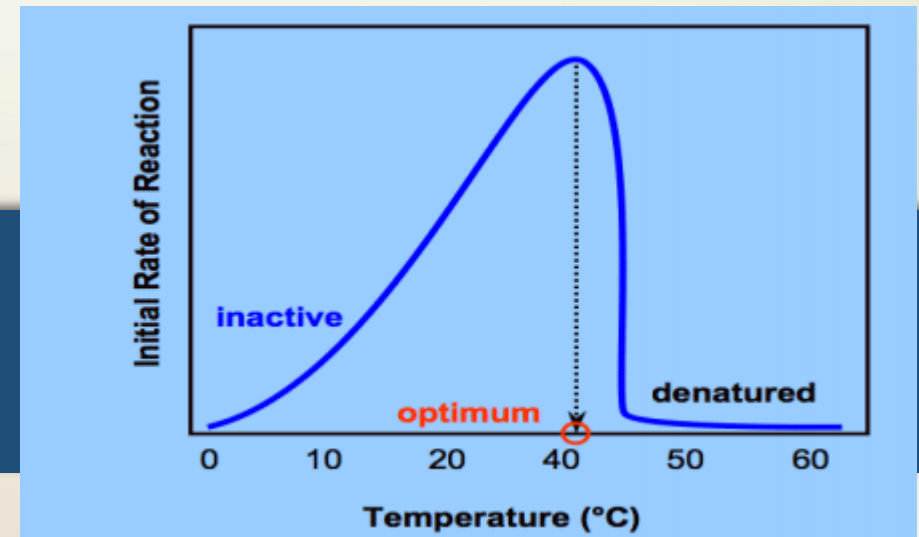
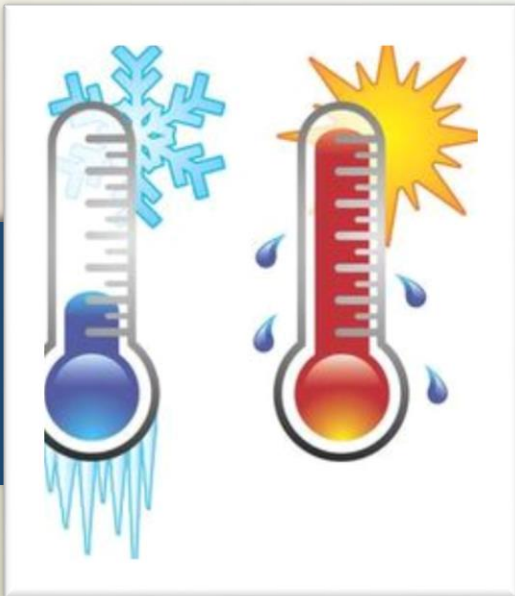
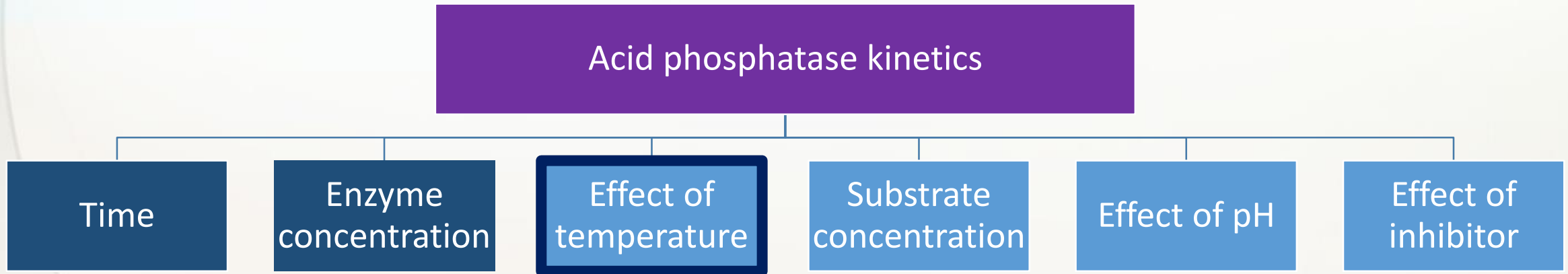


