# Enzymes

How enzymes work?

$$\text{Sucrose} + O_2 \rightarrow CO_2 + H_2O + ATP$$

$$\Delta G < 0$$

How long can a box of chocolate last ?

All chemical reactions in life are catalyzed by enzymes.

# Enzymes

P. 184

1. Catalytic RNA (Ch 26)
2. Proteins (in their native conformations)

Enzyme
= Protein
= Protein + cofactor (inorganic ions, e.g. Table 6-1)
= Protein + coenzyme (organic molecules, e.g. Table 6-2)

Tightly bound to Enz. → Prosthetic group

Holoenzyme = Apoenzyme + cofactor/coenzyme

Complete,
catalytically active

# Enzyme classification (p.185)

**TABLE 6–3 International Classification of Enzymes**

| Class no. | Class name | Type of reaction catalyzed |
|---|---|---|
| 1 | Oxidoreductases | Transfer of electrons (hydride ions or H atoms) |
| 2 | Transferases | Group transfer reactions |
| 3 | Hydrolases | Hydrolysis reactions (transfer of functional groups to water) |
| 4 | Lyases | Addition of groups to double bonds, or formation of double bonds by removal of groups |
| 5 | Isomerases | Transfer of groups within molecules to yield isomeric forms |
| 6 | Ligases | Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor |

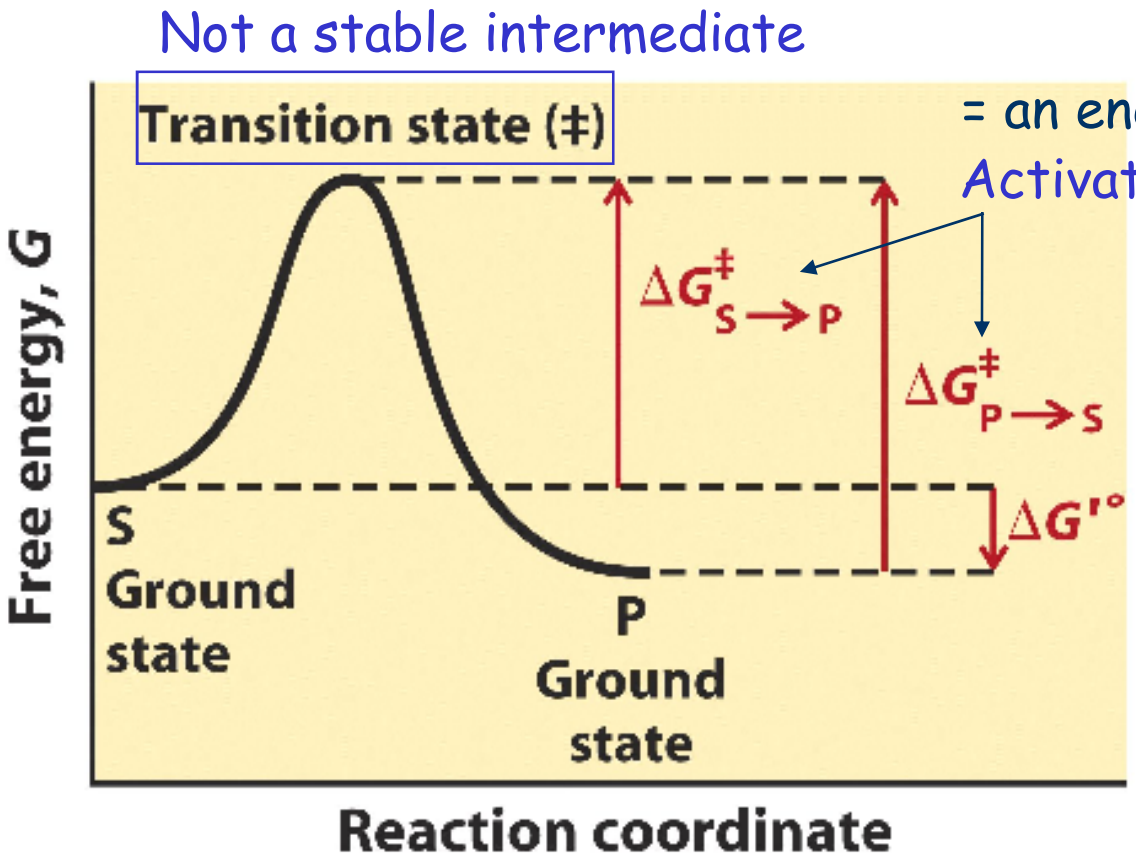
# Naming of enzymes

p. 185

- Reactant + *-ase*
- 6 classes (Table 6.3), based on the reaction type
  - Oxidoreductase, 氧化還原酶, ($A^- + B \rightleftharpoons A + B^+$)
  - Transferase, 轉移酶, ($A\text{-}B + C \rightleftharpoons A + B\text{-}C$)
  - Hydrolase, 水解酶, ($A\text{-}B + H_2O \rightleftharpoons A\text{-}H + B\text{-}OH$)
  - Lyase, 裂解酶, ($A\text{-}B \rightleftharpoons A{=}B + X\text{-}Y$)
  - Isomerase, 異構酶, ($A\text{-}B \rightleftharpoons A\text{-}B$)
  - Ligase, 接合酶, (synthetase) ($A + B \rightleftharpoons A\text{-}B$)

# Energy Diagram of a chemical reaction

- Substrate (S) $\rightleftharpoons$ Product (P), no catalyst




✓Free energy change

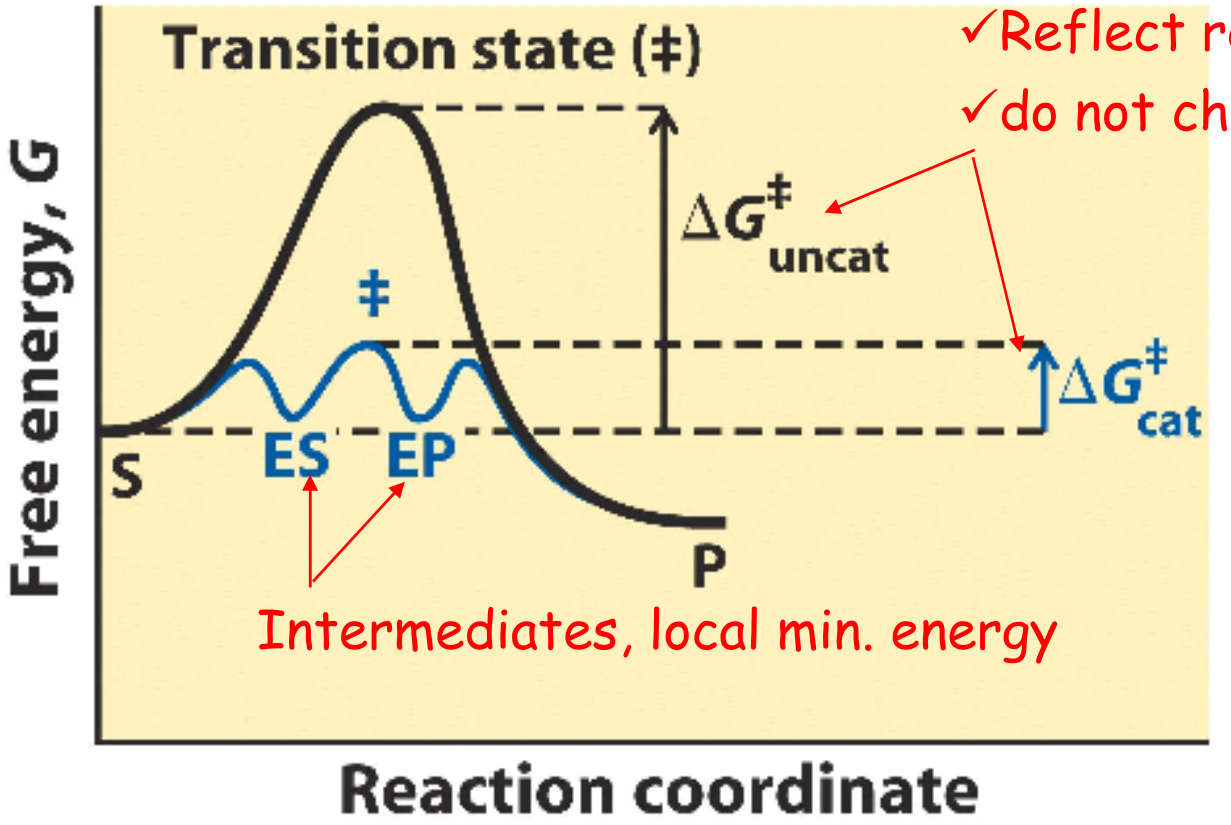
✓Affect equilibrium

✓$\Delta G < 0$, favor P

Fig 6-2, p.186

# Enzymes lowers the activation energy

- $E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$, with catalyst E




✓Reflect reaction rate

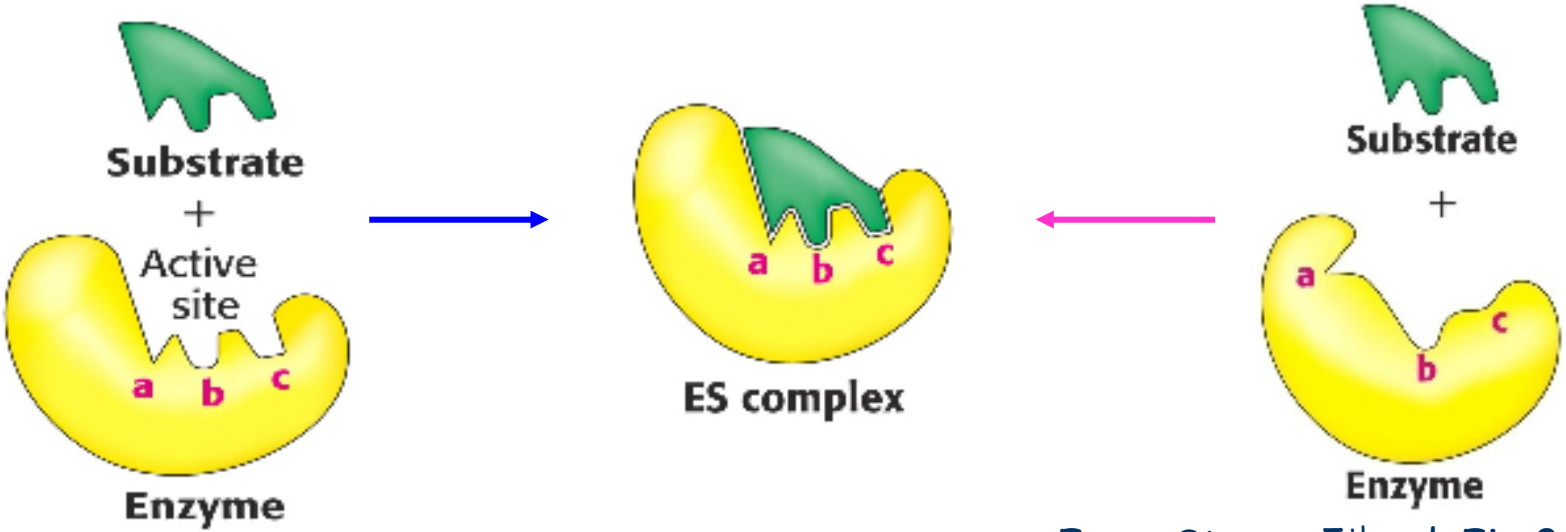
✓do not change equilibrium

Intermediates, local min. energy

Fig 6-3, p.187

# Catalytic power vs. Specificity

- Enzyme-substrate interaction:
  - "Lock and Key" hypothesis
    - Enzymes are structurally complementary to their substrates
  - Induced-fit hypothesis
    - A conformational change of E is induced by initial binding with S, which optimize the ES interaction
- Which hypothesis makes a good enzyme?




