Enzyme kinetics

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Enzyme kinetics

- the quantitative measurement of the rates of enzyme-catalyzed reactions

- the systematic study of factors that affect these rates,

- Catalytic mechanism of a given enzyme
- Diagnostics
- Therapeutics

- Chemical reactions are described using balanced equations
- Changes in free energy determine the direction & equilibrium state of chemical reactions

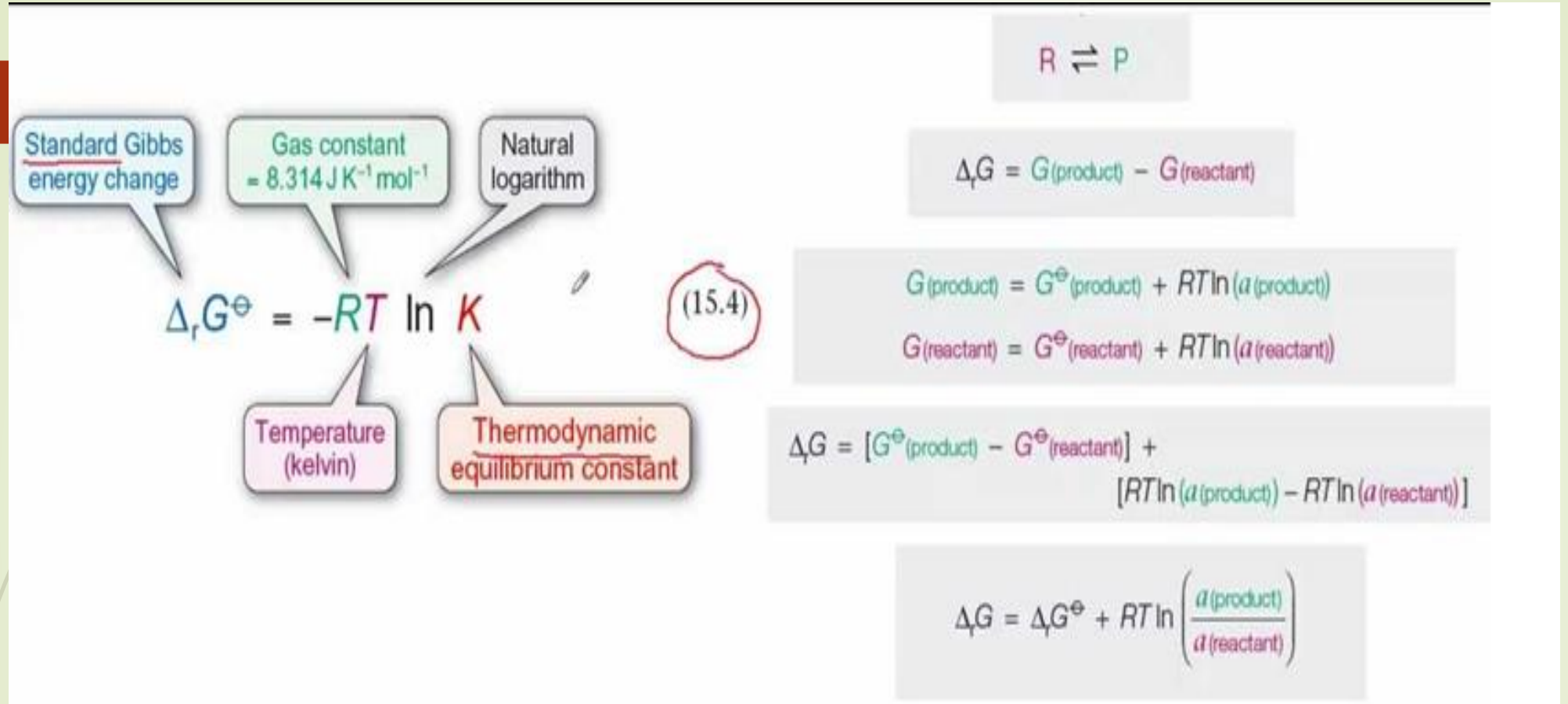
Gibbs Free Energy (G)

$$G = H - TS$$

The Gibbs Free Energy change for a given process is

$$\Delta G = \Delta H - T\Delta S$$

(at constant T and P)



ΔG0 : Standard free energy change under standard conditions: 1atm,1M,25C

ΔG0′, which defines ΔG0 at a standard state of 10−7 M protons, pH 7.0.

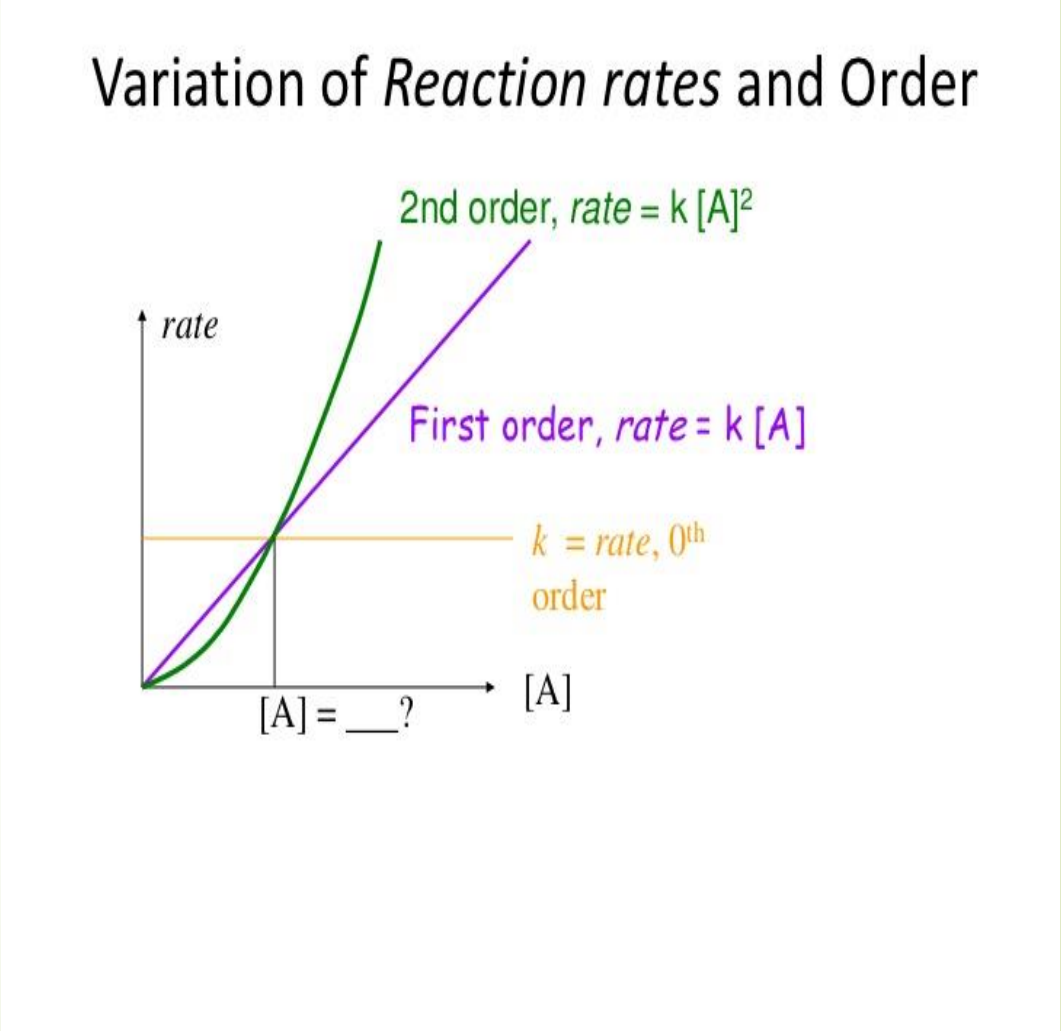
Order of reaction

- **A + B → P**
- **Rate1 = *k*1[A]*n*[B]*m***

$$\frac{k_1}{k_{-1}} = \frac{[P]}{[A]^n[B]^m}$$

- The sum of the molar ratios of the reactants defines the **kinetic order** of the reaction

