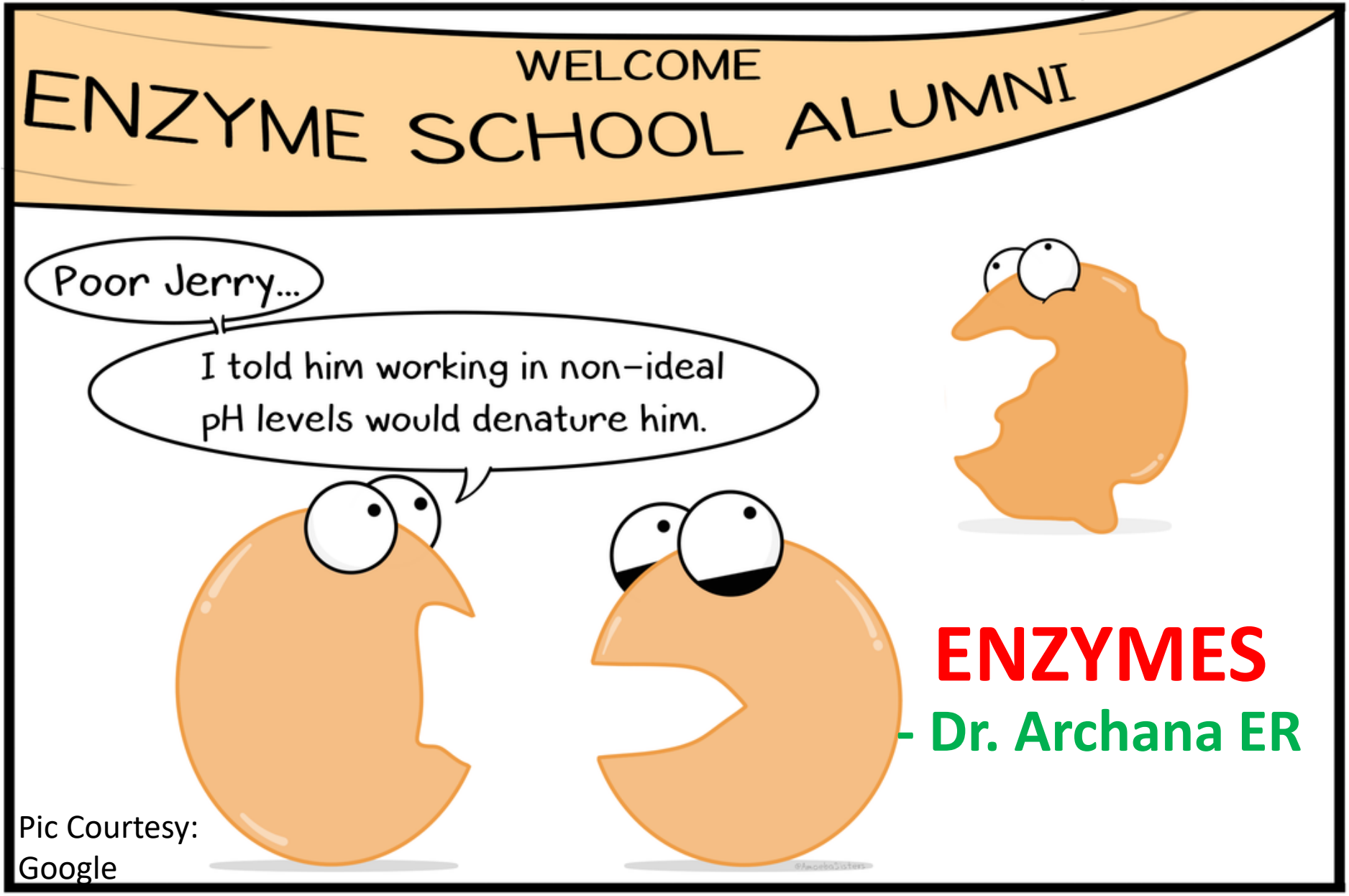


ENZYMES

- Dr. Archana ER

Pic Courtesy: Google

Enzymes

- Biological catalysts
- Accelerates the rate of reaction in a metabolic pathway
- Multi-enzyme systems
- Produced inside the cell
- Action – endo or exoenzymes
- Presence – Constitutive or inducive enzymes
- Extermozymes – high temperature
- Abzymes – Catalytic antibodies

Ribozymes

- RNA as enzymes
- Nobel prize in chemistry for the discovery – Cech and Altman
- Eg. Ribonuclease-P, self-splicing rRNA
- Substrate – mostly RNA
- Might the vestigial remnants of the primordial living systems

Proteinaceous enzymes

- Catalytic proteins
- Charged, high molecular weight, controlled activity

❑**Naming:**

- Trivial name or systematic name
- Trivial – name of substrate, type of reaction and the suffix –ase . Eg. Lactic acid dehydrogenase
- Systemic name – Substrate, type of reaction in a different way. Eg. Lactic acid NAD oxidoreductase

