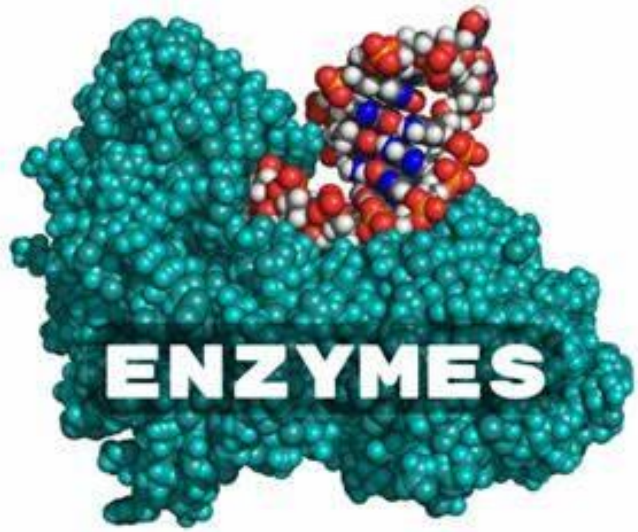
ENZYME ACTION



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Some enzyme require non protein molecule for their activity. So enzymes are conjugated proteins known as **Holoenzyme**.

Holoenzyme = Apoenzyme + Cofactor/coenzyme

Enzyme without non protein part is inactive and called apoenzyme.

Cofactors can be either inorganic or organic compounds .

Holoenzyme:
Catalytically active enzyme–cofactor complex is called a **holoenzyme.**

Apoenzyme:
The enzymatically inactive protein resulting from the removal of a holoenzyme's cofactor is referred to as an **apoenzyme.**

$$\text{Apoenzyme } (\textit{inactive}) + \text{cofactor} \rightleftharpoons \text{holoenzyme } (\textit{active})$$

Flavin (Co-enzyme) + Inactivated Succinate dehydrogenase (Apoenzyme)

$\Downarrow$

Activated Succinate dehydrogenase (Holoenzyme)

Other Examples are DNA Polymerase and RNA polymerase.

Complex (conjugated) Protein :
enzymes formed of two parts:

1) Protein part: called apoenzyme
2) Non- protein: called cofactor

- The whole enzyme is called holoenzyme

The cofactor may be coenzyme or prosthetic group

- Coenzyme: Is organic, thermo-labile , loosely attached to enzyme.
- They are mainly vitamin B derivatives e.g. FAD, NAD.
- Prosthetic group: Is inorganic, thermo-stable, firmly attached to enzyme.
- They are usually metal ions e.g. Ca, Zn

Cofactors

Non-polypeptide things at the active site that help enzymes do their job

- **Cofactors**
  - Are nonprotein enzyme helpers, eg Zn^{++}
- **Coenzymes**
  - Are organic cofactors

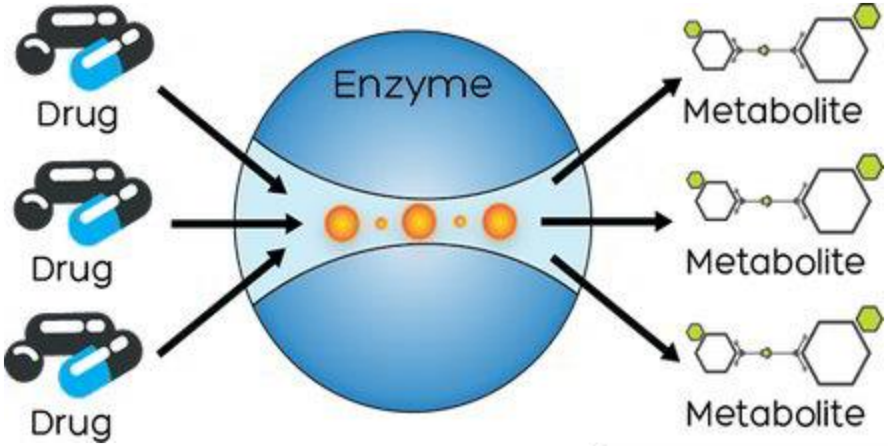
Mechanism of enzyme action

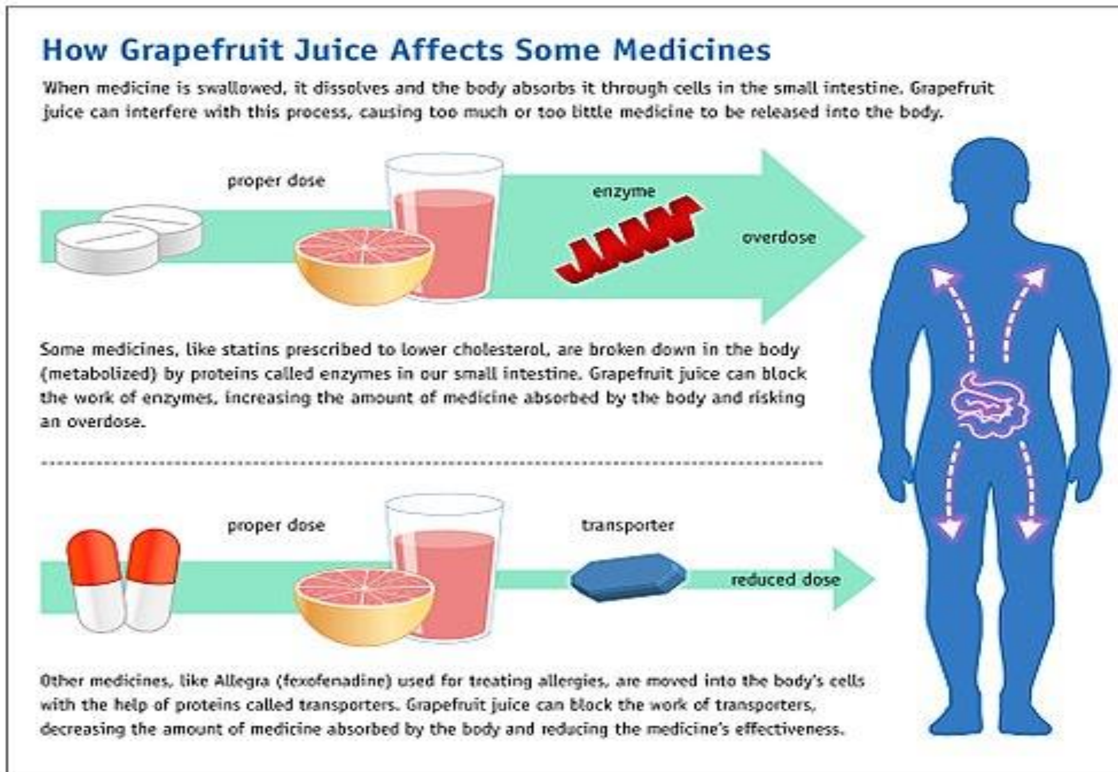
The enzymatic reactions takes place by binding of the substrate with the active site of the enzyme molecule by several weak bonds.

$E + S \longleftrightarrow ES \longrightarrow E + P$

Formation of ES complex is the first step in the enzyme catalyzed reaction then ES complex is subsequently converted to product and free enzyme.

ENZYME FUNCTION IN HUMAN BODY





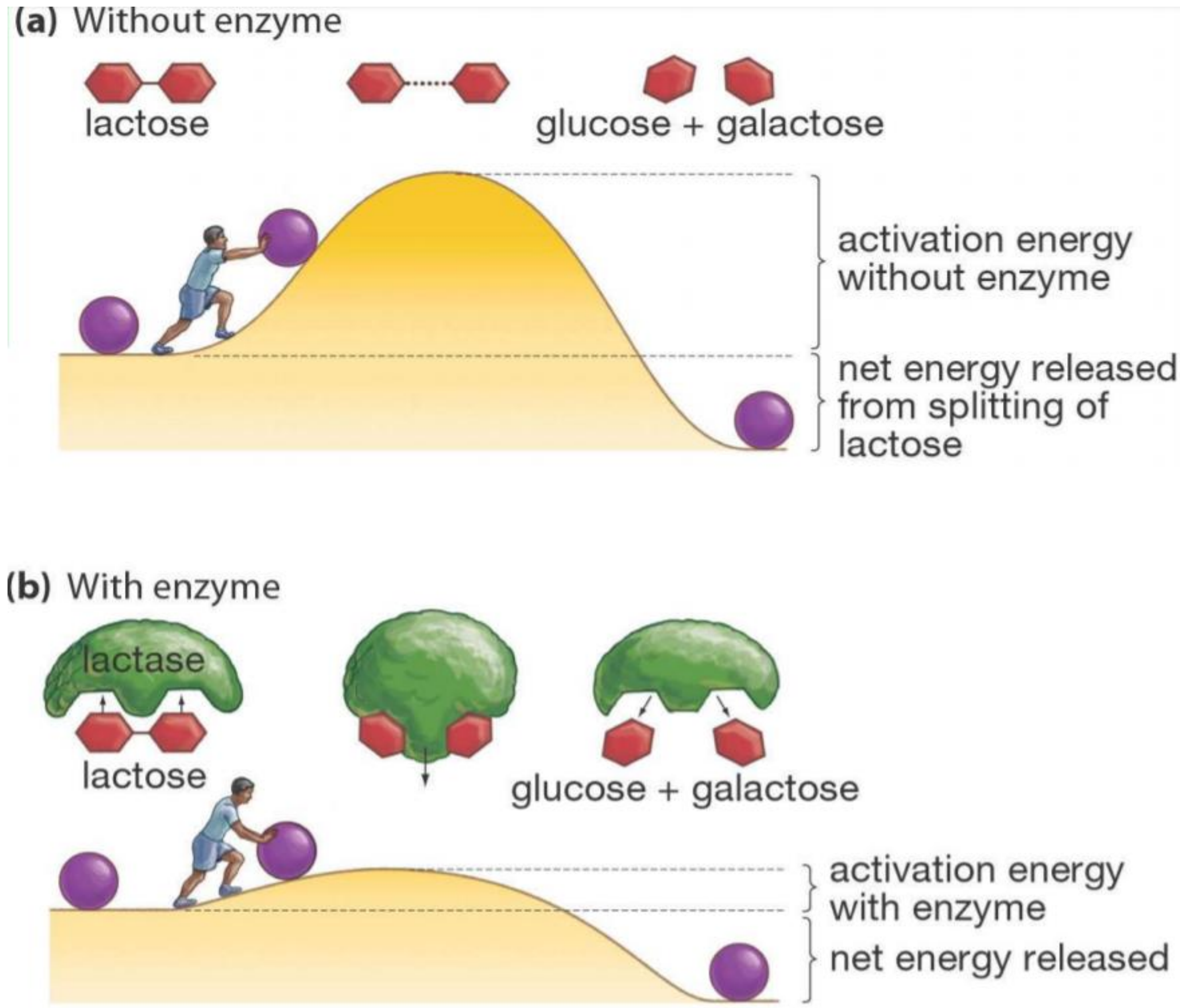
ENZYMES make reactions easier to occur at reasonable temperature by