- **? Pseudo first order reaction**

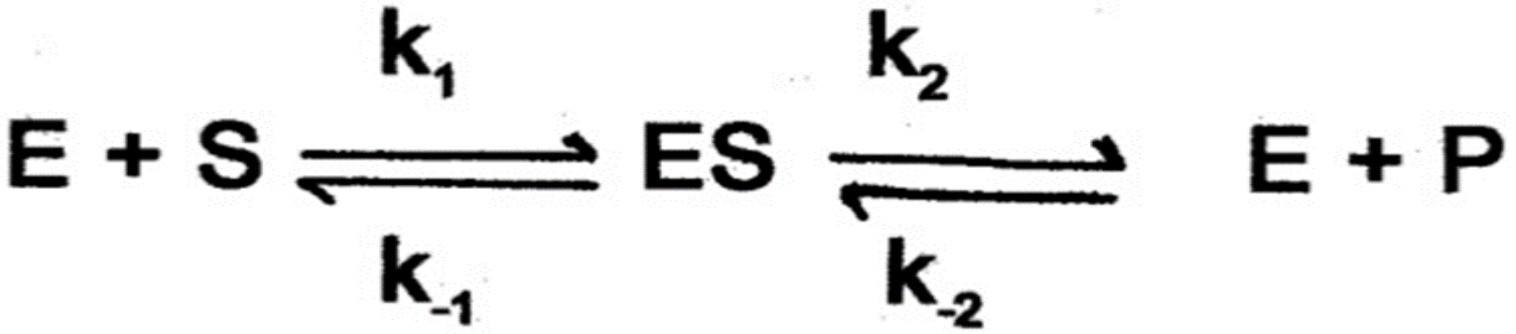


THE KINETICS OF ENZYME CATALYSIS

- Enzymes Lower the Activation Energy Barrier for a Reaction

- MULTIPLE FACTORS AFFECT THE RATES OF ENZYME CATALYZED REACTIONS
- Temperature
- pH
- Substrate concentration