The effect of temperature on the rate of an enzyme catalyzed reaction

Subtitle



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



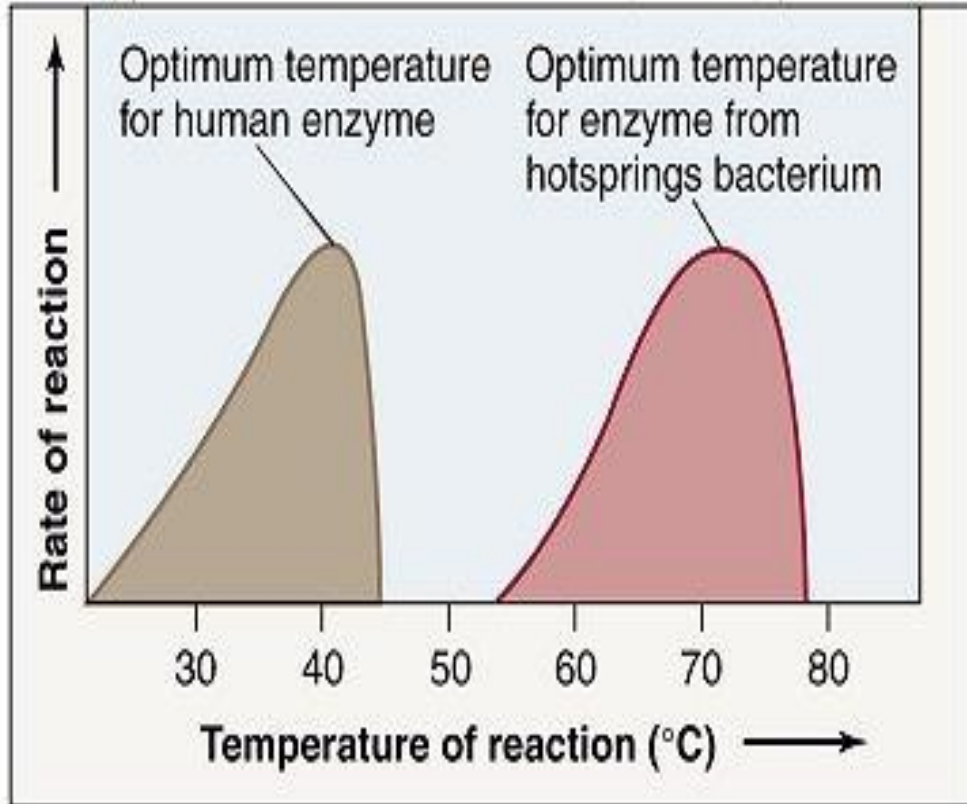
Objectives

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

The effect of Temperature on Enzyme

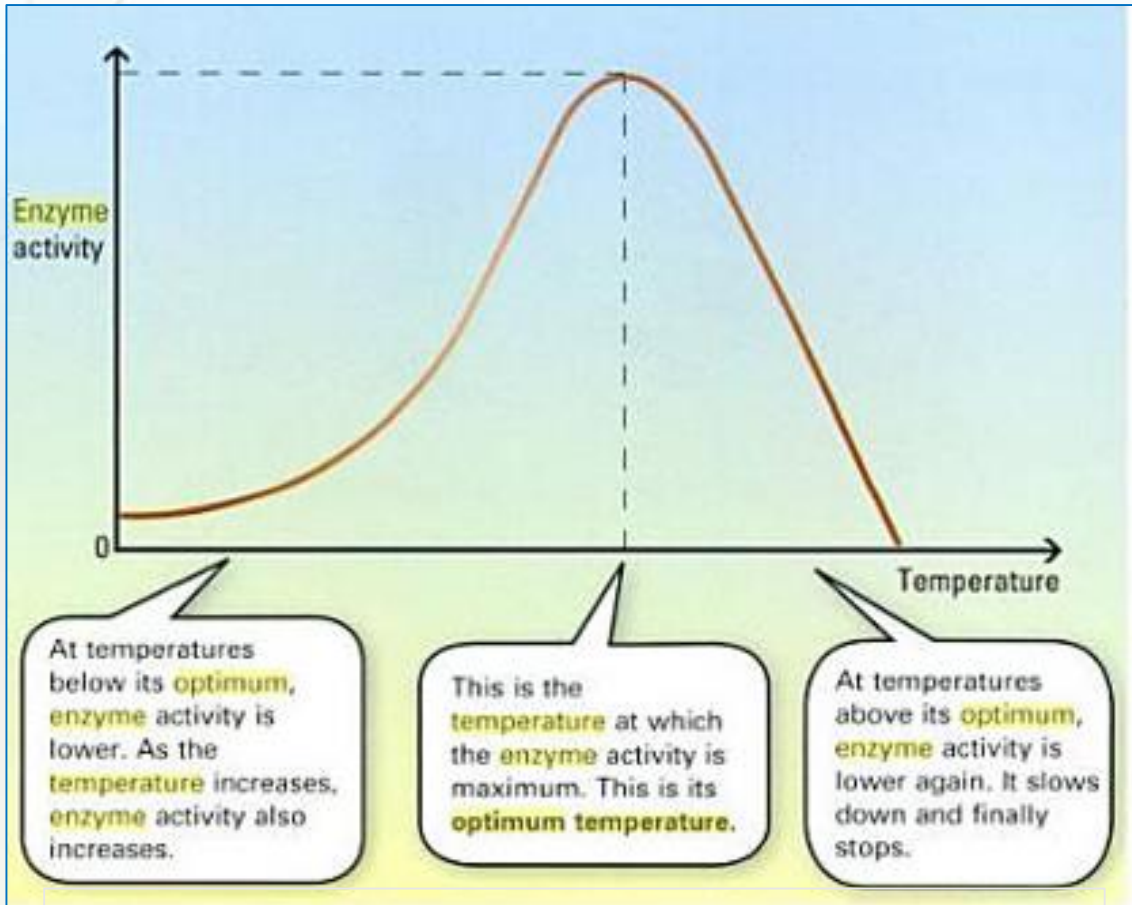
- The rate of an enzyme catalyzed reaction is affected by changes in temperature
- Each enzyme has a temperature that it works optimally in called (**Optimum Temperature**)
- For most enzymes, the optimum temperature is at or above the temperature of the cells in which the enzyme is found in vivo.