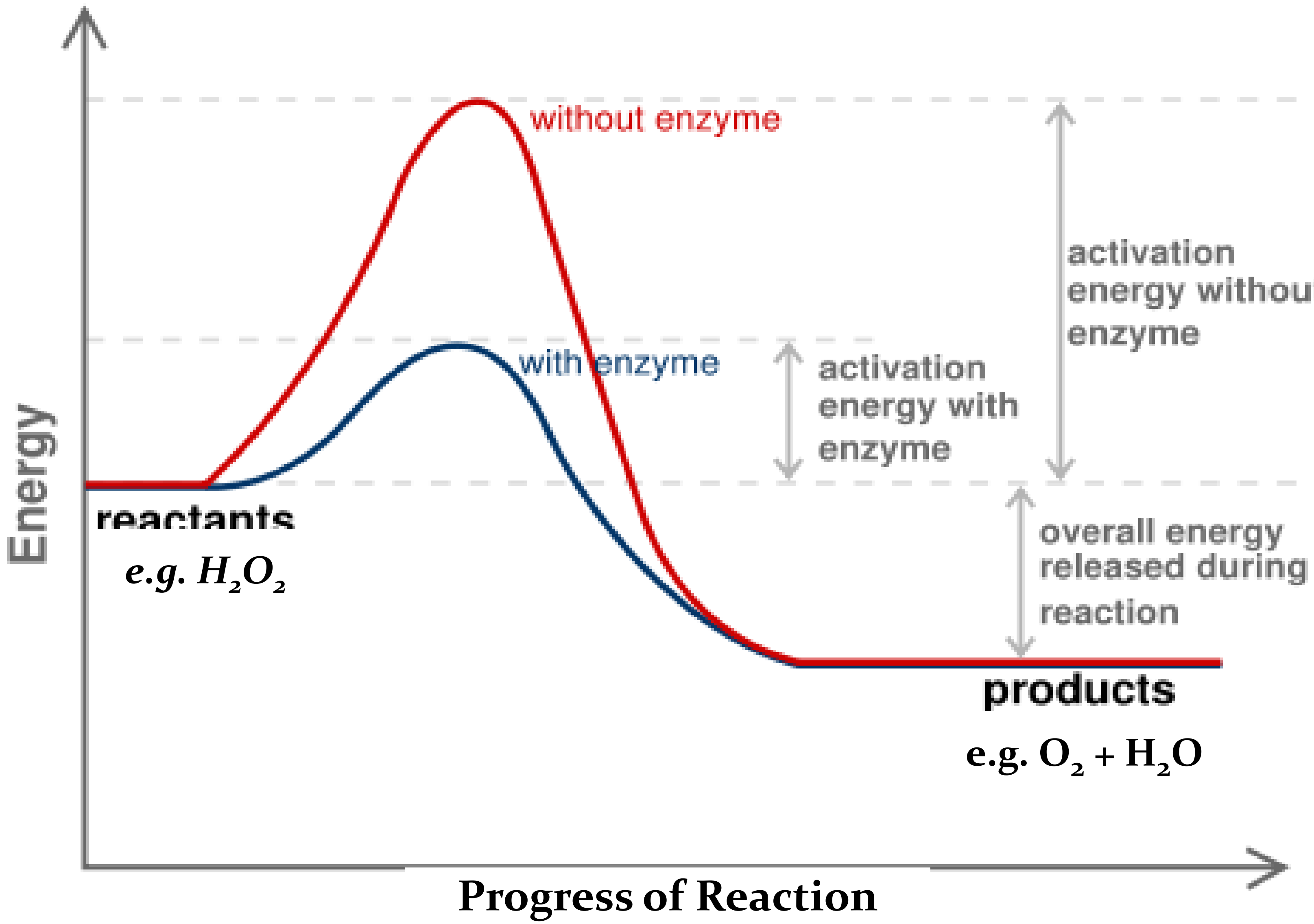
LOWERING the ACTIVATION ENERGY

E_A

of the reaction

Enzyme : Reduced activation Energy (Lactose Example)




without enzyme
with enzyme
activation energy without enzyme
activation energy with enzyme
overall energy released during reaction
Energy
reactants
e.g. H_2O_2
products
e.g. O_2 + H_2O
Progress of Reaction

CATALYSTS:

promote a *specific* reaction

But are NOT consumed in the process

Key concepts:

Promotes - *does not alter* what would normally occur thermodynamically

Specificity - promotes *only one reaction*, only between specific reactants to *give* specific products

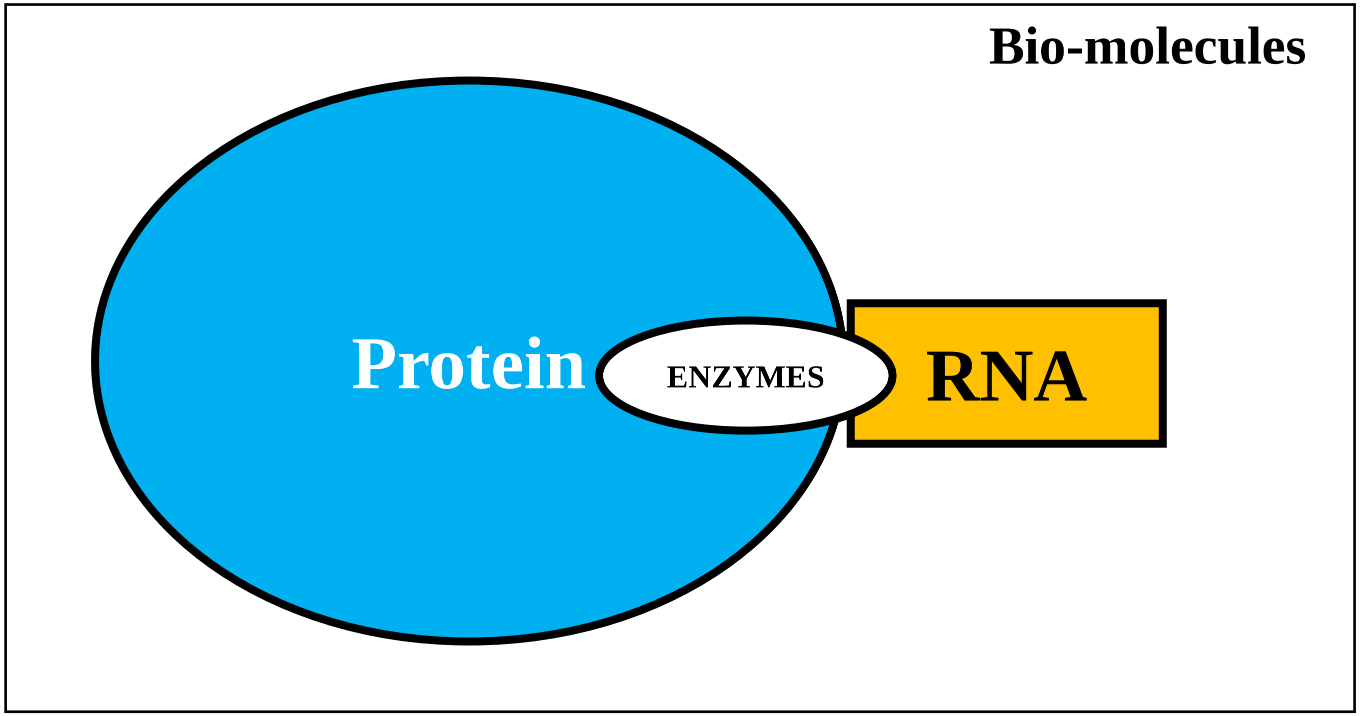
Reusable - regenerated in the process

ENZYMES are biological CATALYSTS

- usually, PROTEINS

- sometimes RNA or
  RNA/protein complexes

What is the difference between an enzyme and protein ?



All enzyme are proteins except some RNAs
Not all proteins are enzymes

What is a substrate?–

A substrate is the compound that is converted into the product in an enzyme catalyzed reaction. For the reaction catalyzed by aldolase, fructose 1,6-phosphate is the substrate.

Chemical nature of enzymes

All enzymes are proteins except some RNAs and not all proteins are enzymes

- It was assumed that all enzymes are proteins until 1982 when Thomas Cech and Sydney Altman discovered catalytic RNAs (Nobel prize in Chemistry, 1989)
- Catalytic RNA, or ribozymes, satisfy several enzymatic criteria: substrate specificity, enhance reaction rate, and emerge from reaction unchanged.

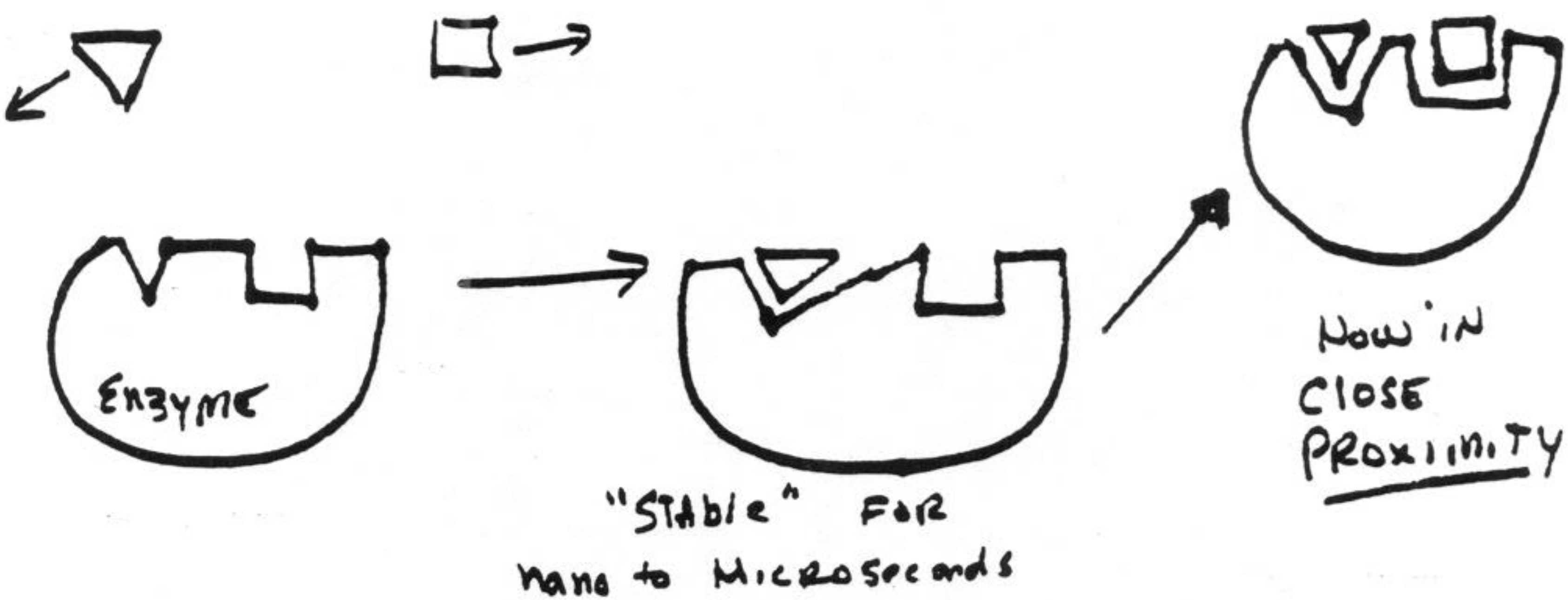
The difference between enzyme catalyzed & uncatalyzed chemical reactions

Enzyme catalyzed reactions are much faster than uncatalyzed reactions.

- Enzyme catalyzed reactions display saturation kinetics with respect to substrate concentration.
- Enzyme catalyzed reactions are optimized for specific temperature and pH values

How do Enzymes do it?

Enzymes have **BINDING AFFINITY** for their reactants = *Substrates*



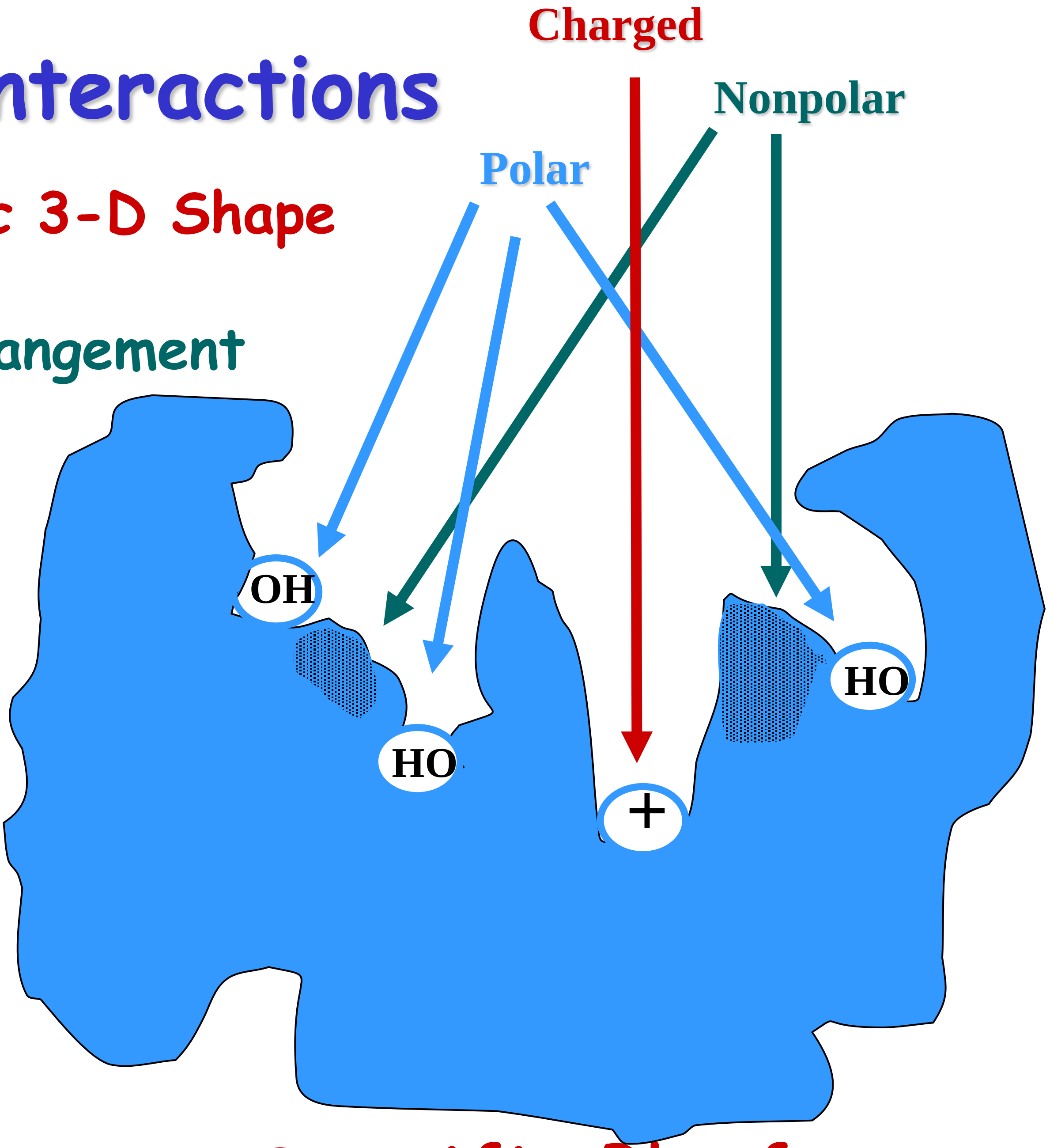
Brings substrates in close proximity: conc