Enzyme Kinetics Equation



S = substrate P = product

E = enzyme

ES = enzyme-substrate complex

k_1, k_{-1}, k_2, k_{-2} are rate constants

***K*eq Is a Ratio of Rate Constants**

THE MICHAELIS MENTEN EQUATION MODEL THE EFFECTS OF SUBSTRATE CONCENTRATION

Michaelis-Menten Equation

$$v_o = \frac{[V_{max}][S]}{Km + [S]}$$

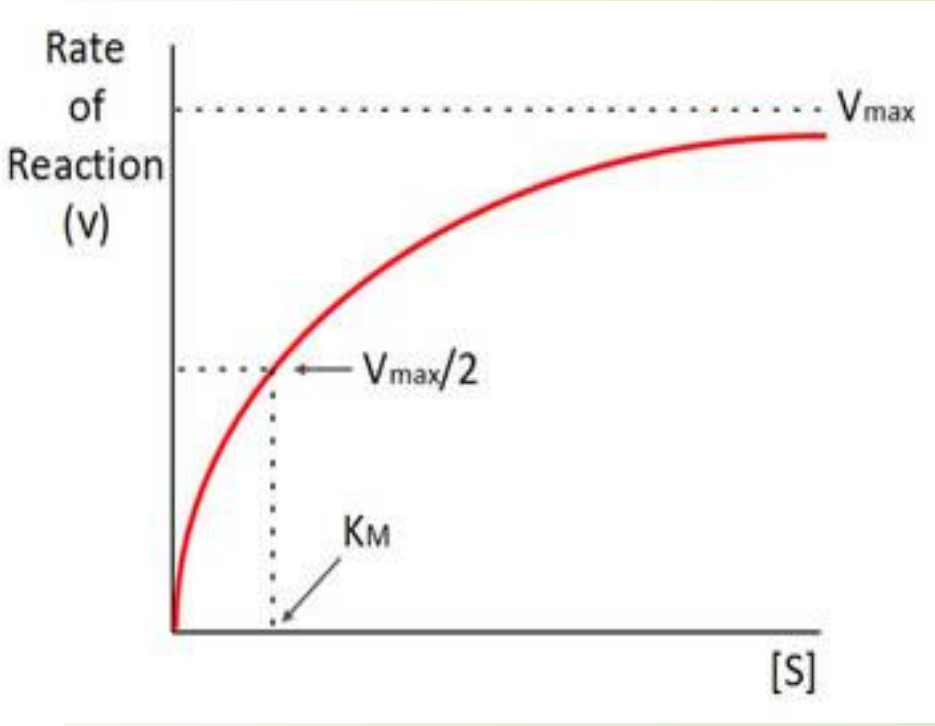
v_o = initial reaction velocity

V_{max} = maximal velocity

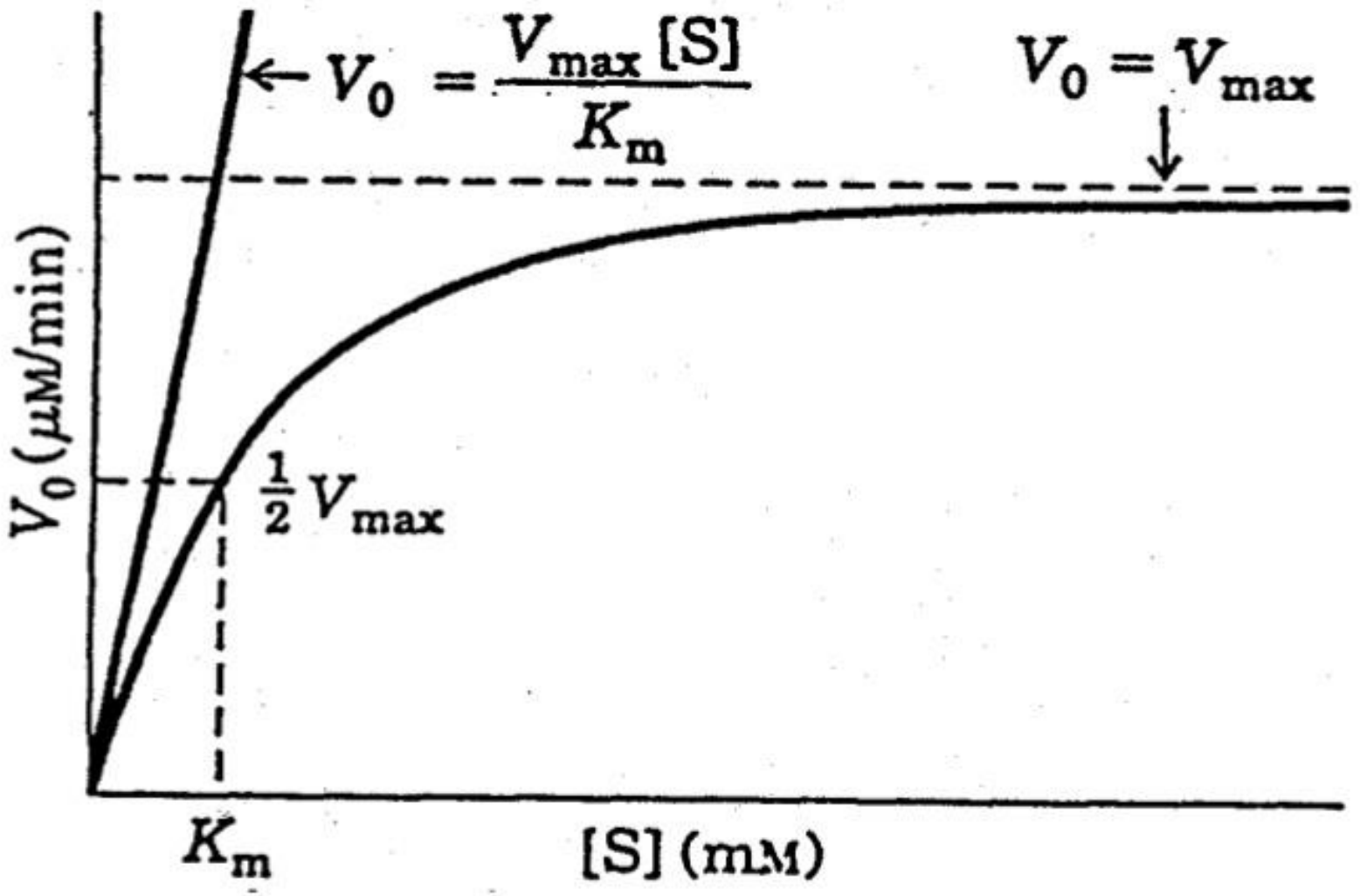
[S] = substrate concentration

$$K_m = \frac{k_{-1} + k_2}{k_1} \quad \text{if } k_{-2} <<<$$

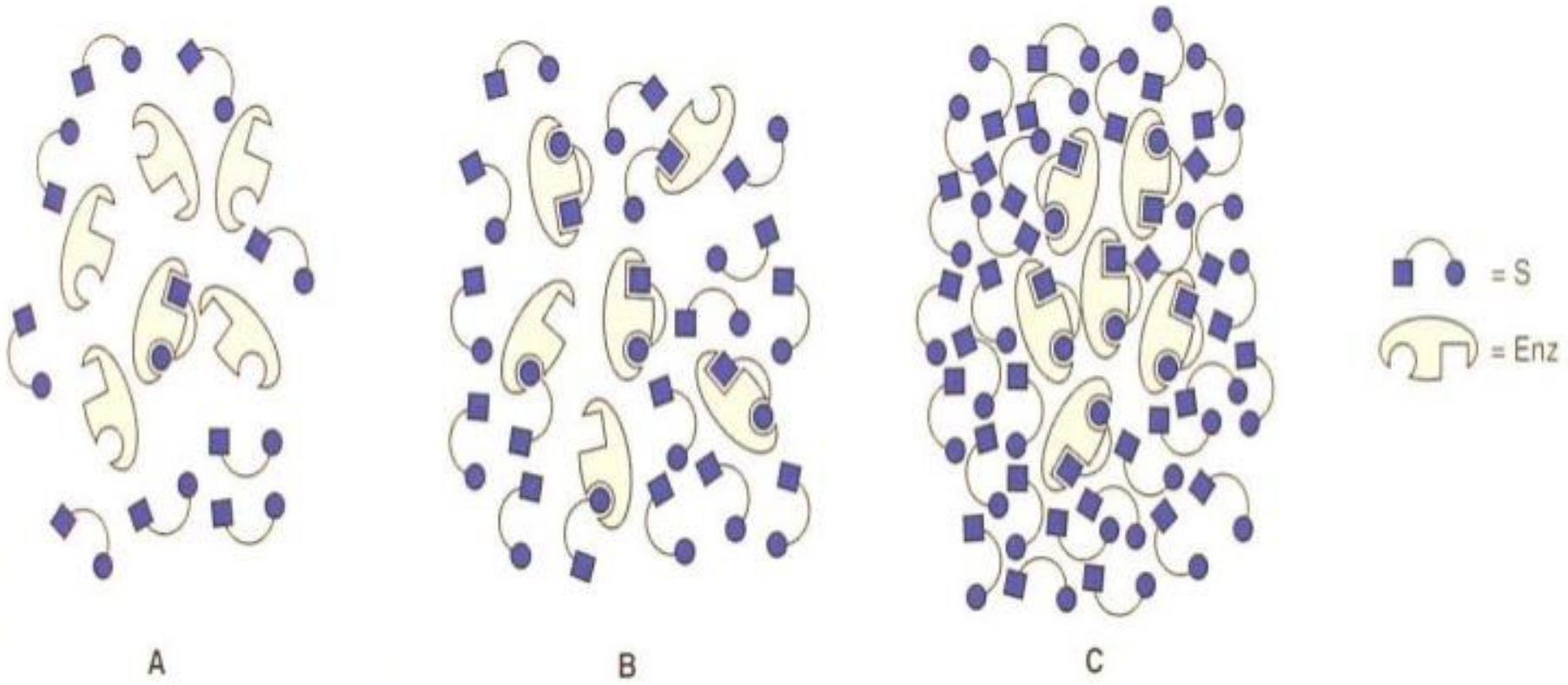
(i.e., E + P back to ES is minimal)