Optimum Temperature



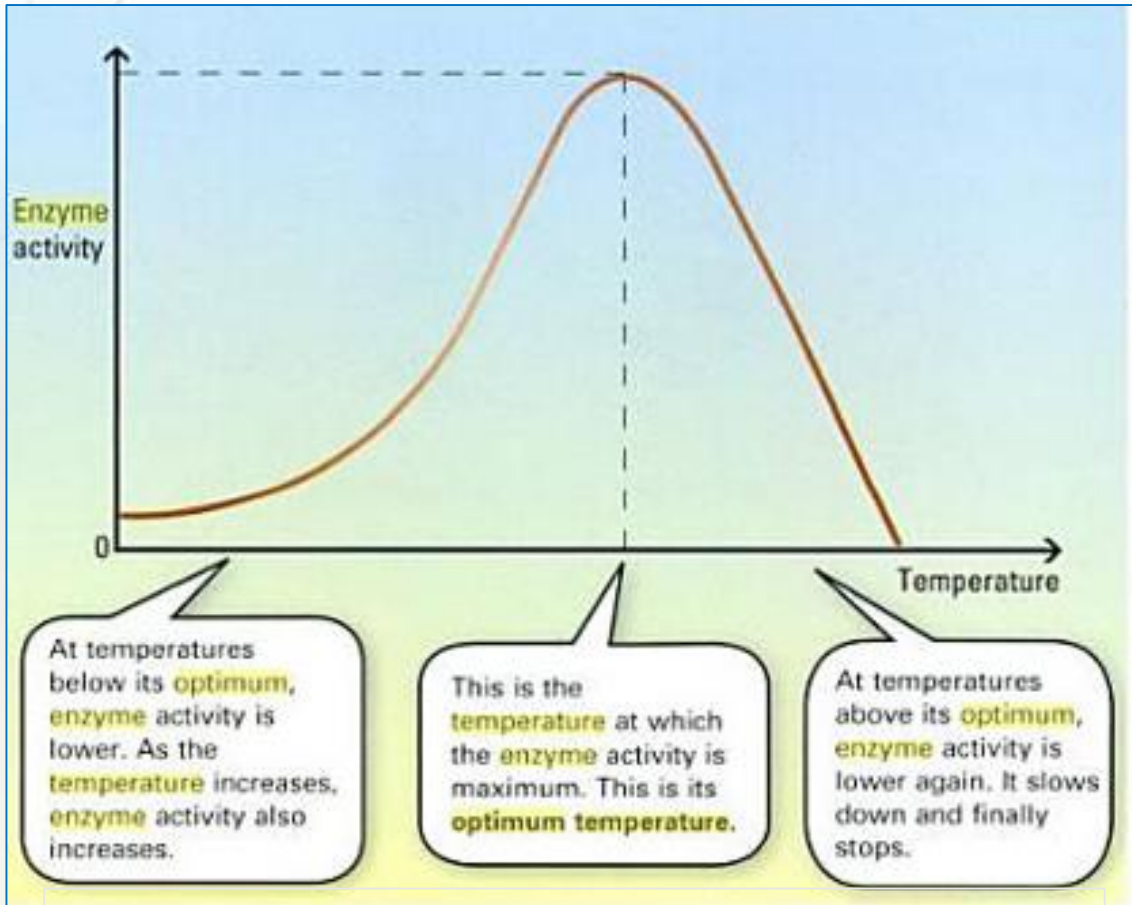
- Each enzyme has a temperature that it works optimally in, which in humans is around 37 degrees Celsius the normal body temperature for humans. However, some enzymes work really well at lower temperatures like 4 degrees Celsius
- and some work really well in higher temperatures like 95 degrees Celsius.