CLASSIFICATION

IUB (International Union of Biochemists) system

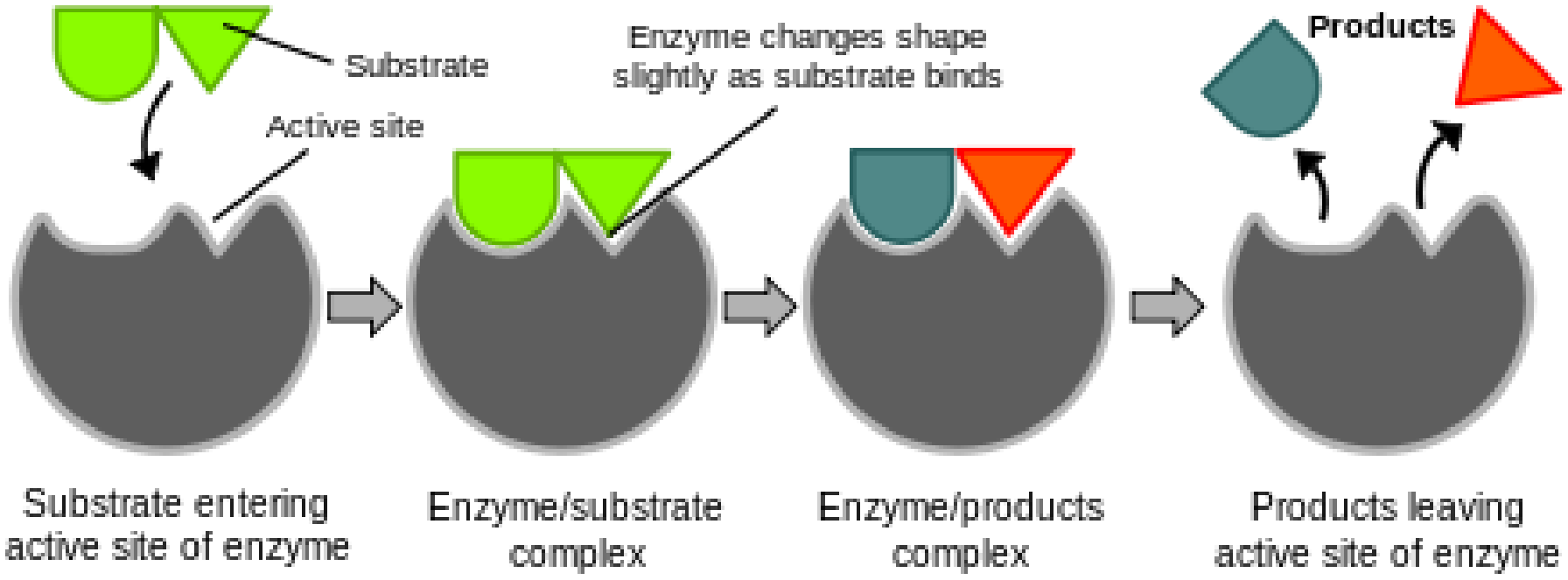
1. Oxidoreductases: Oxdn-Redn – peroxidases, dehydrogenases
2. Transferases: transaminases
3. Hydrolases: Peptidase
4. Lyases: removal of a group – carboxylases
5. Isomerases: Epimerases, mutases
6. Ligases: Synthases- polymerases

- Simple enzymes: Simple proteins
- Complex proteins: proteins conjugated with other factors to form a **holoenzyme**
- Protein part – **apoenzyme**
- Non-protein – **co-factor**
- Co-factor – inorganic ions or organic co-factors
- Some co-enzymes remain tightly bound to apoenzymes – Prosthetic group
- **Isoenzymes –** polymorphic forms of enzymes
- **Metalloenzymes –** requires metal ions for activation

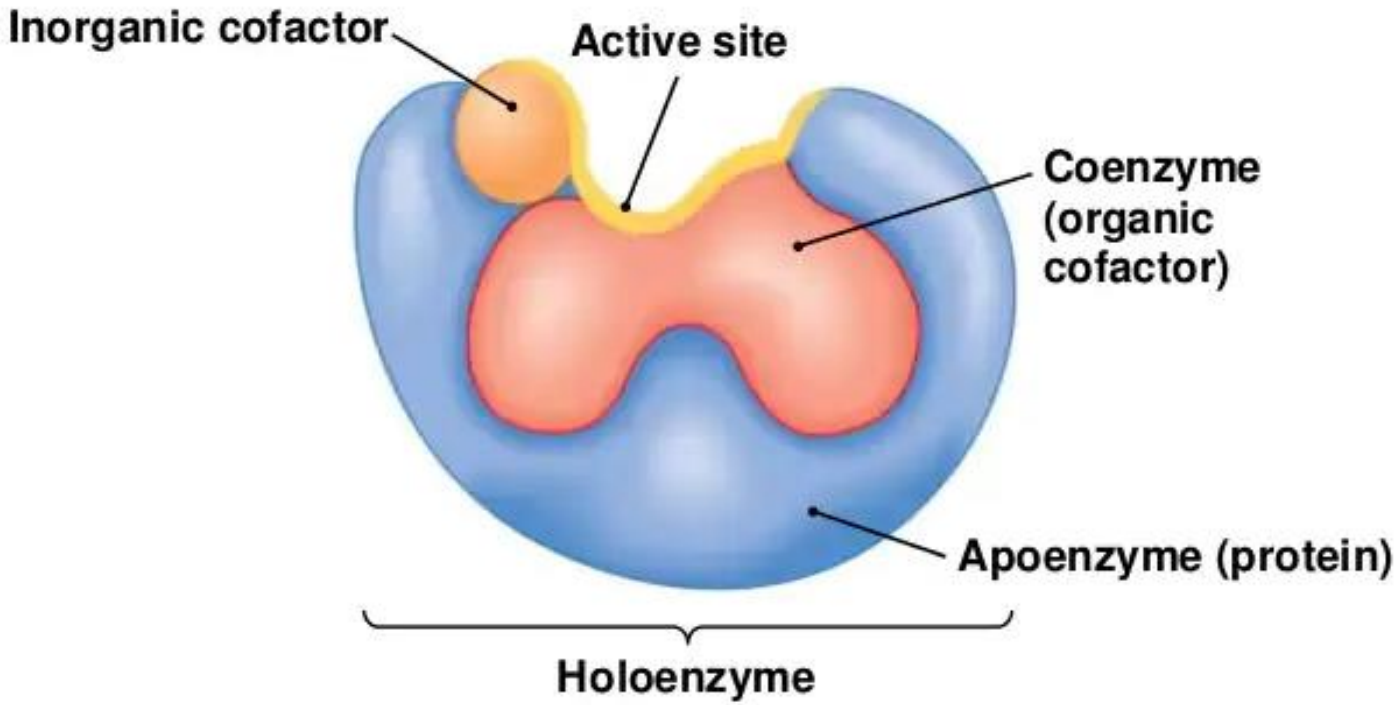
Functional organization

- Active site – has contact site and catalytic site
- Hydrophobic interaction enables the binding of enzymes to the substrate
- Binding depends on the proper alignment of the functional groups
- Allosteric enzymes has regulatory sites – positive regulators are the activators and negative regulation is done by the inhibitors