Michaelis-Menten Curve

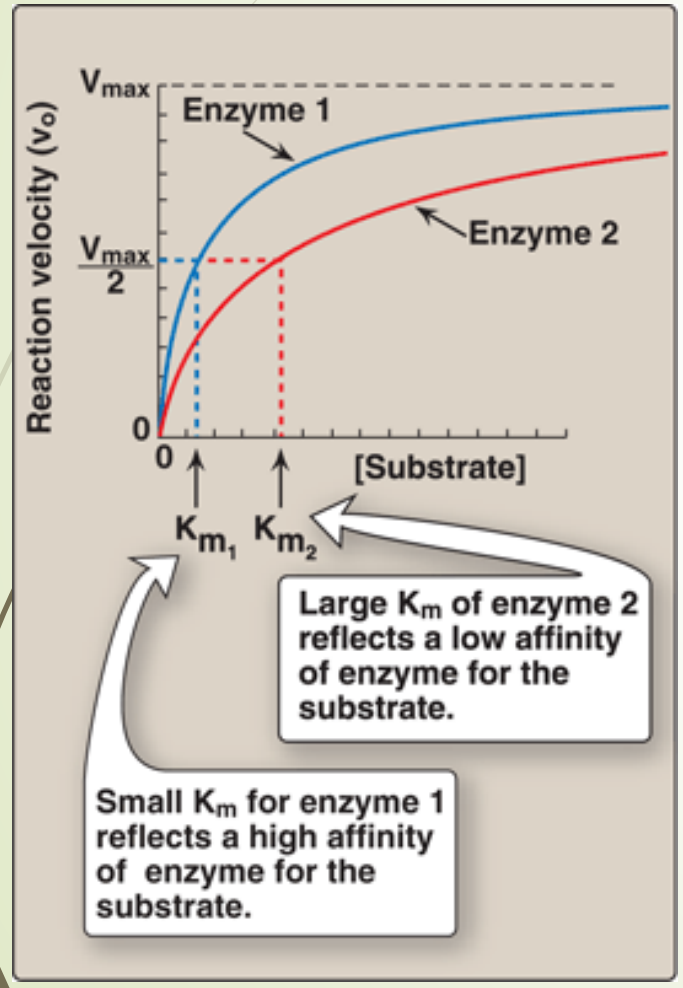


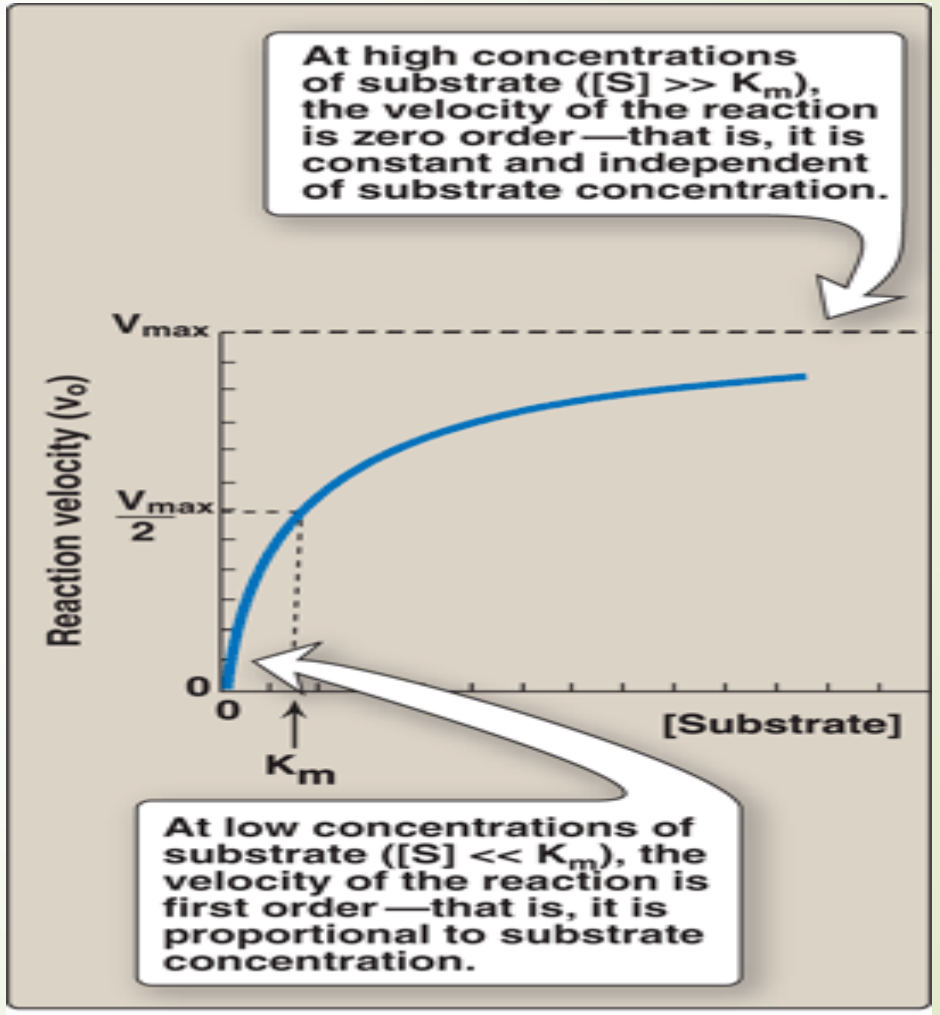
Substrate Saturation of an Enzyme



A. Low [S] B. 50% [S] or K_m C. High, saturating [S]

Effect of substrate concentration on reaction velocities





Important conclusions

- Characteristics of Km
- Relationship of velocity to enzyme concentration
- Order of reaction

Important Conclusions of Michaels - Menten Kinetics

- when $[S] = K_M$, the equation reduces to

$$V = \frac{V_{max}[S]}{K_M + [S]} = \frac{V_{max}[S]}{[S] + [S]} = \frac{V_{max}}{2}$$

- when $[S] >> K_M$, the equation reduces to

$$V = \frac{V_{max}[S]}{K_M + [S]} = \frac{V_{max}[S]}{[S]} = V_{max}$$

- when $[S] << K_M$, the equation reduces to

$$V = \frac{V_{max}[S]}{K_M + [S]} = \frac{V_{max}[S]}{K_M} = \frac{V_{max}}{K_M}[S]$$

- Which of the following describes a characteristic feature of an enzyme obeying Michaelis-Menten kinetics?

- (A) The enzyme velocity is at 1/2 the maximal rate when 100% of the enzyme molecules contain bound substrate.
- (B) The enzyme velocity is at 1/2 the maximal rate when 50% of the enzyme molecules contain bound substrate.
- (C) The enzyme velocity is at its maximal rate when 50% of the enzyme molecules contain bound substrate.
- (D) The enzyme velocity is at its maximal rate when all of the substrate molecules in solution are bound by the enzyme.
- (E) The velocity of the reaction is independent of the concentration of enzyme.

Lineweaver-Burk plot

- Linear Form of the Michaelis-Menten Equation Is Used to Determine *K*m & *V*max