From Stryer 5th ed. Fig 8-9, 8-10

# Breaking of a metal stick (I)

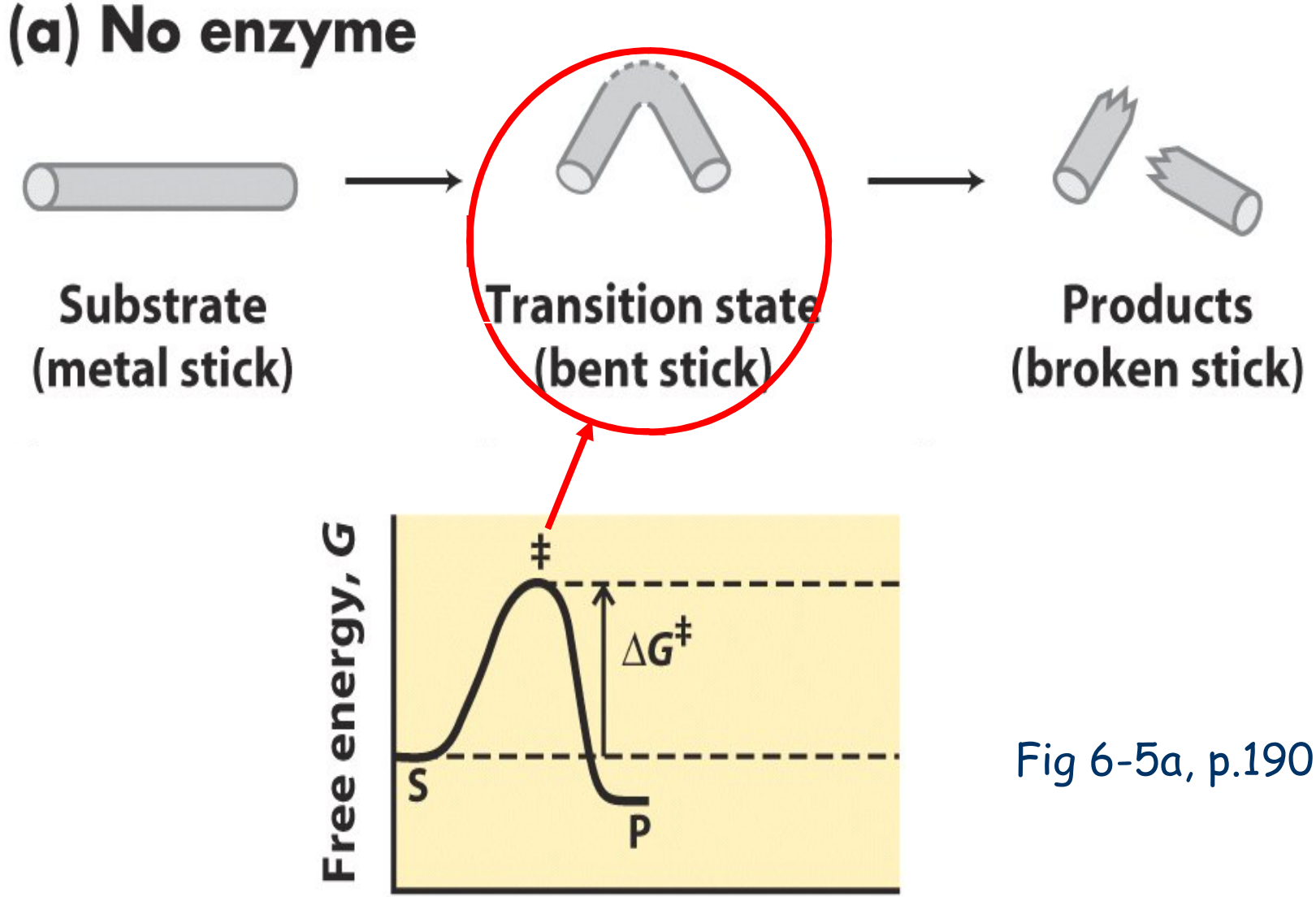
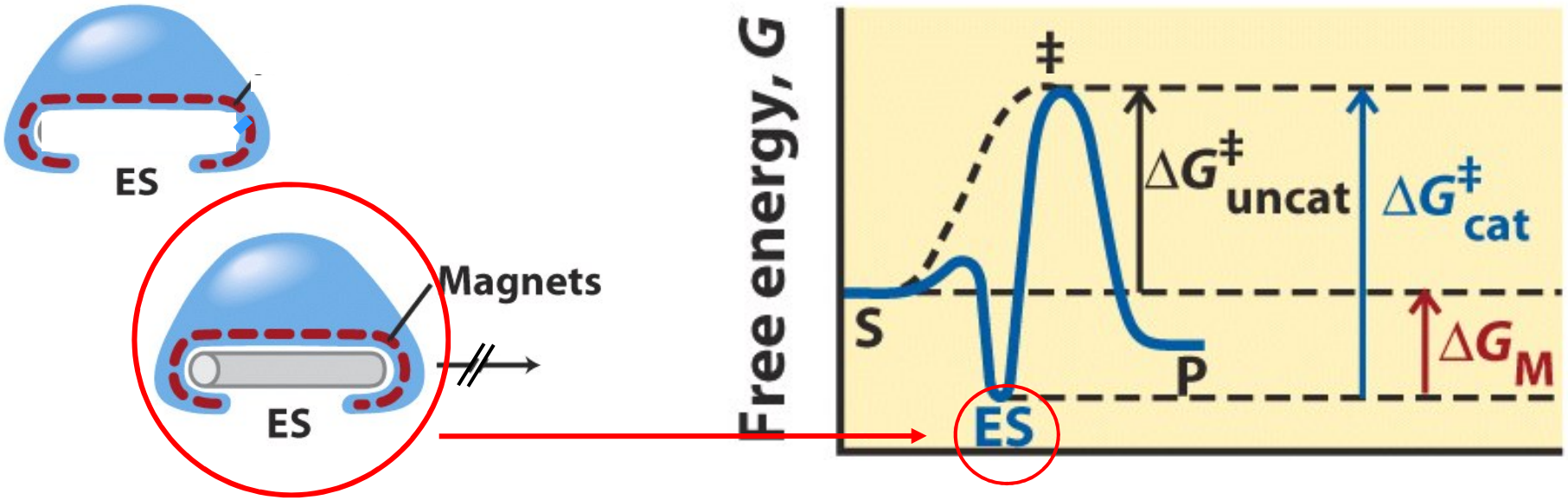



Fig 6-5a, p.190

# Breaking of a metal stick (II)

- With stick*ase*
- An enzyme structurally complementary to substrate (the stick).

**(b) Enzyme complementary to substrate** Fig 6-5b, p.190

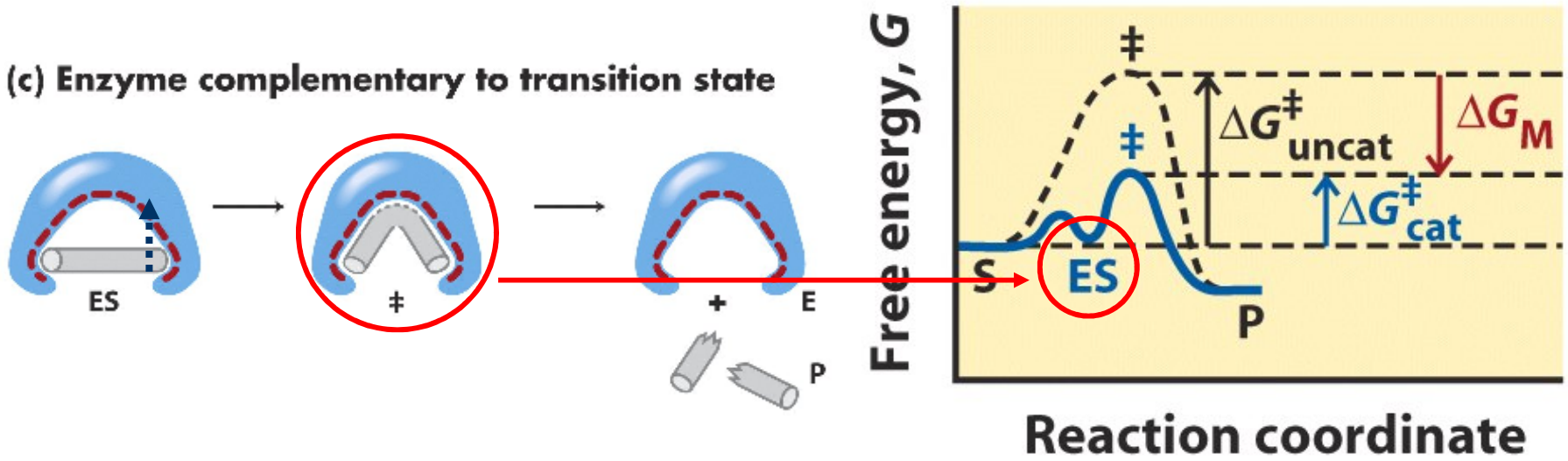



- Stabilize the substrate, impede the reaction.
- An useless enzyme !!

# Breaking of a metal stick (III)

- With another stick*ase*
- An enzyme structurally complementary to the transition state.

Fig 6-5c, p.190




- Stabilize the transition state, allows enzyme to catalyze the reaction.