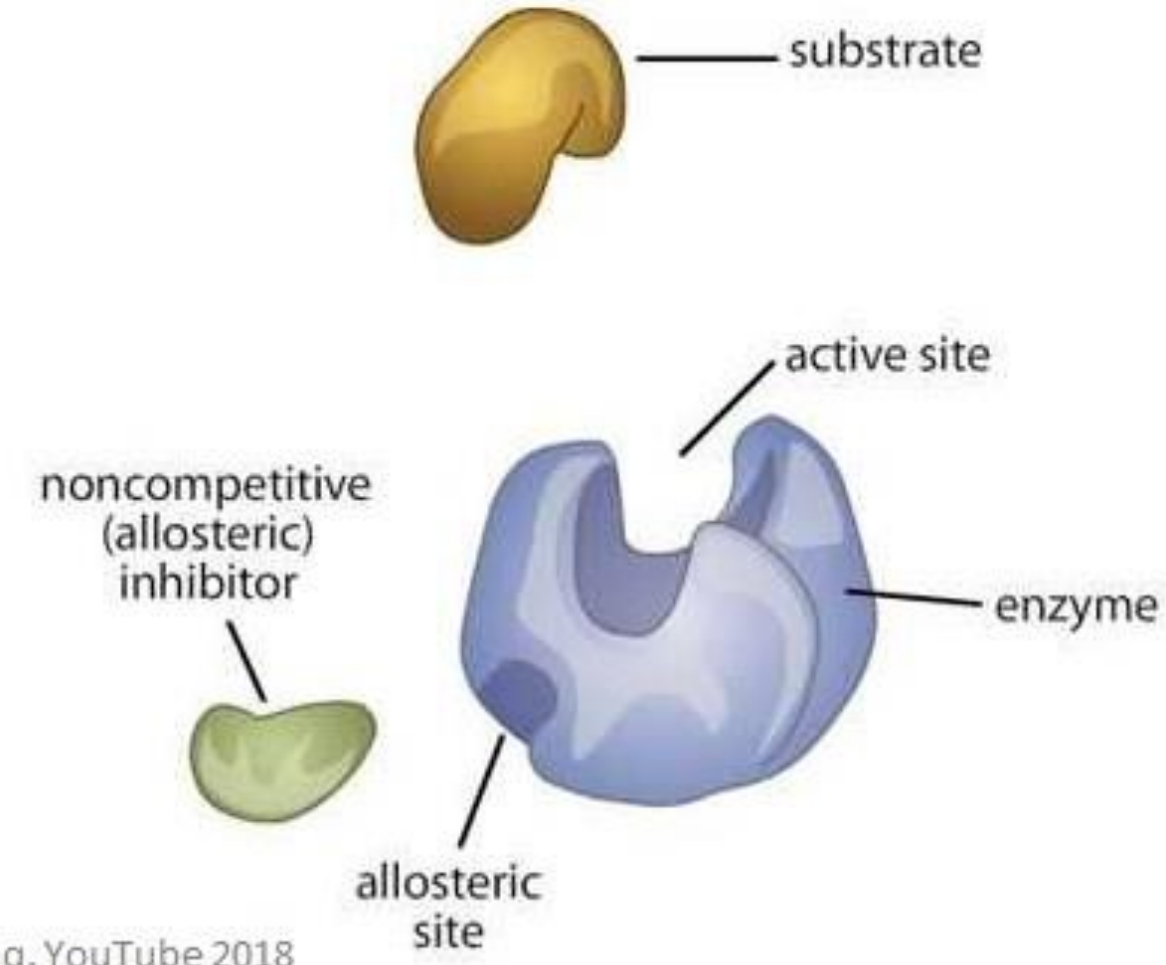
Simple enzyme



Holoenzyme


Inorganic cofactor
Active site
Coenzyme (organic cofactor)
Apoenzyme (protein)
Holoenzyme

Allosteric enzyme



Properties of enzymes

- Controllable activity
- Less stable and thermalabile
- High catalytic efficiency
- Do not alter the thermodynamic properties of the reaction but alters the kinetic properties
- Increase the rate of reaction by lowering the level of activation energy
- Not get altered by the reaction and is thus retained after the process
- Extremely specific
- Synthesis and concentration is genetically controlled

Thermodynamic quantities

1. **Enthalpy:** Total heat content of a system

- Represents the number and kinds of bonds in a system
- **H=E+PV**, where E – internal energy, PV is the product of volume and pressure
- Positive ΔH – Endothermic reaction
- Negative ΔH – Exothermic reaction

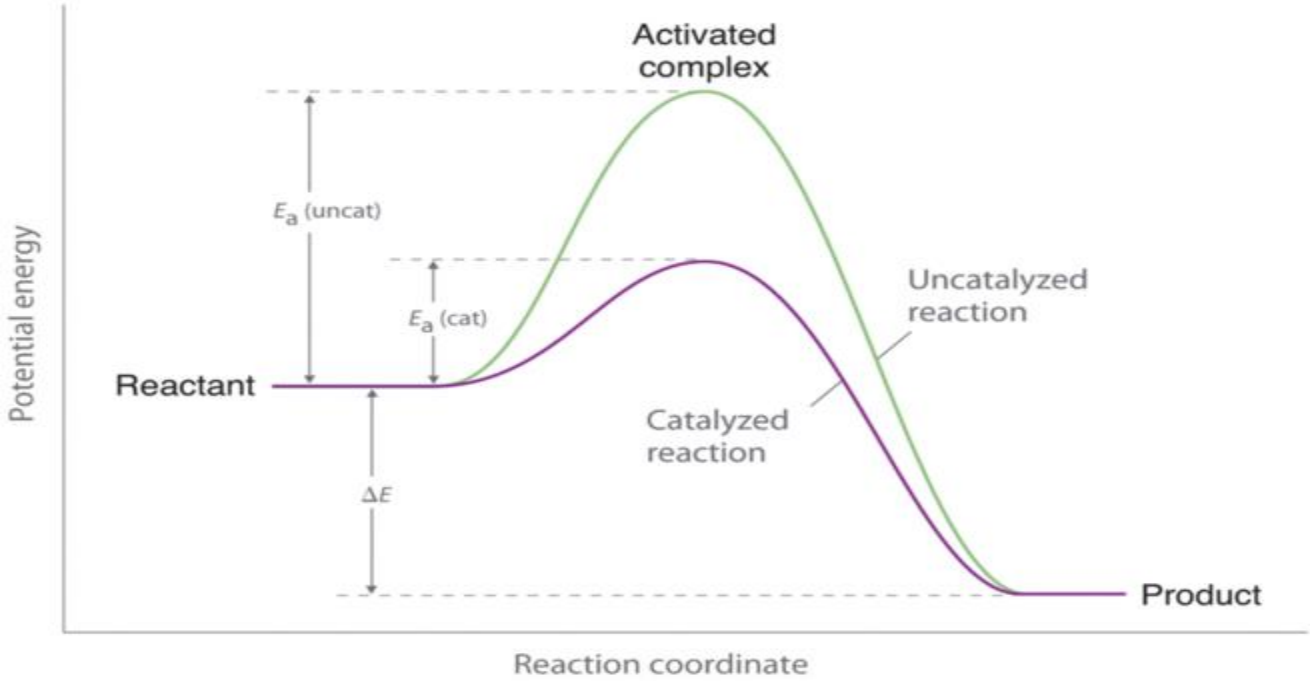
2. Entropy: Energy that is not available for useful work or the degree of energetic randomness

