


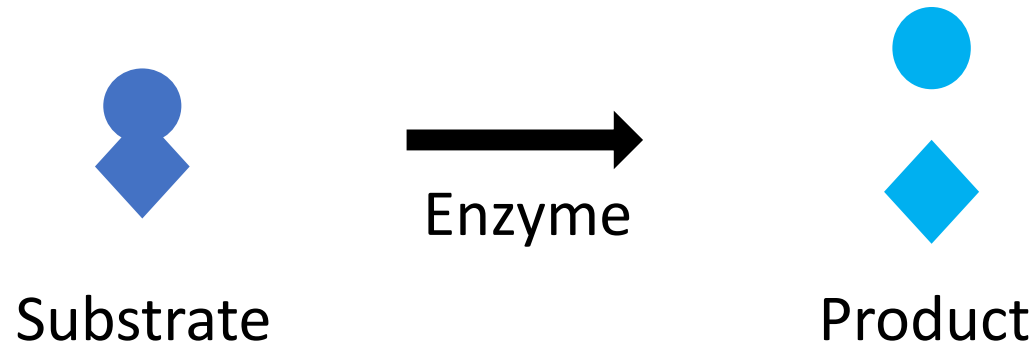
# Enzymes

# Enzymes


Prior knowledge

# Enzymes


- Globular proteins 
- Can be found in living cells and sometimes outside (secreted)
- Work as catalysts → speed up chemical reactions
- Turn substances, the enzyme's **substrate**, into specific **products**




One enzyme can only use one substrate (enzyme-substrate-specificity)