# Role of binding energy ($\Delta G_B$)

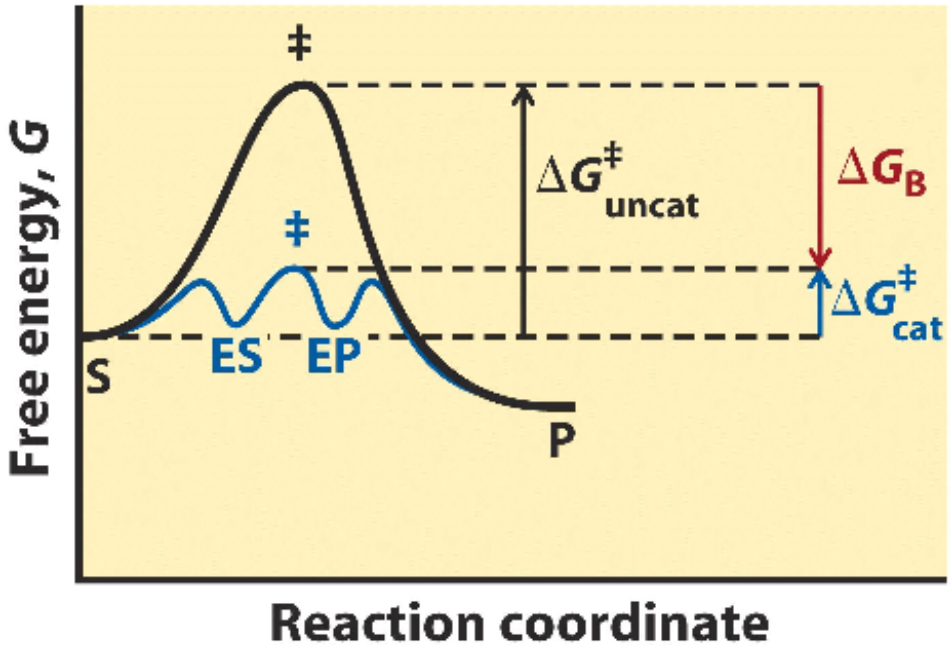


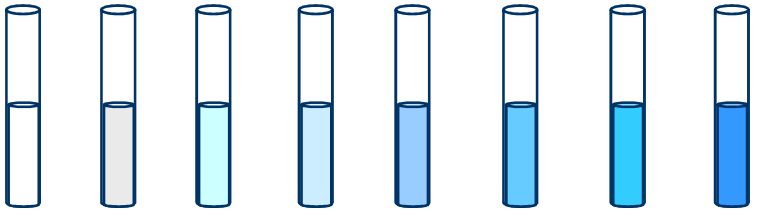

Fig 6-6, p.191

- The binding energy ($\Delta G_B$) released results in lowering the activation energy
- $\Delta G_B$: from multiple weak E-S interactions
- Results in **catalysis** and **specificity**

# Enzyme kinetics

- $S \overset{E}{\rightleftharpoons} P$
- Experiment:
  - [E]: fixed
  - [S]: increasing
  - At beginning, $\Delta$[S] ~ 0, [S] remains unchanged
  - Measure $V_o$ at different [S]
  - $V_o$ = Initial velocity (rate)
  - $V_o$ = [P]/time

$[S_0][S_1][S_2][S_3][S_4]$ .....



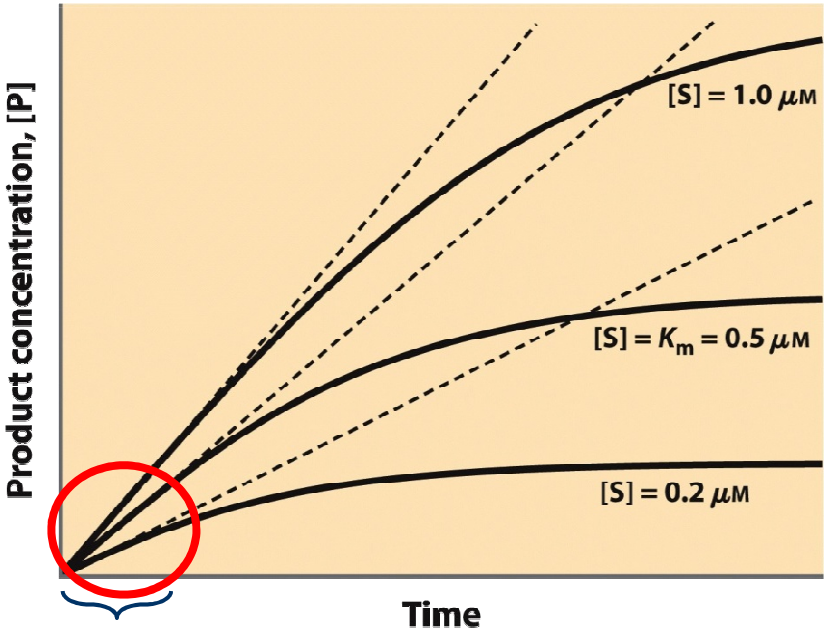


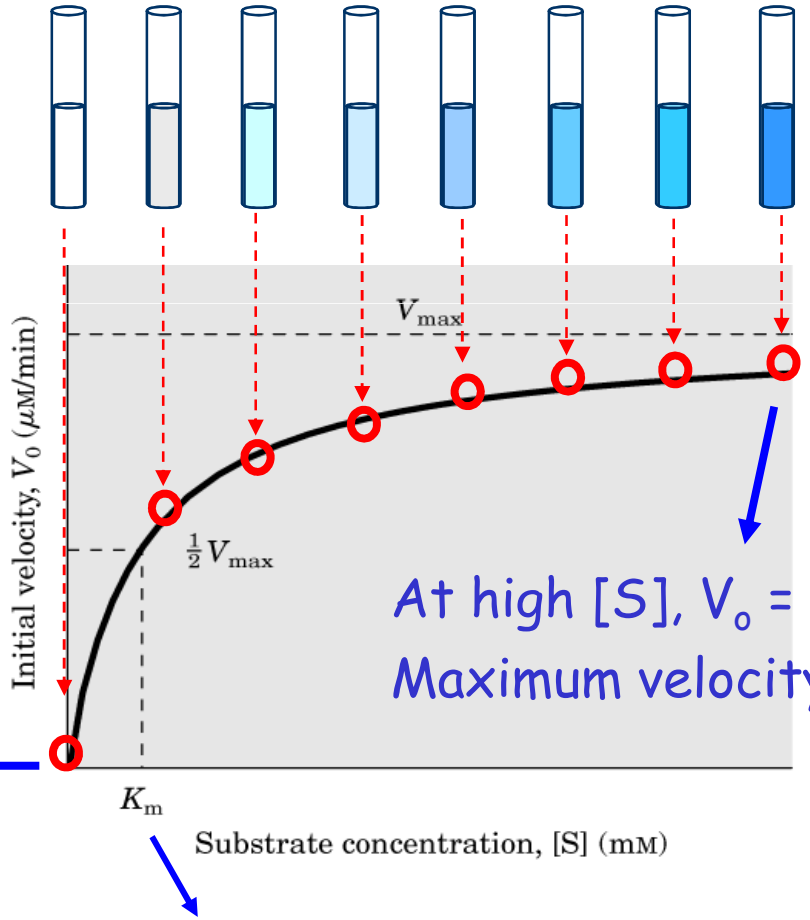

Fig 6-10, p.194

# Enzyme kinetics

- S $\overset{E}{\rightleftharpoons}$ P, measure the initial rate ($V_o$)
- Results:
  - Plot $V_o$ vs. [S]

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Michaelis-Menten equation




At high [S], $V_o = V_{max}$
Maximum velocity

At low [S], $V_o \propto [S]$

When $V_o = \frac{1}{2} V_{max}$, $[S] = K_m$

Fig 6-11, p.195

# Kinetic model

- [S], $V_o$, $V_{max}$, and $K_m$ can be determined by exp.
- Michaelis-Menten kinetics
- Steady-state kinetics
  - Before ES builds up: pre-steady state
  - After [ES] reaches const. : steady state

$$V_0 = \frac{V_{\max}[\mathrm{S}]}{K_{\mathrm{m}} + [\mathrm{S}]}$$

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{E} + \mathrm{P}$$

p. 195, (6-7, 6-8)

**fast** **Slow ← Rate limiting step**

# Steady-state kinetics (I)

p. 196

- Early in the reaction, [P] is negligible, and $k_{-2}$ is ignored for simplicity:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P \qquad (6\text{-}10)$$

- $V_o$ is determined by the breakdown of ES:

$$V_o = k_2[ES]$$

- At steady-state: [ES] constant
  - Rate of ES formation = Rate of ES breakdown

$$k_1[E][S]$$

$$k_1([E_t]-[ES])[S] = k_{-1}[ES] + k_2[ES]$$

# Steady-state kinetics (II)



- Rearrange: $k_1[E_t]\,[S] = (k_{-1} + k_2)[ES] + k_1[ES][S]$

$= (k_1[S] + k_{-1} + k_2)[ES]$

- Solve for [ES] $= k_1[E_t]\,[S]/(k_1[S] + k_{-1} + k_2)$

$= [E_t]\,[S]/([S] + \boldsymbol{(k_{-1} + k_2)/\, k_1})$

$= [E_t]\,[S]/([S] + \mathbf{K_m})$

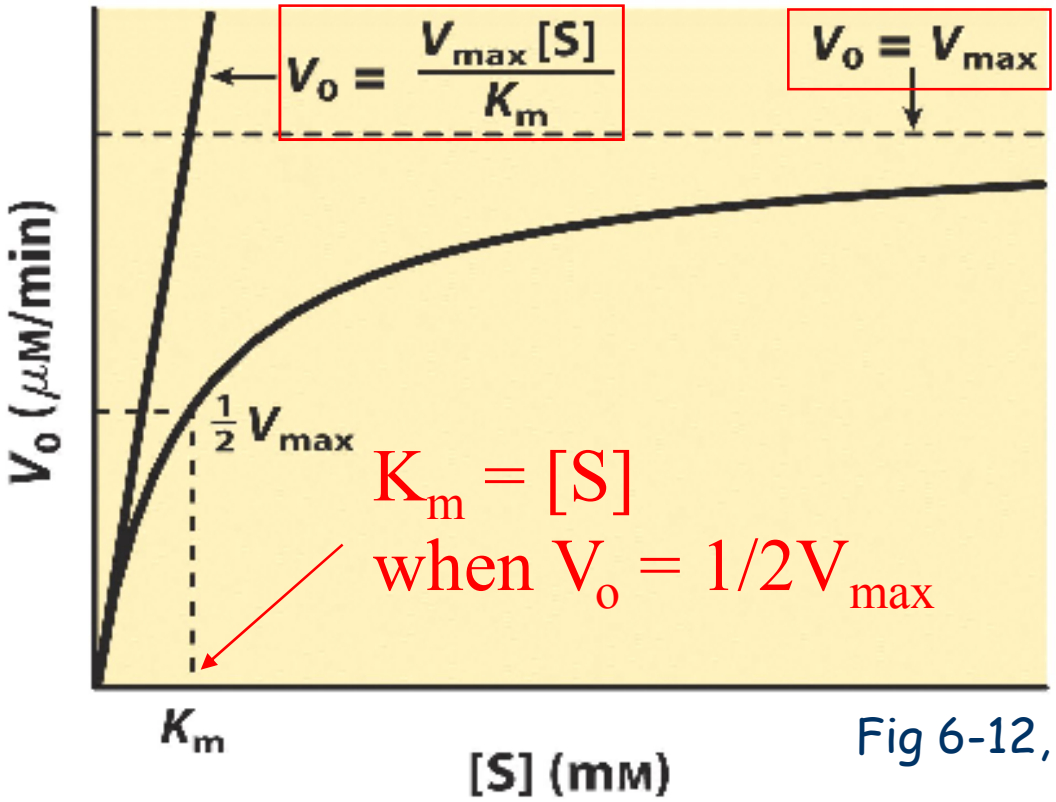
$V_o = k_2[ES] = k_2\,[E_t]\,[S]/([S] + K_m)$

