Chapter 6

Enzymes

6.1 An introduction to enzymes

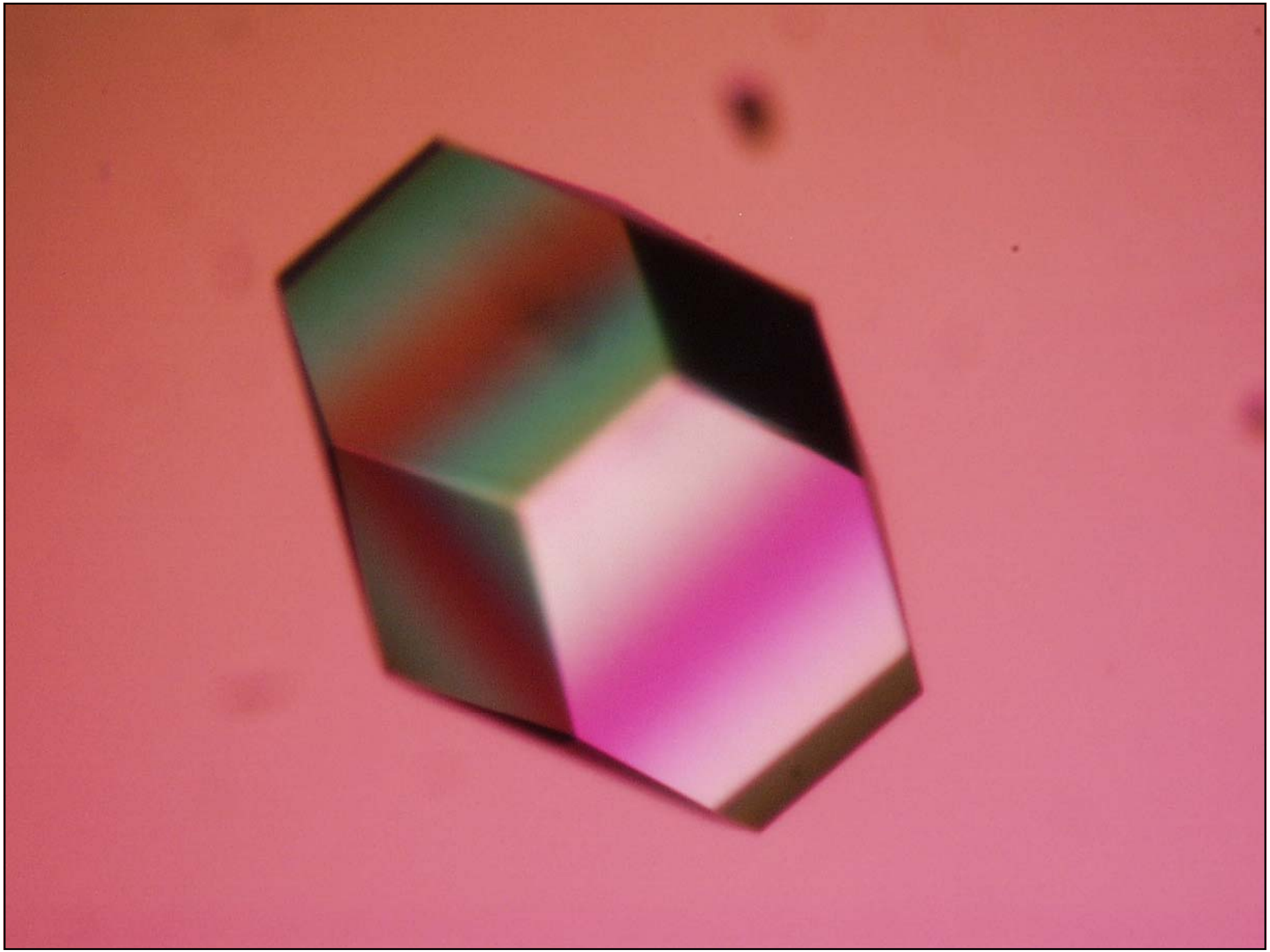
6.2 How enzymes work

6.3 Enzyme kinetics as an approach to understanding mechanism

6.4 Examples of enzymatic reactions

6.5 Regulatory enzymes

- In the **1850s**, **Louis Pasteur** came to the conclusion that **the fermentation of sugar to alcohol by yeast was catalyzed by a vital force contained within the yeast cells called "ferments", which were thought to be inseparable from the organisms**. This view, called **vitalism**, prevailed for decades.
- In **1877**, German physiologist **Wilhelm Frederick Kühne** first used the term ***enzyme***, which comes from Greek, "in yeast", to describe this process. Notably, in 1876, Kühne discovered the protein-digesting enzyme trypsin.
- In **1897**, **Eduard Buchner** found that the **sugar was fermented even when there were no living yeast cells in the mixture**. He named the enzyme that brought about the fermentation of sucrose "zymase". **In 1907, he received the Nobel Prize in Chemistry** *"for his biochemical research and his discovery of cell-free fermentation"*.
- In **1926**, **James B. Sumner** showed that the enzyme **urease** was a pure protein and **crystallized** it. The conclusion that pure proteins can be enzymes was definitively proved by **Northrop and Stanley**, who worked on the digestive enzymes **pepsin** (1930), **trypsin and chymotrypsin**. These three scientists were awarded the 1946 Nobel Prize in Chemistry. *"for his discovery that enzymes can be crystallized" and "for their preparation of enzymes and virus proteins in a pure form".*
- **Lysozyme** was the second protein structure and the **first enzyme structure to be solved via X-ray diffraction methods** by a group led by **David Chilton Phillips** and published in 1965. This high-resolution structure of lysozyme revealed how enzymes work at an atomic level of detail.
- Many enzymes have been named by adding the suffix ***-ase*** to the name of their substrates (***e.g.***, urease catalyzes the hydrolysis of urea) or the type of reaction (***e.g.***, DNA polymerase forms DNA polymers).



