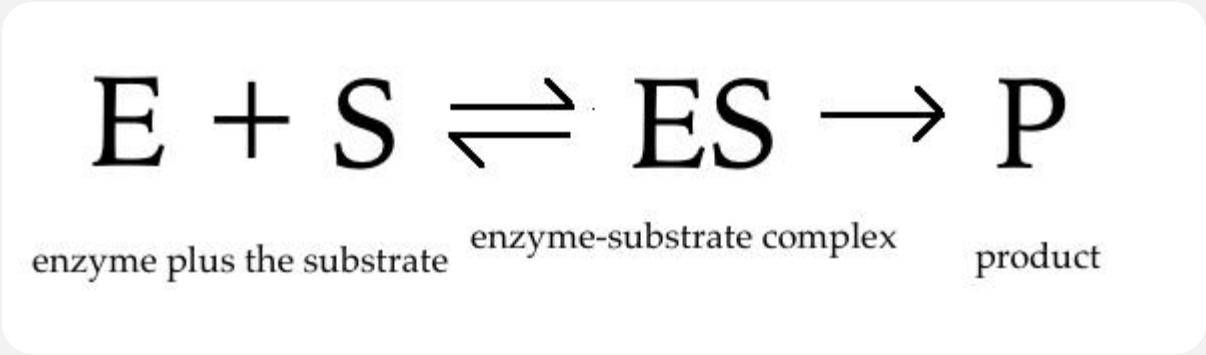
• This reaction commonly occurs in nature and accounts for the "**browning**" of peeled potatoes and bruised fruits.



1) To demonstrate activity of polyphenol oxidase enzyme

Principle:

- In this reaction we are looking for enzyme substrate reaction in general
- The intensity of this color **(brown)** will be **proportional** to the enzyme's activity in the tube under observation.
- You must notice the color change with the time.



Method:

a. Label three clean test tubes A, B and C.

b. Prepare each tube as follows:

Tube	Addition
Tube a	15 drops of enzyme extract. 15 drops of 0.01M catechol solution
Tube b	15 drops of enzyme extract. 15 drops of distilled water.
Tube C	15 drops of catechol solution. 15 drops of distilled water.

c. Place all three tubes in a water bath at 37 ºC.

d. Shake each tube every 5 minutes to aerate, thereby adding oxygen to the solution.

e. Every 5 minutes, after shaking, hold the tubes up to the light and examine.

Results:

Record the color in each tube, according to the scheme described, in the table. Continue for 25 minutes.

Incubation time (Minutes)	Degree of color intensity (Symbol: −, +, ++ or +++)		
	Tube A	Tube B	Tube C
0			
5			
10			
15			
20			
25			

Degree of color intensity symbol

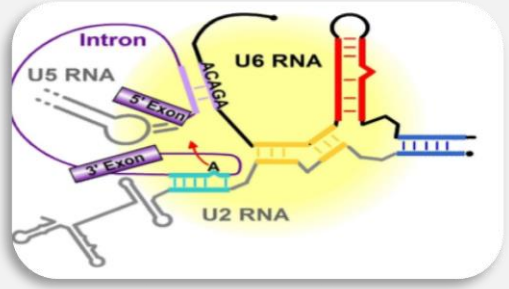
No color change (colorless) −
Faint color (just detectable) +
Definite color ++
Dark (deep) color +++

What do you noticed about the color at the end, is there any further change at 25 min?

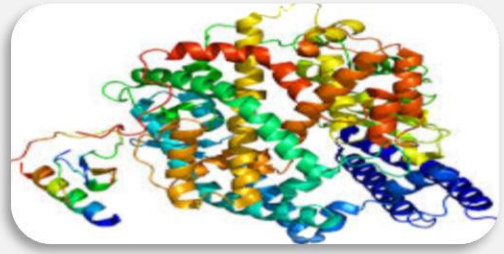
2) To demonstrate the Chemical nature Of Polyphenol oxidase:

- **Majority of enzymes** are proteins. Some are made of RNA

- **The nature of polyphenol oxidase** will be examined, wither is it protein or not



RNA(catalytic)



Protein (Enzyme)

Principle: This test depend on affecting solubility of the protein as a function of changes in pH. In highly acidic media, the protein precipitate.

