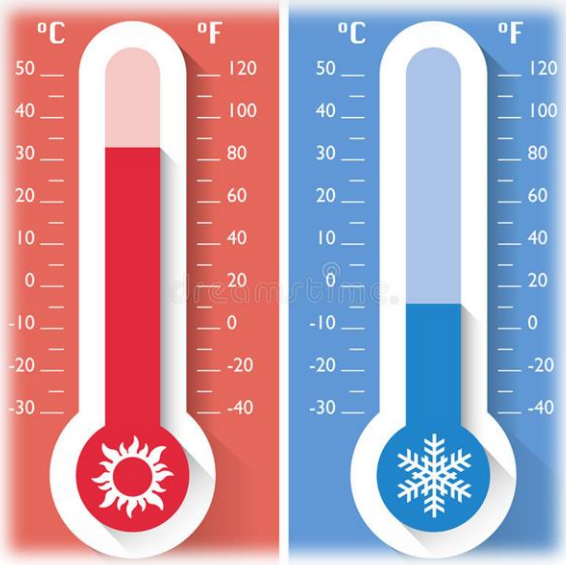
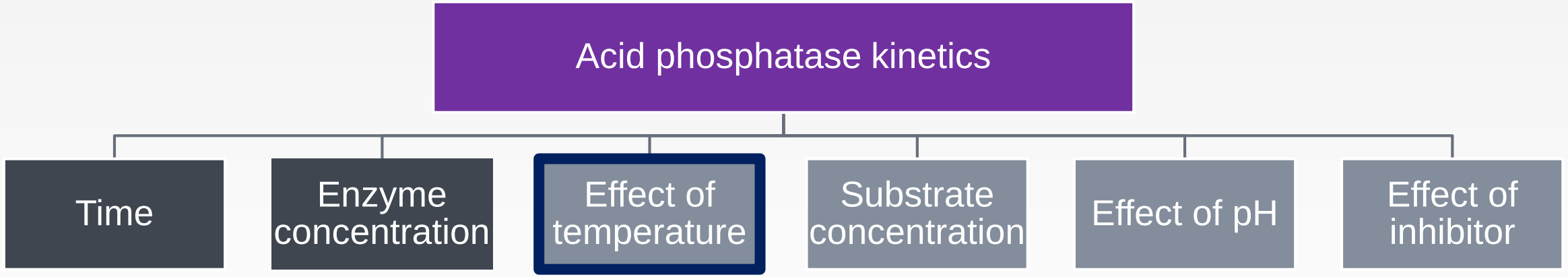
The effect of temperature on the rate of an enzyme catalyzed reaction



- **In this experiment, we will continue to study acid phosphatase kinetics**

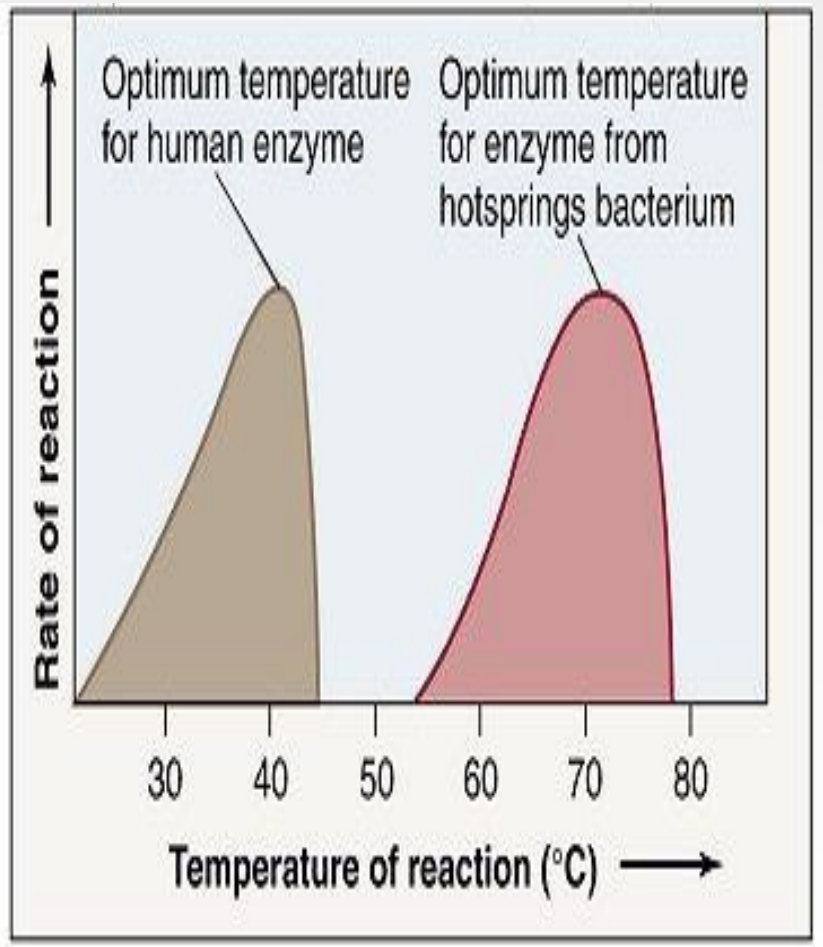


Objectives

- To establish the relationship between temperature and the rate of an enzyme catalyzed reaction.
- To determine the optimum temperature for such a reaction.