3. Free energy:

- The sum total of **available energy in the system**
- The difference in the free energy of the reactant and the free energy of the product is called the **standard free energy change** (ΔG)
- Negative ΔG – Exergonic (High free energy of the reactants)
- Positive ΔG – Endergonic (High free energy of the products)
- **ΔG = ΔH – TΔS**; where, H – heat consumed or produced, T – abs temp., S - Entropy

Energy of Activation

- Ea is the energy required to convert the reactants to products
- Enzymes lowers the activation energy by stabilizing E-S complex

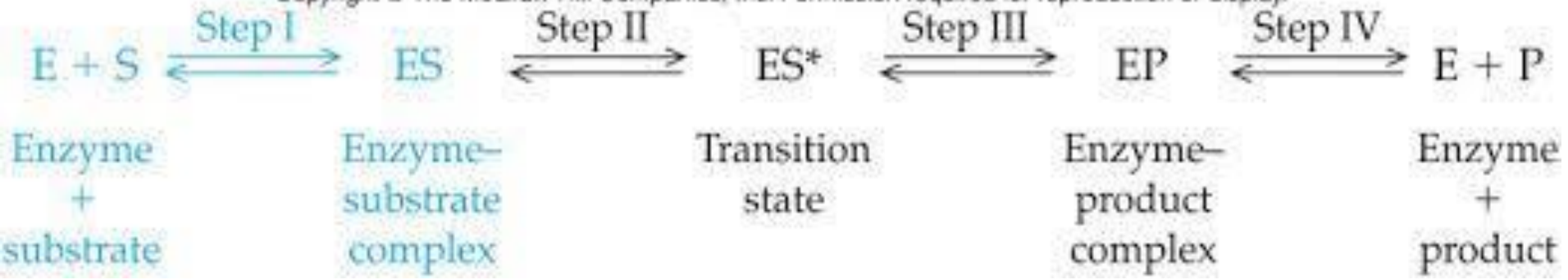


Mechanism of enzyme action

Enzyme-Substrate complex formation (Michaelis-Menten hypothesis)

Stages involved:

1. Substrate binds to the active site on the enzyme stereospecifically to form primary E-S complex
2. Conversion of primary E-S to one or more activated E-S complexes
3. Formation of E-P complex
4. Detachment of product from the active site of enzyme

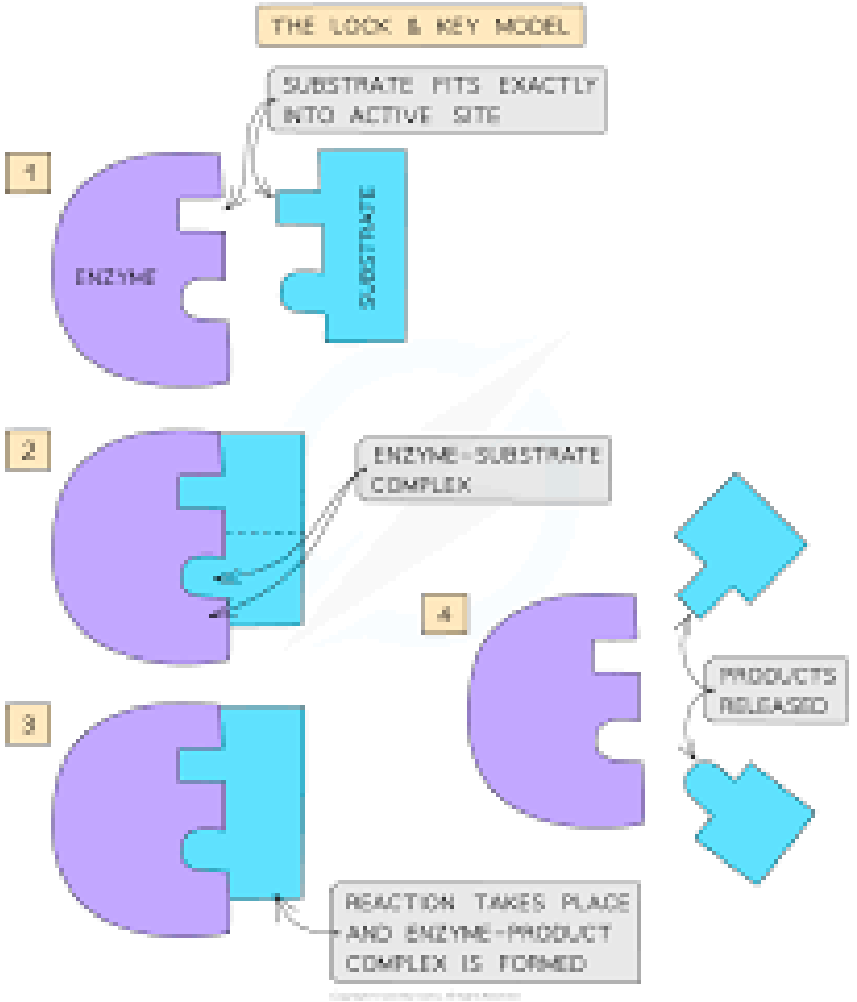


- **Step I** – depends on the conc. of the substrate, lowering of Ea
- **Step II –** Slow process, breaking of the substrate bonds and creation of new bonds, enzyme properties might change
- **Step III** – Rate limiting step as this involves the formation and decay of reaction intermediates
- **Step IV-** slow and short, depends on the concentration of ES and rate of diffusion of the product to the environment

Enzyme – Substrate interaction

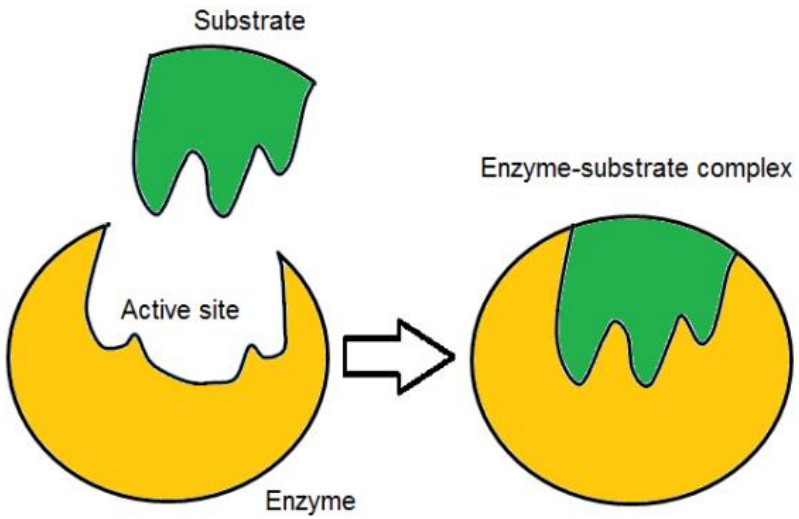
1. Lock and key hypothesis:

- Structural complimentarity between E and S
- Enzyme is a rigid template with pre-shaped active site to which substrate binds
- Accounts for extreme specificity
- But do not support the flexibility of allosteric interactions



2. Induced fit model:

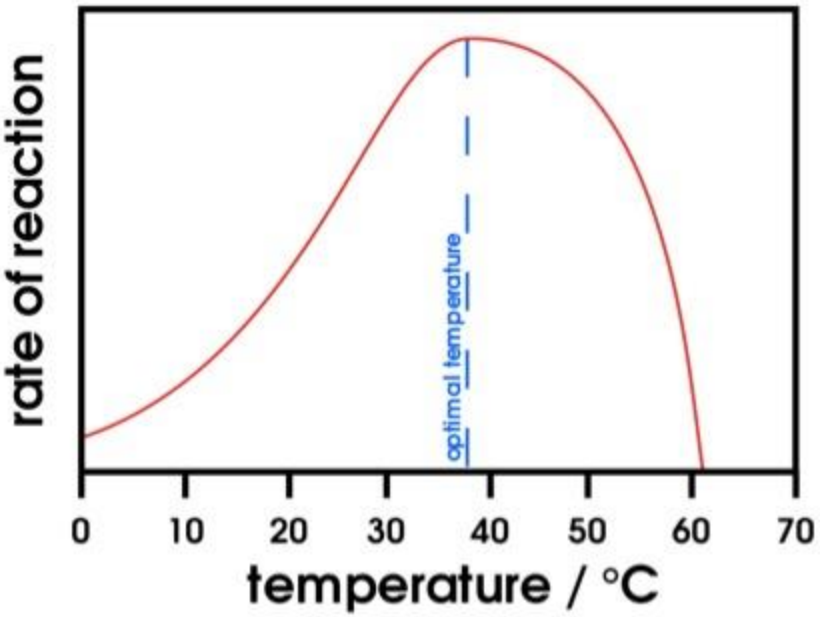
- Substrate binding causes conformational changes in the active site of the enzyme
- Precise orientation of the functional groups of the enzyme is necessary for the ES complex formation
- Stereospecific binding is the key process
- If analogues binds, the conformational changes happening might not occur in the reuired orientation. Thus, it will not work.



Factors affecting the velocity of enzyme action

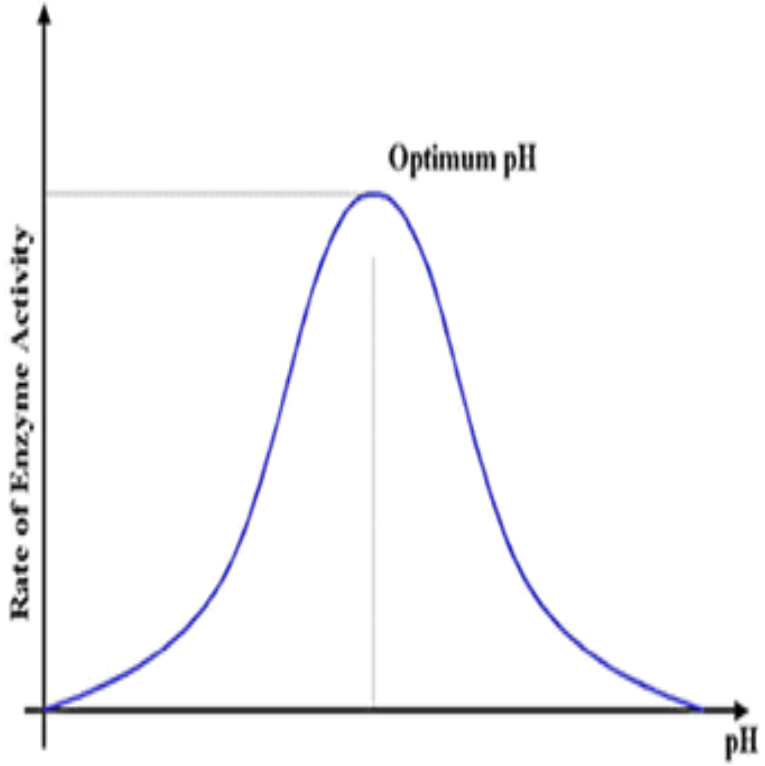
1. Temperature:

- Enzyme activity increases with increase in temperature until it reaches the optimum point
- Beyond the optimum temperature it drops to zero
- Denatures the structure of the polypeptide chain
- Extremely low level of temperature also deactivate the enzymes
- Exception: Adenylate kinase, enzymes of thermophilic bacteria