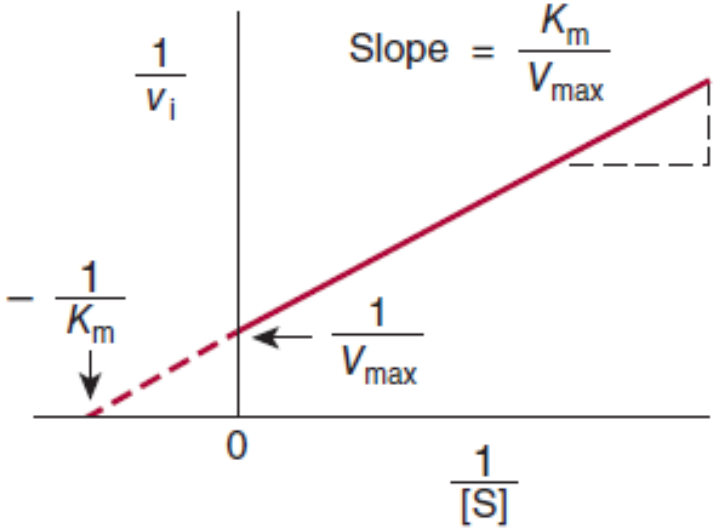
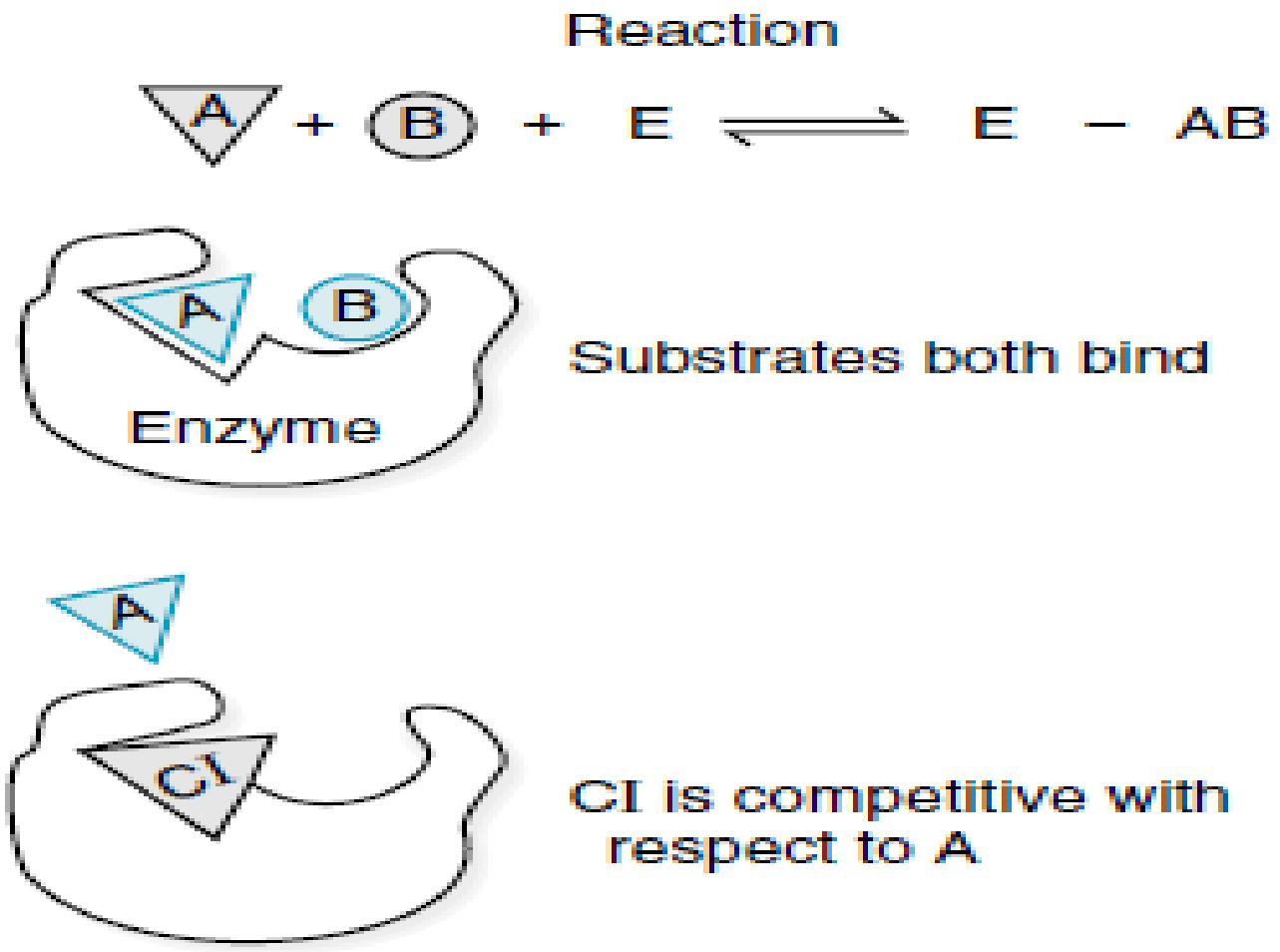
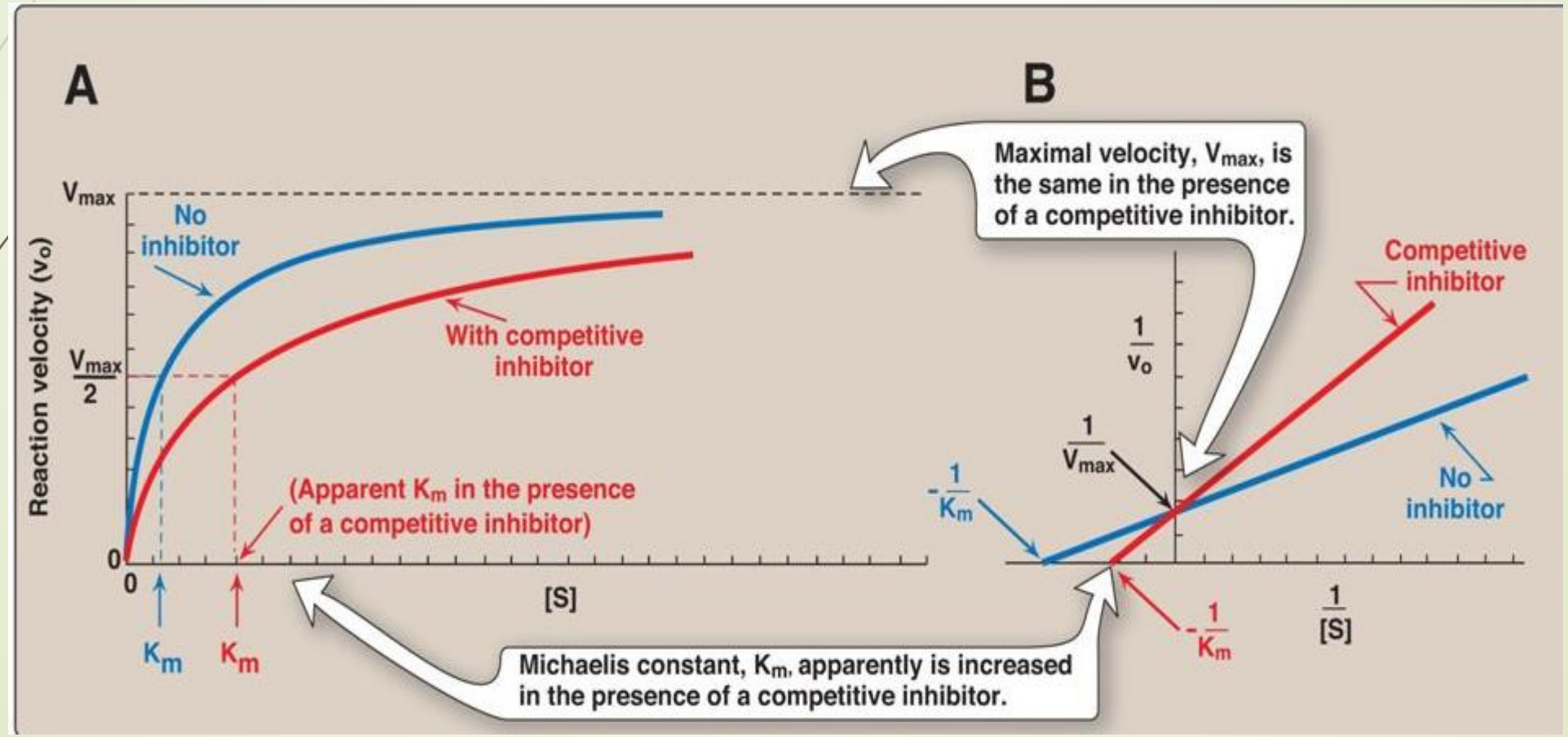


FIGURE 8–6 Double-reciprocal or Lineweaver-Burk plot of $1/v_i$ versus $1/[S]$ used to evaluate K_m and V_{max}.