Most enzymes are proteins

- With the exception of a small group of catalytic RNA molecules (**Ribozyme , and ribosomal RNA**), all enzymes are proteins.
- Their catalytic activity depends on the integrity of their native protein conformation.
- Some enzymes require no chemical groups for activity other than their amino acid residues. Others require an additional **chemical component called a cofactor—either one or more inorganic ions such as Fe^{2+}, Mg^{2+}, Mn^{2+}, or Zn^{2+}**, or **a complex organic or metalloorganic molecule called a coenzyme**.

TABLE 6–1 Some Inorganic Ions That Serve as Cofactors for Enzymes

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

TABLE 6–2 Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Note: The structures and modes of action of these coenzymes are described in Part II.

- A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a **prosthetic group**.
- A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a **holoenzyme**.
- The protein part of such an enzyme is called the **apoenzyme** or **apoprotein**.
- Some enzyme proteins are modified covalently by phosphorylation, glycosylation, and other processes.

Enzymes are classified by the reactions they catalyze

- Many enzymes have been named by adding the suffix “-ase” to the name of their substrate or to a word or phrase describing their activity.
- Other enzymes were named by their discoverers for a broad function, before the specific reaction catalyzed was known.

Enzyme Commission number (E.C. number)

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

What is enzyme kinetics?

- **Kinetics** is the study of the rate at which compounds react
- Rate of enzymatic reaction is affected by
  - Enzyme
  - Substrate
  - Effectors
  - Temperature

Why study enzyme kinetics?

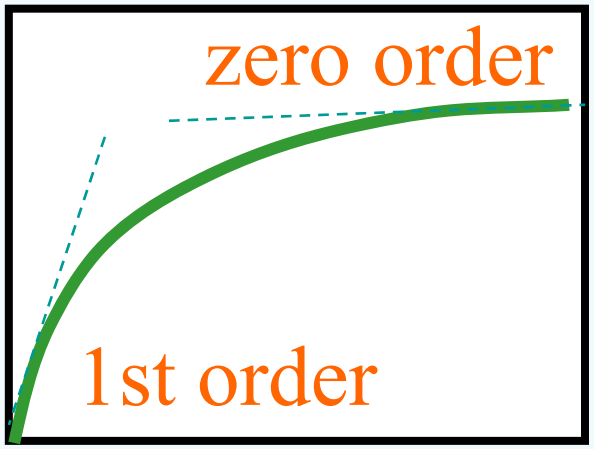
- Quantitative description of biocatalysis
- Determine the order of binding of substrates
- Elucidate acid-base catalysis
- Understand catalytic mechanism
- Find effective inhibitors
- Understand regulation of activity

動力學公式的意義

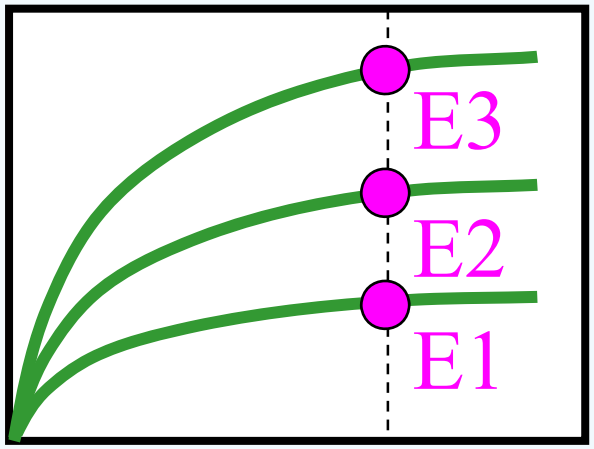
$v_0 = V_{max} \times K = k_3 [Et] \times K$