2. pH

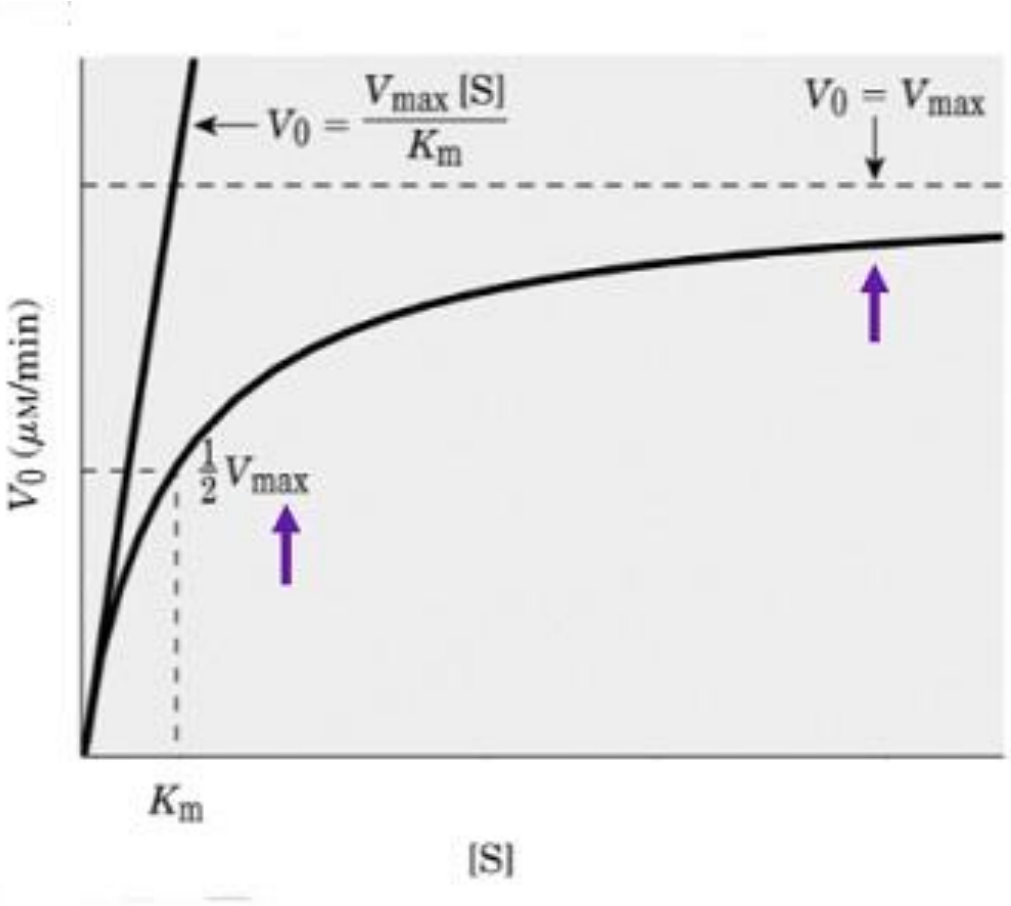
- Enzymes are highly sensitive to pH changes
- Increased pH irreversibly alters the enzyme structure
- Affects the ionization of substrate
- Affects the enzyme-substrate binding and reactivity of the enzyme
- Neutral pH is the most favorable one, though optimum pH is different for different enzymes



3. Concentration

- Concentration of enzymes and substrate
- Velocity is directly proportional to the enzyme conc.
- Enzyme conc. - Rate limiting factor
- Dependent on substrate concentration (directly proportional) but the rate of reaction remains unchanged after a particular point

Michaelis – Menten constant



$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

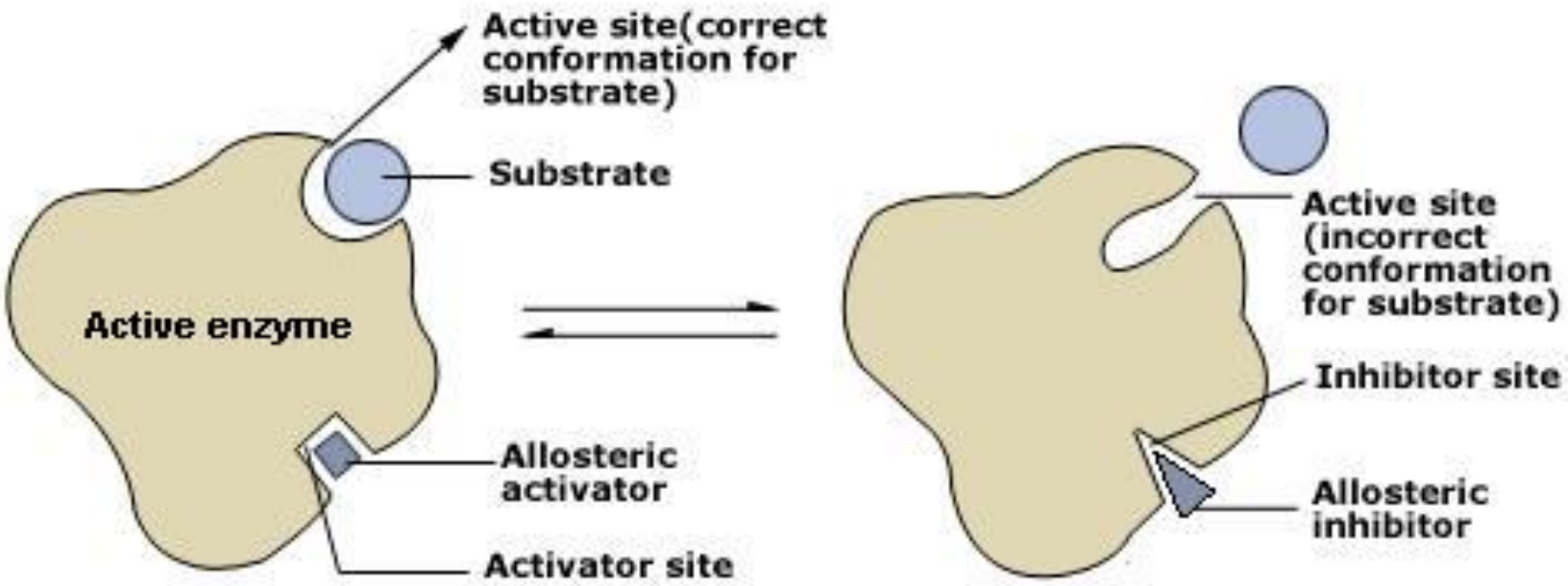
$K_m = [S]$ when $V_o = \frac{V_{max}}{2}$

Lower the Km – High affinity of the enzyme to substrate
Higher Km – weaker affinity of the enzyme to substrate

Regulation of enzyme action

1. Allosteric modulation:

- Allosteric effectors binds to the allosteric sites of the enzyme
- Includes: hormones, coenzymes and metal ions
- Allosteric activators: promotes the enzyme action by causing favorable conformation of the active site to bind to the substrate
- Allosteric inhibitors: Blocks the binding of enzyme to substrate by altering the active site
- Significant in regulating the rate of metabolism



Schematic representation of allosteric enzyme activity

2. Covalent modification:

- Modification of an enzyme by the addition or removal of chemical groups
- Eg. Phosphorylation, dephosphorylation, methylation, demethylation etc.