Enzyme can only catalyse one biochemical reaction



In the cell thousands of biochemical reactions take place



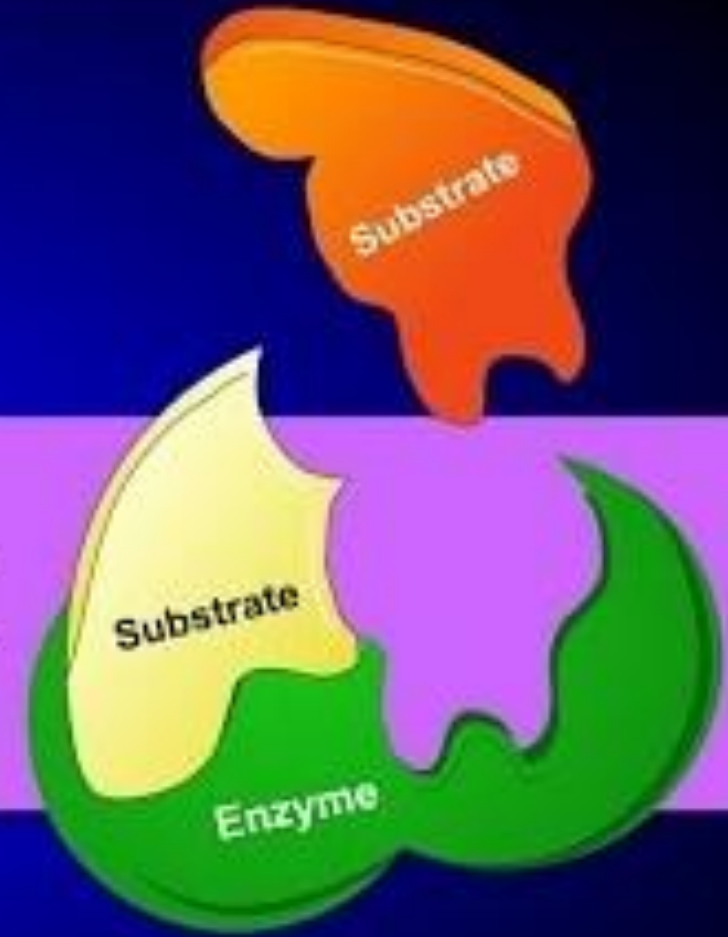
Many different enzymes are needed

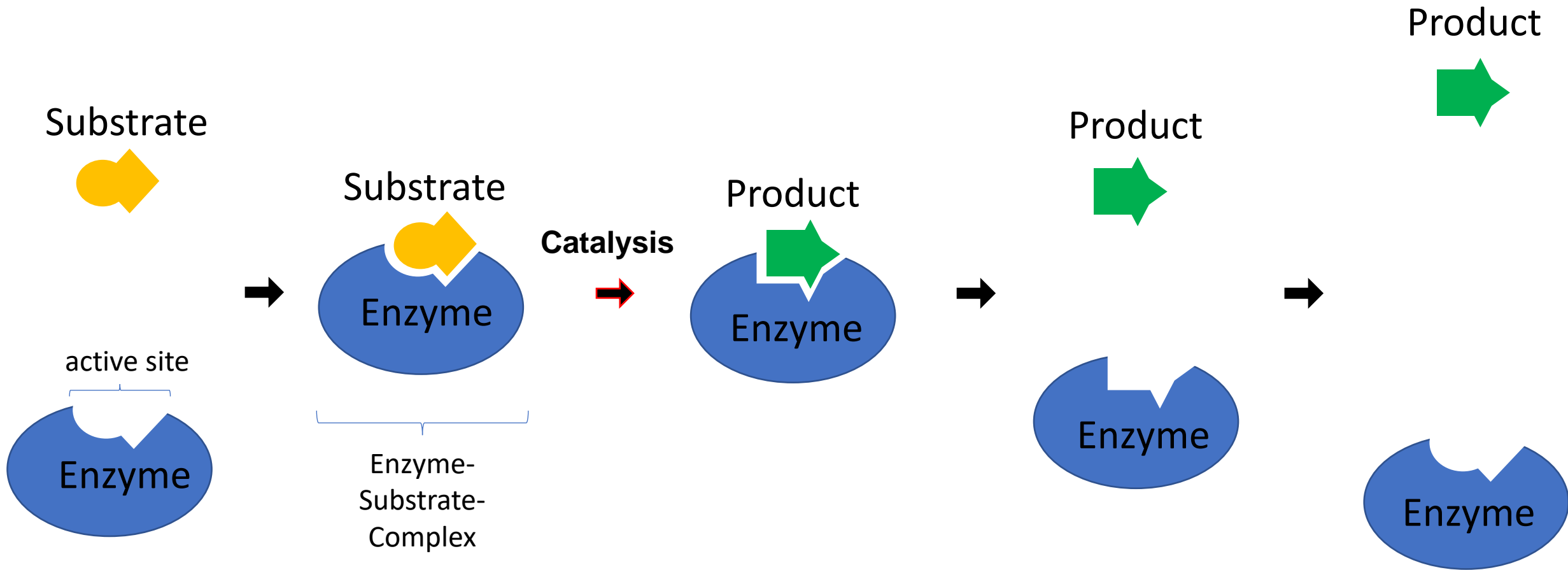
# Questions:

1. What is an enzyme?
2. Define the active site.
3. Explain how an enzyme attaches to a substrate using the lock and key hypothesis.



# How Enzymes Work





# Enzyme activity

- <http://www.kscience.co.uk/animations/ectopeptidase.swf>
- <http://www.kscience.co.uk/animations/enzyme.swf>
- <http://www.kscience.co.uk/animations/specific.swf>



# Enzyme activity

- [http://biomodel.uah.es/en/lab/abs/activ\\_enz.htm](http://biomodel.uah.es/en/lab/abs/activ_enz.htm)



# Factors affecting enzyme activity

- Which factors can you think of?

# Factors affecting enzyme activity

- Temperature
- pH
- Substrate concentration

# Poll

How does the temperature affect enzymes?

- It increases enzyme activity.
- It decreases enzyme activity.

# Factors affecting enzyme activity

- [http://biomodel.uah.es/en/lab/abs/activ\\_enz.htm](http://biomodel.uah.es/en/lab/abs/activ_enz.htm)

The objective is to find optimal **pH** and **temperature** conditions for this enzyme.