Stabilized Interactions

Have a *very Specific 3-D Shape*

With a *Specific Arrangement of Functional Groups*

Flexible

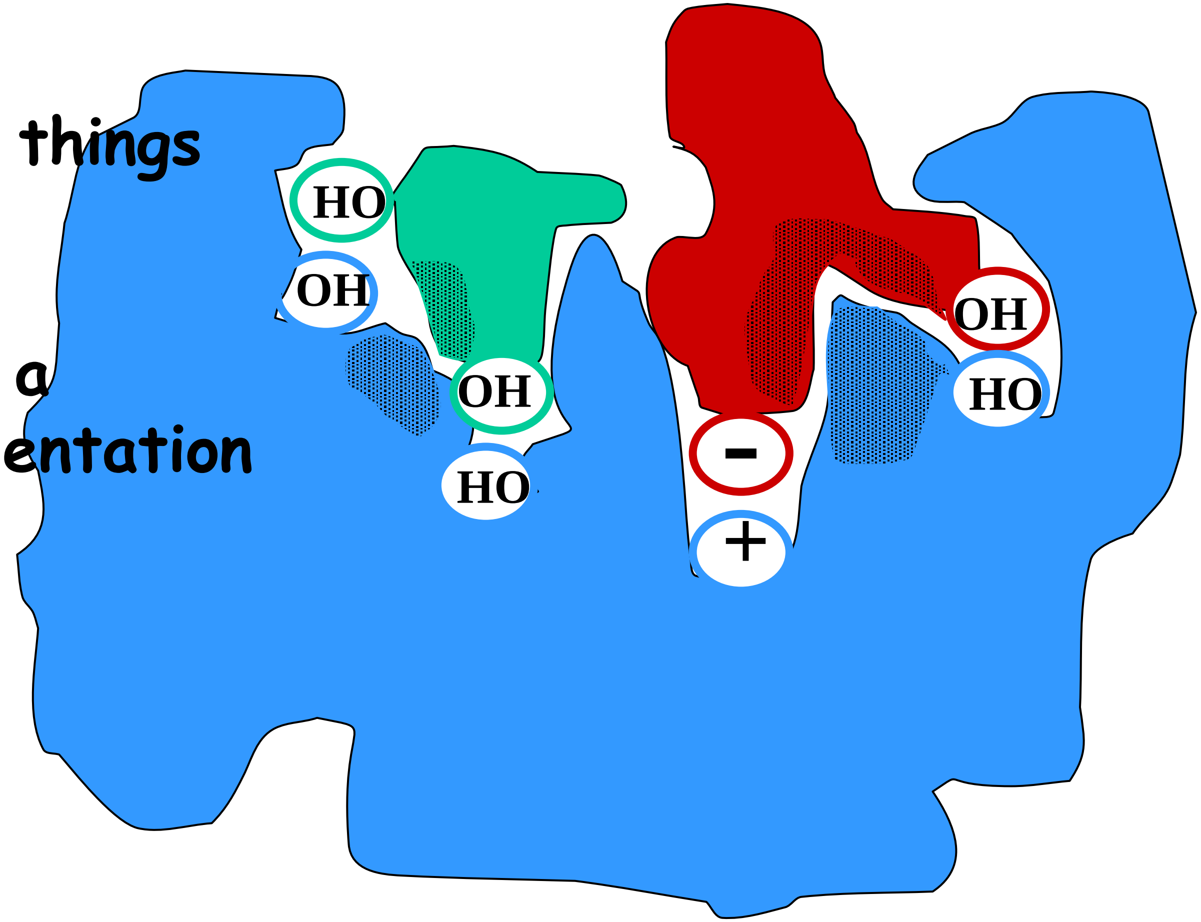


Enzymes act as a Specific Platform

ENZYMES:

Bind ONLY specific things

Bind them ONLY in a
Specific 3-D Orientation

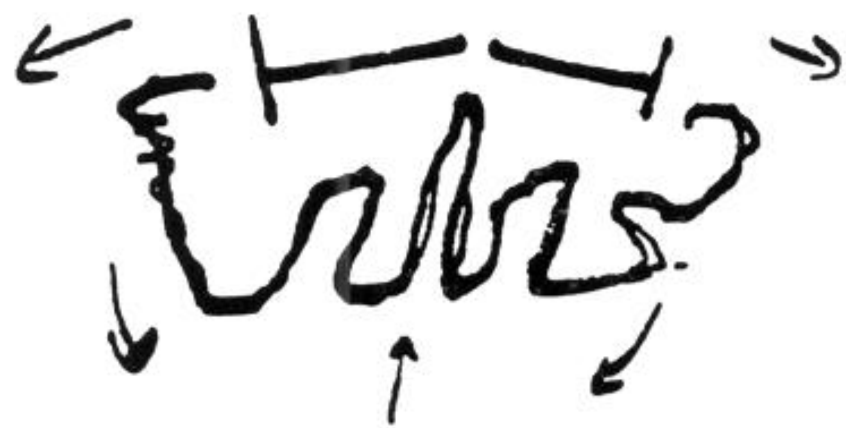


<u>SPECIFICITY</u> is the Key to Enzyme Action

Enzymes cause BOND STRAIN

- destabilize existing bonds

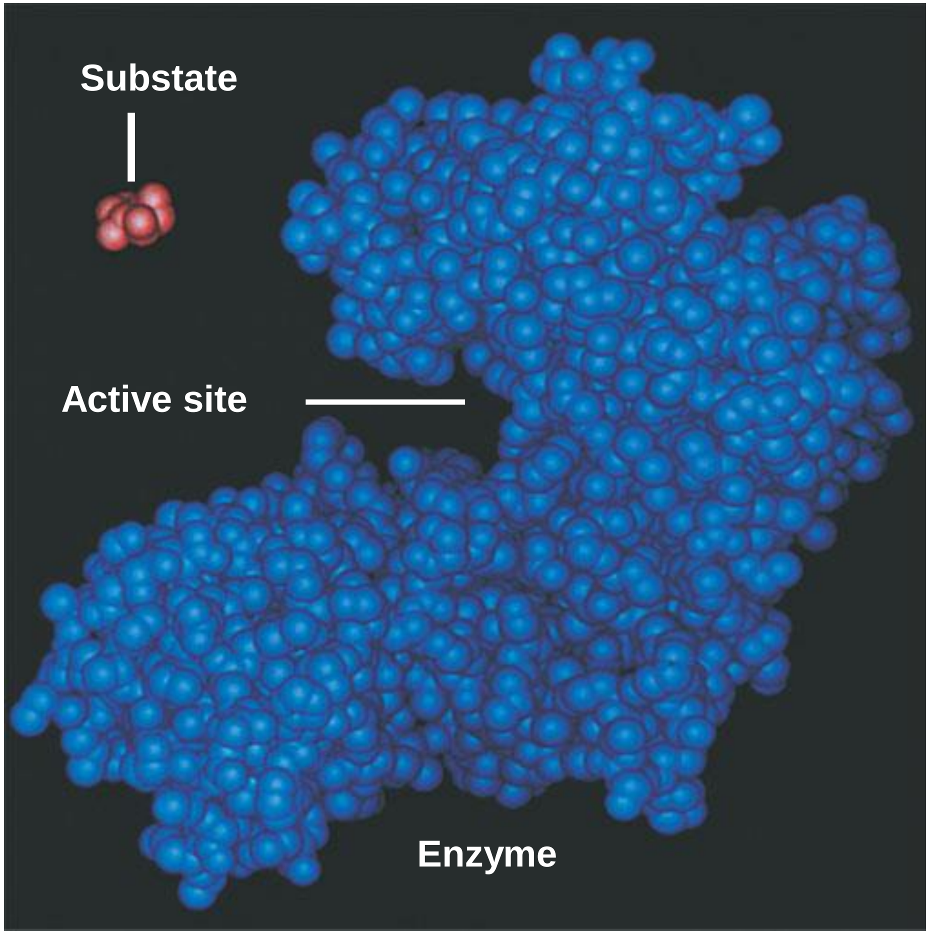
Physical Strain



Chemical Strain

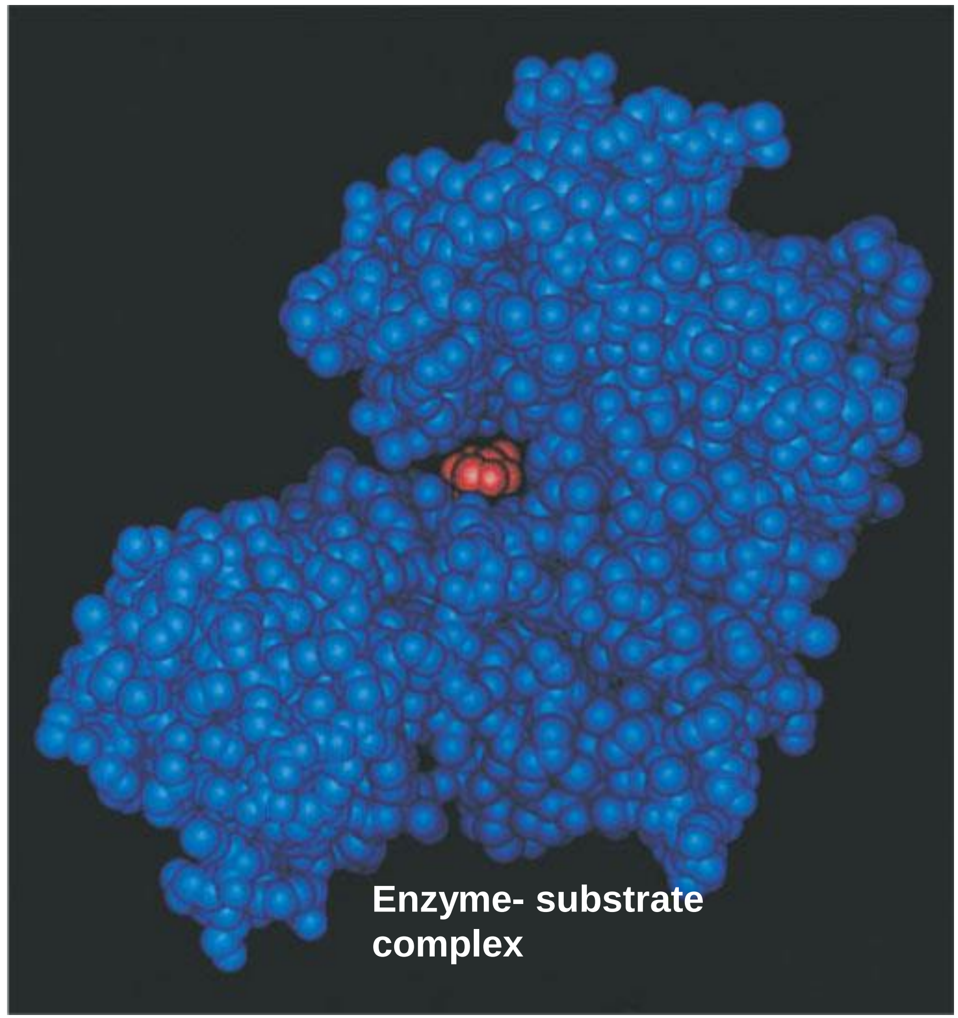
The active site

- Is the region on the enzyme where the substrate binds



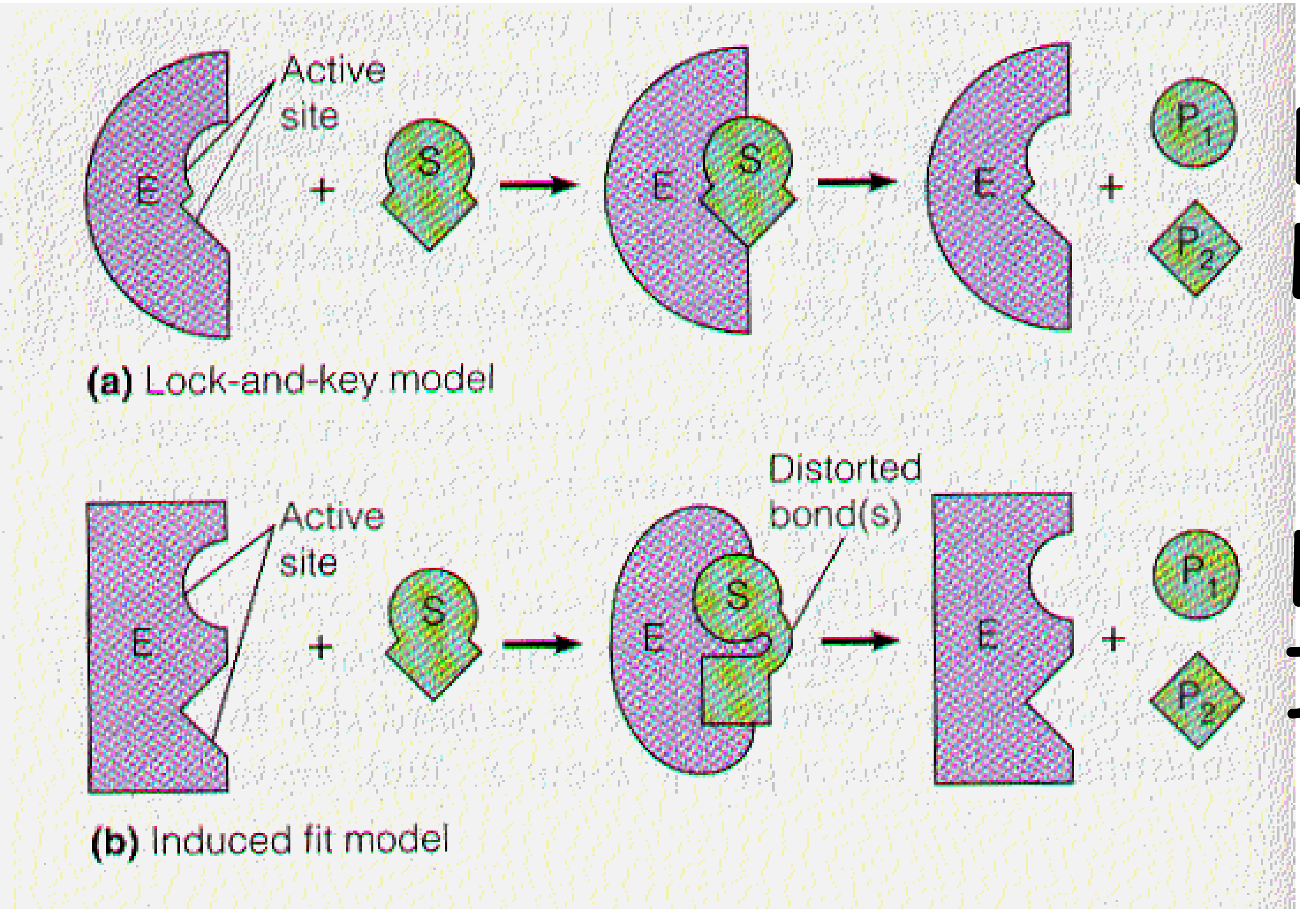
(a)

Induced fit of a substrate



(b)