- When [S] >> [E], $[E_t] = [ES]$, $V_{max} = k_2[E_t]$

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

$K_m$, the Michaelis constant

# Michaelis-Menten kinetics




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

Fig 6-12, p.197

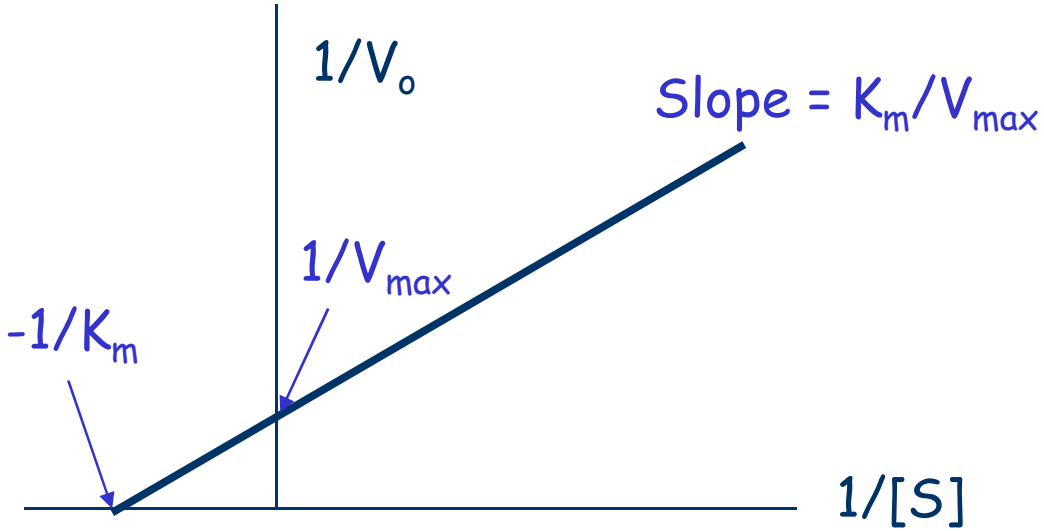
- $K_m$: Michaelis constant
  - The conc. of substrate that will produce $\frac{1}{2}V_{max}$.

# Lineweaver-Burk equation

$$\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

Plot 1/V vs. 1/[S]

- y-intercept: $1/V_{max}$
- x-intercept: $-1/K_m$
- Slope: $K_m/V_{max}$




**Double-reciprocal plot**

See Box 6-1, p.197

# Exercise

A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetic.

a) Please make a Michaelis-Menten plot.

b) Please make a Lineweaver-Burk plot (double reciprocal plot).

c) $V_{max}$ for the enzyme is _____.

d) $K_m$ for the enzyme is _____.

| Substrate conc. [S], μM | Initial velocity $V_o$ (μmole/min) |
|---|---|
| 1 | 49 |
| 2 | 96 |
| 8 | 349 |
| 50 | 621 |
| 100 | 676 |
| 1,000 | 698 |
| 5,000 | 699 |

# Rate constant: $k_{cat}$

p. 198

- The limiting rate of any enzyme-catalyzed reaction *at saturation.*

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightleftharpoons} E + P \qquad (6\text{-}10)$$

$$k_{cat} = k_2$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \overset{k_3}{\rightleftharpoons} E + P \qquad (6\text{-}25)$$

$$k_{cat} = k_3$$

# $k_{cat}$ = turnover number

p. 199

$V_{max} = k_{cat}[E_t]$ → Michaelis-Menten equ.

$$V_o = \frac{k_{cat}[E_t][S]}{K_m + [S]} \qquad (6\text{-}27)$$

$$k_{cat}\,(s^{-1}) = V_{max} / [E_t]$$

- First-order rate constant ($s^{-1}$) in M-M eq.
- Turnover number
  - The number of S → P in a given unit of time when the E is saturated with S.

# Specificity constant: $k_{cat}/K_m$

p. 199

- The rate constant for E+S $\rightarrow$ E+P.

$$V_o = \frac{k_{cat}[E_t][S]}{K_m + [S]} \qquad (6\text{-}27)$$

- When [S] << $K_m$:
  - $V_o \propto [E_t][S]$ ←second-order equation
  - $k_{cat}/K_m$ ← second-order rate constant ($M^{-1}s^{-1}$)
  - Used to compare different enzymes
  - Upper limit: $10^8$-$10^9$ $M^{-1}s^{-1}$, diffusion-controlled

# Reaction types

- Zero-order reaction (V ~ constant)
- First-order reaction (V ∝ [S])
- Second-order reaction (V ∝ $[S_1]$ and $[S_2]$ )

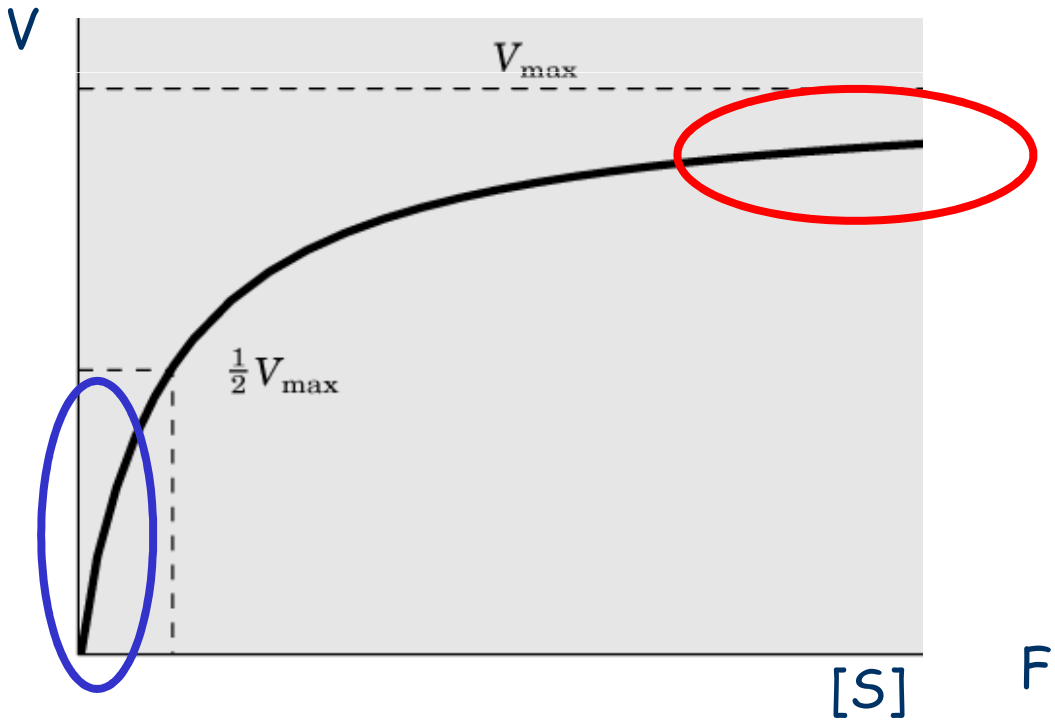
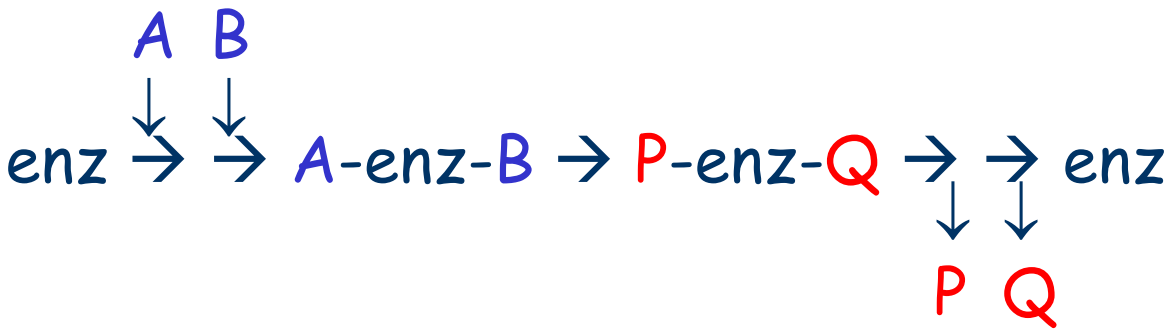



Fig 6-11

# Second-order reaction (I)



- $A + B \underset{}{\overset{E}{\rightleftharpoons}} P + Q$ (bi-substrate)
- **Single-displacement (sequential)** reaction
  - Ternary complex formation
  - Both substrates must bind to the enzyme before any products are released
  - The addition of A and B may be **ordered or random**, so is the release of products P and Q (Fig 6-13a, 6-14a)



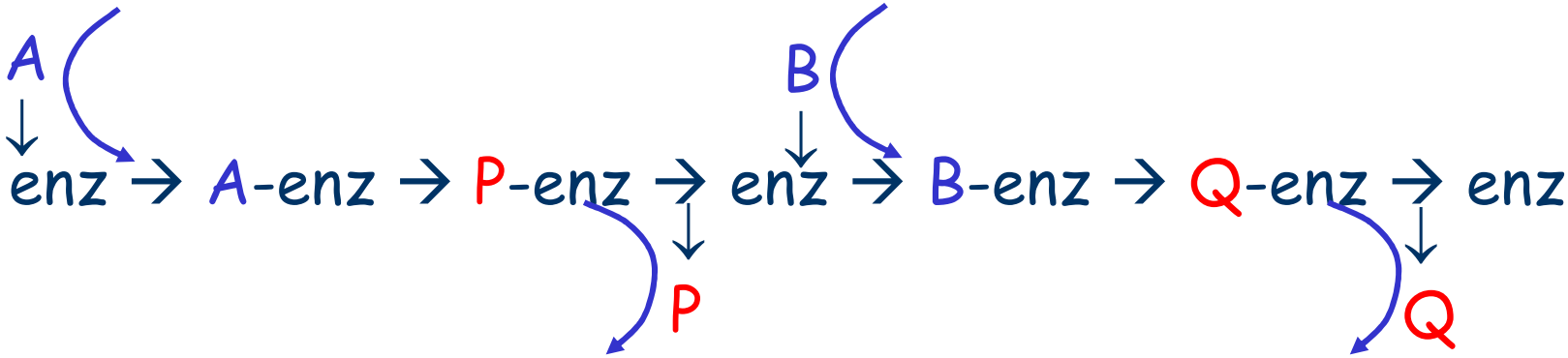

Compulsory order (Ordered Bi Bi)