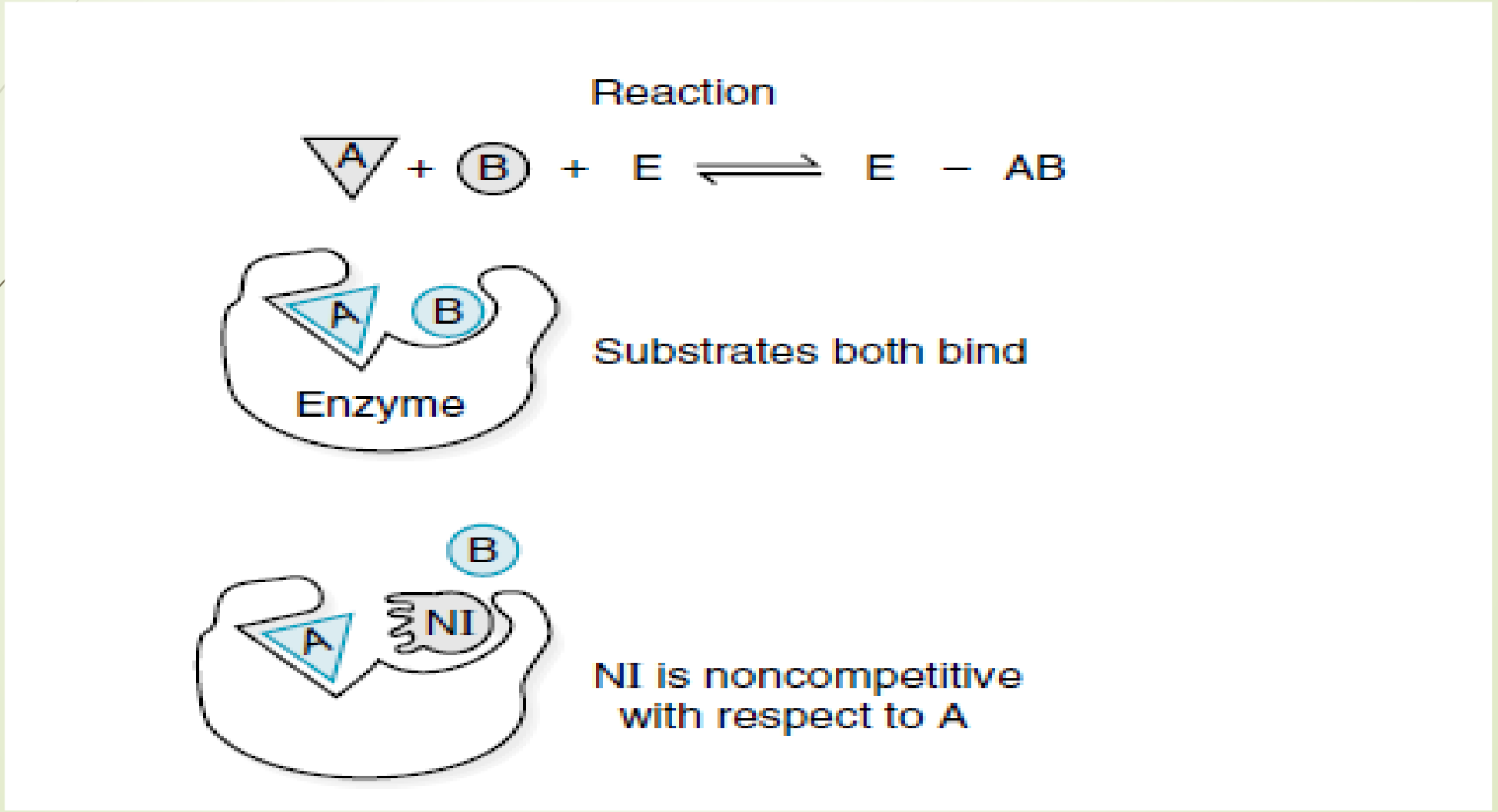
Competitive inhibition



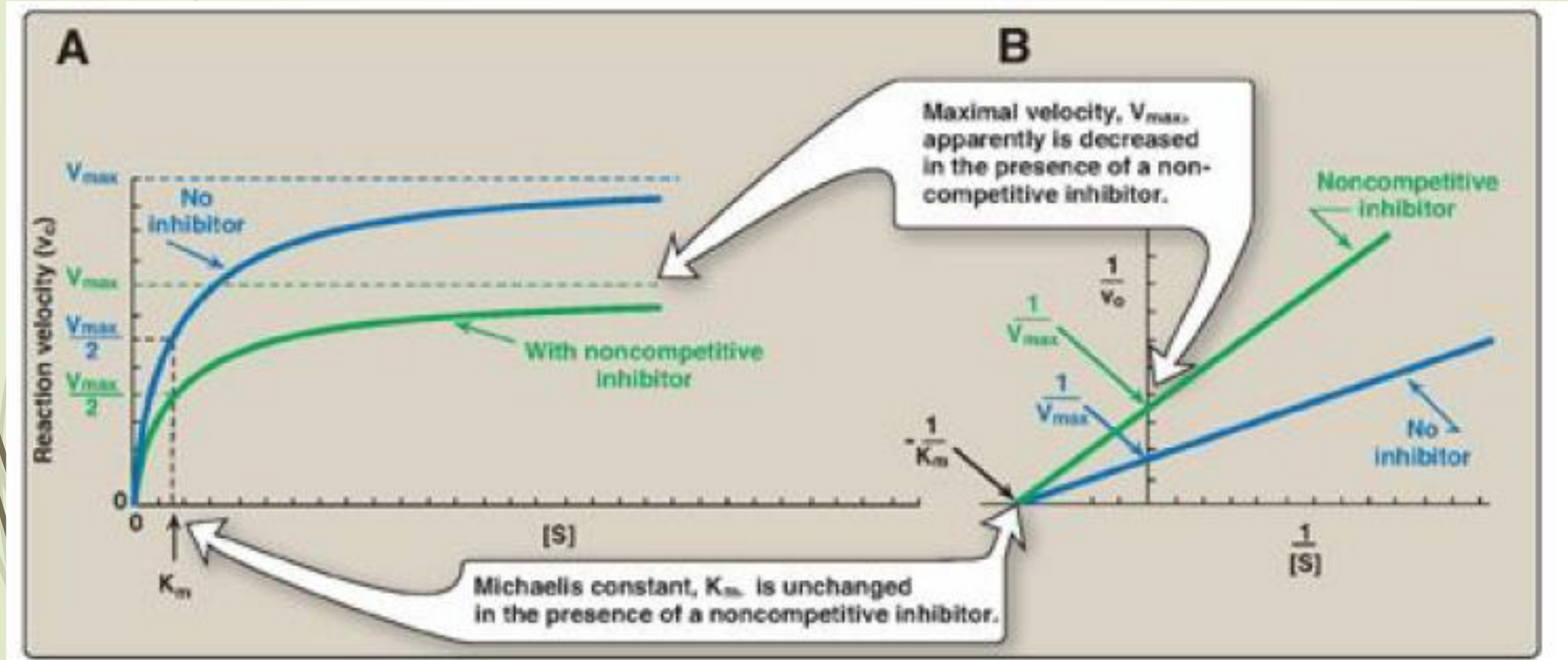
KINETIC ANALYSIS DISTINGUISHES COMPETITIVE FROM NONCOMPETITIVE INHIBITION



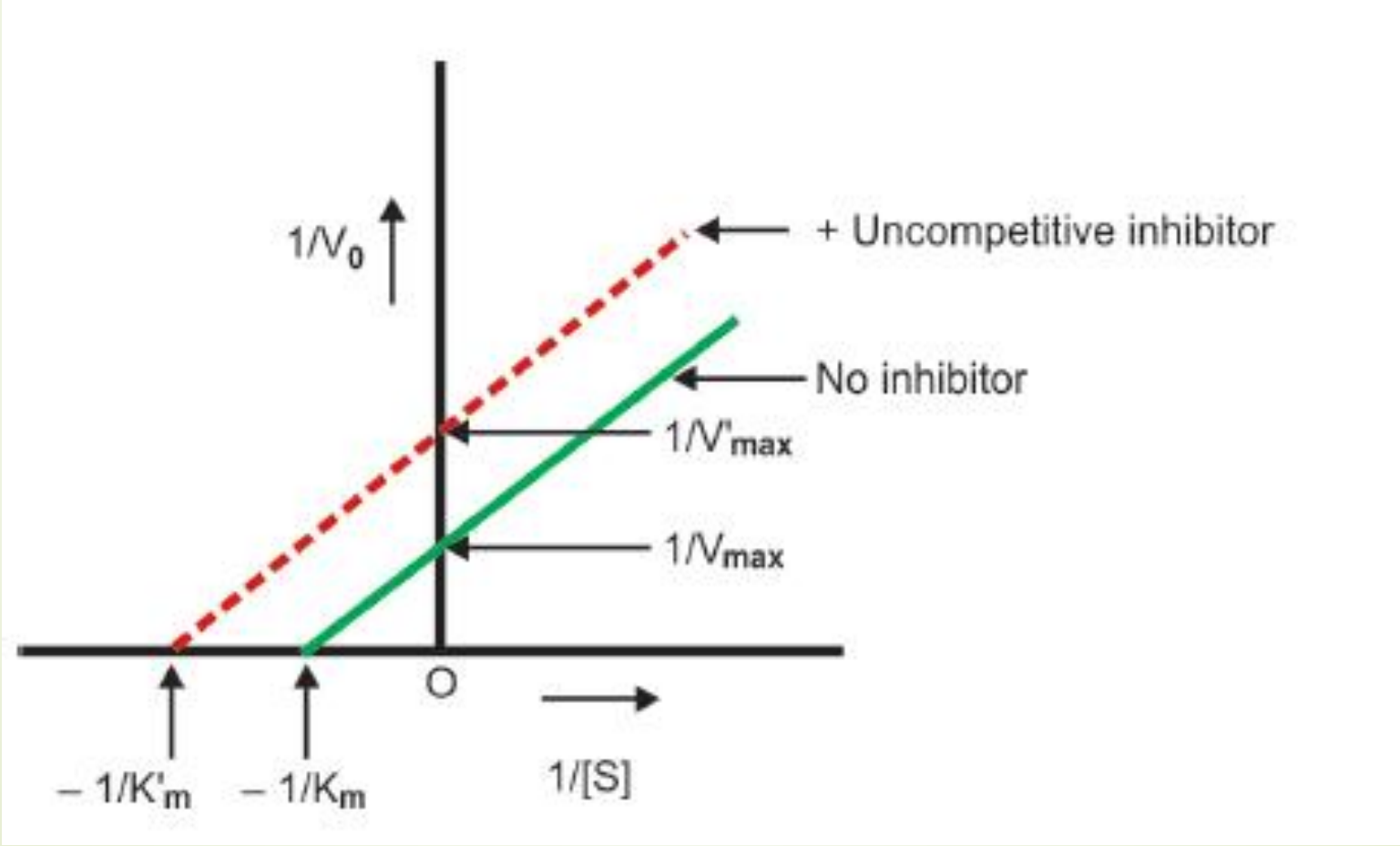
Non- competitive inhibition



Non competitive inhibition



Uncompetitive inhibition



- Methanol (CH_3OH) is converted by alcohol dehydrogenases to formaldehyde (CHO), a compound that is highly toxic in the human. Patients who have ingested toxic levels of methanol are sometimes treated with ethanol (CH_3CH_2OH) to inhibit methanol oxidation by alcohol dehydrogenase. Which of the following statements provides the best rationale for this treatment?

- (A) Ethanol is a structural analog of methanol, and might therefore be an effective noncompetitive inhibitor.

- (B) Ethanol is a structural analog of methanol that would be expected to compete with methanol for its binding site on the enzyme.