Enzyme-substrate interactions

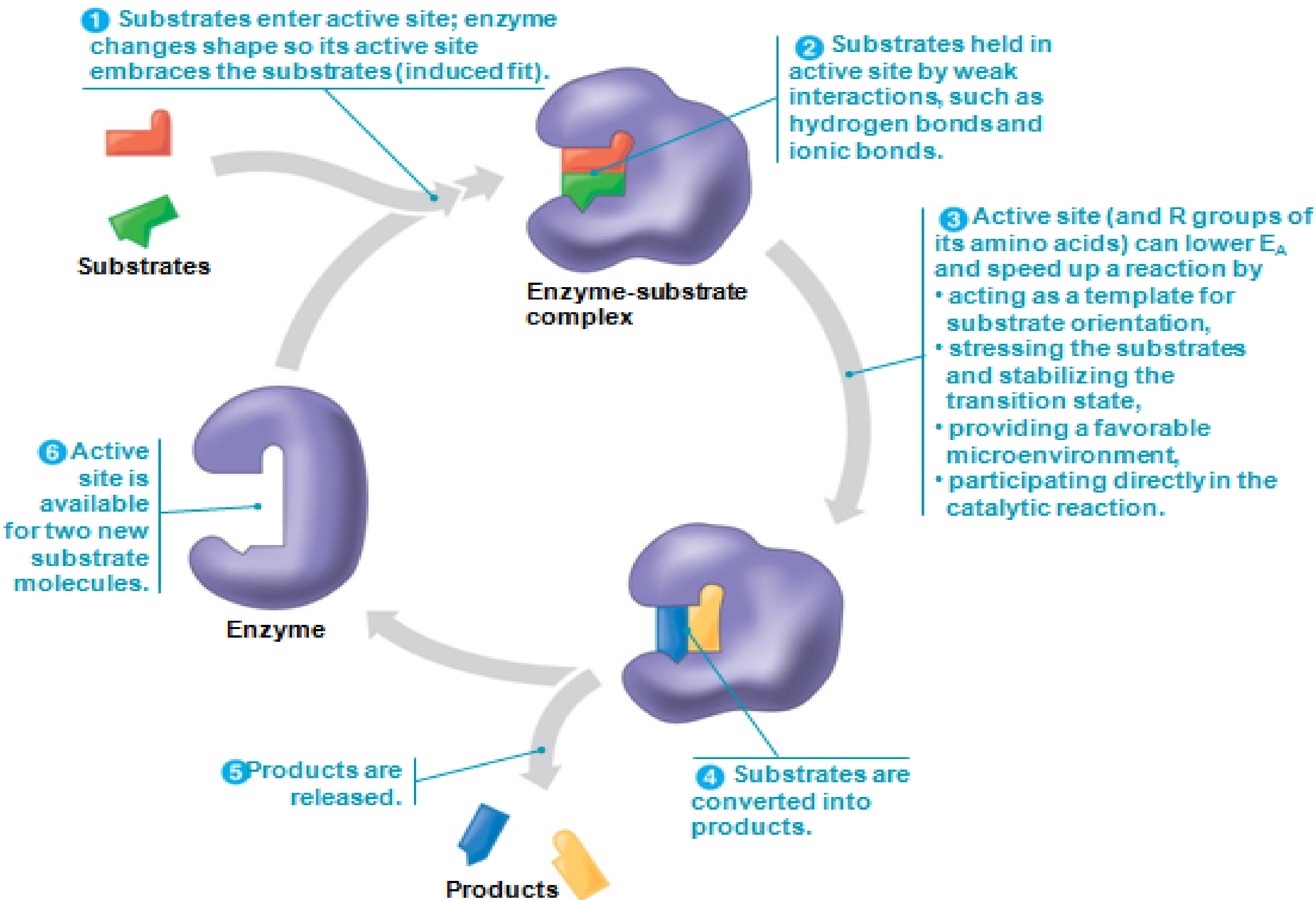


Fischer:
Lock & key

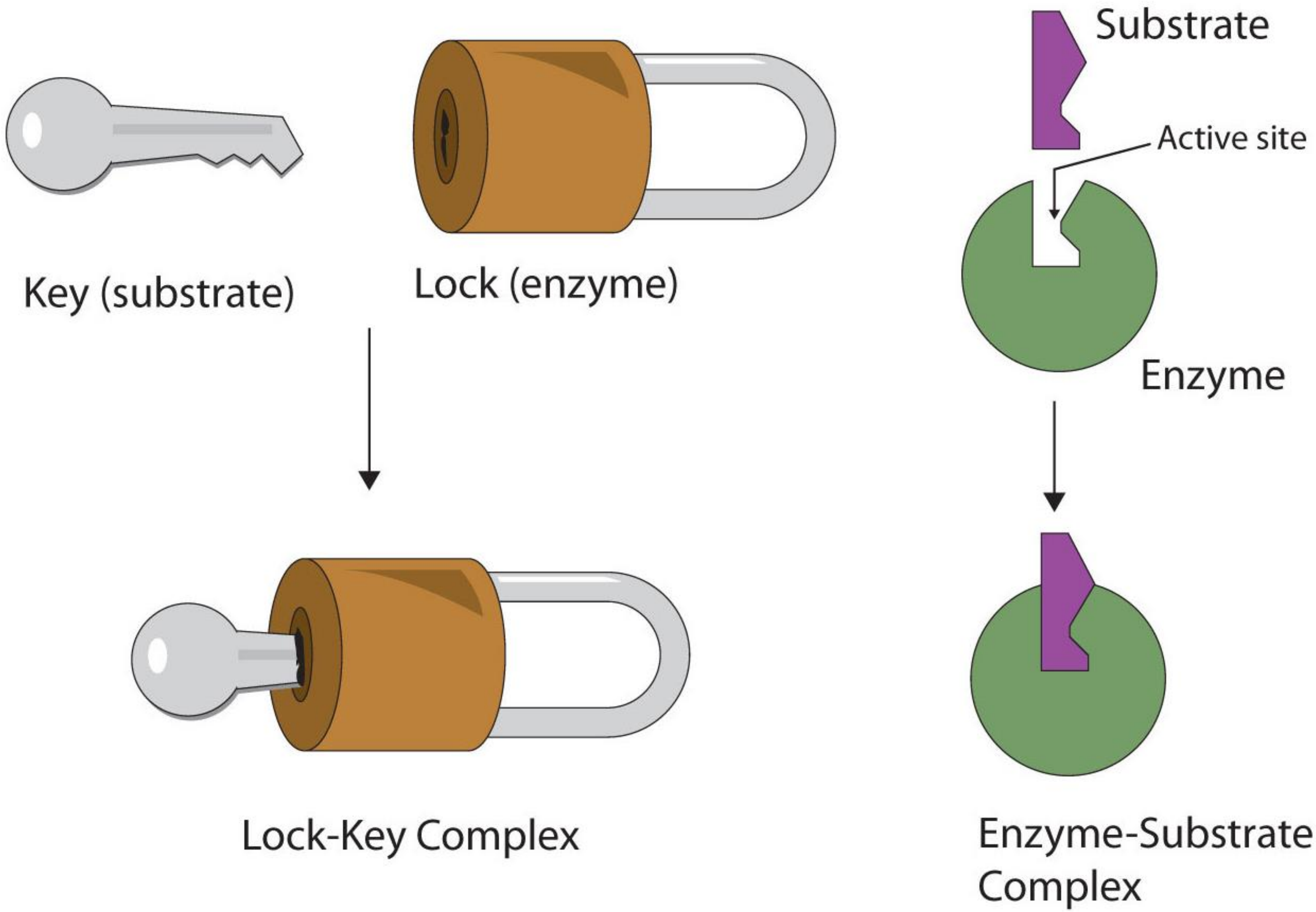
Koshland:
Induced fit

Physical bond strain

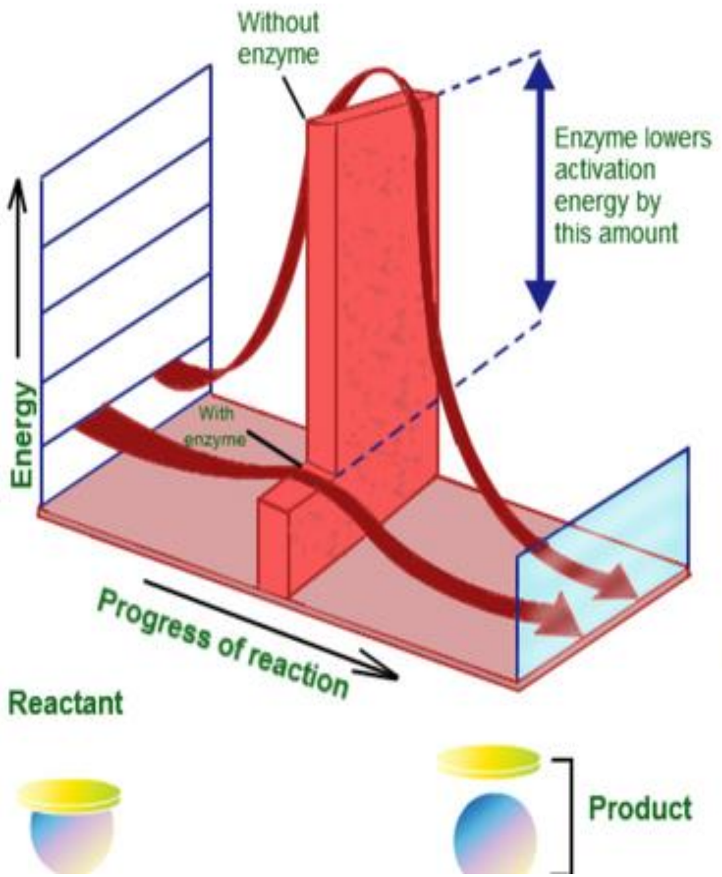
Induced-fit model

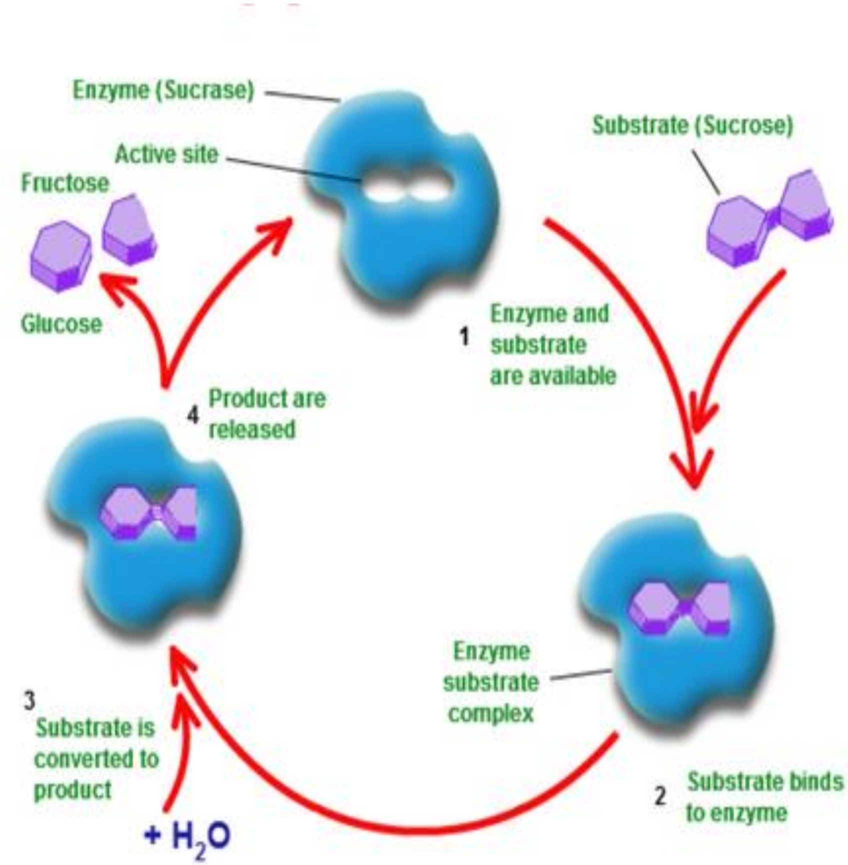


Enzyme working model : Lock and Key

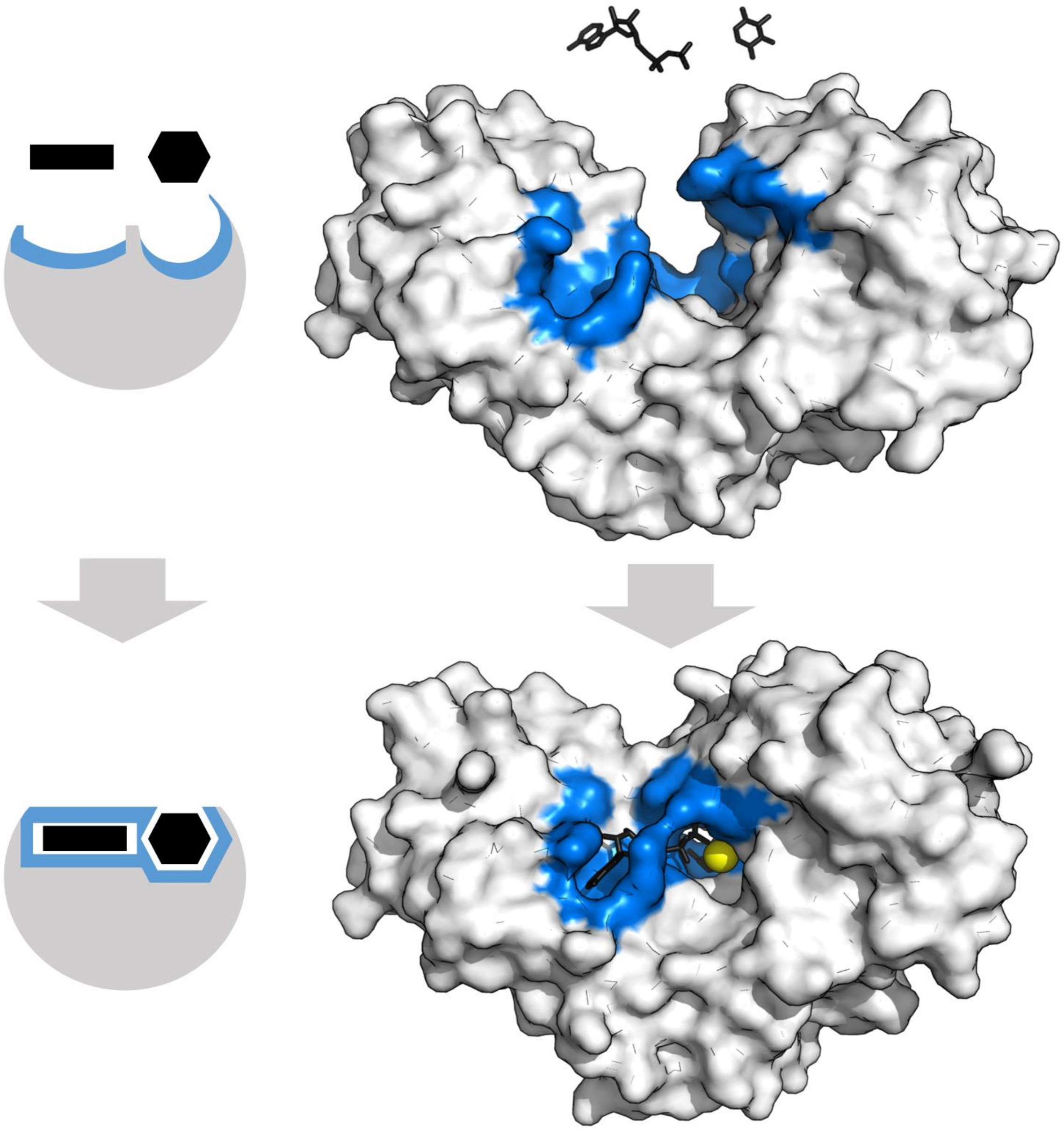


Enzyme Working Model :

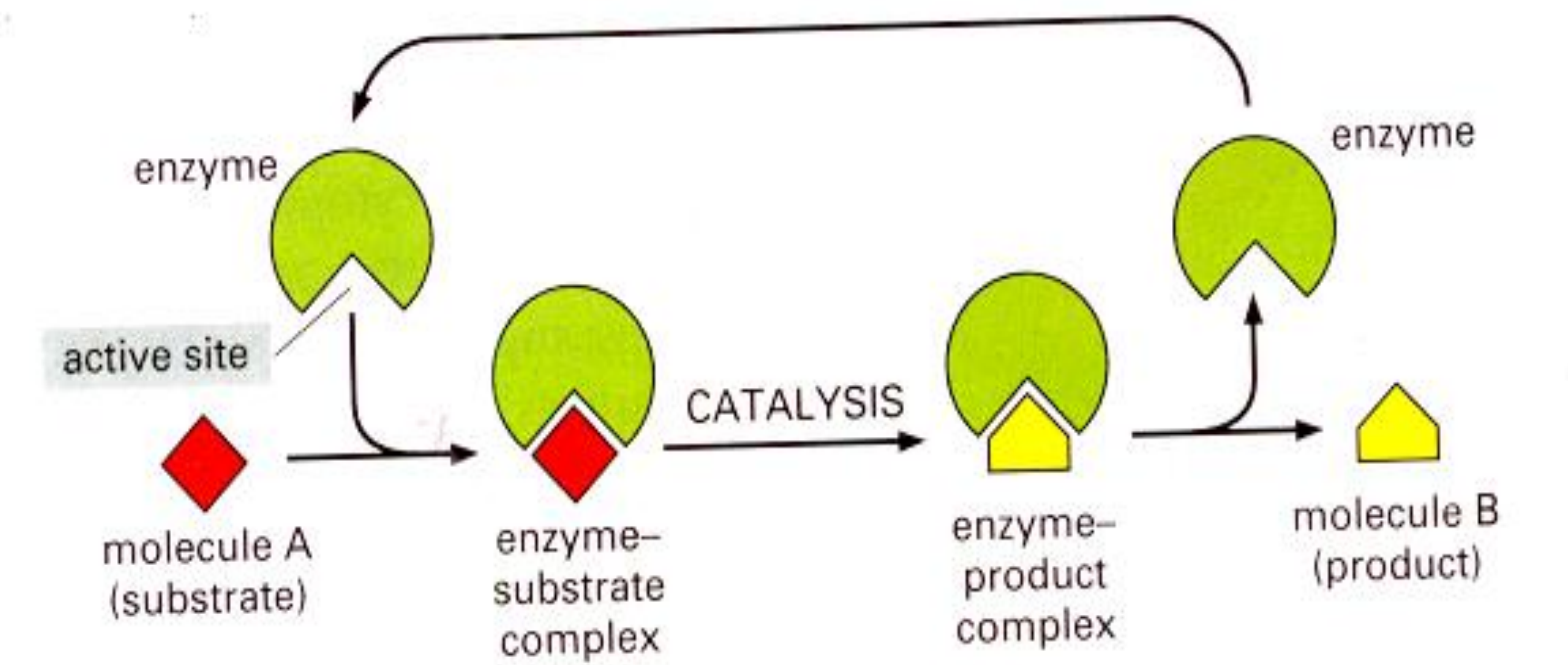




ENZYME & SUBSTRATE INTERATION



Enzymes "*partake*" in reactions but are not consumed in them

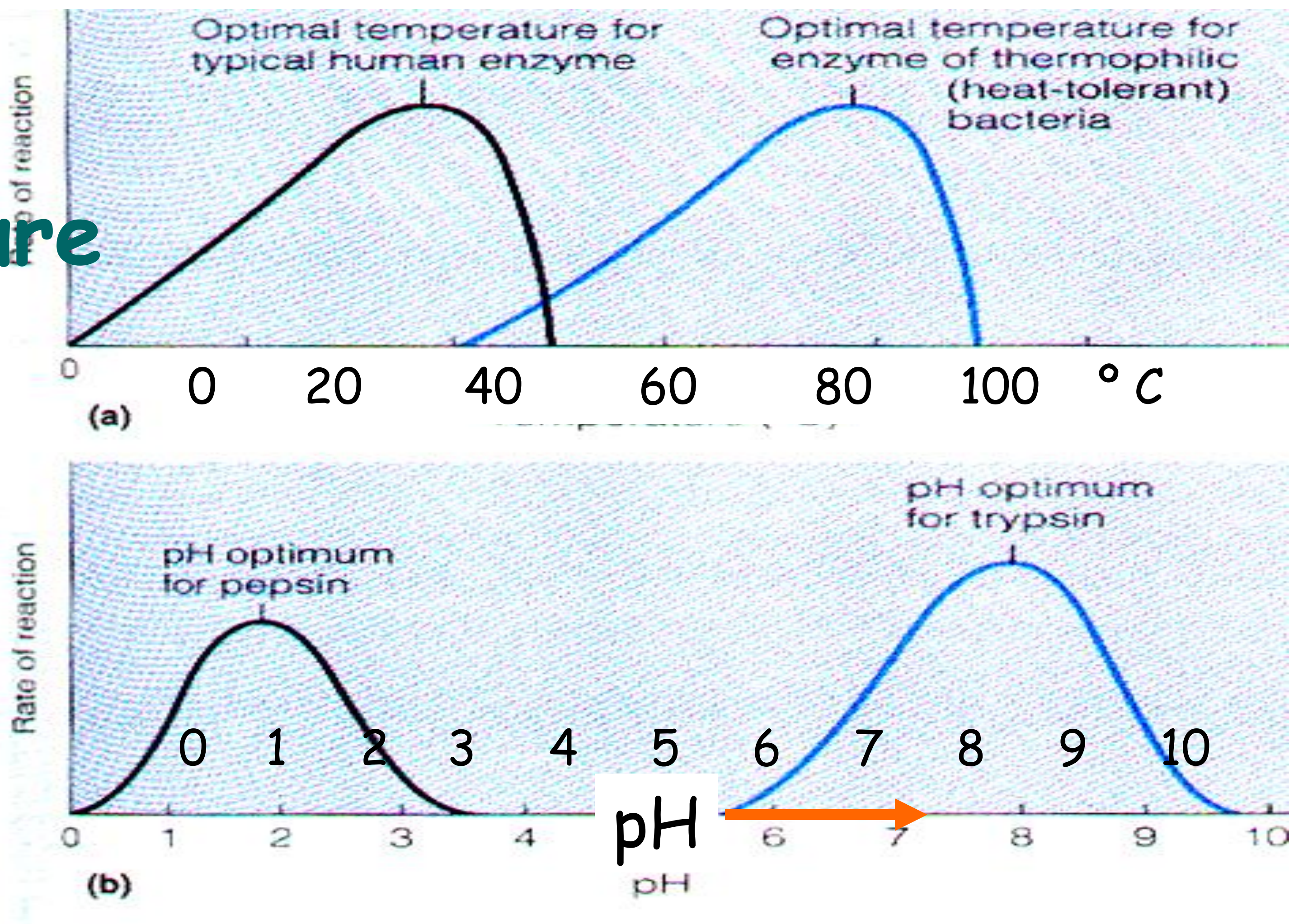


Converts MANY "A's" into "B's"