Random order (Random Bi Bi)

# Second-order reaction (II)

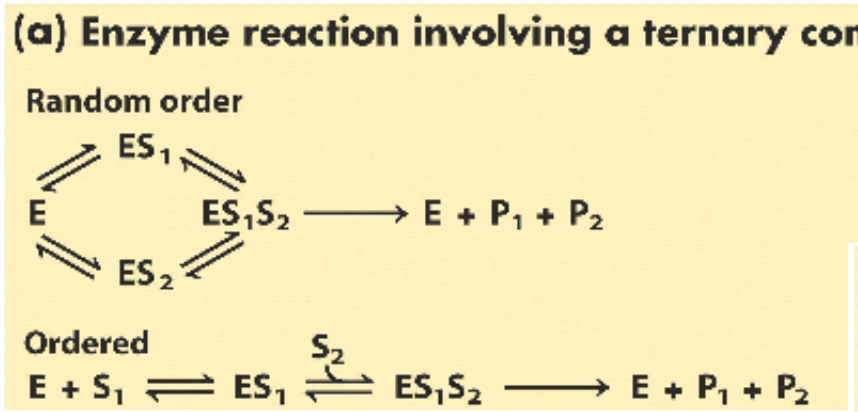


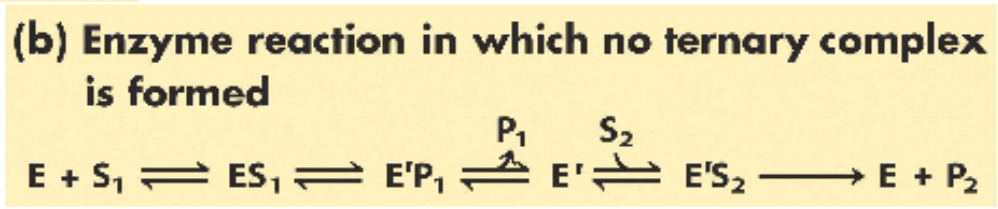
- $A + B \overset{E}{\rightleftharpoons} P + Q$ (bi-substrate)
- **Double-displacement** (***ping-pong***) reaction
  - One substrate binds to the enzyme and one product is released before the second substrate binds (no ternary complex formed) (Fig 6-13b, 6-14b)

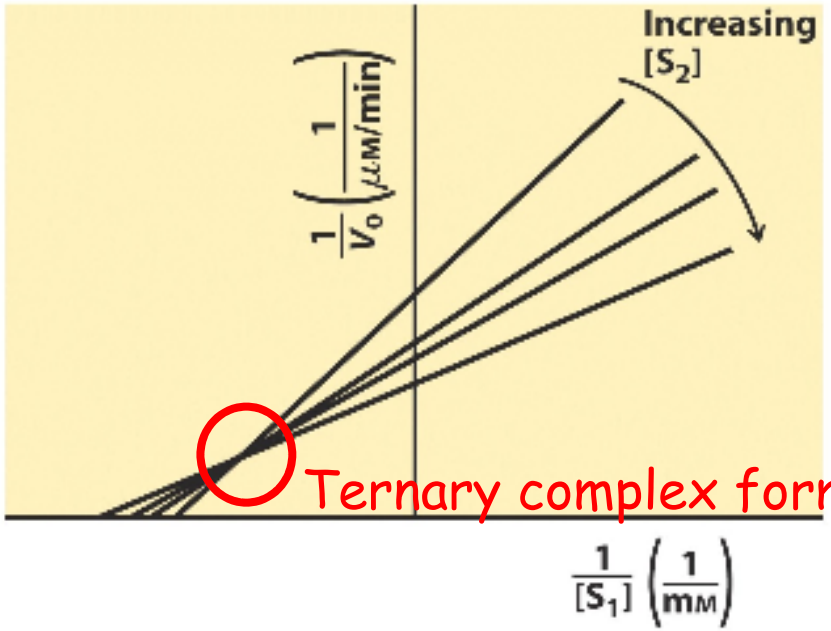


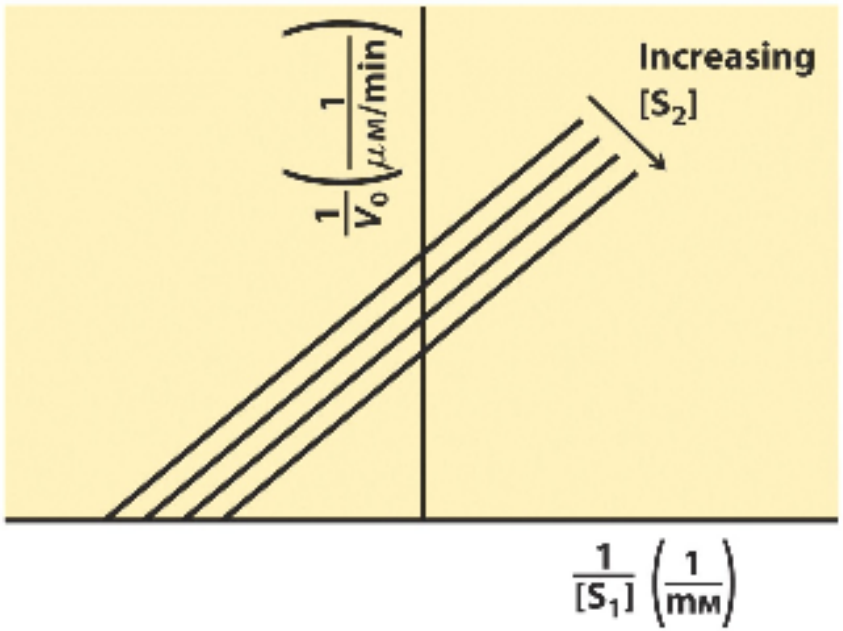
# Bisubstrate reactions

Fig 6-13, -14, p.200



# Enzyme and inhibitors

- Irreversible inhibition (p. 203)
  - Inhibitors bind and destroy the active sites
  - e.g. Nerve gas (DIFP) and ACE
    - ACE: acetylcholinesterase, catalyze the hydrolysis of acetylcholine (a neurotransmitter)
    - Chymotrypsin (Fig 6-16)
  - e.g. Asprin and prostaglandin synthet*ase*
    - Prostaglandin => pain …
  - Suicide or mechanism-based inactivators
    - Drug design
- Reversible inhibition (p. 201)
  - Competitive
  - Uncompetitive
  - Mixed

# Competitive inhibition

- Inhibitor (I) competes with S for the same active site on E to form EI
- I has similar structure as S

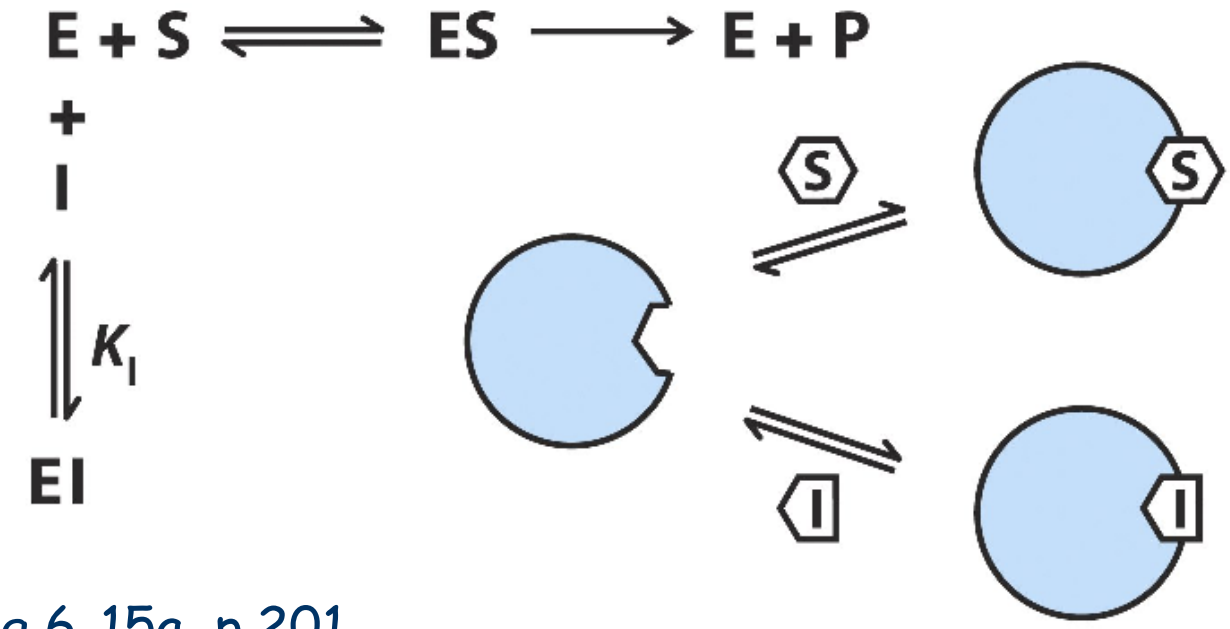
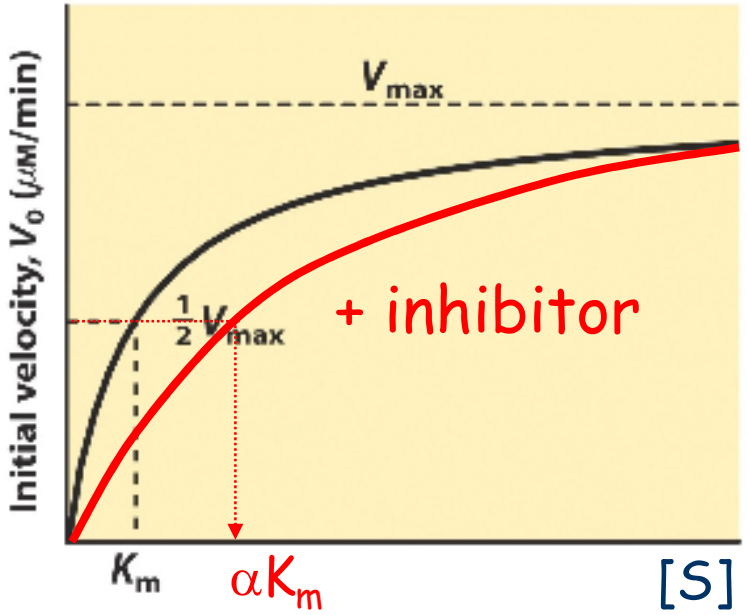