Method:

a. Label four clean test tubes A, B, C and D.

b. Prepare, and teat, each tube as follows:

Tube	(1)	(2)	(3)
Tube A	-15 drops of enzyme -15 drops of 0.01M catechol solution	- Shake tube and place in water bath at 37 ºC for 10 minutes. • **As a Control!**	
Tube B	• Add 10 drops of enzyme extract. • Add 10 drops of 5% trichloroacetic acid.	Shake tube thoroughly and wait 5 minutes.	Add 10 drops of 0.01M catechol solution. Place in water bath at 37 ºC for 10 minutes
Tube C	- Add 15 drops of enzyme - Add few crystals of phenylthiourea	Shake tube thoroughly and continue shaking it frequently during a period of 5 minutes.	Add 15 drops of 0.01M catechol solution. Place in water bath at 37 ºC for 10 minutes

C. Examine and compare with tube A.

Results:

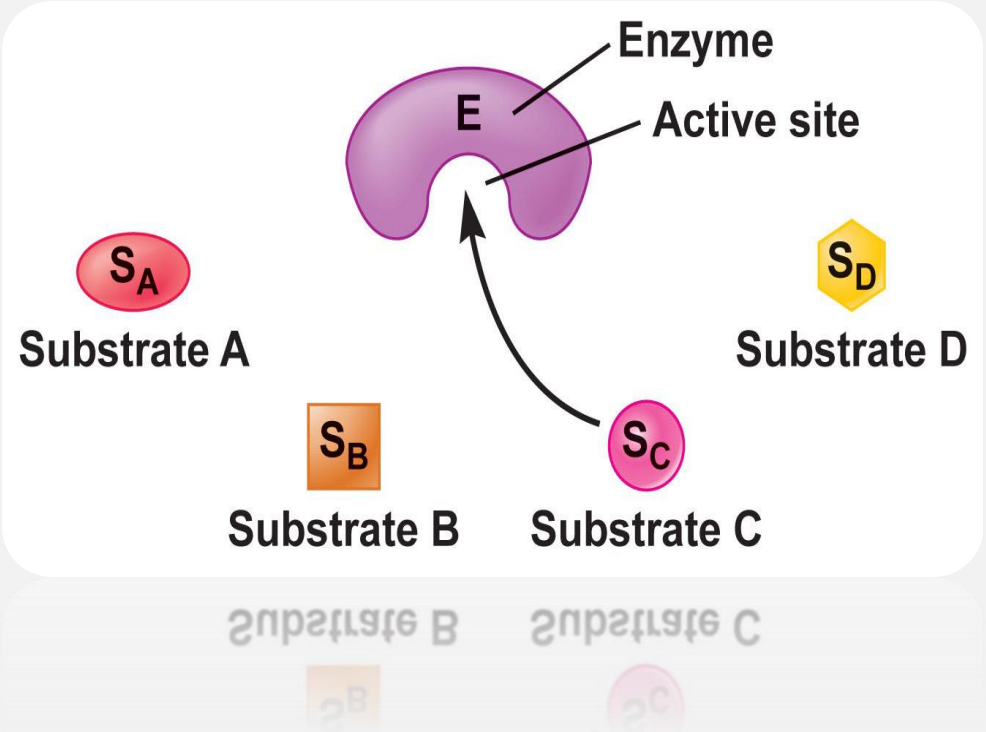
Tube	Treatment	Degree of color intensity (Symbol: −, +, ++ or +++)
A	Control	
B	TCA	
C	Phenylthiourea	

- **TCA:** an acid (Low pH) that precipitate and denature proteins
- **Phenylthiourea**: Phenylthiourea has a very strong chemical affinity for the element copper (PPO's cofactor).

3) To investigate the substrate specificity of the enzyme.

The substrate binds to the enzyme at the **active site**.

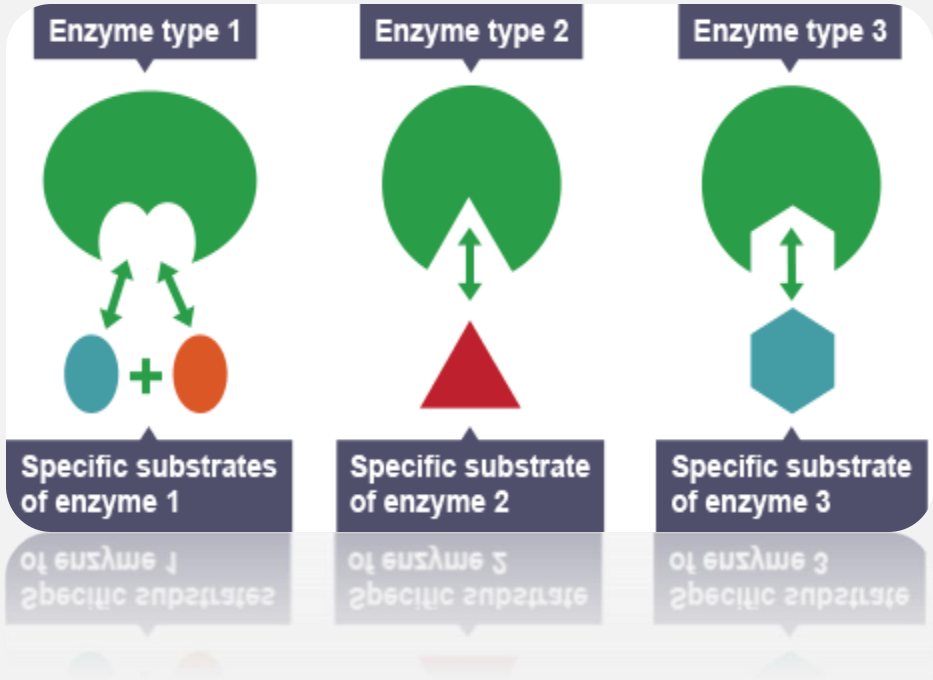
Since enzymes are proteins, this site is composed of a unique combination of amino acid residues (side chains or R groups). Which play a role in the specificity of the enzyme toward substrate