可求得 V_{max} 及 K_m

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



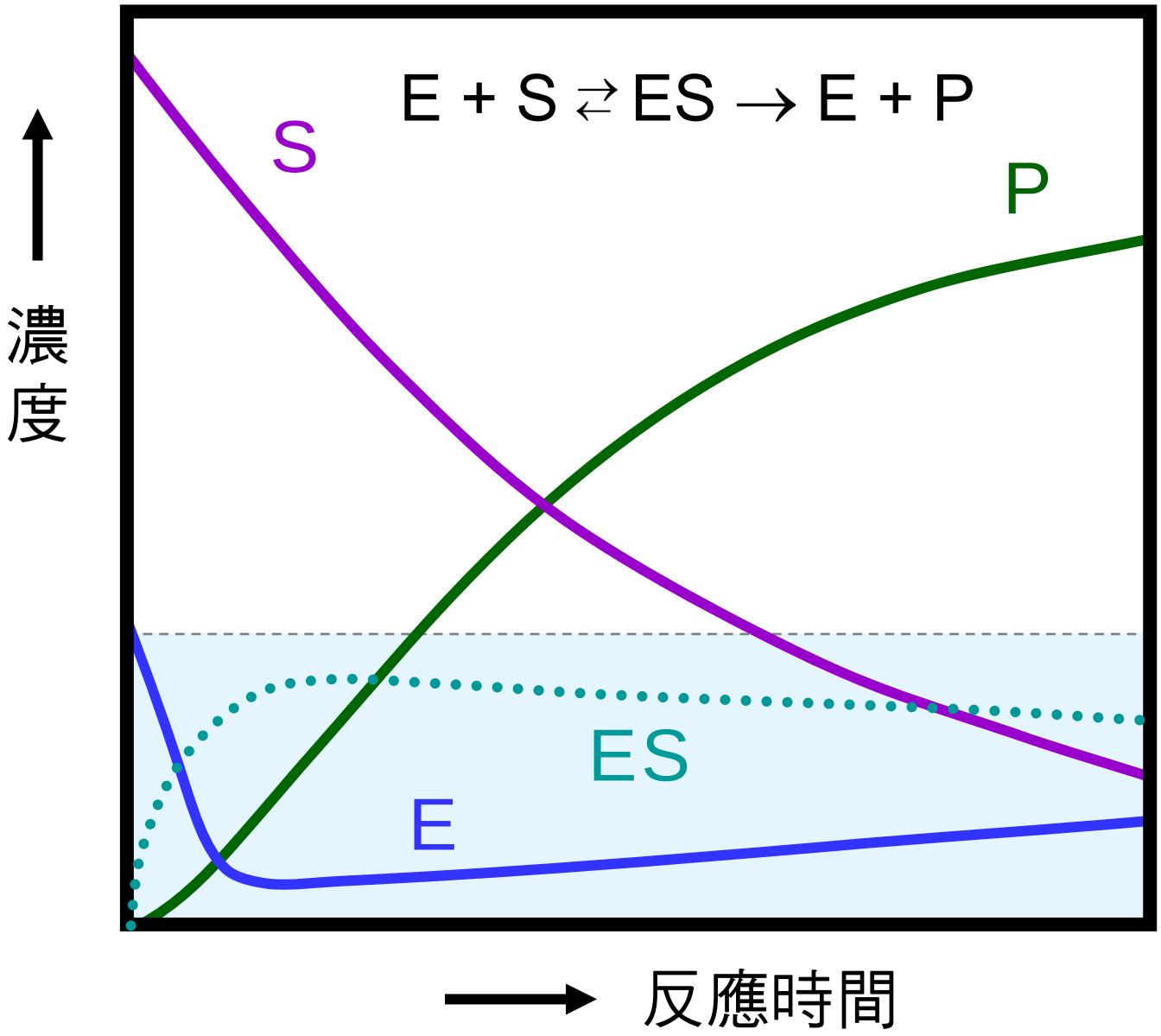
[S] = 低濃度 → 高濃度



[S] = 固定濃度

與酵素量成正比

Steady State 時 ES 的濃度恆定



K_m 是基質親和力的指標

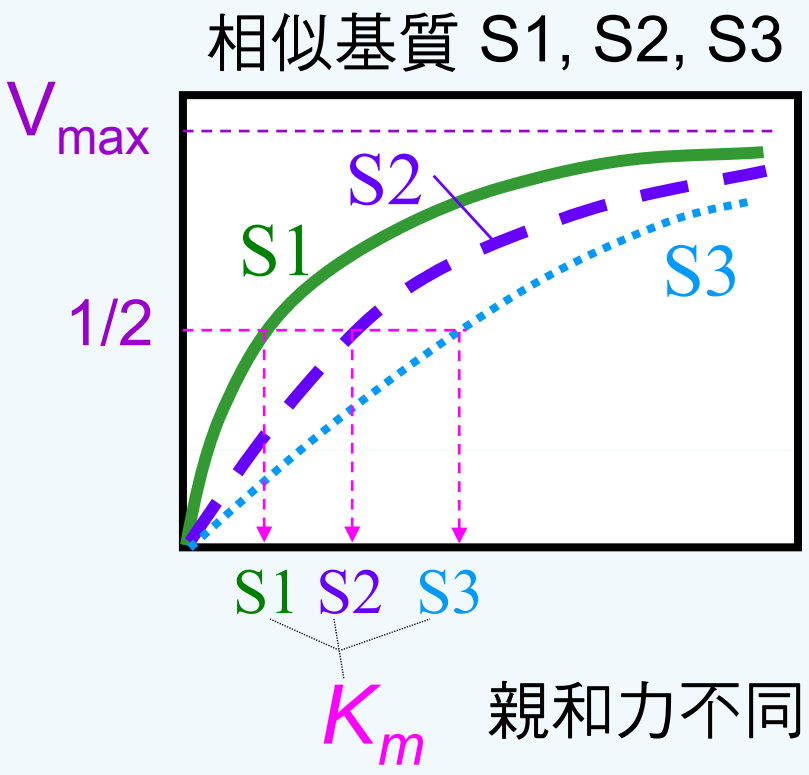