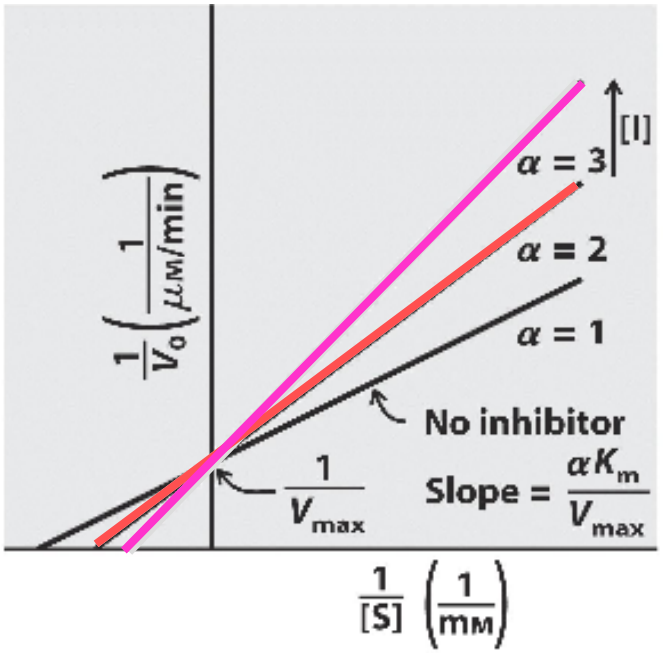

Fig 6-15a, p.201

# Competitive inhibition

In presence of a competitive inhibitor, [E] constant

- $V_{max}$ unchanged, $K_m$ increase




$$V_o = \frac{V_{max}[S]}{\alpha K_m + [S]} \qquad (6\text{-}28)$$

Apparent $K_m$ (exp. determined)

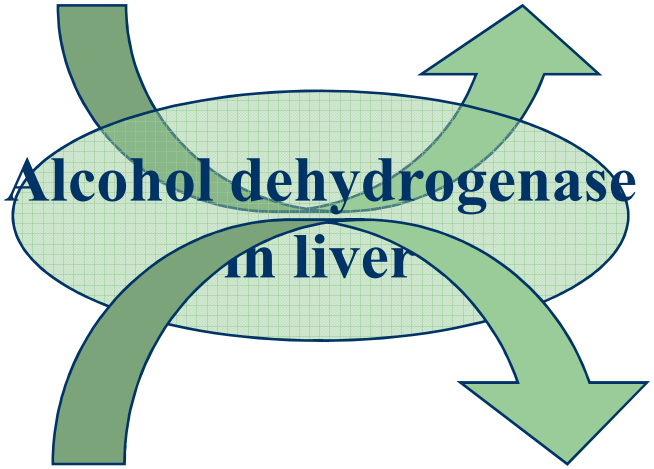
Box 6-2, Fig 1, p.202

# Competitive inhibition

- Medical application

Methanol → Formaldehyde → Tissue damage

e.g. blindness




Ethanol → Acetaldehyde

$CH_3CH_2OH + NAD^+ \rightarrow CH_3CHO + NADH + H^+$

Acetaldehyde dehydrogenase

- Mitochondrial: low $K_m$
- Cytosolic: high $K_m$

$CH_3COO^- + NADH + 2H^+$

# Uncompetitive inhibition

- Inhibitor (I) binds to a different site from S
- I binds ES complex to form ESI

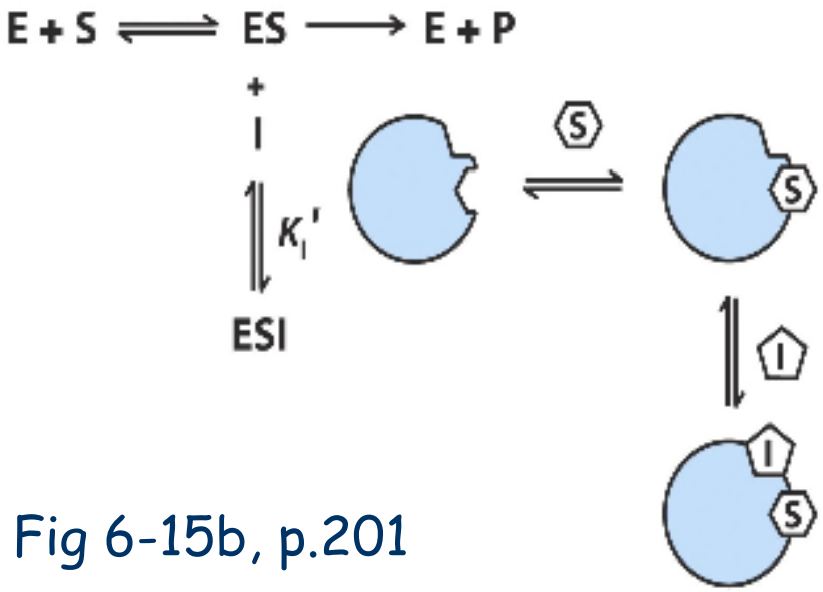
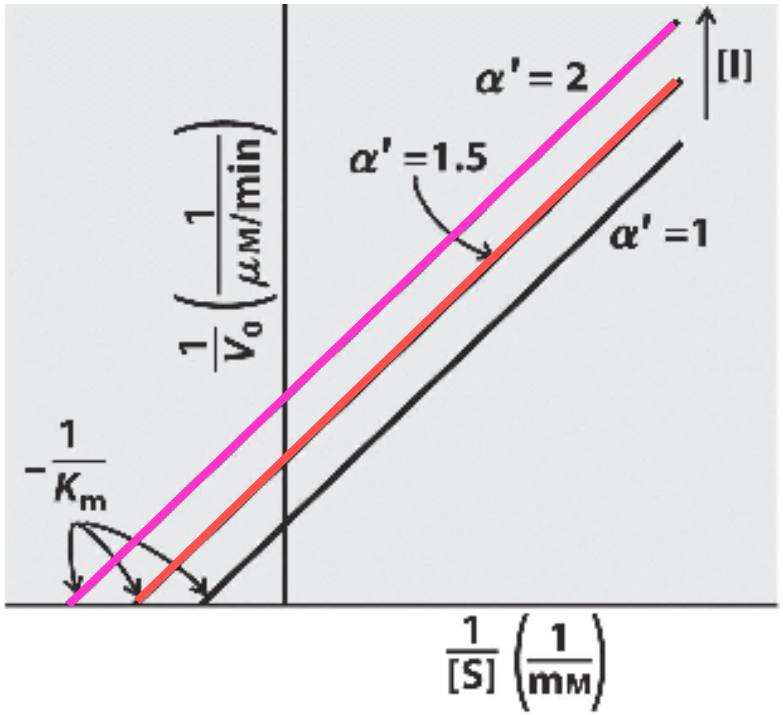



Fig 6-15b, p.201




Box 6-2, Fig 2, p.202

- Both $K_m$ and $V_{max}$ change ( ↓ )
- Parallel lines

# Mixed inhibition

- Inhibitor (I) binds a different site from S
- I binds both E and ES
  - Noncompetitive inhibition (a special case)

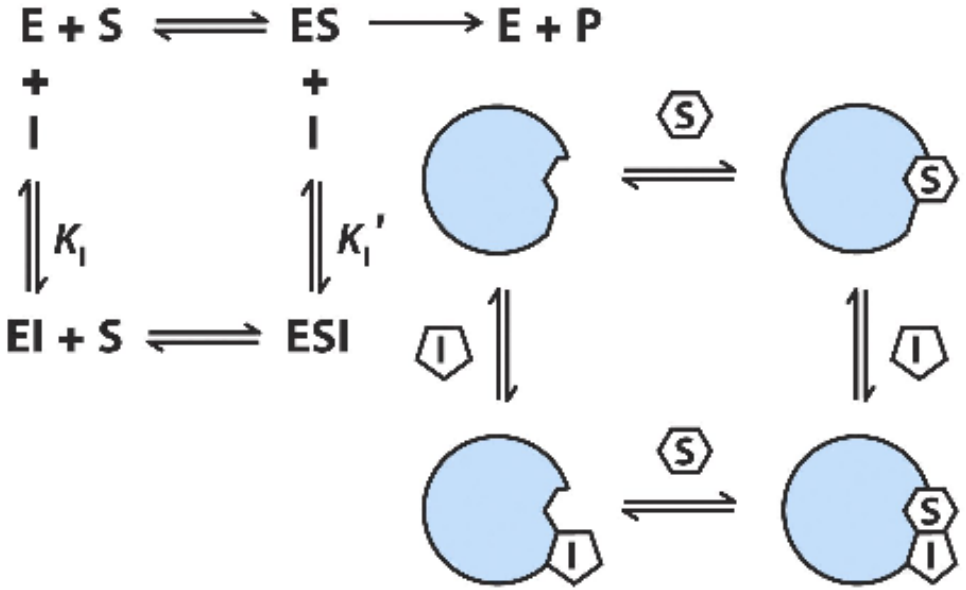
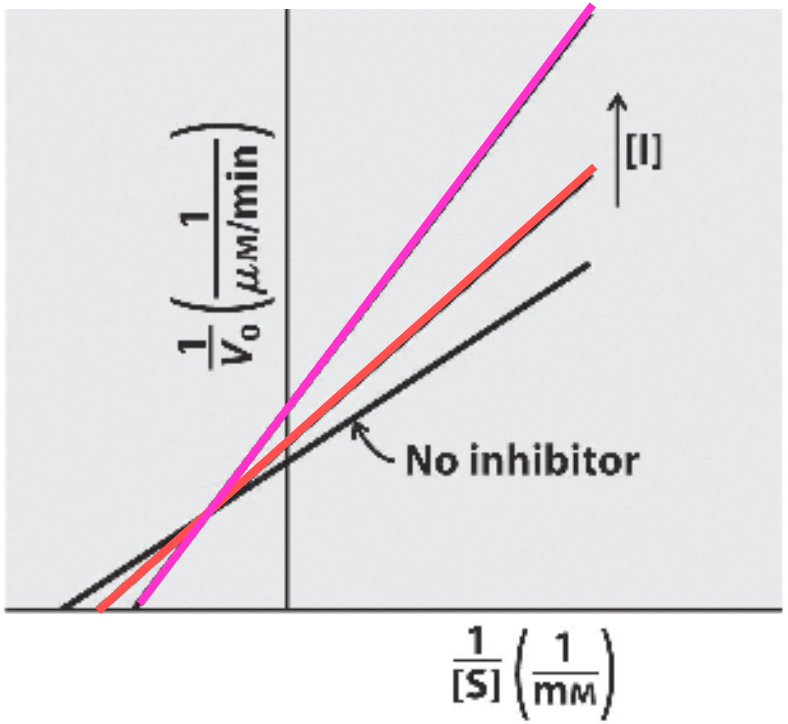


