Enzymes

王春光

chunguangwang@tongji.edu.cn

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Learning objectives:

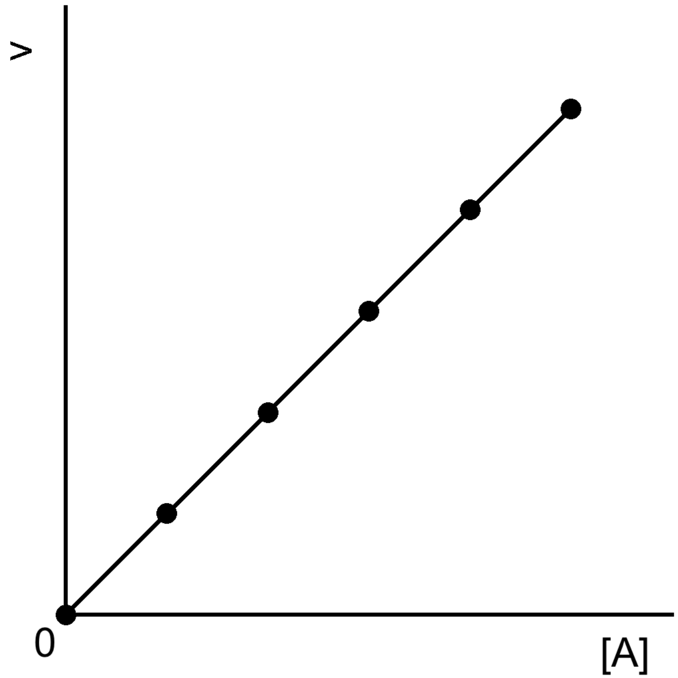
- Memorize the Michaelis-Menten equation;
- Interpret the meaning of K_m and k_{cat};
- Apply the equation to predict the expected reaction rate.