1. Slide the controls to pick a pH and a temperature for the assay.
2. Press, in order, the buttons to add components of the reaction mixture into the cuvette.  
The "add substrate" button will first empty the cuvette. The "add sample" button will start the incubation.
3. Once the colour has developed, press the button in the spectrophotometer to measure absorbance, and write down its value.
4. Systematically change either pH or temperature and write down each absorbance value in your lab notebook, or in the table on the right.

pH 7 T 50°C

1

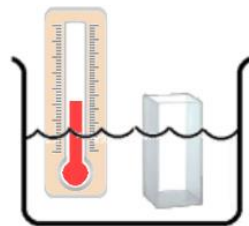
buffer and substrate add (2a)

sample (2b)

incubate for 1h

DNS (2c)

and 10 min at 100°C



Lab notebook:  
(write down the measurements)

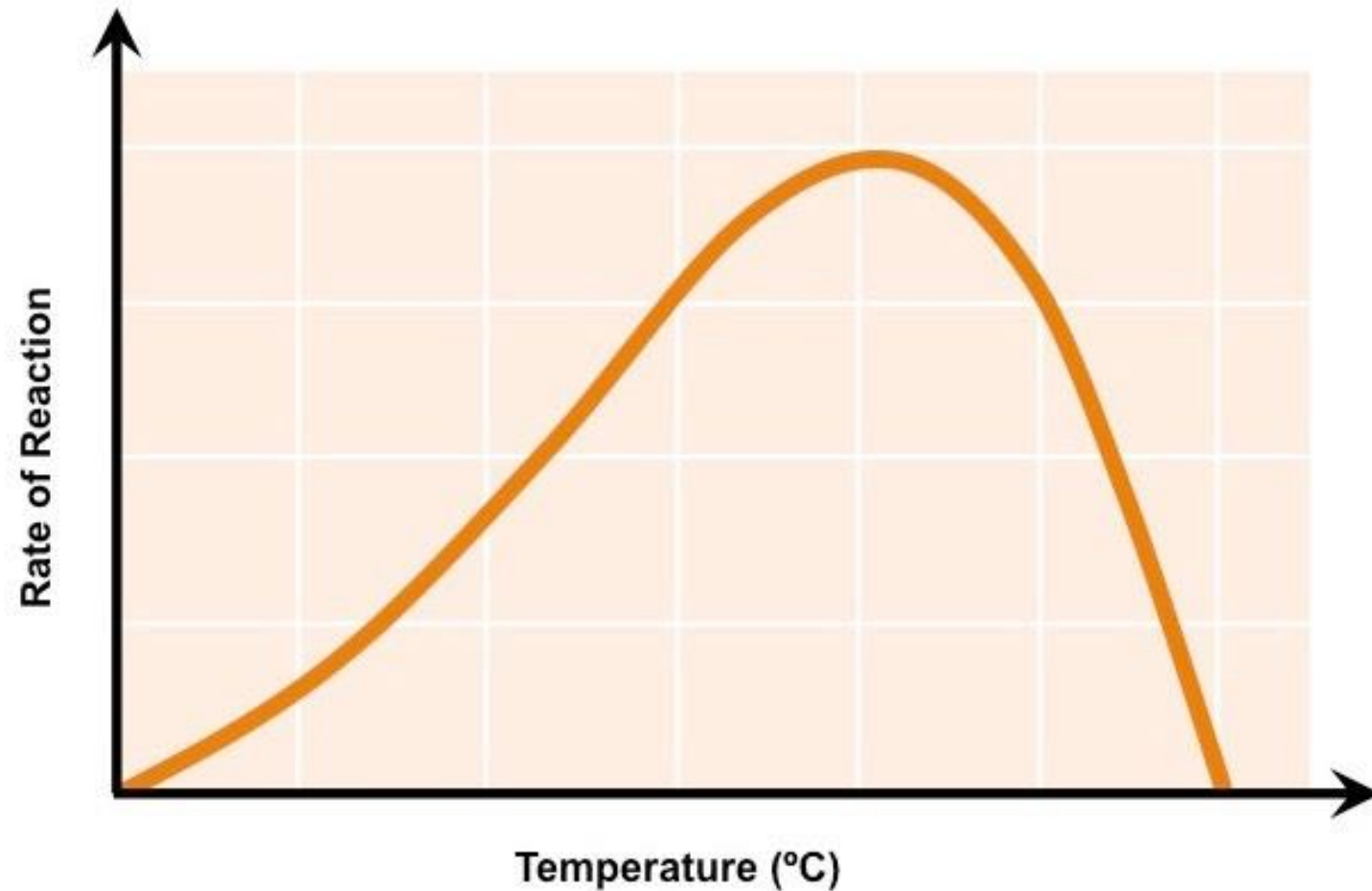
add another row

4)