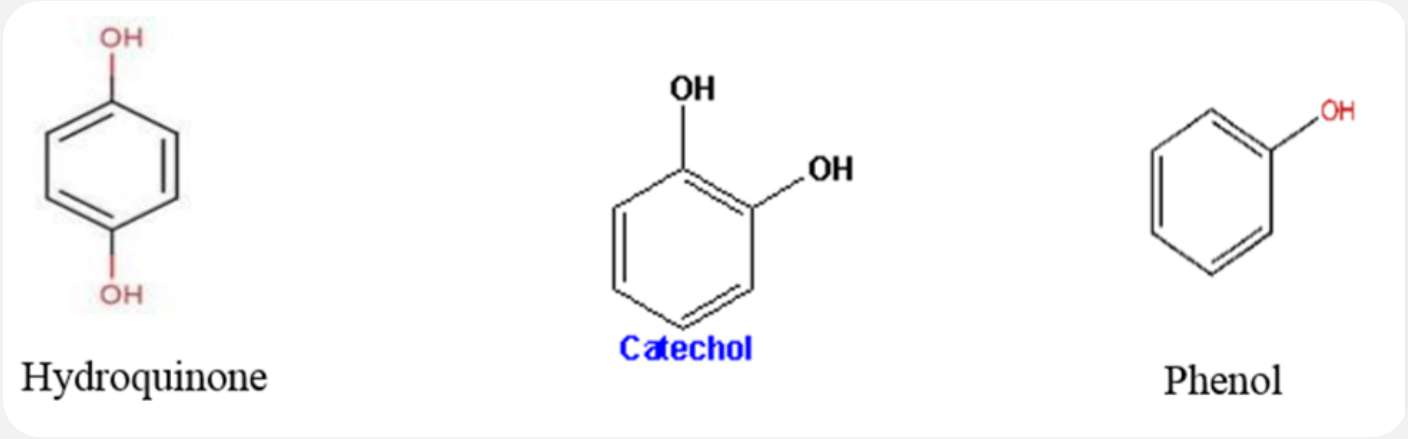
Types of Enzyme Specificity

There are four distinct types of specificity:

- **Absolute specificity** - the enzyme will catalyze only one reaction.
- **Group specificity** - the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.
- **Linkage specificity** - the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.
- **Stereochemical specificity** - the enzyme will act on a particular steric or optical isomer.



These three compounds will be used to find out which one of them is a substrate for PPO



Method:

a. Label three clean test tubes A, B and C.

Tube	(1)	(2)
Tube A	15 drops of enzyme extract	Add 15 drops of 0.01M catechol solution
Tube B	15 drops of enzyme extract	Add 15 drops of 0.01M phenol solution
Tube C	15 drops of enzyme extract	Add 15 drops of 0.01M hydroquinone

b. Shake the tubes gently and place them in a water bath at 37 ºC.

c. Examine the tubes after 5 minutes and after 10 minutes. Record the color in each tube.

d. The three compounds used as substrates in this part of the experiment are structurally related.

Results:

Substrate	Degree of color intensity (Symbol: −, +, ++ or +++)	
	5 Minutes	10 Minutes
Catechol		
Phenol		
Hydroquinone		

•**Phenol** is a mono-hydroxyl phenol, and the enzyme only works on di- or tri- hydroxyl phenol.

• **Hydroquinone** is a di-hydroxyl phenol that can slightly change the active site configuration depending on “induced fit model” and gives a fait color reacting with PPO.