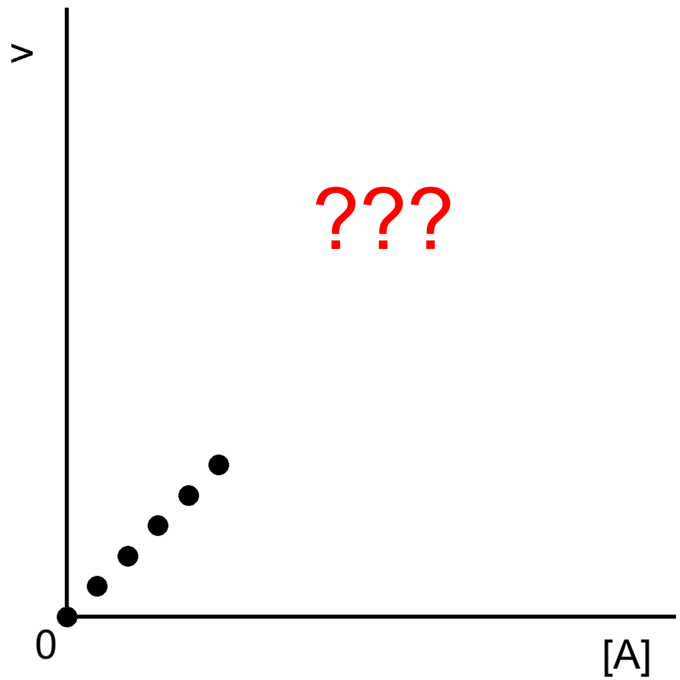
$A \rightarrow P$

$A \xrightarrow{\text{Enzyme}} P$

$$v = \frac{d[\mathrm{P}]}{dt} = k[\mathrm{A}]$$

$$v = \frac{d[\mathrm{P}]}{dt} = ??[\mathrm{A}]$$





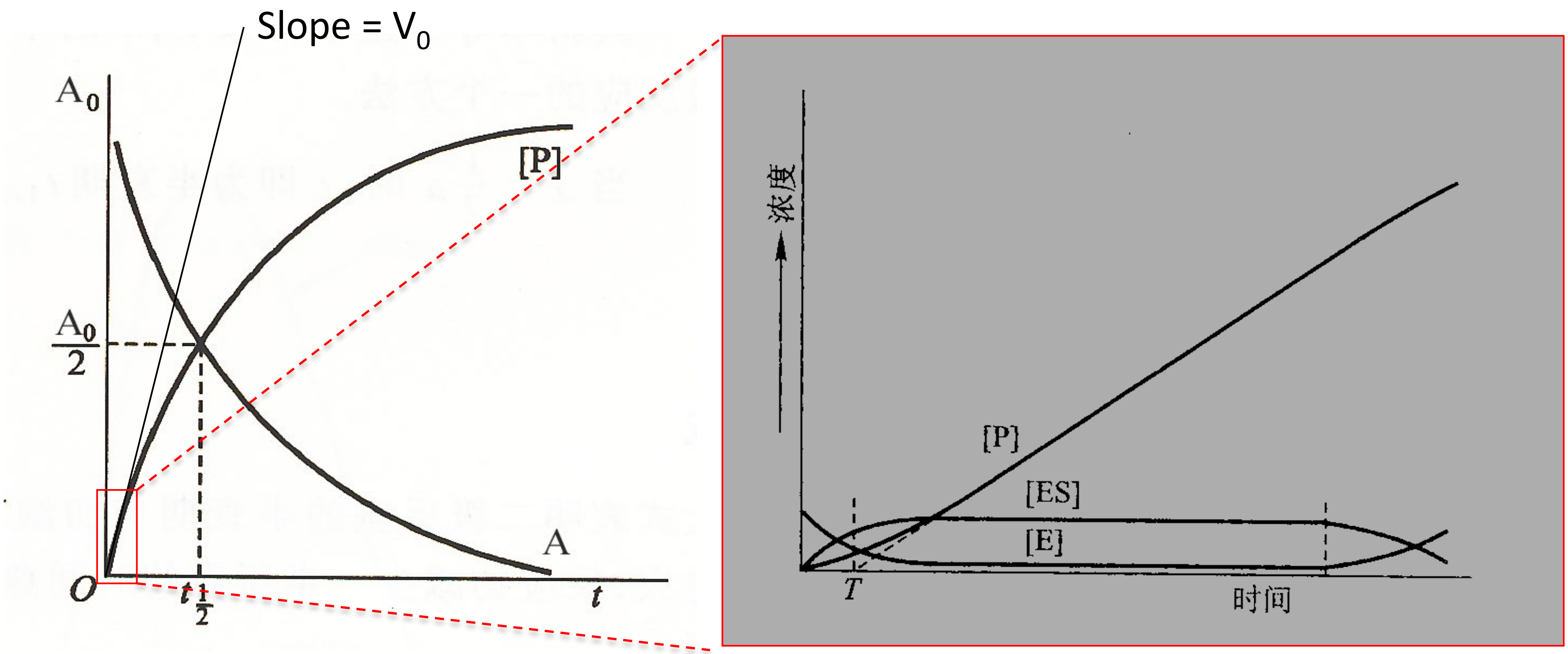
$$A \xrightarrow{\text{Enzyme}} P$$

$$S + E \rightarrow P + E$$

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

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

$$v = \frac{d[\mathrm{P}]}{dt} = k_2[\mathrm{ES}] = ??[\mathrm{S}]$$



complete reaction initial period

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

$$\boxed{\text{Steady state:} \quad \frac{d[\mathrm{ES}]}{dt} = 0}$$

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

In most cases, $[S]_0 >> [E]$, then $[S]_0 - [ES] \approx [S]_0$

$$k_1 \cdot [\mathrm{S}] \cdot ([\mathrm{E}]_0 - [\mathrm{ES}]) = k_2 \cdot [\mathrm{ES}] + k_{-1} \cdot [\mathrm{ES}]$$