- (C) Ethanol would be expected to alter the Vmax of alcohol dehydrogenase for the oxidation of methanol to formaldehyde.

- (D) Ethanol would be expected to inhibit the enzyme by binding to the formaldehyde binding site on the enzyme, even though it cannot bind at the substrate binding site for methanol.

Mechanism based inhibition

- **Covalent**
- **Transition state analogues**

Table 6.5: Effect of inhibitors on kinetic properties of enzymes

Type of inhibitor	*Km*	V_{max}
Irreversible	No effect	Decreased
Reversible competitive	Increased	No effect
Reversible noncompetitive	No effect	Decreased
Reversibe uncompetitive	Decreased	Decreased

Many Drugs Are Enzyme Inhibitors

- **Sulfa Drugs:** competition with **paminobenzoic acid** (PABA),
- **Methotrexate:** competition with dihydrofolate for dihydrofolate reductase.
- **Fluorouracil**: irreversible inhibitor of thymidylate synthetase

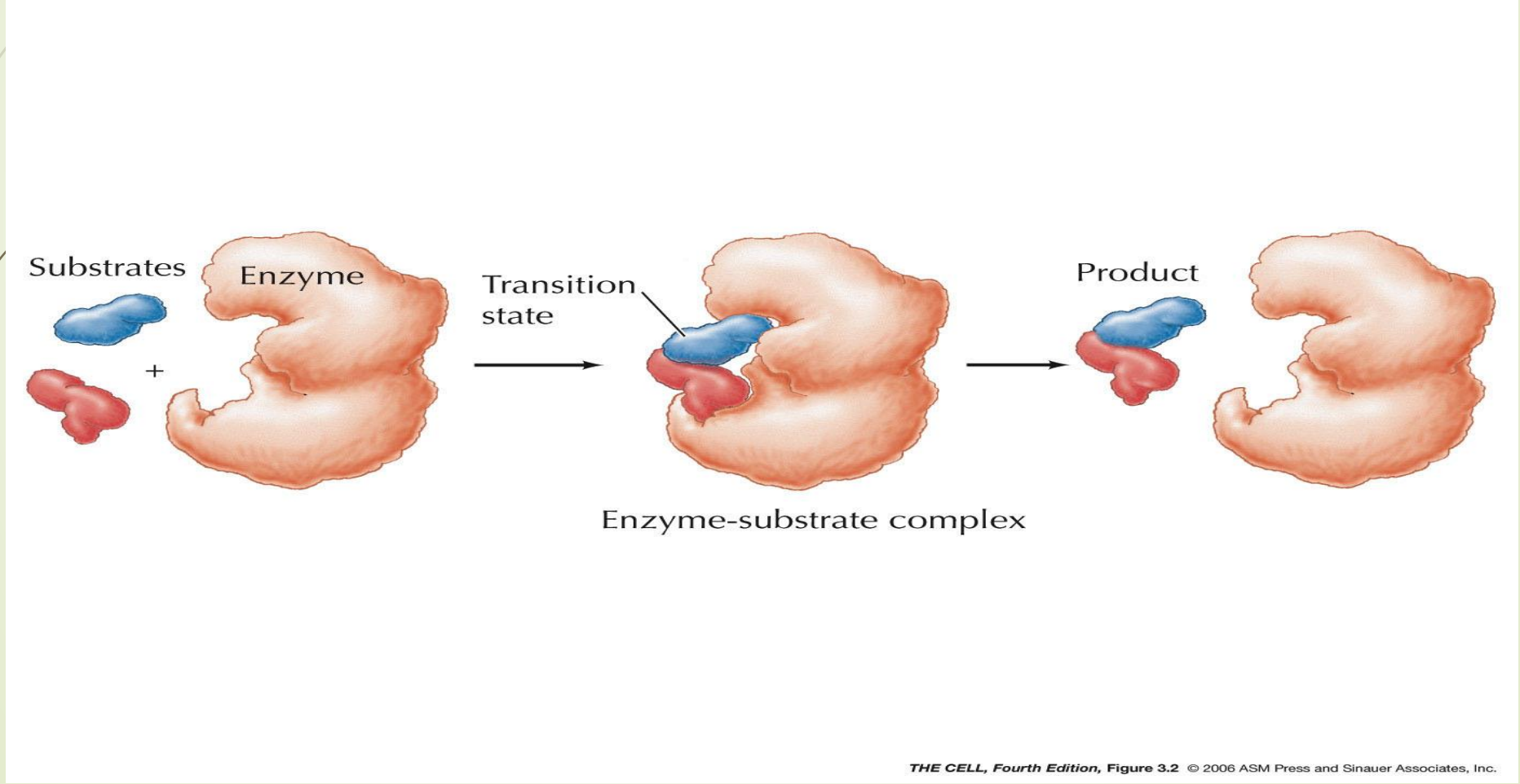
Table 6.4: Commonly used drugs that are enzyme inhibitors

