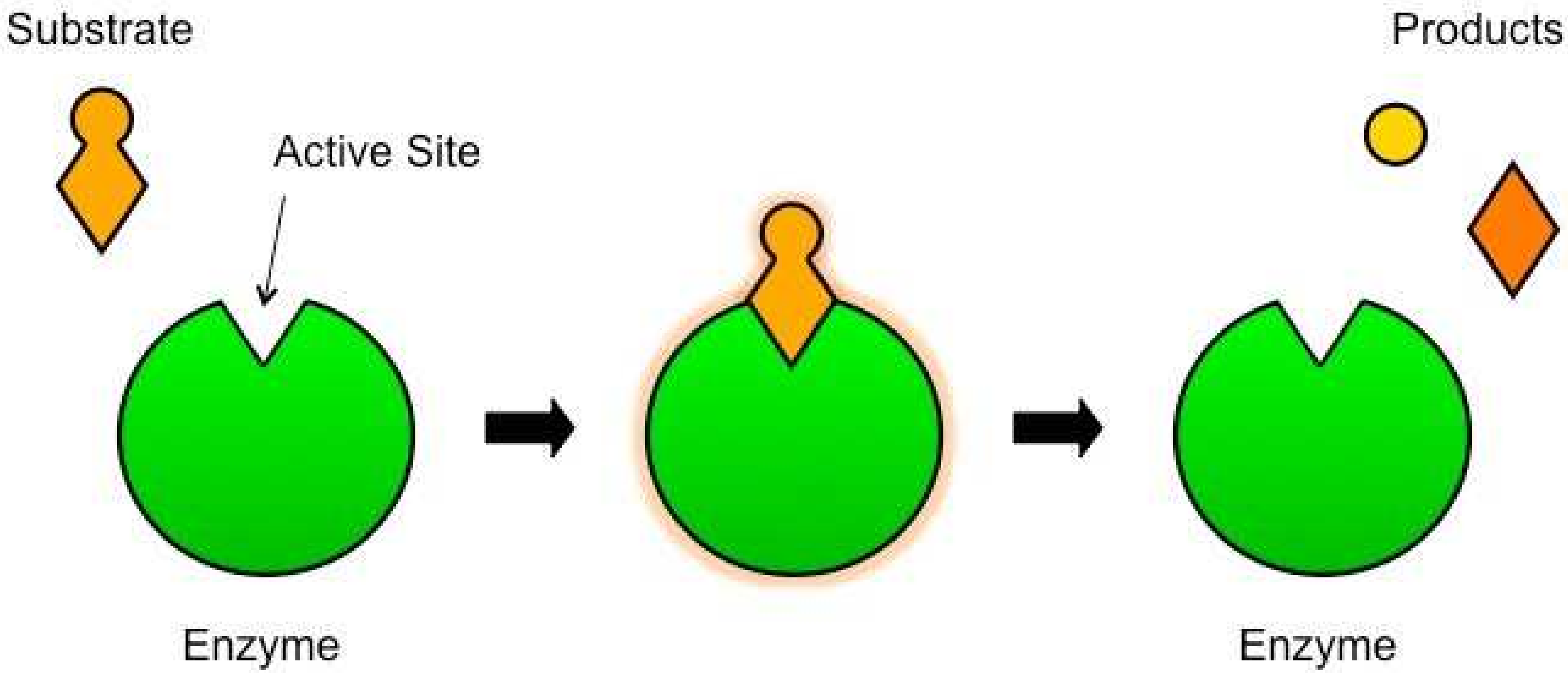
Enzyme reactivity and inhibition

Today's goal

- Derive and understand the Michaelis-Menten eq.
- Effect of (reversible) inhibitions on the rate of enzyme reactions
- Understand how biochemical mechanisms can be represented by mathematical models

Enzyme reactivity

- Works in lock-and-key fashion



#1

Modeling enzyme reactions

Step 1: The free enzyme (E) reacts with substrate (S) to form an enzyme-substrate complex (ES)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$$