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

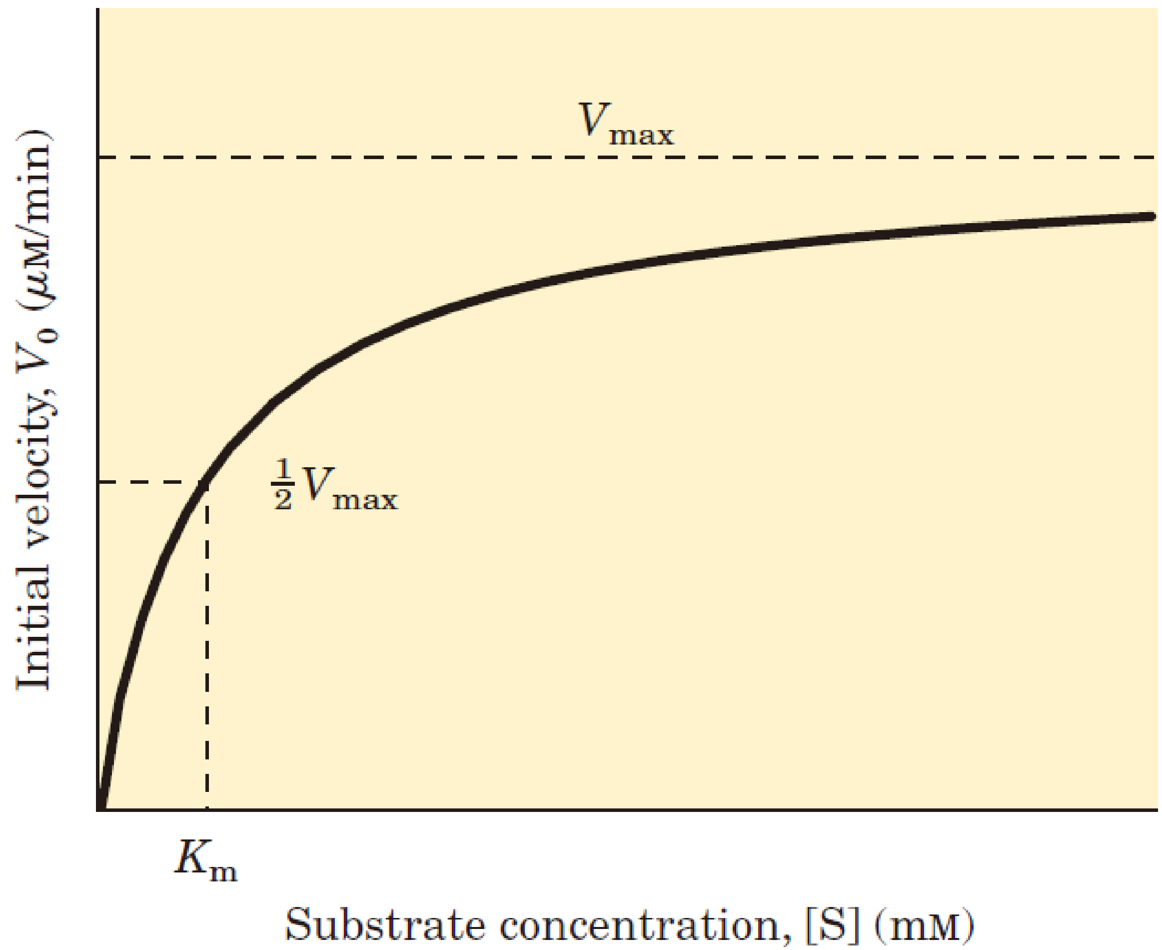
$$[\mathrm{ES}] = \frac{[\mathrm{S}]\cdot[\mathrm{E}]_0}{[\mathrm{S}] + \dfrac{k_{-1}+k_2}{k_1}}$$

$$\text{Let } \frac{k_{-1}+k_2}{k_1} = K_\mathrm{m}, \qquad [\mathrm{ES}] = \frac{[\mathrm{S}]\cdot[\mathrm{E}]_0}{[\mathrm{S}]+K_\mathrm{m}}$$

$$v = k_2\cdot[\mathrm{ES}] = \frac{k_2\cdot[\mathrm{E}]_0\cdot[\mathrm{S}]}{K_\mathrm{m}+[\mathrm{S}]}$$