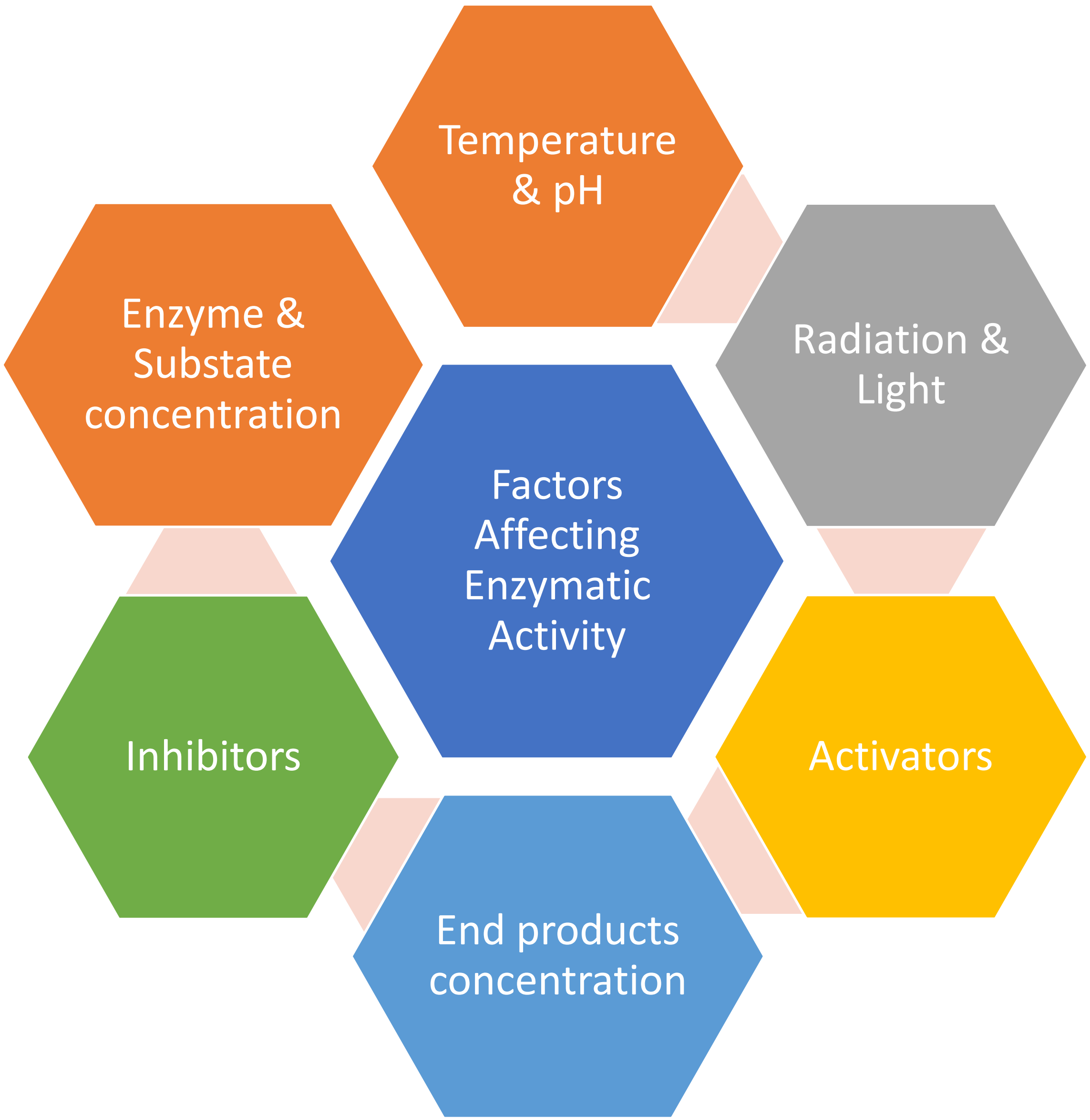
Things that affect protein structure often affect enzyme activity

temperature

pH



Factors Affecting Enzymatic Activity



Effect of Temperature

- Each enzyme is is most active at a specific temperature which is called it's *optimum temperature*.

↑ Temperature

⇩

↑ Molecular collision

⇩

↑ Interaction

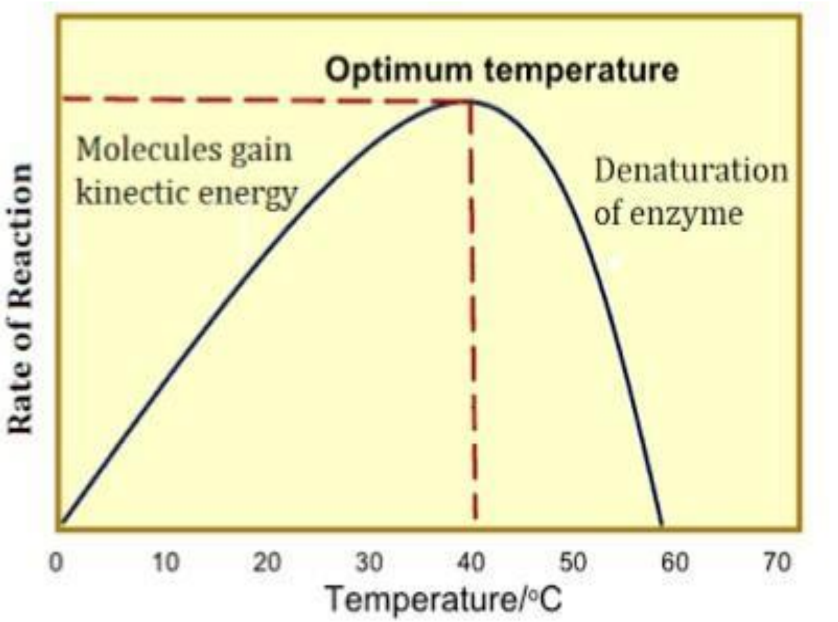
⇩

Faster reaction

- Activity of enzyme progressively decreases when the temperature of reaction is below or above the optimum temperature.

- However, increase in temperature also causes denaturation of enzyme.

- The shape of the curve is bell-shape, Most of the enzymes of human system have and optimum temperature within the range of 34-40ºC. Thus, the optimum temperature is the temperature at which the activity of the enzyme is maximum.



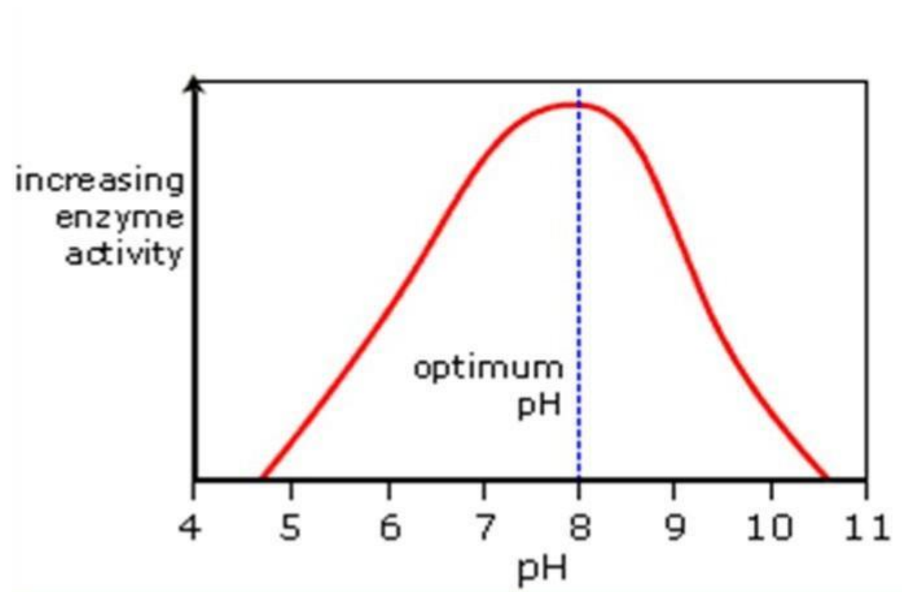
Effects of temperature on enzymatic reaction.

Effect of pH

pH change alters:

- Ionization states of the amino acid residue present in the active site.
- May dissociate apoenzyme from cofactor.
- Drastic change denatures the enzyme protein

Optimum pH is different for different enzymes
mostly 6-8
Exception: Pepsin 1-2
Acid phosphatase 4-5



Effect of pH on enzymatic reaction

Effect of concentration of products

- Products formed as a result of enzymatic reaction may accumulate and this excess of product may lower the enzymatic reaction by occupying the active site of the enzyme.

↓ Product concentration = ↓ Reaction rate

SUMMARY

Enzymes:

1. Bring reactants (substrates) in close proximity
2. Align substrates in proper orientation
3. Can act as a Lever: a press or an anvil
   small shape change translates to large force
4. Release products when reaction done
   rebind more substrates
5. Many small steps, each easily achieved
   rather than one huge leap

Enzyme regulation:

Activity controlled

Continually adjusted

Principal Ways of Regulating Enzymes

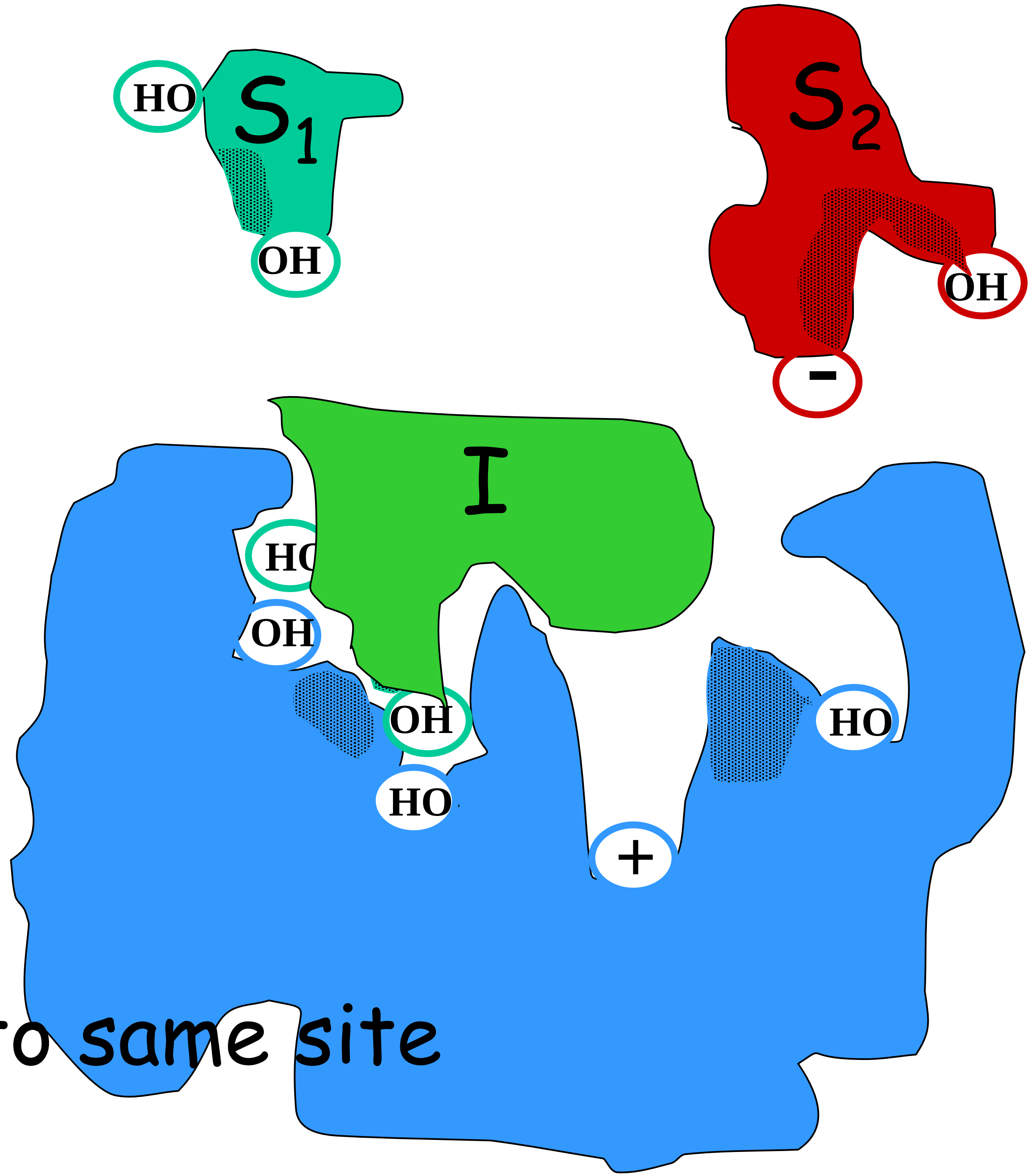
Competitive Inhibition

Allosteric Inhibition

Covalent Modification (phosphorylation)