Optimum Temperature

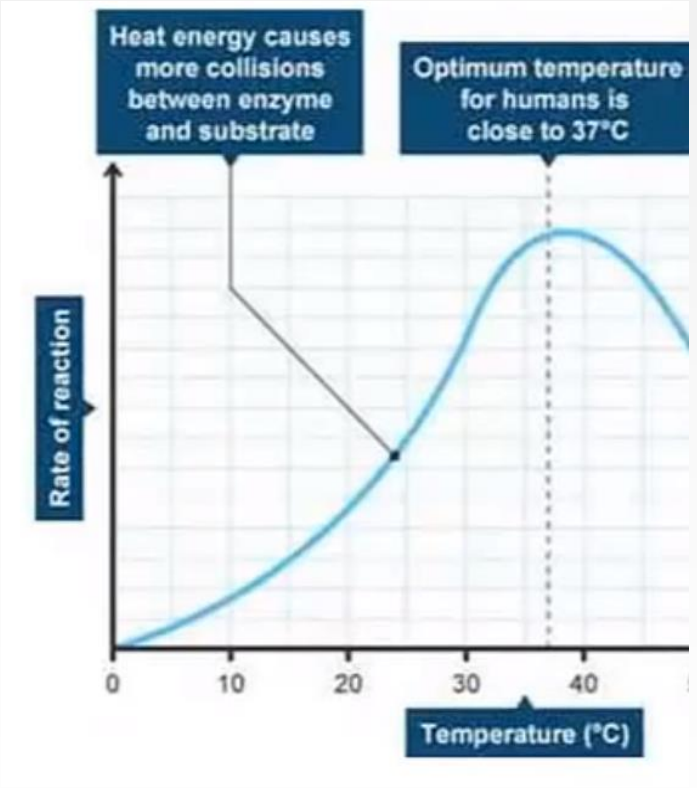
- The rate of an enzyme catalyzed reaction is affected by changes in temperature.
- Each enzyme has a temperature that it works optimally in called (**Optimum Temperature**).
- For most enzymes, the optimum temperature is at the temperature of the cells in which the enzyme is found in **vivo**.
- For example, humans' enzymes works optimally **at around 37** degrees Celsius.
- However, some enzymes from hot spring bacteria work really well in higher temperatures like 95 degrees Celsius.



What is The effect of Temperature on the activity of Enzyme?

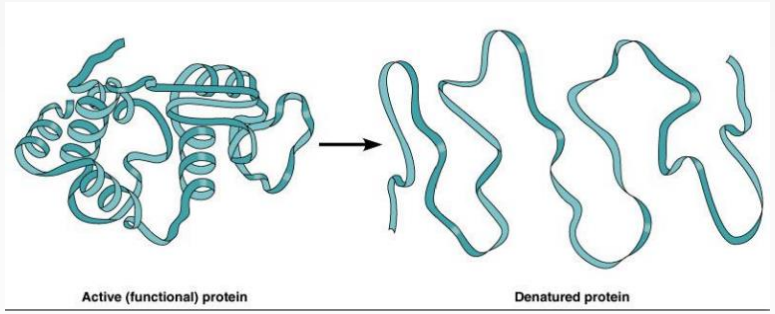
1-At suboptimal temperature, increasing temperature increase the kinetics energy of the reactant. As they move faster, they move likely to collide and interact with each other and the enzyme.