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

Michaelis-Menten equation: $$v = \frac{V_{\max} \cdot [S]}{K_m + [S]}$$



Michaelis-Menten equation: $$v = \frac{V_{\max} \cdot [S]}{K_m + [S]}$$

When [S] << K_m,

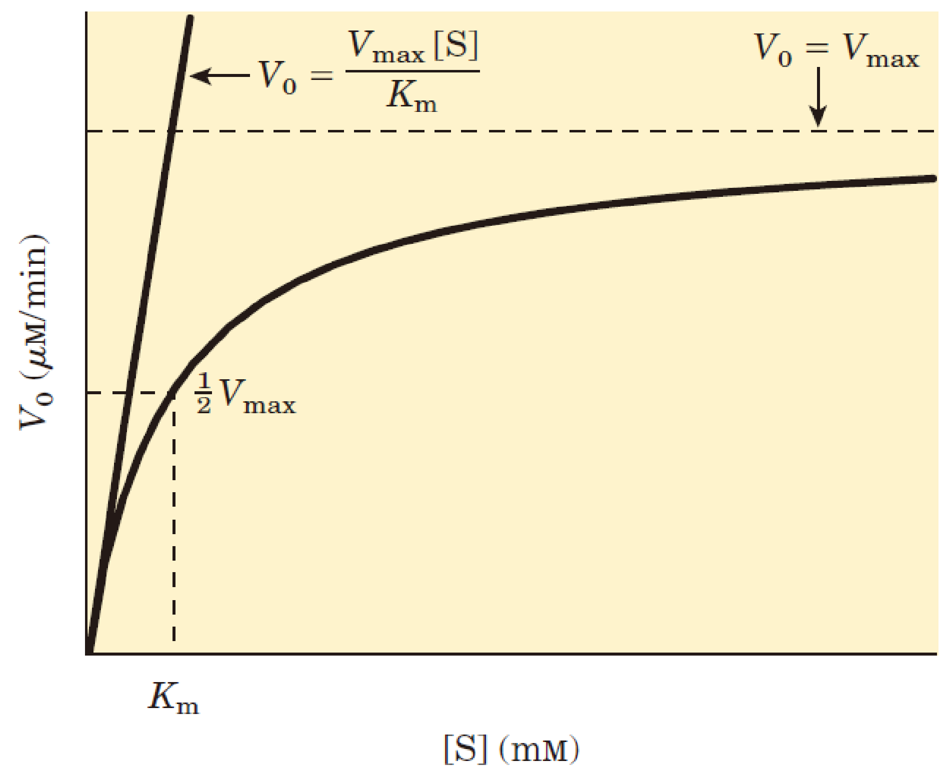