Enzyme activation

1. **By structural changes :** pro-enzyme to active enzyme by removal of polypeptide chains
2. **By inorganic ions:** Forms the bridge bond between enzyme and substrate
3. **By kinases:** Removal of peptide using ATP
4. **By co-factors:** Activation of the conjugates enzymes

Enzyme inhibition

Might be reversible or irreversible

Types:

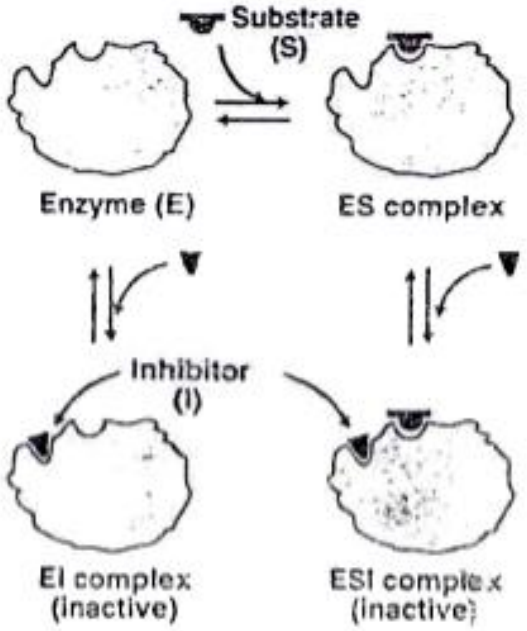
1. Competitive
2. Non-competitive
3. Uncompetitive
4. Substrate – linked
5. Product inhibition
6. End product/Feed-back inhibition

1. Competitive inhibition

- Inhibitor is a structural homologue of substrate and binds to the active site of the enzyme
- Blocks the formation of E-S complex and forms E-I complex
- Eg. Malonic acid inhibits the enzyme succinic acid dehydrogenase

2. Non-competitive inhibition

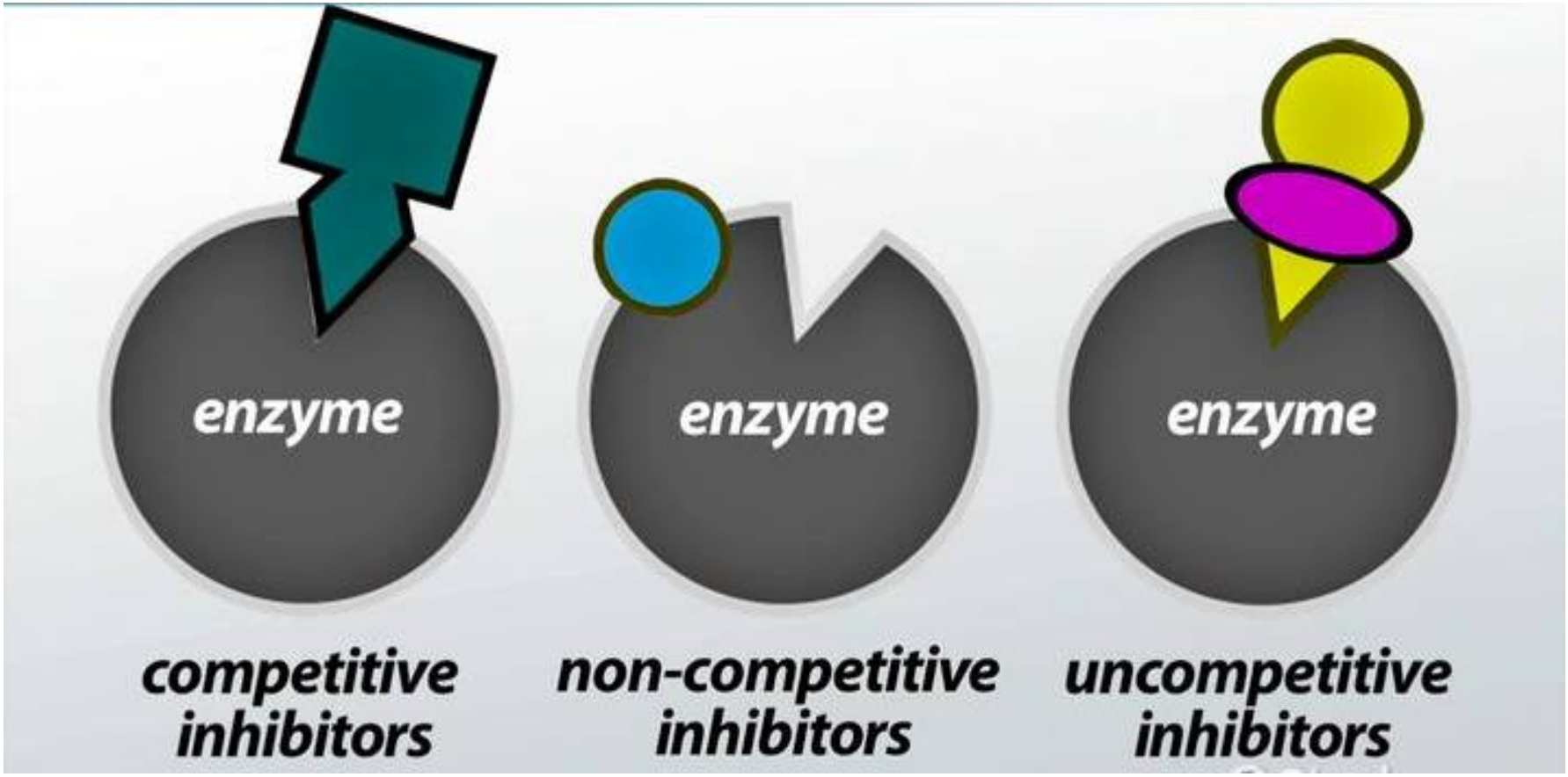
- Inhibits the conversion of E-S to E-P complex instead forms a E-S-I complex or E-I
- Inhibitor binds to the site other than the active site
- Do not affect the Km of the reaction but decreases V
- Eg. Cyanide can inhibit cytochrome oxidase



A noncompetitive inhibitor binding to both free enzyme and enzyme-substrate complex

3. Uncompetitive inhibition

- The inhibitor combines with E-S complex alone and not with free enzyme
- E-S complex is formed and I binds to the Substrate part of the complex
- Cannot overcome by increasing substrate conc.
- Eg. Inhibition of placental alkaline phosphate by phenyl alanine


enzyme
enzyme
enzyme
competitive inhibitors
non-competitive inhibitors
uncompetitive inhibitors