Drug	*Type of inhibition*	*Target enzyme*	*Therapeutic use*
Mevinolin Lovastatin	Competitive	HMG-CoA reductase (3-Hydroxy-3-Methyl-Glutaryl CoA-reductase)	Hypercholesterolemia
Allopurinol	Competitive	Xanthine oxidase	Gout
Methotrexate	Competitive	Dihydrofolate reductase	Cancer
5-Fluorouracil	Suicide	Thymidylate synthase	Cancer
Aspirin	Suicide	Cyclo-oxygenase	Anti-inflammatory
Penicillin	Suicide	Bacterial transpeptidase	Antibacterial

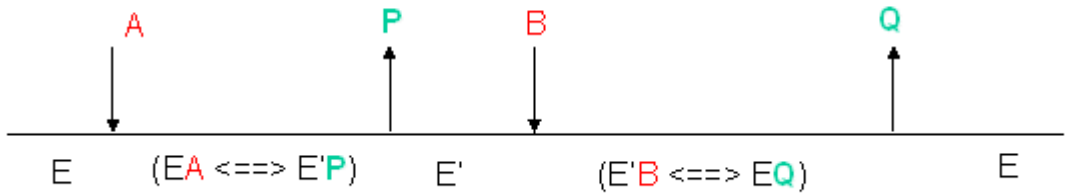
Double displacement reactions

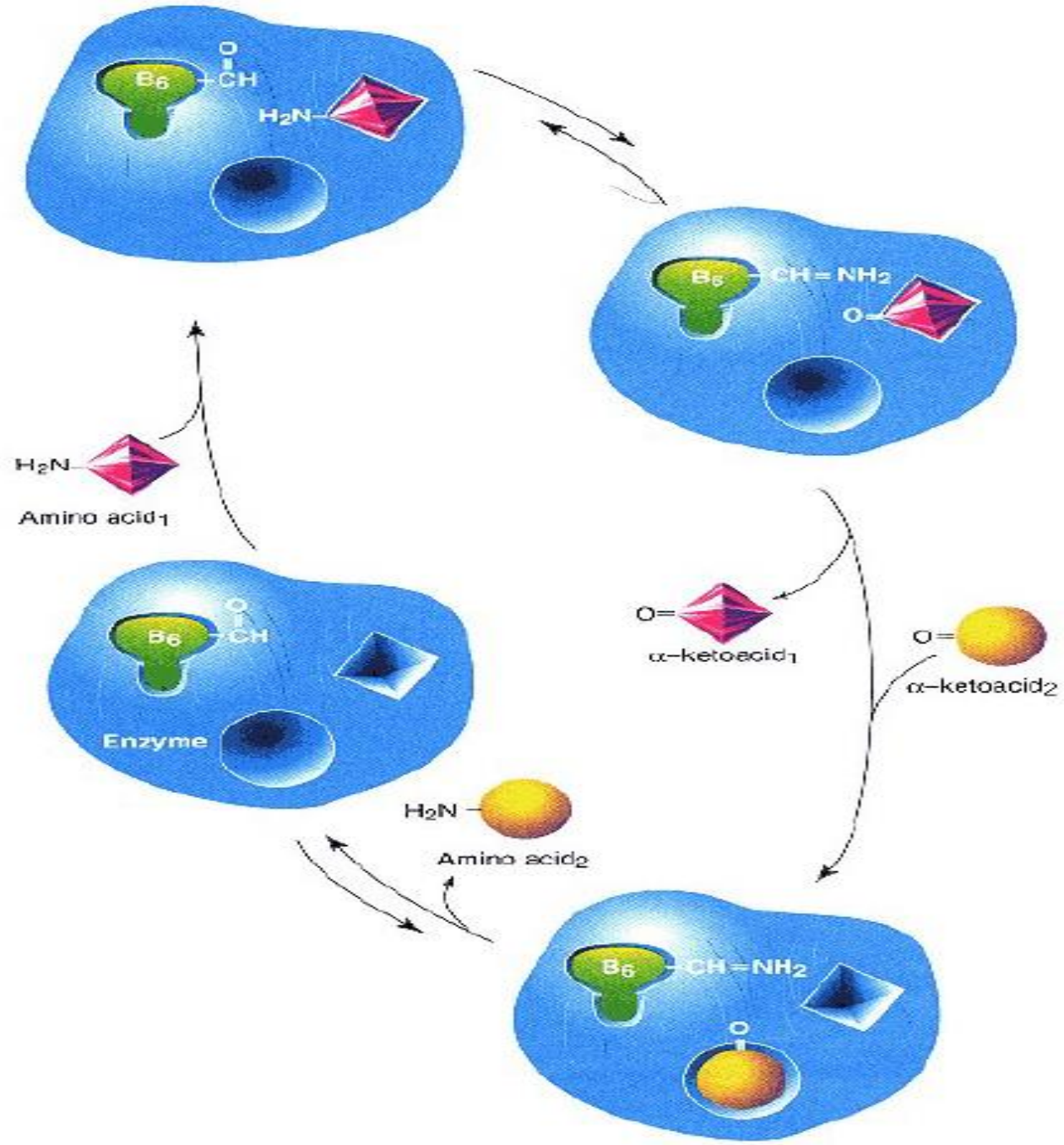
- **Sequential:**
- Random
- Ordered
- **Ping pong/Double displacement**

Sequential/single displacement reactions



Ping-Pong: Reactant A binds, followed by release of product (P), followed by binding reactant B, then release of product Q.




B6
O
CH
H2N
B6
CH=NH2
O
H2N
Amino acid1
O=
α–ketoacid1
O=
α–ketoacid2
B6
O
CH
Enzyme
H2N
Amino acid2
B6
CH=NH2
O

Parameters used to compare the relative activity of different enzymes or of different preparations of the same enzyme.

- *Specific activity*
- *Turnover number*
- *Catalytic constant, k*cat
- Catalytic Efficiency, *k*cat/*K*m