Step 2: The enzyme-substrate complex (ES) breaks down to form free enzyme and products (P)

$$ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Assumptions

1) *The total concentration of enzyme ($[E]_{total}$) in the system is constant*
2) *Step 2 is essentially irreversible*
3) *[ES] does not change over time (pseudo-steady state)*

Use assumption 2) & 3):

rate of ES-producing reactions = rate of ES-consuming reactions

$$k_1[E][S] = k_{-1}[ES] + k_2[ES] \quad \Rightarrow \quad [E] = K_M \frac{[ES]}{[S]}$$

where $K_M = \dfrac{k_{-1} + k_2}{k_1}$

The rate of enzyme reaction (v)

= The rate of the reaction to produce "P"

(step 2 forward reaction)

$$v = k_2[ES]$$

Maximum "v" achievable in the system (v_m):

$$v_m = k_2[E]_{total}$$

Mass balance for enzyme in the system

$$[E]_{total} = [E] + [ES]$$

$[E]_{total}$ is constant by assumption 1)

Compare v and v_m:

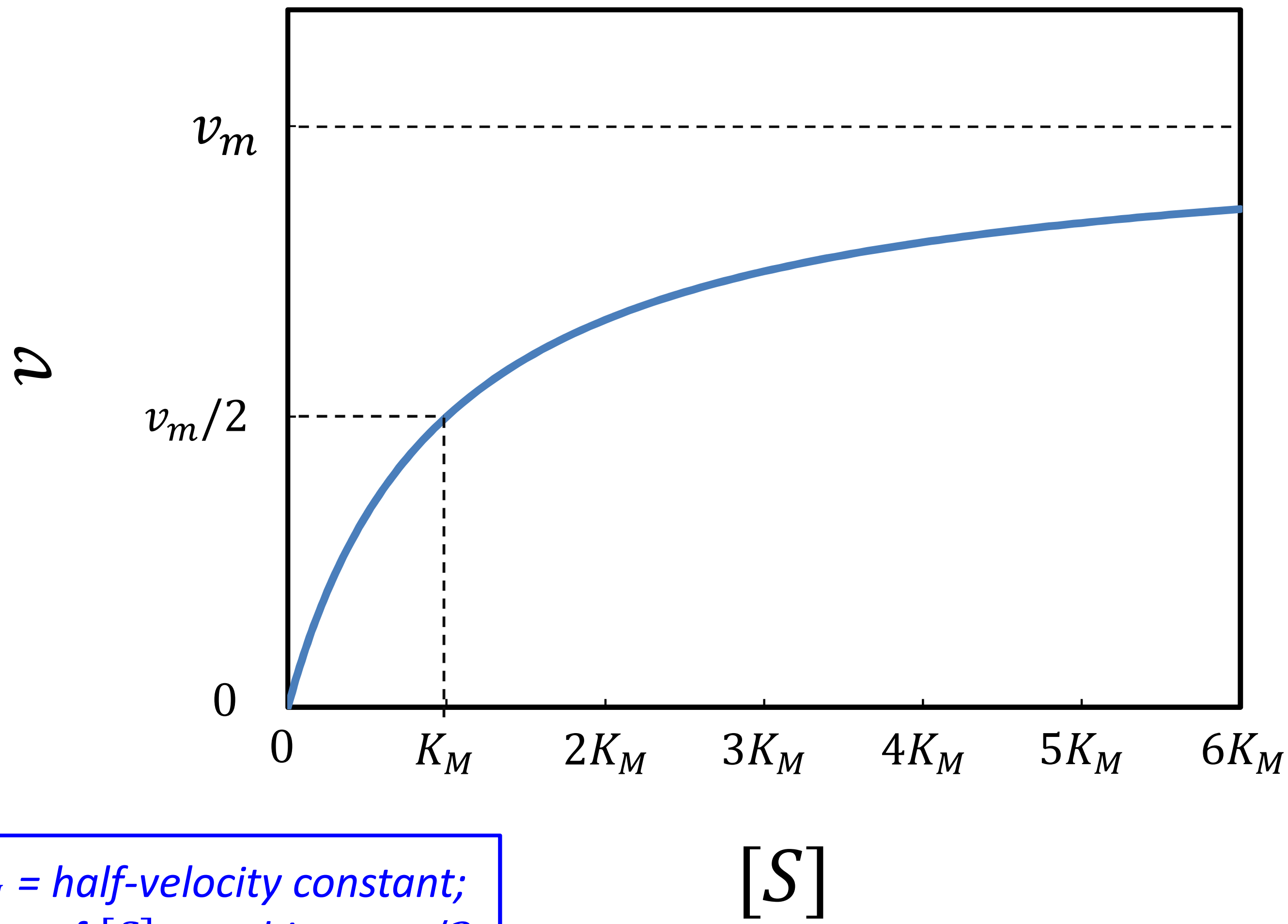
$$\frac{v}{v_m} = \frac{k_2[ES]}{k_2[E]_{total}} = \frac{[ES]}{[E]_{total}} = \frac{[ES]}{[E] + [ES]} = \frac{[ES]}{K_M \frac{[ES]}{[S]} + [ES]} = \frac{[S]}{K_M + [S]}$$