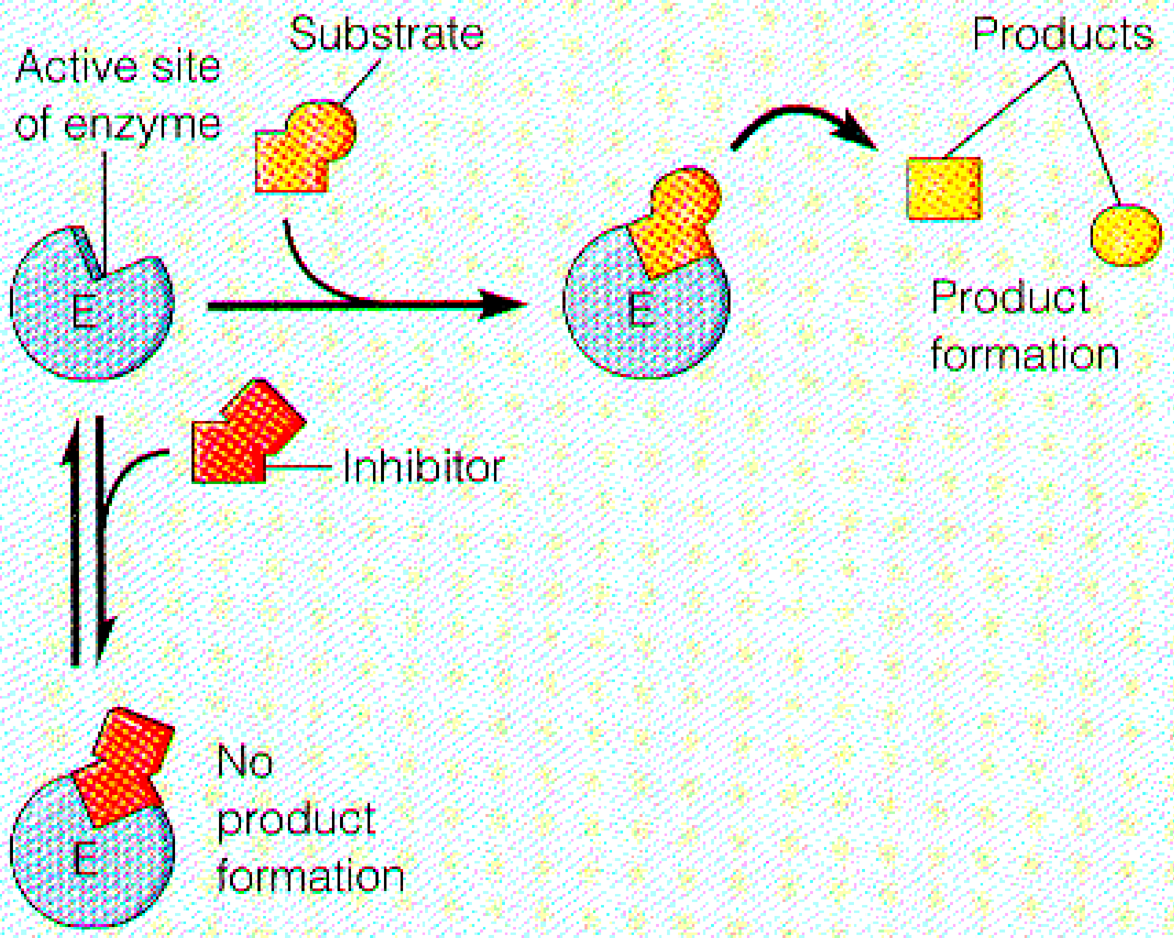
Competitive Inhibitors:

bind to active site "unproductively" and block true substrates' access



S & I bind to same site

Competitive inhibition



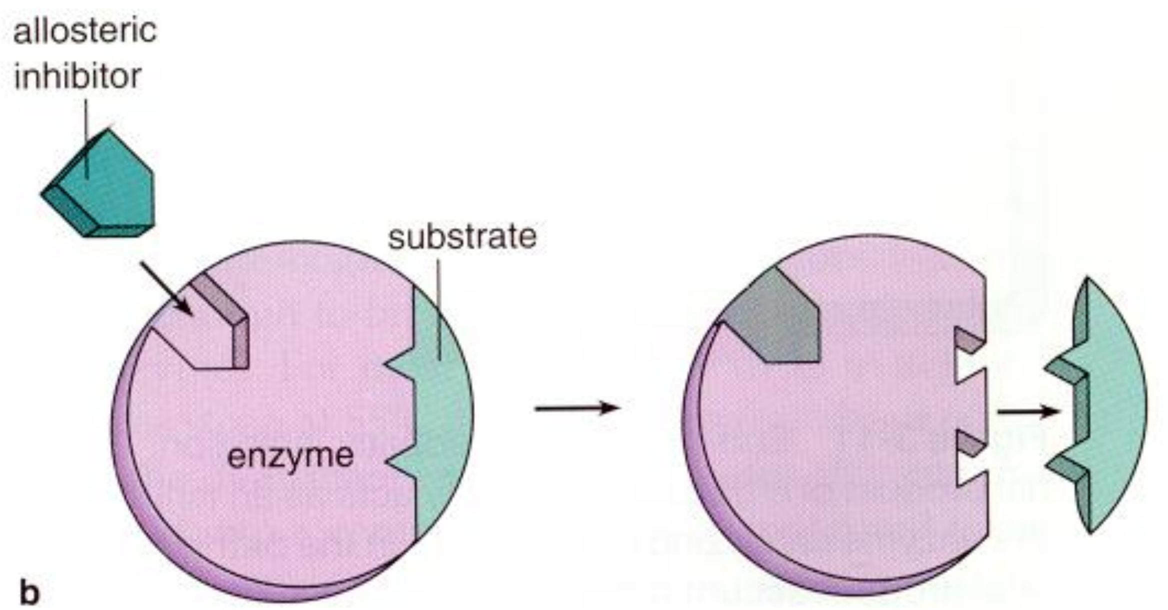
(a) Competitive inhibition. Inhibitor and substrate both bind to the active site of the enzyme. Binding of an inhibitor prevents substrate binding.

Allosteric Inhibitors

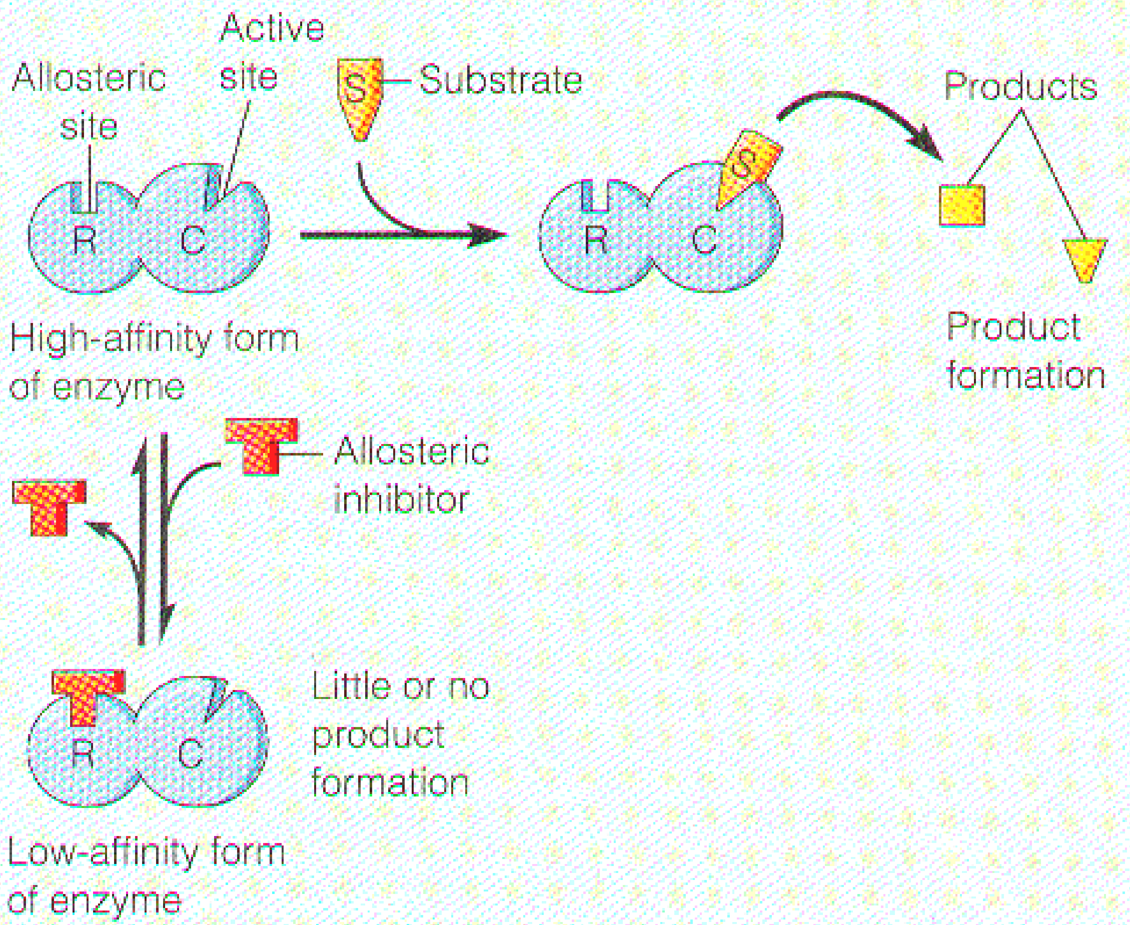
"other" "site"

Distorts the conformation of the enzyme

Negative allosteric regulator

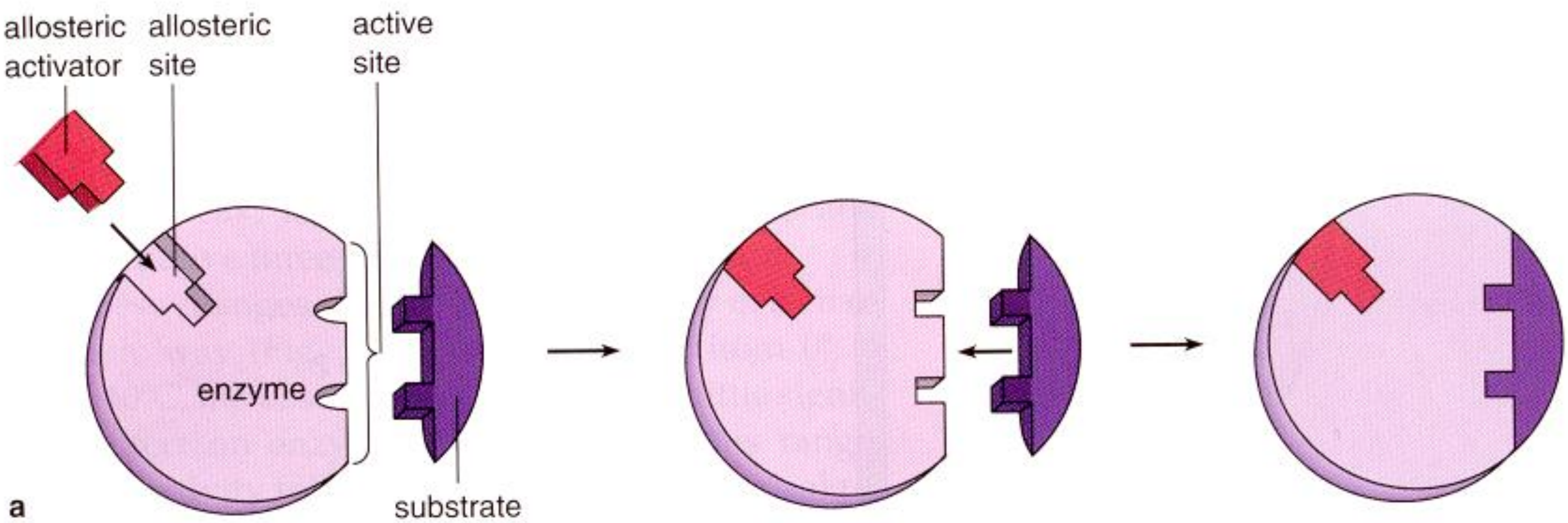


Allosteric inhibition



(a) Allosteric inhibition. An enzyme subject to allosteric inhibition is active in the uncomplexed form, which has a high affinity for its substrate (S). Binding of an allosteric inhibitor (red) stabilizes the enzyme in its low-affinity form, resulting in little or no activity.