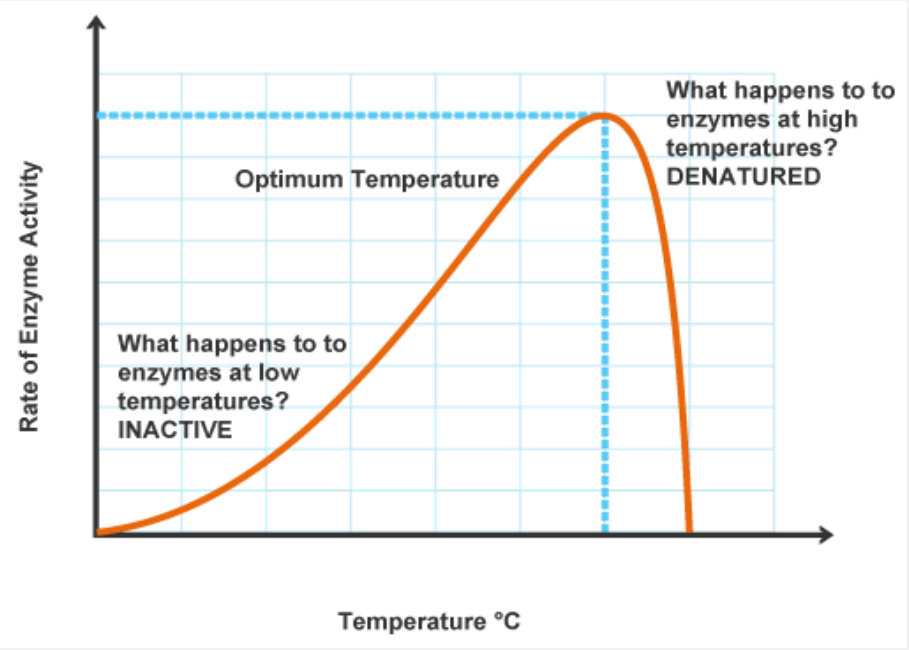
4) To investigate the effects of temperatures on the enzyme activity

Optimum Tm:

is the temperature at which the activity of the enzyme is maximum.

Principle:

Non-covalent bond can be broken by heating, leading to protein denaturation and the precipitation, and enzyme is no longer work.



Method:

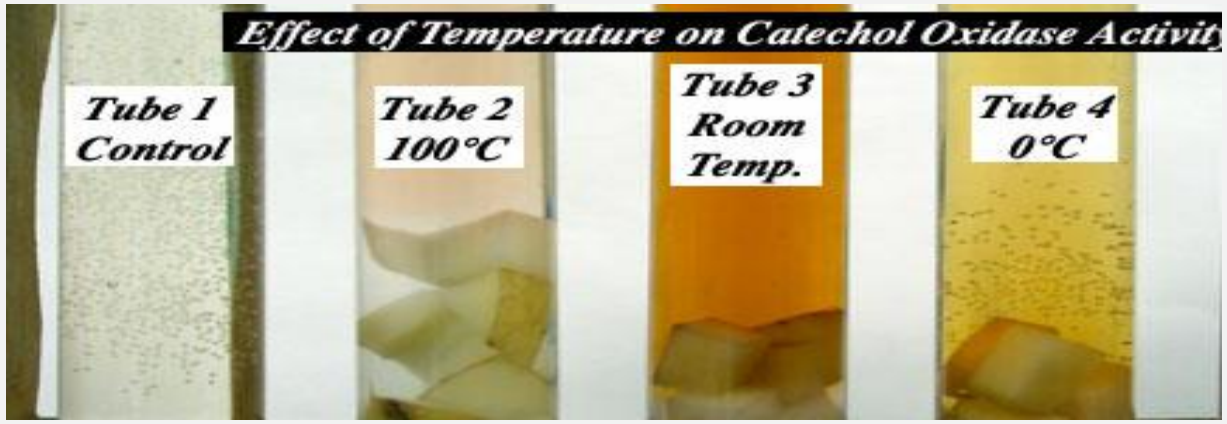
a. Label three clean test tubes A, B and C.

Tube	(1)	(2) Incubate at:	(3)
Tube A	Add 15 drops of enzyme extract to each tube.	0 ºC for 10 min	Add 15 drops of 0.01M catechol solution to each tube
Tube B		37 ºC for 10 min	
Tube C		95 ºC for 10 min	

b. Shake each tube gently and quickly return it to its proper temperature condition.
c. Wait for 15 minutes. Then, examine each tube without removing it from its temperature condition, and record the color in each tube, according to the scheme described.

Results:

Temperature (ºC)	Degree of color intensity (Symbol: −, +, ++ or +++)
0	
37	
95	



Discussion:

-Introductory paragraph

-Discuss in each experiment what you observed and the reasons behind the findings in details

Questions:

- **What type of specificity does PPO have?**

- **If we treated the enzyme with trypsin before adding the catechol, will the reaction happen or not? WHY?**