Finally we get:

$$v = v_m \frac{[S]}{K_M + [S]}$$

Michaelis-Menten equation

M-M eq.: [*S*] vs. *v*



K_M = *half-velocity constant;*
value of $[S]$ *to achieve* $v_m/2$

Inhibition of enzyme reactions

- Chemical agents can reduce the activity of an enzyme by binding to it

- Reversible vs. irreversible inhibition
  - Reversible inhibition
    - An inhibitor binds to enzymes with non-covalent interactions
    - The effect of inhibition disappears when the inhibitor is removed
  - Irreversible inhibition
    - An inhibitor binds to enzymes with covalent interactions
    - The enzymes are made permanently inactive

Reversible inhibitions

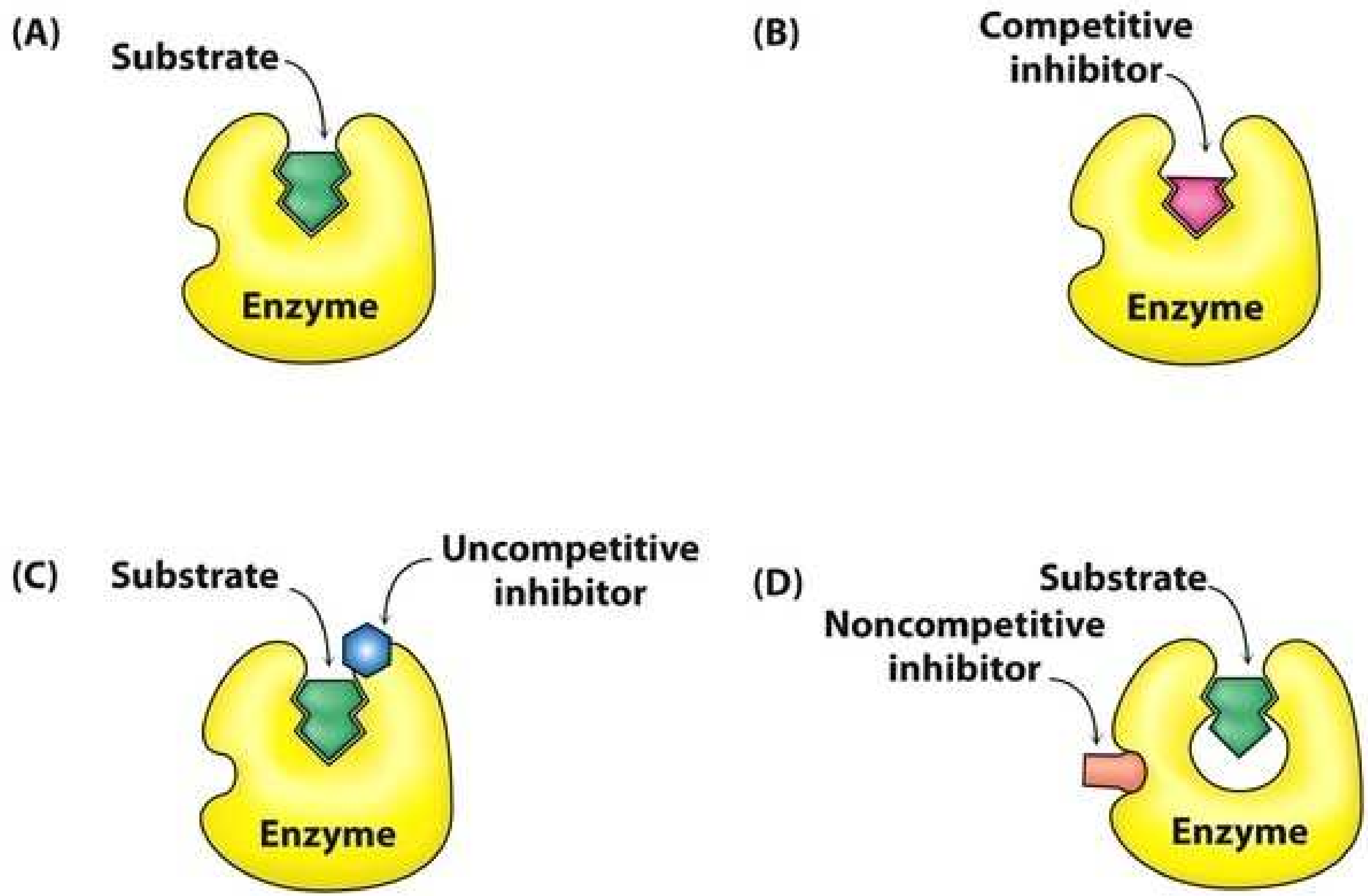


Figure 8.14
Biochemistry, **Seventh Edition**

Types of reversible inhibition (1)

1) Competitive inhibition

- **E** (free enzyme) binds to **I** (inhibitor)
- max. velocity unchanged; half-saturation const. increased

Competitive Inhibition: Derivation

Now we have

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \qquad \& \qquad E + I \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} EI$$

$$ES \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} E + P$$

Here we again assume [ES] = constant, so

rate of ES-producing reactions = rate of ES-consuming reactions

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

$$[E] = K_M \frac{[ES]}{[S]}$$

$$E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI$$

As we did for [ES], we assume [EI] = constant, so

$$k_3[E][I] = k_{-3}[EI]$$

$$[EI] = \frac{k_3}{k_{-3}}[E][I] = \frac{k_3}{k_{-3}}\left(K_M\frac{[ES]}{[S]}\right)[I] = \frac{K_M}{K_I}\left(\frac{[ES]}{[S]}\right)[I]$$

where $K_I = \frac{k_{-3}}{k_3}$

From enzyme mass balance

$$[E]_{total} = [E] + [ES] + [EI]$$

$$= K_M\frac{[ES]}{[S]} + [ES] + \frac{K_M}{K_I}\left(\frac{[ES]}{[S]}\right)[I]$$