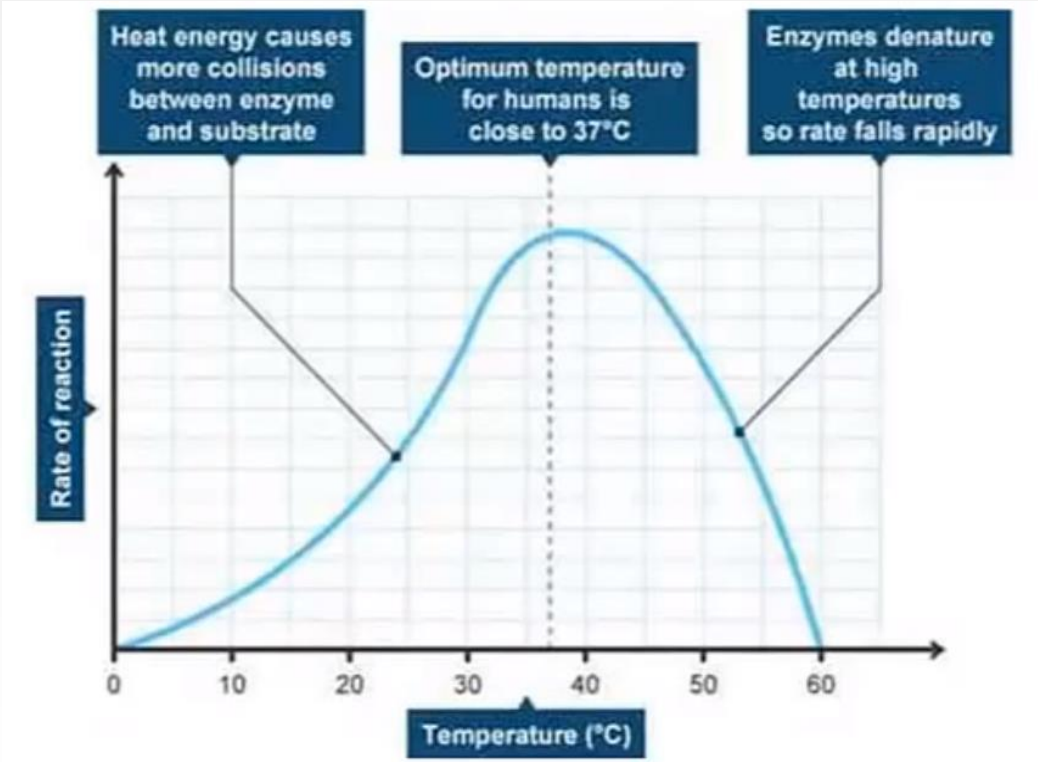
2-The rate continues to rise until reaches a peak at the optimum temperature



What is The effect of Temperature on the activity of Enzyme?

3-Above this temperature, the rate usually falls dramatically. This is because the increase energy cause bonds that maintain the enzymes shape to break , and the enzyme becomes **denatured**.(three dimensional structure will change)





- **The changed shape means that the substrate can no longer fit into the active site, and enzyme activity is lost.**

Method:

- This exercise illustrates the effect of increasing enzyme concentrations on reaction rate.
- You will perform a series of **5-minutes assays**, in which different temperature will be used

In order to detect the effect of Temperature you must fix all the component except the temperature	
Time (**5 minutes**)	constant
Enzyme concentration	constant
Temperature (degree)	**Variable**
pH (5.7)	constant

Method:

- The effect of the following temperature will be studied on Acid phosphatase:

Desired temperature (˚C)	Method of preparation
4˚C	Ice plus tap water in an ice bucket
25˚C	Room temperature
37˚C	Thermos-tatted water bath
65˚C	Thermos-tatted water bath
80˚C	Thermos-tatted water bath
100˚C	Thermos-tatted water bath

Method:

- Label 12 assay tubes as the following:

Each should have its own Blank	
Blank-4C°	Test-4 C°
Blank-25 C°	Test-25 C°
Blank -37 C°	Test-37 C°
Blank-65 C°	Test-65 C°
Blank-80 C°	Test-80 C°
Blank-100 C°	Test-100 C°

Method:

- You must prepare the following for each tube(Blank + Test):

Chemical	Volume
1.0M sodium acetate buffer (pH 5.7)	0.5 ml
0.1M $MgCl_2$	0.5 ml
p-nitrophenyl phosphate	0.5 ml
Water	5 ml

- Place the tubes in the labeled temperature and let the temperature equilibrate for 5 min

Method

- For the blank you must first add 0.5 ml KOH then add the enzyme(we do not want any product to appear in the blank)

- For **TEST** , Add **0.5 ml of enzyme** extract to TEST and allow the reaction to proceed for **5 min**, using all the water bath temperatures described in the previous table

- Stop the reaction by the addition of **0.5ml of KOH** .

- ***When all of the reaction mixtures have returned to room temperature***, determine the absorbance at 405 nm of each experimental tube against its own blank.

Tube no.	Start the reaction (min.)	Stop the reaction (min.)
4 C	0	5
25	2	7
37	4	9
65	6	11
80	8	13
100	10	15

Time	Addition

Results :

Temperature	Absorbance 405 nm	Velocity (µmole of PNP/min)
0		
25		
37		
65		
80		
100		

Plot a graph illustrating the effect of different temperatures on the rate of the reaction.

Calculations:

Velocity (V) = $(A \times 10^6) / (E \times \text{time}) =$ **µmole of PNP/min**

A= absorbance

E= extension coefficient=18.8×10^3

Time = 5 min

Discussion

- Discuss the shape of the graph. And From the curve, explain and discuss the relationship between the activity of acid phosphatase and temperature.

- Define the optimum temperature and determine it from the curve.