$$v = \frac{V_{\max}}{K_m}[S]$$

When [S] = K_m,

$$v = \frac{V_{\max}}{2}$$

When [S] >> K_m,

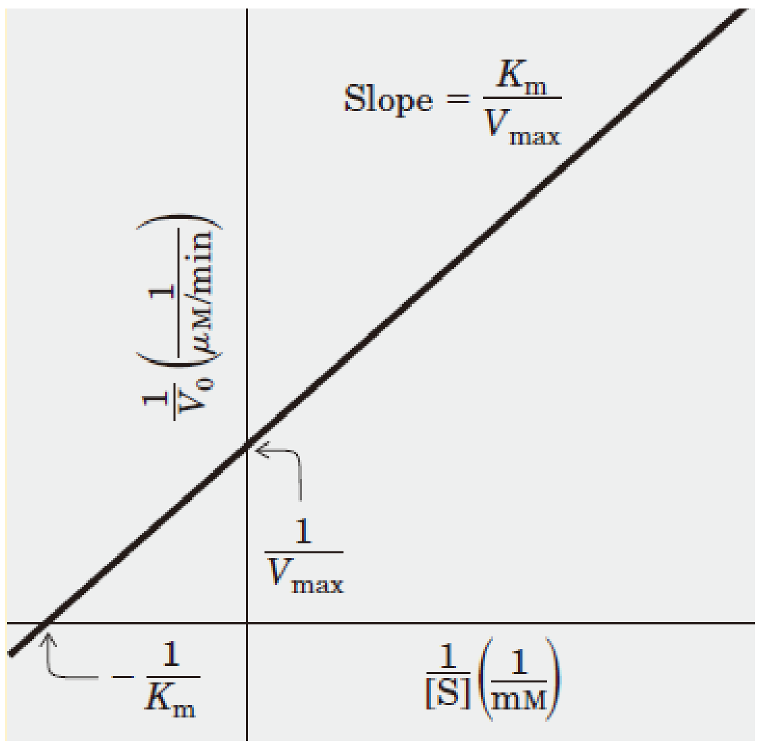
$$v = V_{\max}$$



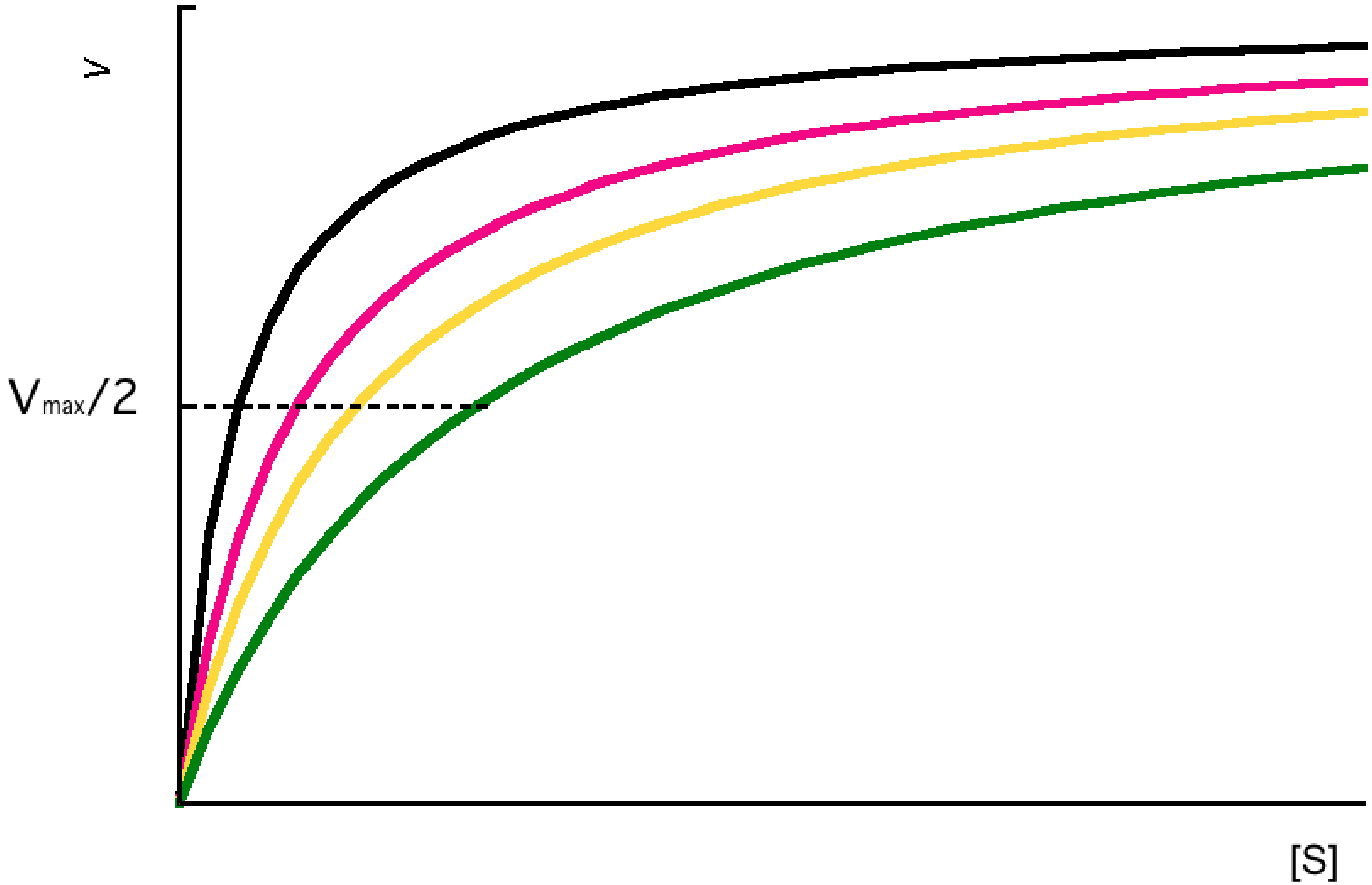
Lineweaver-Burk double-reciprocal plot 双倒数作图法