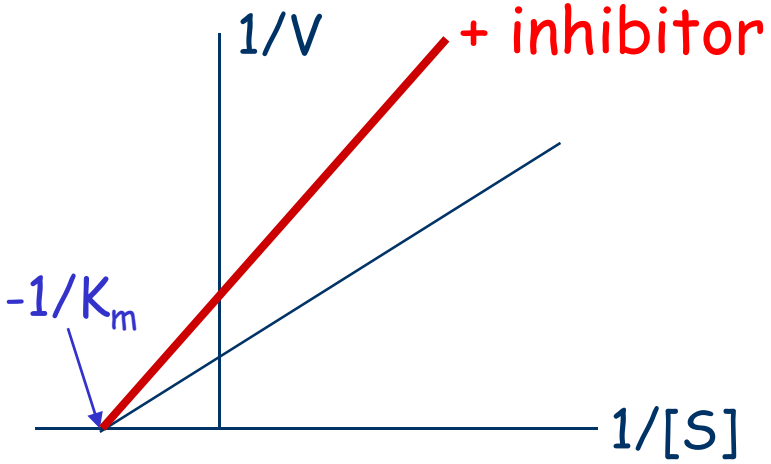

Fig 6-15c, p.201




Box 6-2, Fig 3, p.202

# Non-competitive inhibition

In presence of a non-competitive inhibitor

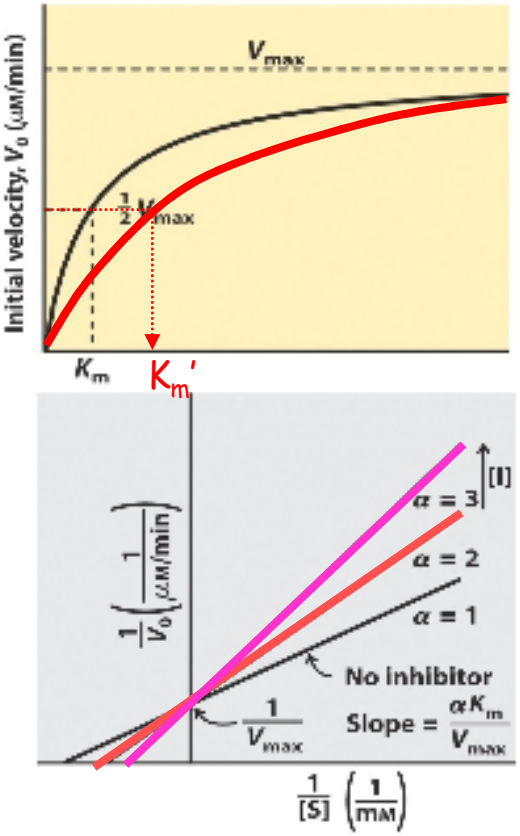



- A special case of mixed inhibition
- $K_m$ unchanged, $V_{max}$ decreased

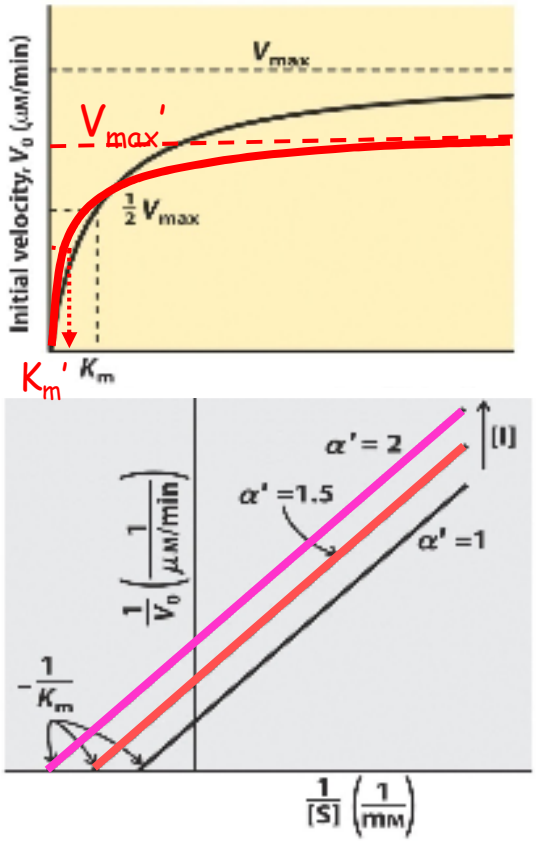
p.203

# Reversible inhibition

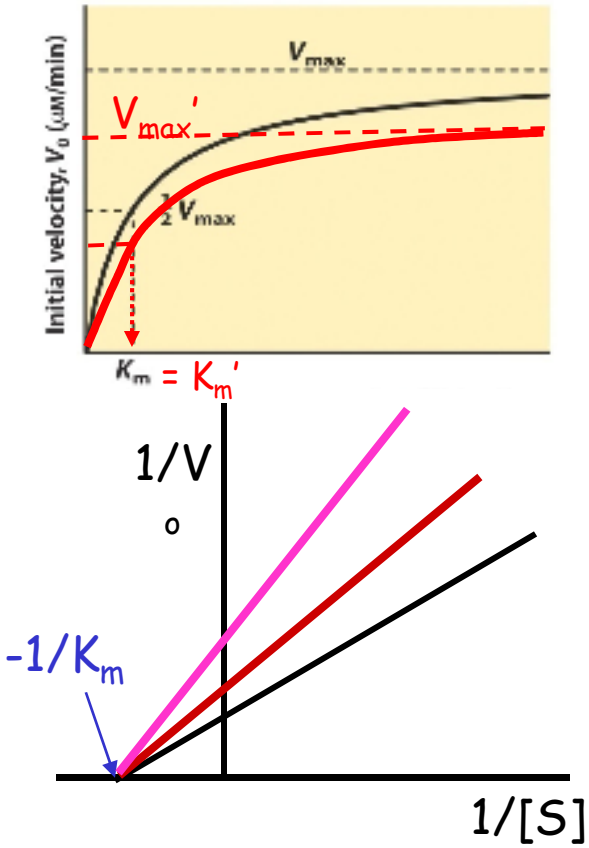
- Competitive, Uncompetitive, Noncompetitive




$V_{max}$ unchanged, $K_m$ ↑




$V_{max}$ and $K_m$ ↓




$V_{max}$ ↓ , $K_m$ unchanged

# Table 6-9, p.203

- Effects of reversible inhibitors on apparent $V_{max}$ and $K_m$

| Inhibitor type | Apparent $V_{max}$ | | Apparent $K_m$ | |
|---|---|---|---|---|
| None | $V_{max}$ | | $K_m$ | |
| Competitive | $V_{max}$ | – | $\alpha K_m$ | ↑ |
| Uncompetitive | $V_{max}/\alpha'$ | ↓ | $K_m/\alpha'$ | ↓ |
| Mixed | $V_{max}/\alpha'$ | | $\alpha K_m/\alpha'$ | |
| Non-competitive | $V_{max}/\alpha'$ | ↓ | $K_m$ | – ($\alpha=\alpha'$) |

# Regulatory enzymes (I), p.220

- Allosteric enzyme (p. 220…)
  - Conformational change (Fig 6-31)
  - non-covalent modification
    - Homotropic: substrate = modulator,
      - e.g. $O_2$ binding of Hb
    - Heterotropic: substrate ≠ modulator
      - e.g. feedback inhibition (Fig 6-33)