4. Substrate-linked inhibition

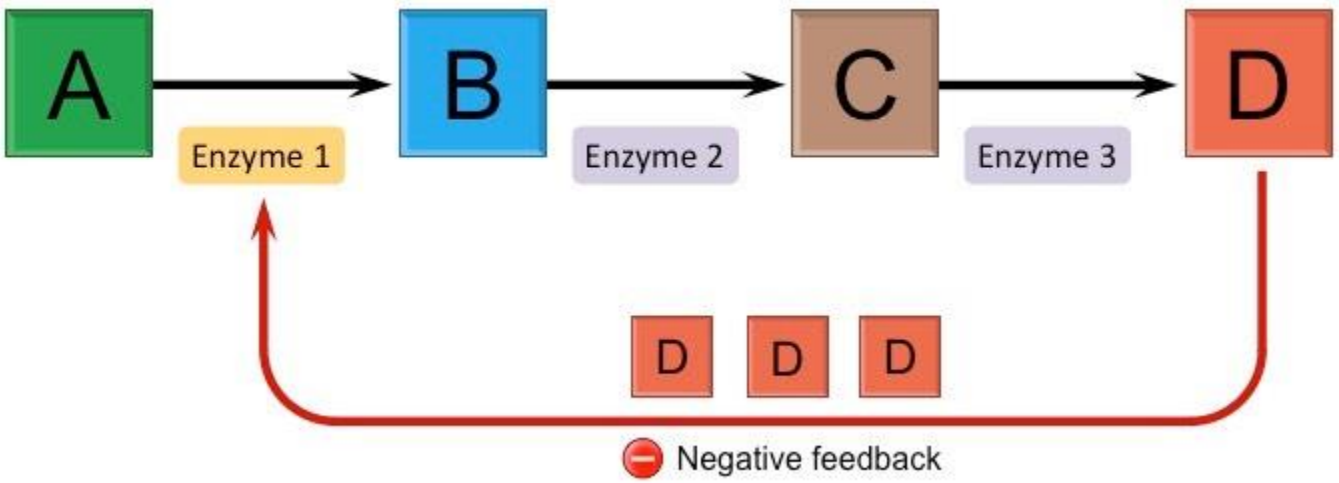
- Less common
- Excessive amounts of substrate causes the accumulation and binding on enzymes at different sites other than the active sites
- Inhibits the whole action

5. Product inhibition

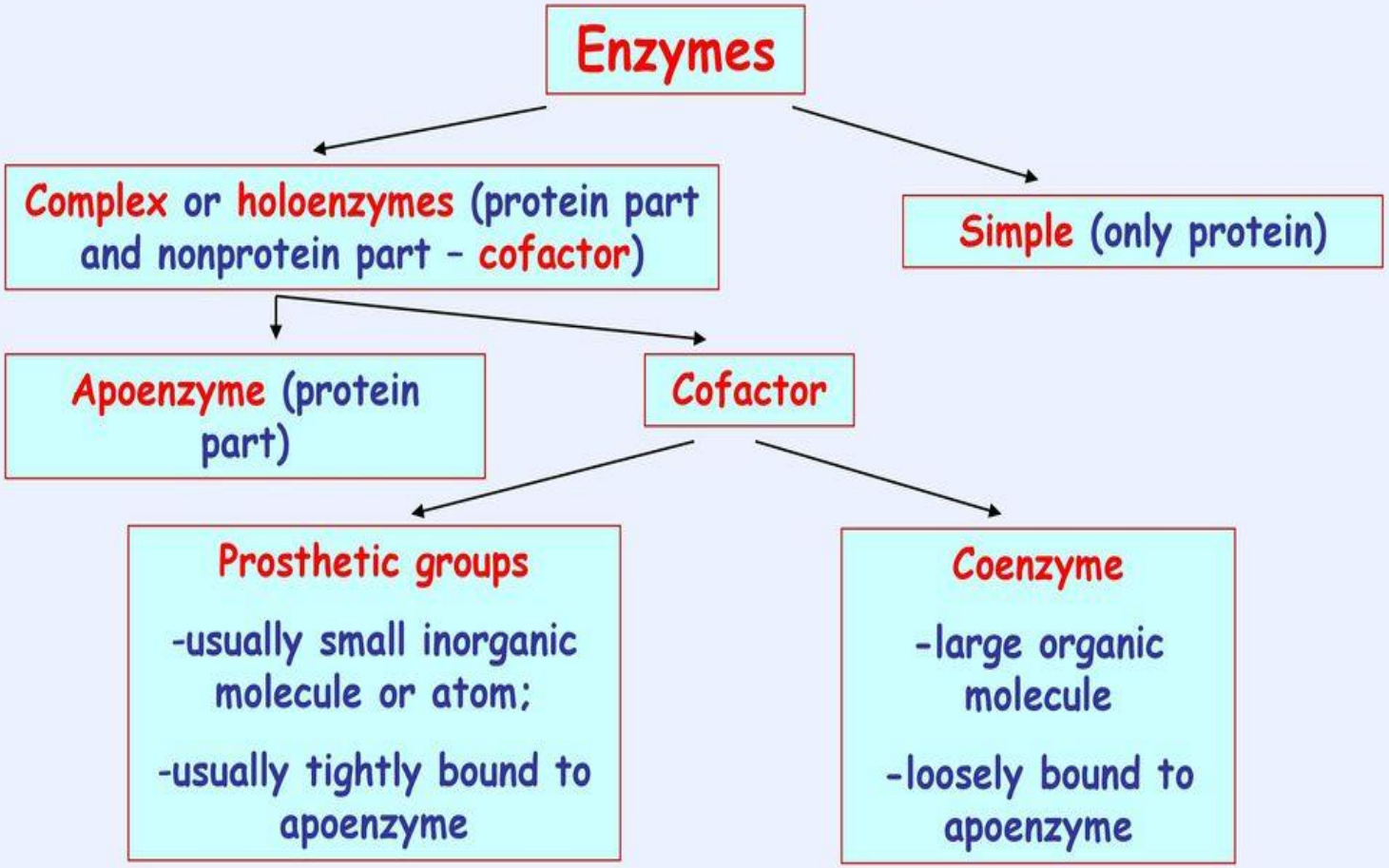
- The product of a particular reaction down-regulates the action of Enzyme

6. Feed back inhibition

- The **end** product of a particular reaction down-regulates the action of Enzyme



Structure of enzymes



Coenzymes

- Organic molecule that binds to the active sites of certain enzymes
- Difficult to differentiate it from the substrate as these are very closely associated to each other

Roles:

1. Provides a binding portion for the substrate
2. Helps E-S interactions by donating or accepting chemical groups
3. Acts as intermediates in the group transfer reactions

Eg. NAD, Cytochromes, ATP, CoA, Biotin

Isoenzymes

- Multiple forms of an enzyme that differs structurally and physically
- Catalyzes the same reaction
- Differ in nucleotide sequences