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

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



Same V_{max}, different K_m



Lower K_m → better substrate

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

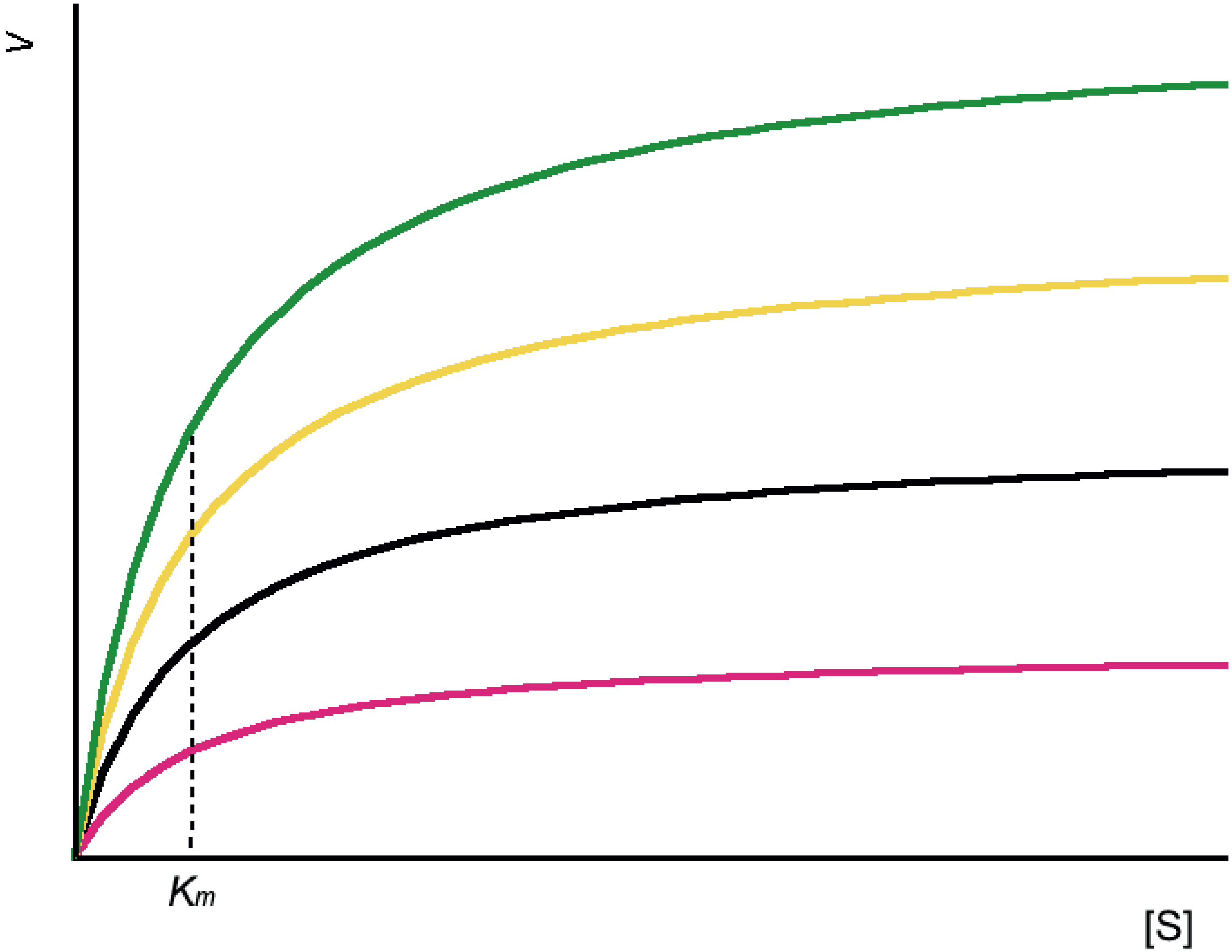
$$K_m = \frac{k_{-1} + k_2}{k_1}$$

When k_2 is rate-limiting（限速步骤）, $k_2 << k_{-1}$, then

$$K_m = \frac{k_{-1}}{k_1} = K_d$$

In many cases, K_m is a very complex function of many rate constants.

Same K_m, different V_{max}



$$V_{max} = k_2 \cdot [E]_0$$

$$V_{max} = k_{cat} \cdot [E]_0$$

TABLE 6–7 Turnover Numbers, k_{cat}, of Some Enzymes

Enzyme	*Substrate*	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

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

$$= \frac{k_{cat} \cdot [\mathrm{E}]_0 \cdot [\mathrm{S}]}{K_{\mathrm{m}} + [\mathrm{S}]}$$