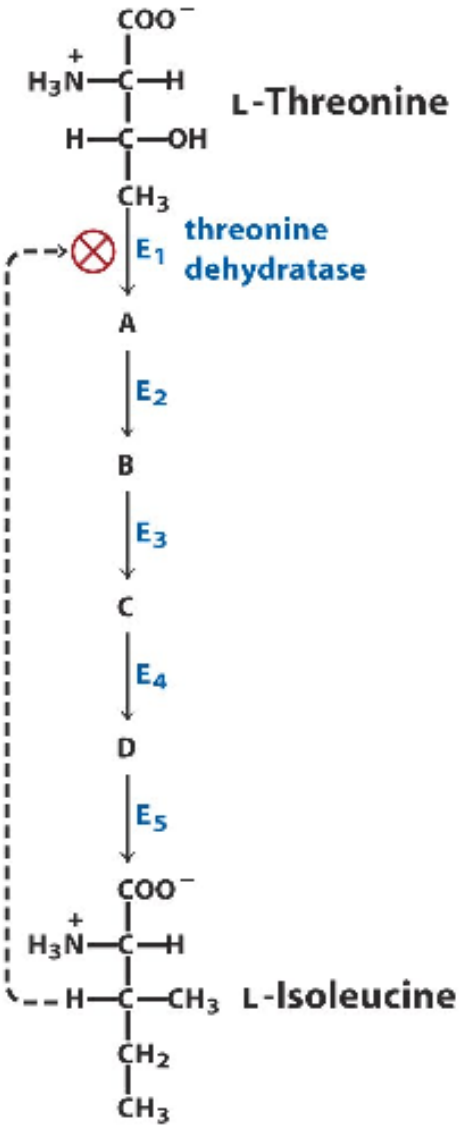


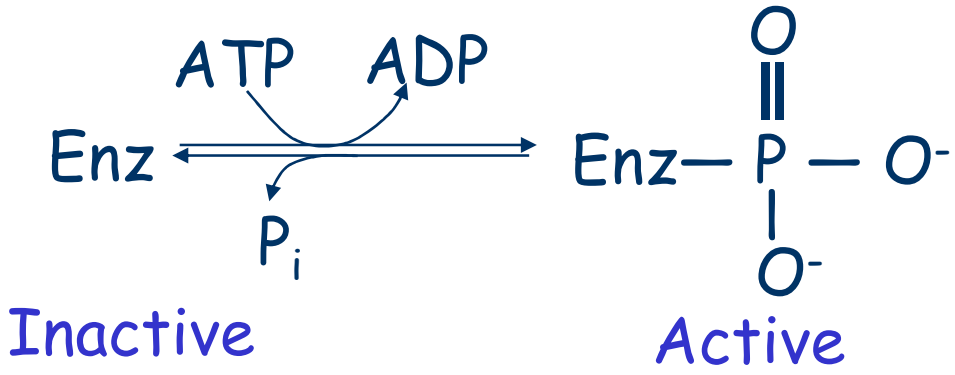

Fig 6-33, p.221

$E_1$ is an allosteric enzyme:
S = Thr, M = Ile

# Regulatory enzymes (II)

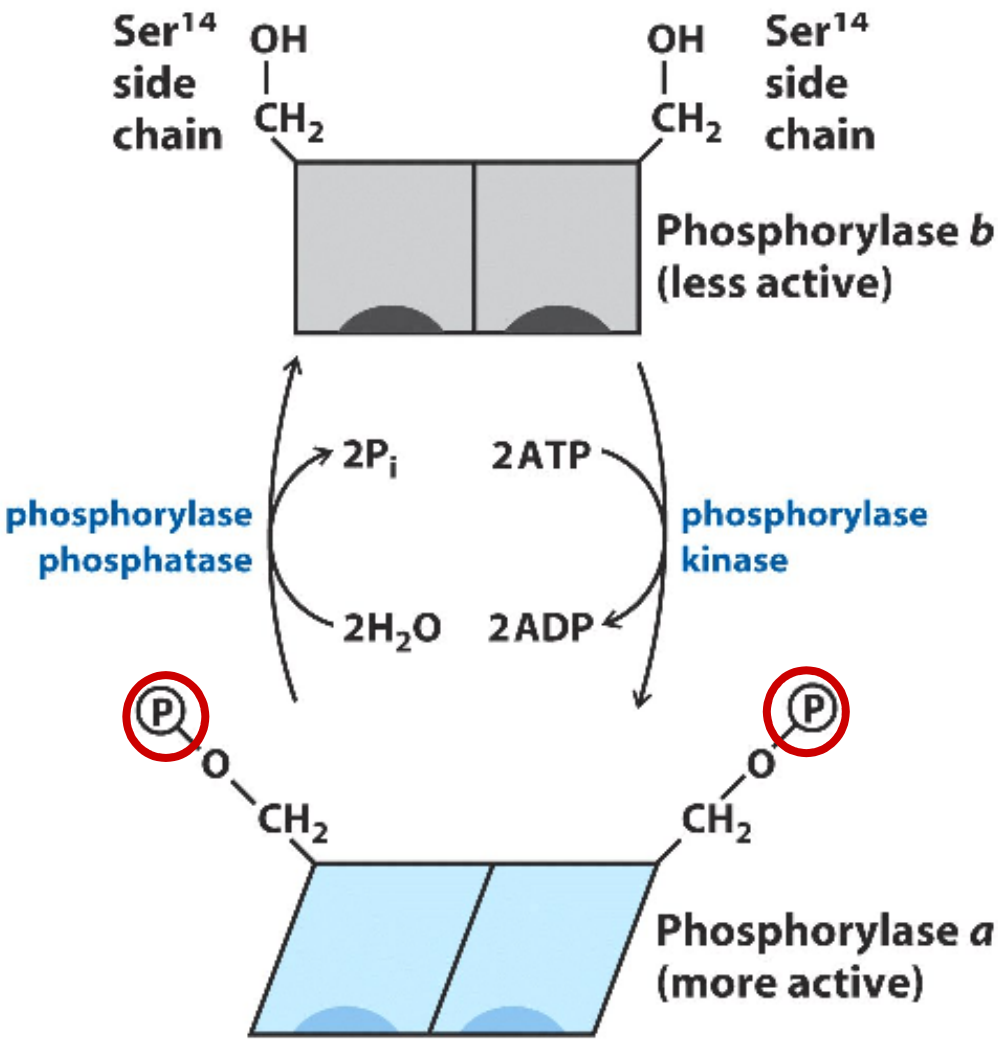
- Covalent modification (p. 223…)
  - All-or-none (Fig 6-30)
    - Reversible
    - e.g. phosphorylation/dephosphorylation (Fig 6-35)

Fig 6-35 (1)



# Phosphoryl group vs. Enz. activity

- Phosphorylation/dephosphorylation
- kinase/phosphatase




4th ed. Fig 6-31

Or 5th ed. Fig 6-36, p.224, central part

# Glycogen phosphorylase in muscle

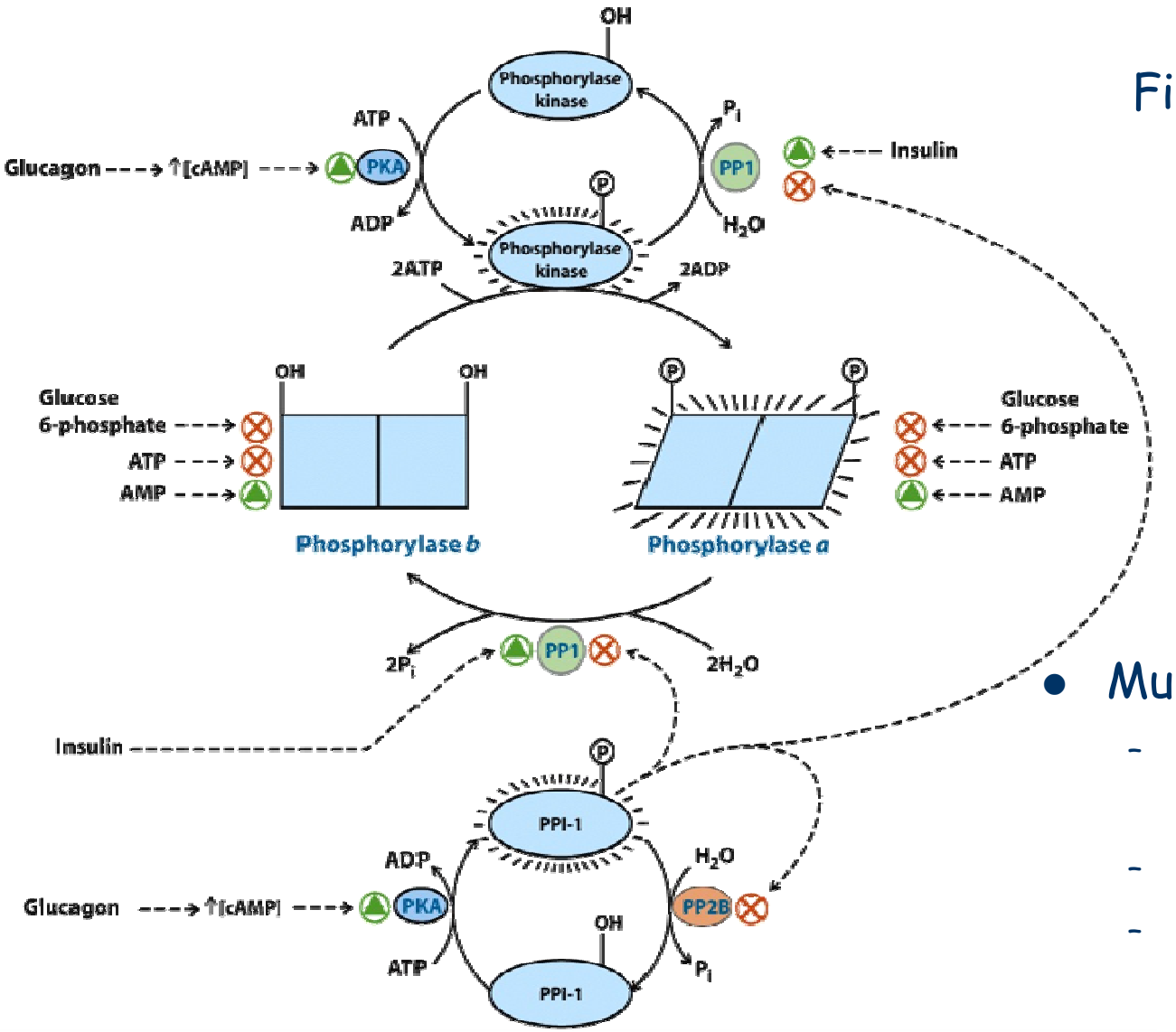


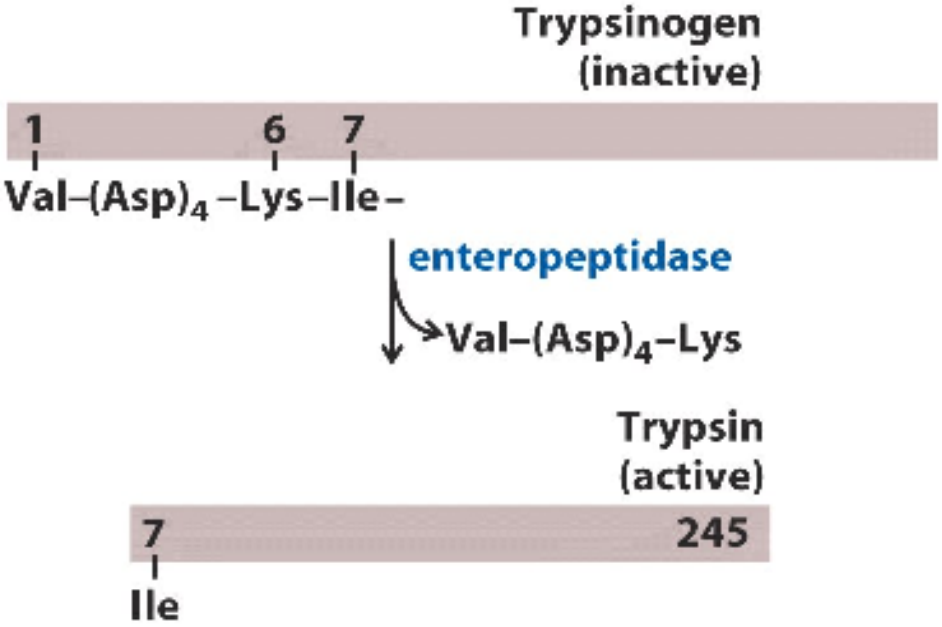

Fig 6-36, p.224

- Multiple regulations
  - Covalent modification
    - Phosphorylation
  - Allosteric regulation
  - Regulatory cascade
    - Hormonal level

# Regulatory enzymes (III)

- Polypeptide cleavage (p.226-7)
  - Inactive form → active form
    - e.g. chymotrypsinogen → chymotrypsin
    - e.g. trypsinogen → trypsin
  - Inactive precursor: zymogen, proenzyme, proprotein
  - Irreversible activation ➔ inactivated by inhibitors

p. 227, Fig 6-38, right



# Summary

- Energetics
- Kinetics
  - Michaelis-Menten equation and plot
  - Lineweaver-Burk equation and plot (double-reciprocal)
  - $V_o$, $V_{max}$, $K_m$, $k_{cat}$ (turnover number), $k_{cat}/K_m$
  - Reaction type
- Inhibition
  - Reversible [competitive, uncomp., mixed (non-competitive)]
  - Irreversible
- Regulation
  - Allosteric enzyme (homotropic, heterotropic)
  - Covalent modification
  - Polypeptide cleavage
- Problems: 8, 10, 16