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

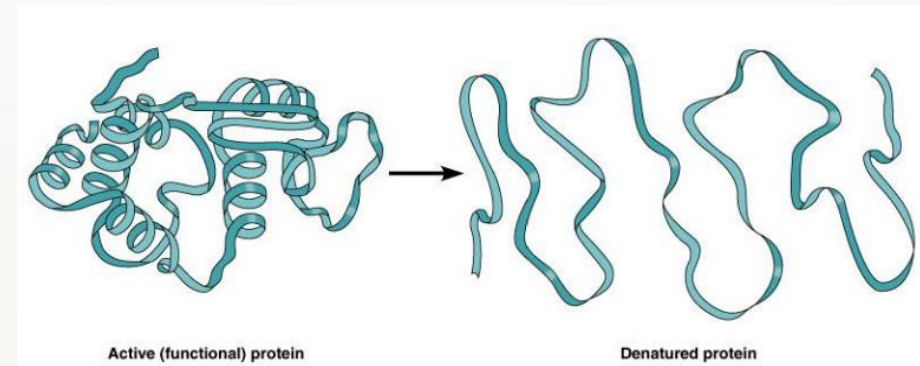


- The rate of an enzyme catalyzed reaction increase with temperature up to maximum called optimum temperature
- 1-At suboptimal temperature**, increasing temperature increase the kinetics energy of the reactant. As they move faster, they move likely to collide and interact with each other and the enzyme.
 - 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 of most enzymes?



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 (37 C 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
30°C	Room temperature
37°C	Thermostatted water bath
50°C	Thermostatted water bath
80°C	Thermostatted water bath
100°C	Thermostatted water bath

Method-Cont

- Label 14 assay tubes as the following:

Each should have its own Blank	
Blank-4C ^o	Test-4 C ^o
Blank-30 C ^o	Test-30 C ^o
Blank -37 C ^o	Test-37 C ^o
Blank-50 C ^o	Test-50 C ^o
Blank-80 C ^o	Test-80 C ^o
Blank-100 C ^o	Test-100 C ^o

Method-Cont

- 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-Cont

- 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.
- Stop the reaction by the addition of 0.5ml of KOH .
- This must be done using all the water bath temperatures described in the previous table. *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.) by Enzyme	Stop the reaction (min.)
4 C	0	5
20	2	7
37	4	9
60	6	11
80	8	13
100	10	15

Time	addition
0	0.5 Enzyme on 4 C
2	0.5 Enzyme on tube 20
4	0.5 Enzyme on tube 37
5	0.5 KOH on tube 4 C
6	0.5 Enzyme on tube 60
7	0.5 KOH on tube 20
8	0.5 Enzyme tube 80
9	0.5 KOH tube 37
10	0.5 Enzyme on 100
11	0.5 KOH on tube 60
13	0.5 KOH on tube 80
15	0.5 KOH on tube 100

Results :

Temperature	Absorbance 405 nm	Velocity (μ mole of PNP/min)
0		
30		
37		
50		
80		
100		

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

Calculations:

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

A= absorbance

E= extension coefficient= 18.8×10^3

Time = 5 min

Discussion

- 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.

Animation

- <http://www.austincc.edu/biocr/1406/laba/enzymes/>