	pH	T (°C)	A <sub>540</sub>
1			
2			
3			
4			
5			
6			

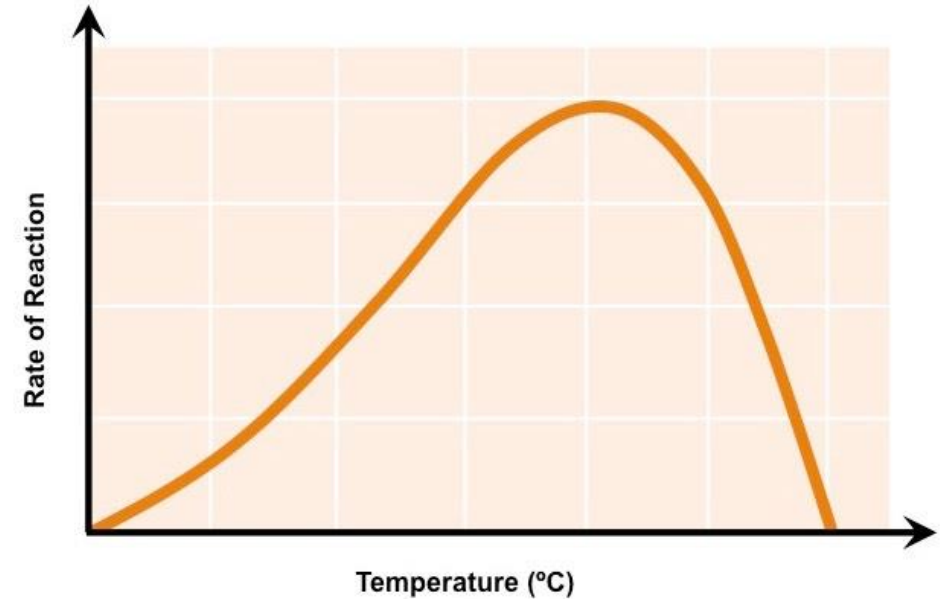
print save

# Factors affecting enzyme activity

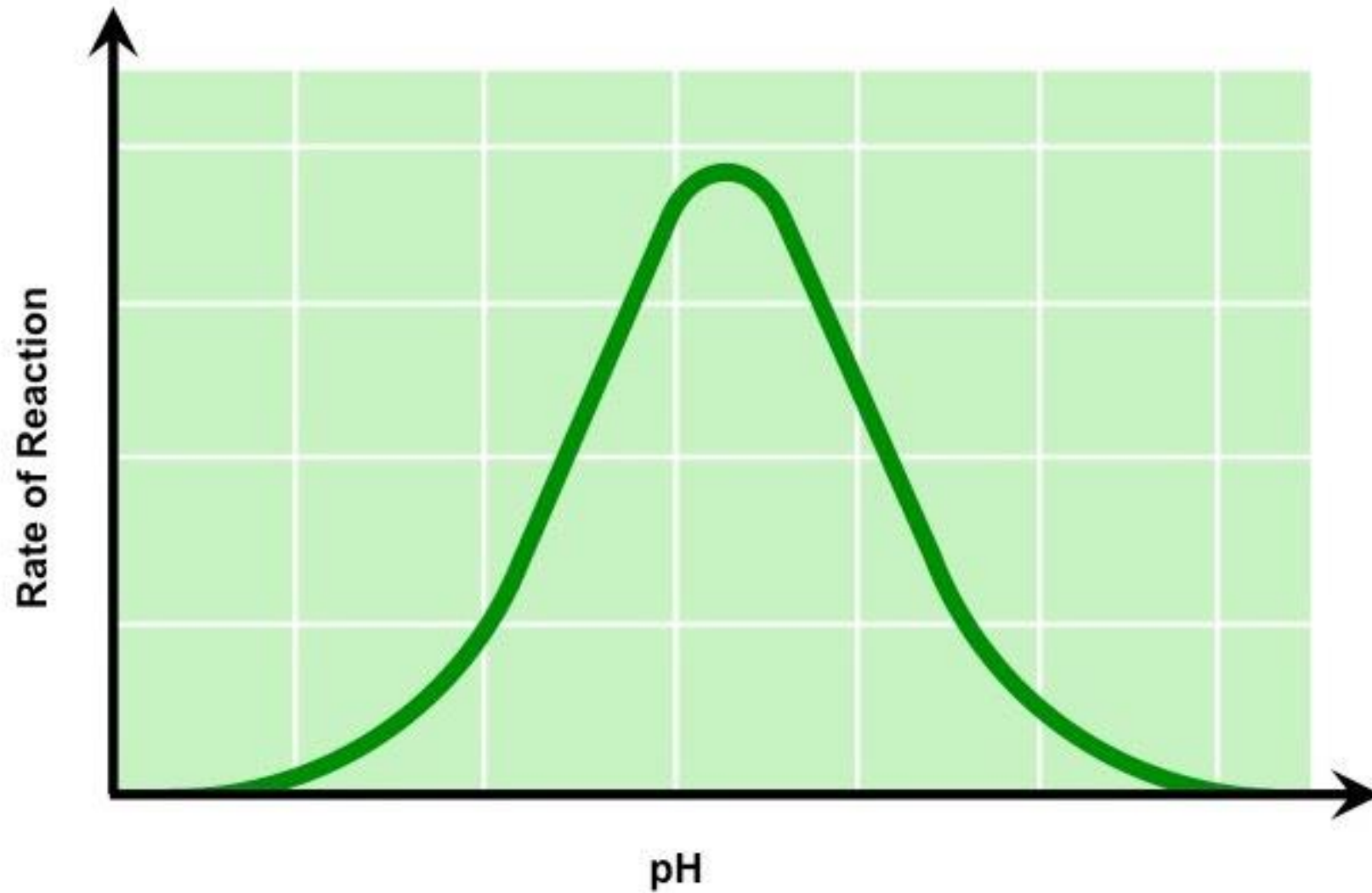


# Factors affecting enzyme activity

- Temperature:
  - With higher temperatures particles move faster and thus the collision of enzymes and substrate is more likely → enzyme activity increases
  - At a certain temperature proteins begin to denature → activity decreases