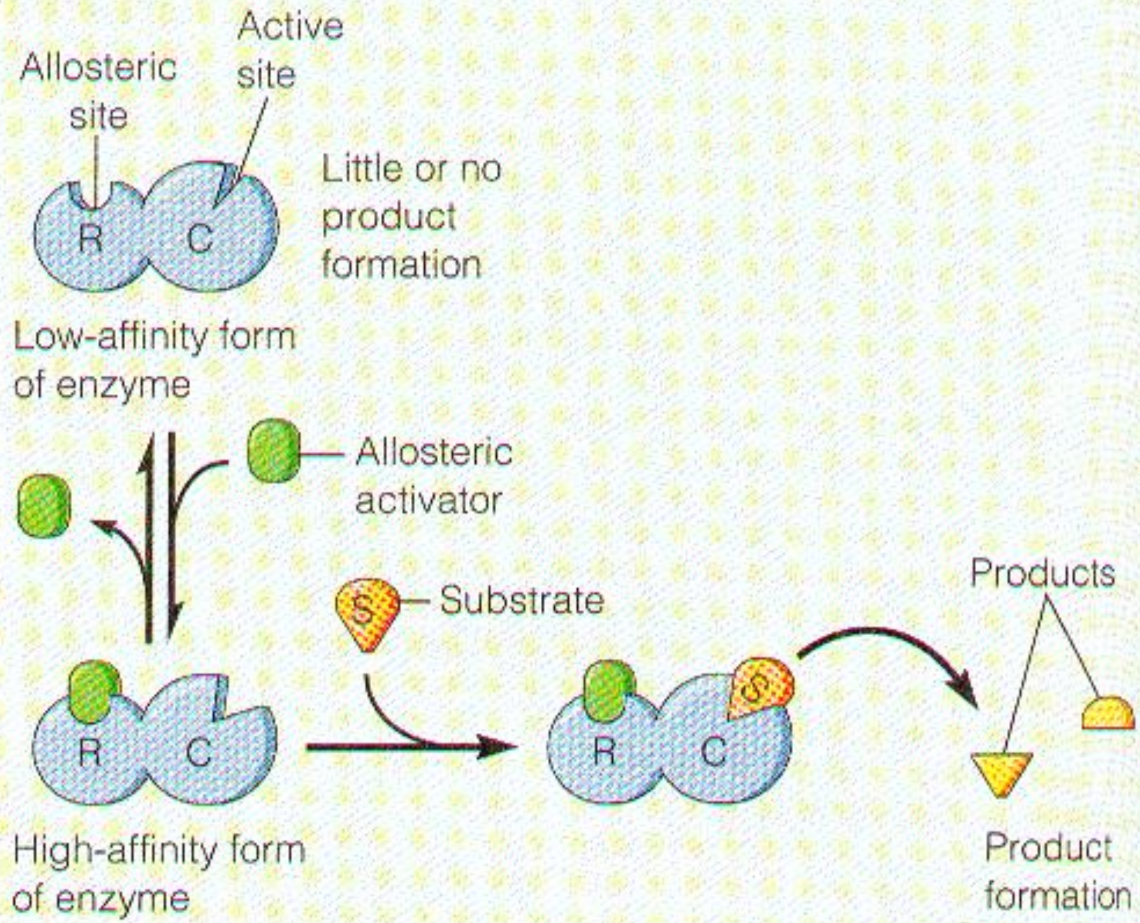
Positive *allosteric regulators*



Helps enzyme work *better*

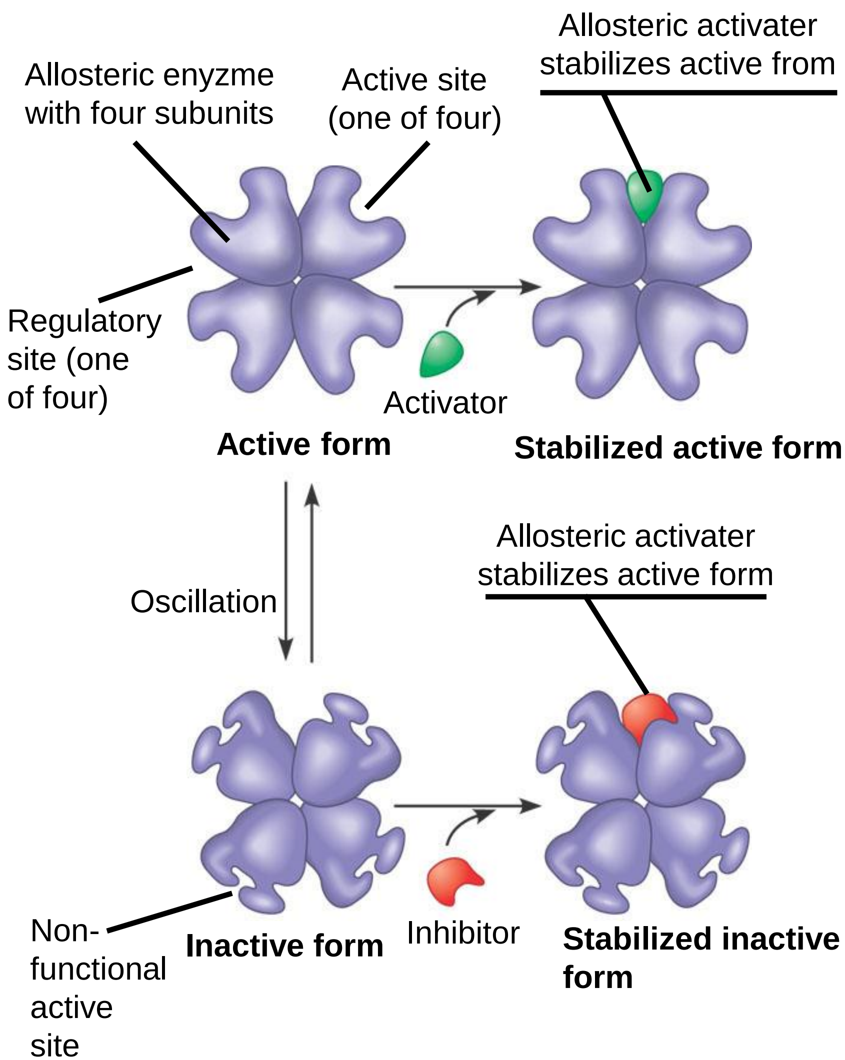
promotes/stabilizes an “active” conformation

Allosteric activation



(b) Allosteric activation. An enzyme subject to allosteric activation is inactive in its uncomplexed form, which has a low affinity for its substrate. Binding of an allosteric activator (green) stabilizes the enzyme in its high-affinity form, resulting in enzyme activity.

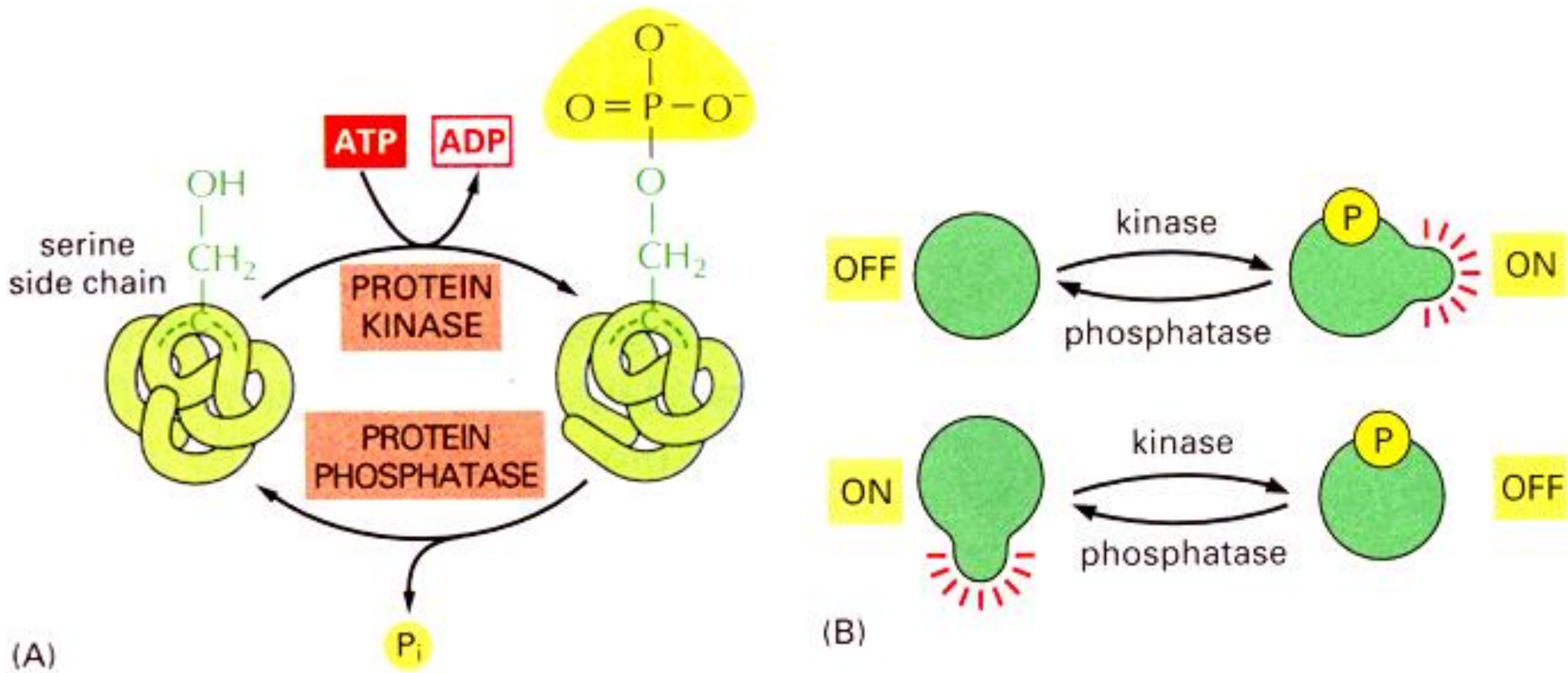
Allosteric regulators *change the shape conformation* of the enzyme



(a) Allosteric activators and inhibitors. In the cell, activators and inhibitors dissociate when at low concentrations. The enzyme can then oscillate again.

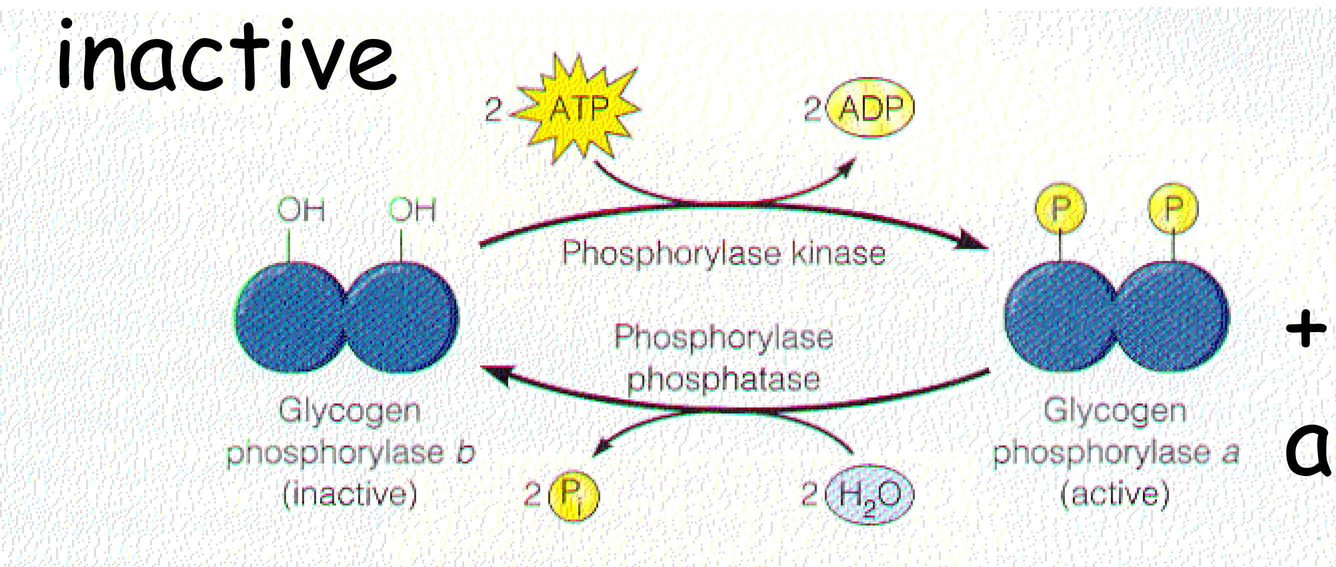
A *frequent* regulatory modification

Phosphorylation of enzymes



Phosphorylase kinase

inactive



+ P

active

Summary

1. enzymes are catalysts
2. Lower activation energy E_A
3. Mechanism of action …
4. Enzyme kinetics-
5. Regulation of enzyme activity
   - competitive, allosteric
     phosphorylation


Thank You!