when $[\mathrm{S}] << K_m$, $$= \frac{k_{cat}}{K_{\mathrm{m}}}[\mathrm{E}]_0[\mathrm{S}]$$

A + B → P

$v = k[\mathrm{A}][\mathrm{B}]$

k_{cat}/K_m is the apparent second-order rate constant, also termed specificity constant.

$$\frac{k_{cat}}{K_m} = \frac{k_2 \cdot k_1}{k_{-1} + k_2} = \frac{k_2}{k_{-1} + k_2} k_1 \leq k_1$$

k_1 is limited by diffusion to 10^8~10^9 $M^{-1}s^{-1}$.

TABLE 6–8 Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

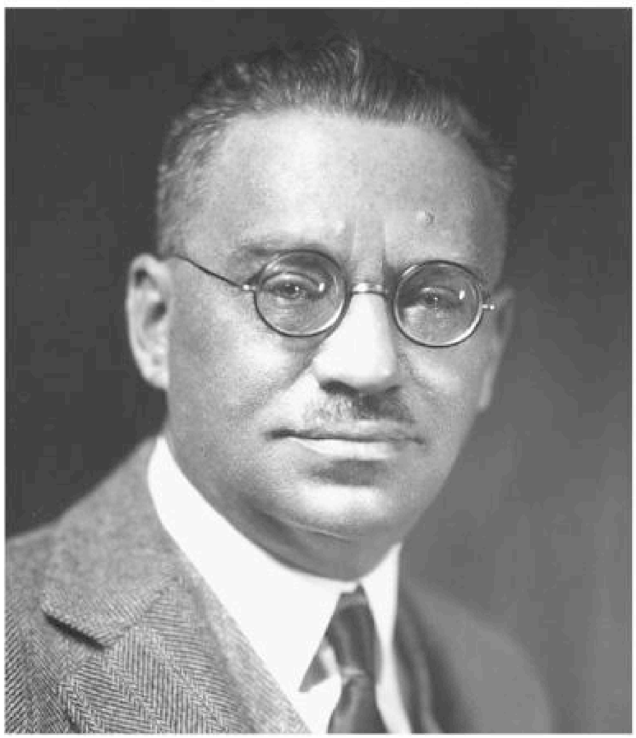
Enzyme	*Substrate*	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1×10^0	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8

Source: Fersht, A. (1999) *Structure and Mechanism in Protein Science*, p. 166, W. H. Freeman and Company, New York.

Michaelis-Menten equation

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

Leonor Michaelis (1875-1949)



Maud Menten (1879-1960)