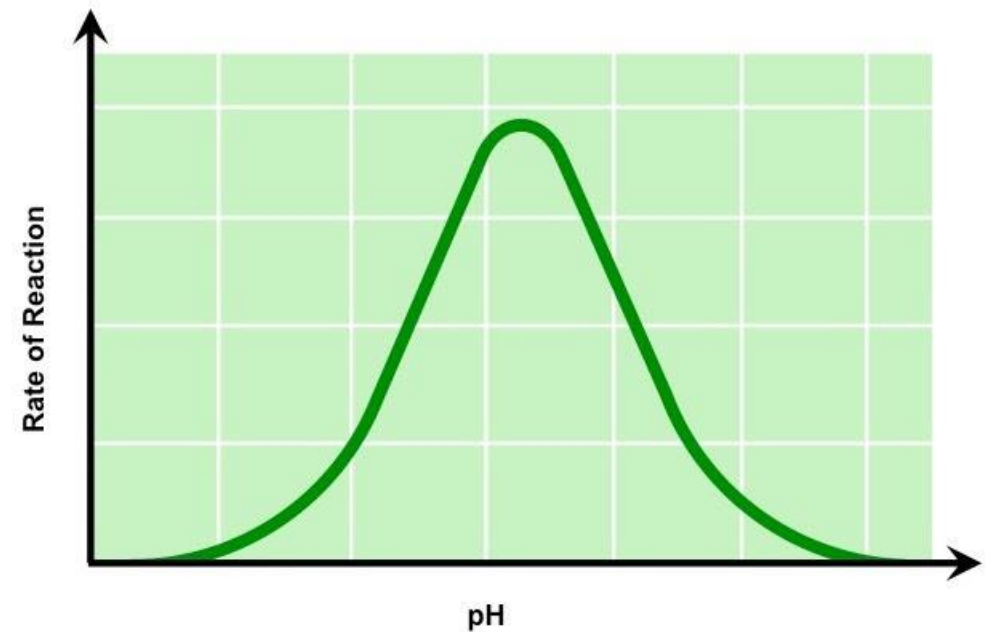
# Factors affecting enzyme activity



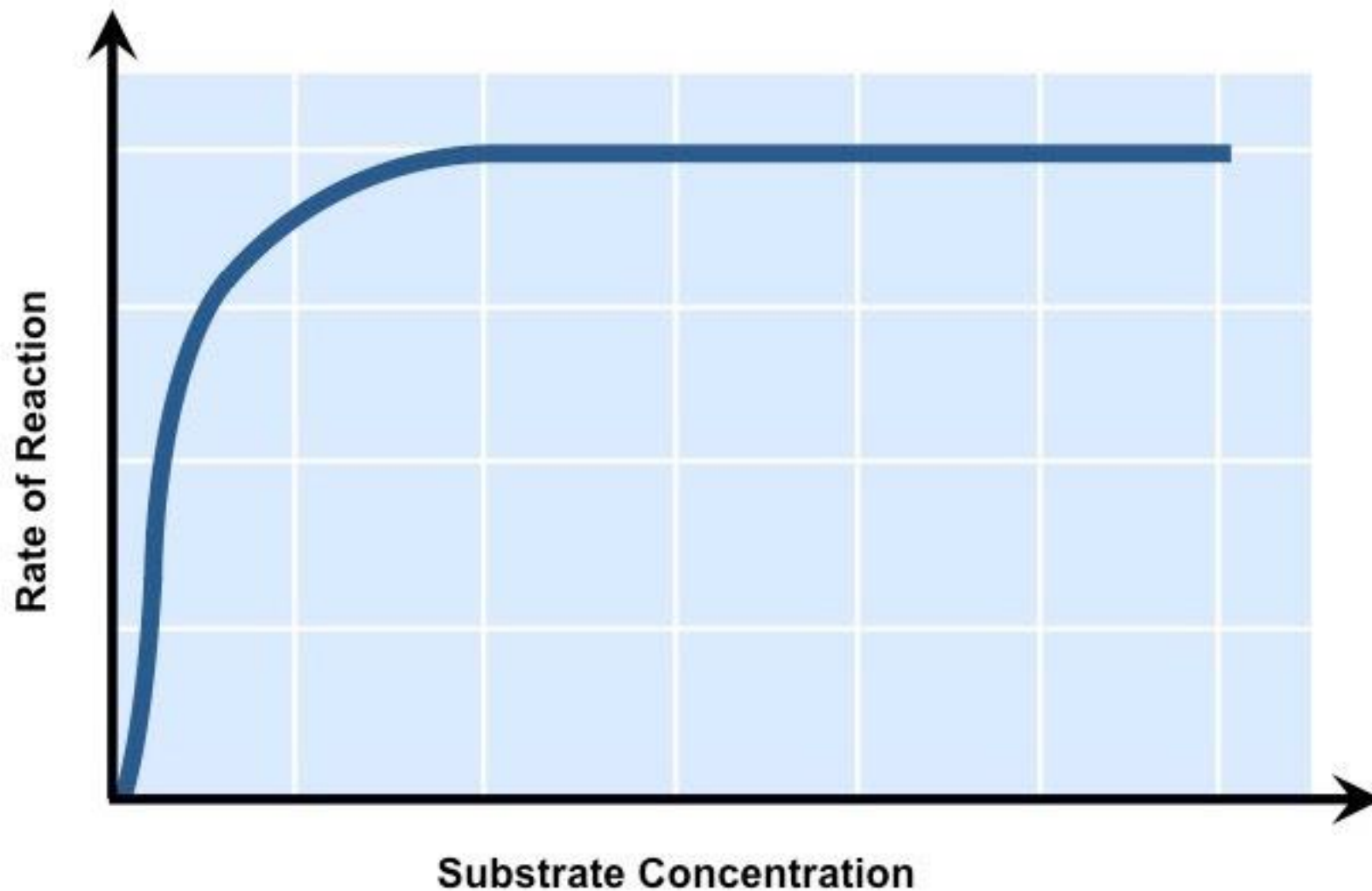


# Factors affecting enzyme activity

- pH:
  - Enzymes have an optimum pH, at which their activity is the highest
  - Different enzymes have a different pH optimum
  - Deviation from the optimum: enzyme activity decreases or stops

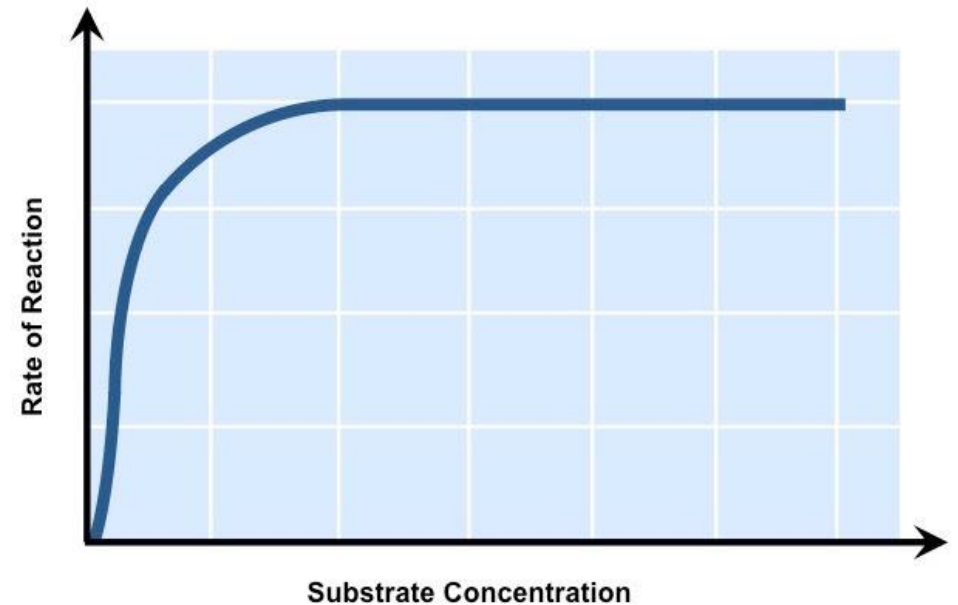


# Factors affecting enzyme activity



# Factors affecting enzyme activity

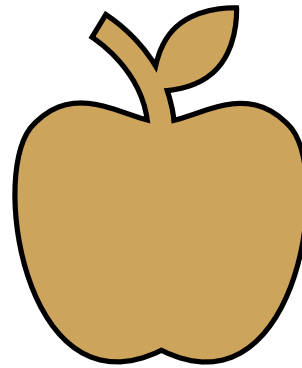
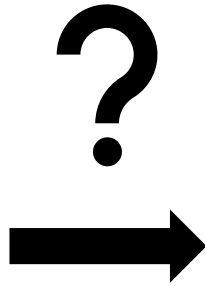
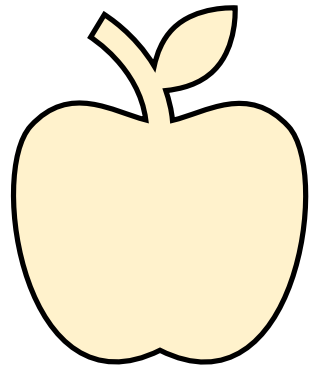
- Substrate concentration:
  - Higher concentration: more frequent collision of enzyme and substrate  
→ higher enzyme activity
  - Even higher concentration: the active sites of all enzymes are occupied → no further increase in enzyme activity



# Enzyme simulation

- <https://sites.google.com/site/biologydarkow/enzymes/enzyme-diversity-simulation>

Why does an apple turn brown  
when you cut it?