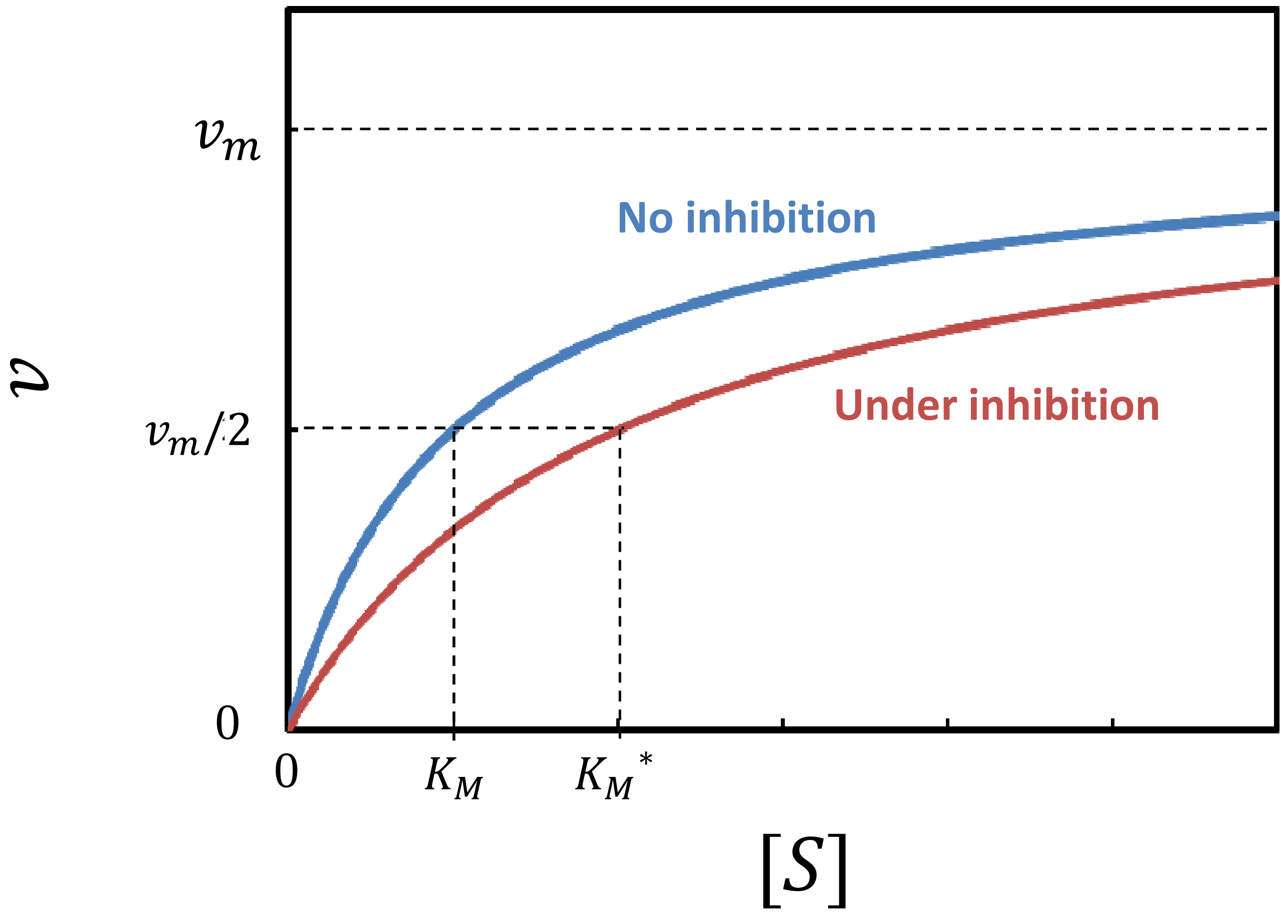
Comparing v and v_m:

$$\frac{v}{v_m} = \frac{[ES]}{[E]_{total}} = \frac{[ES]}{[E] + [ES] + [EI]} = \frac{[ES]}{K_M \frac{[ES]}{[S]} + [ES] + \frac{K_M}{K_I}\left(\frac{[ES]}{[S]}\right)[I]}$$

$$= \frac{[S]}{\left(1 + \frac{[I]}{K_I}\right) K_M + [S]}$$

$$= \frac{[S]}{{K_M}^* + [S]}$$

Effect of competitive inhibition



Types of reversible inhibition (2)

2) Uncompetitive inhibition

- **I** binds only to **ES** (enzyme-substrate complex)
- max. velocity decreased; half-saturation const. decreased

3) Noncompetitive inhibition

- **I** binds to both **ES** and **E**
- max. velocity decreased; half-saturation const. unchanged

4) Product inhibition

- **P** (product) binds to **ES**
- max. velocity decreased; half-saturation const. decreased

References

#1) https://ib.bioninja.com.au/standard-level/topic-2-molecular-biology/25-enzymes/enzyme--substrate.html

#2) Berg, J. M., Tymoczko, J. L., Stryer, L. (2010) Biochemistry, 7th ed. W.H. Freeman & Company.