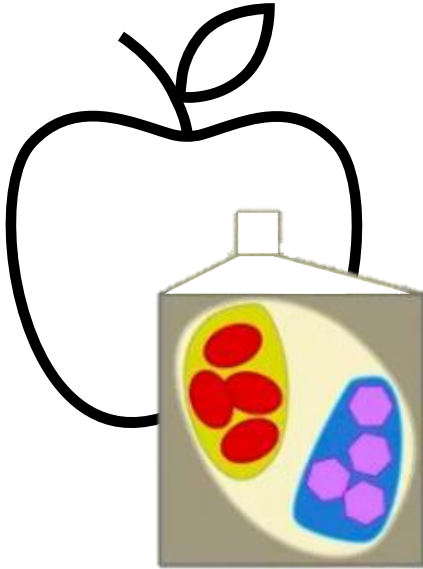


# Polyphenol oxidase

- Enzyme
- Can be found in the chloroplasts and mitochondria of the cells in plants
- Major enzyme responsible for enzymatic browning of apples (and other fruit)

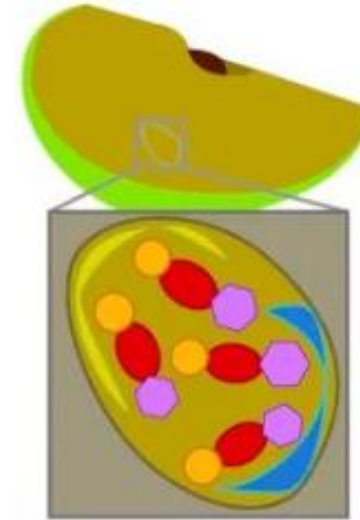
# Apple browning

The Three Players: PPO poly phenol O<sub>2</sub>



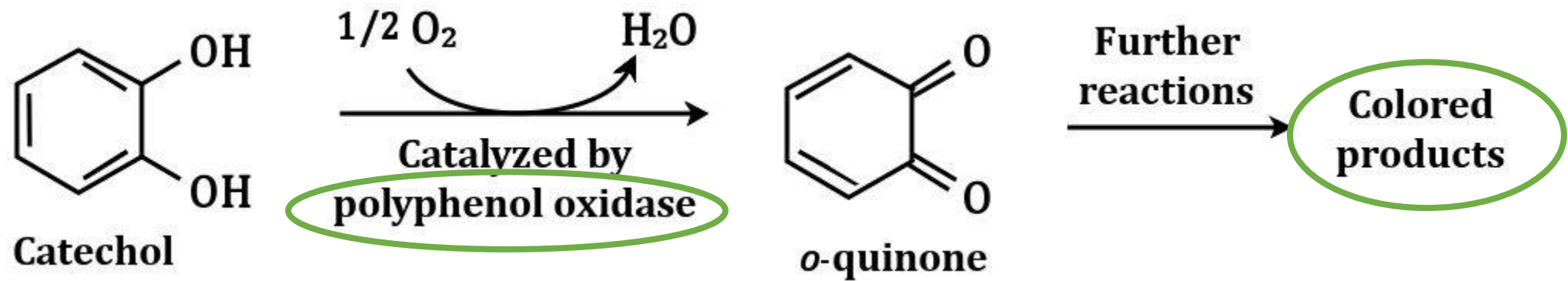
Polyphenol oxidase and polyphenols are separated in the cell. They are not in contact with oxygen.

Cut apple



Cellular compartments are disrupted. Polyphenol oxidase reacts with polyphenols and oxygen.

# Apple browning





What could we do to prevent the browning?

# Enzyme experiment

- Cut an apple into 0.5 cm slices
- Treat slices in different ways:
  - Approach 1: put one slice on a plate at room temperature
  - Approach 2: put lemon juice on one slice, leave slice at room temperature
  - Approach 3: bathe one slice in hot water for a minute, rub it dry and leave slice at room temperature
  - Approach 4: put one slice into the fridge
  - Approach 5: soak one slice in water
  - Approach 6: put slice on a plate with the sliced side down
  - Approach 7: Come up with your own idea
- After one hour have a look which slices have turned brown and also pay attention to the intensity of the colour