$$v_o = \frac{V_{max}[S]}{K_m + [S]}$$

若 $v_o = \frac{V_{max}}{2}$

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

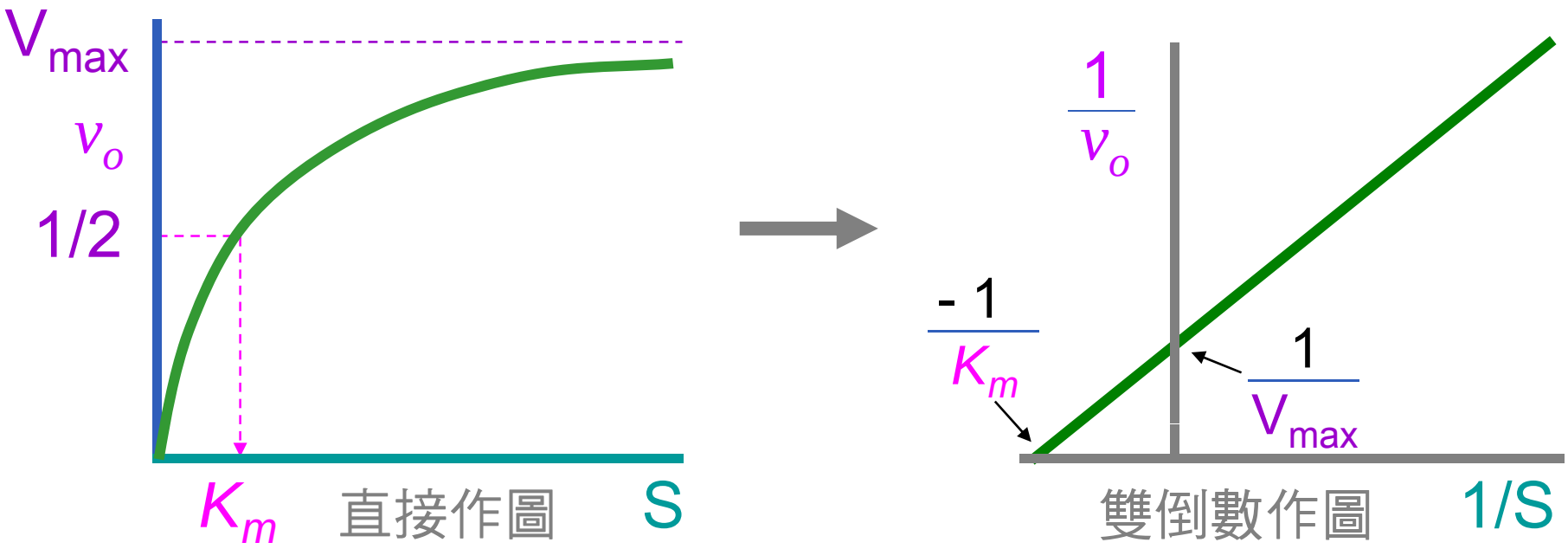
$$K_m + [S] = 2[S]$$

$$K_m = [S]$$

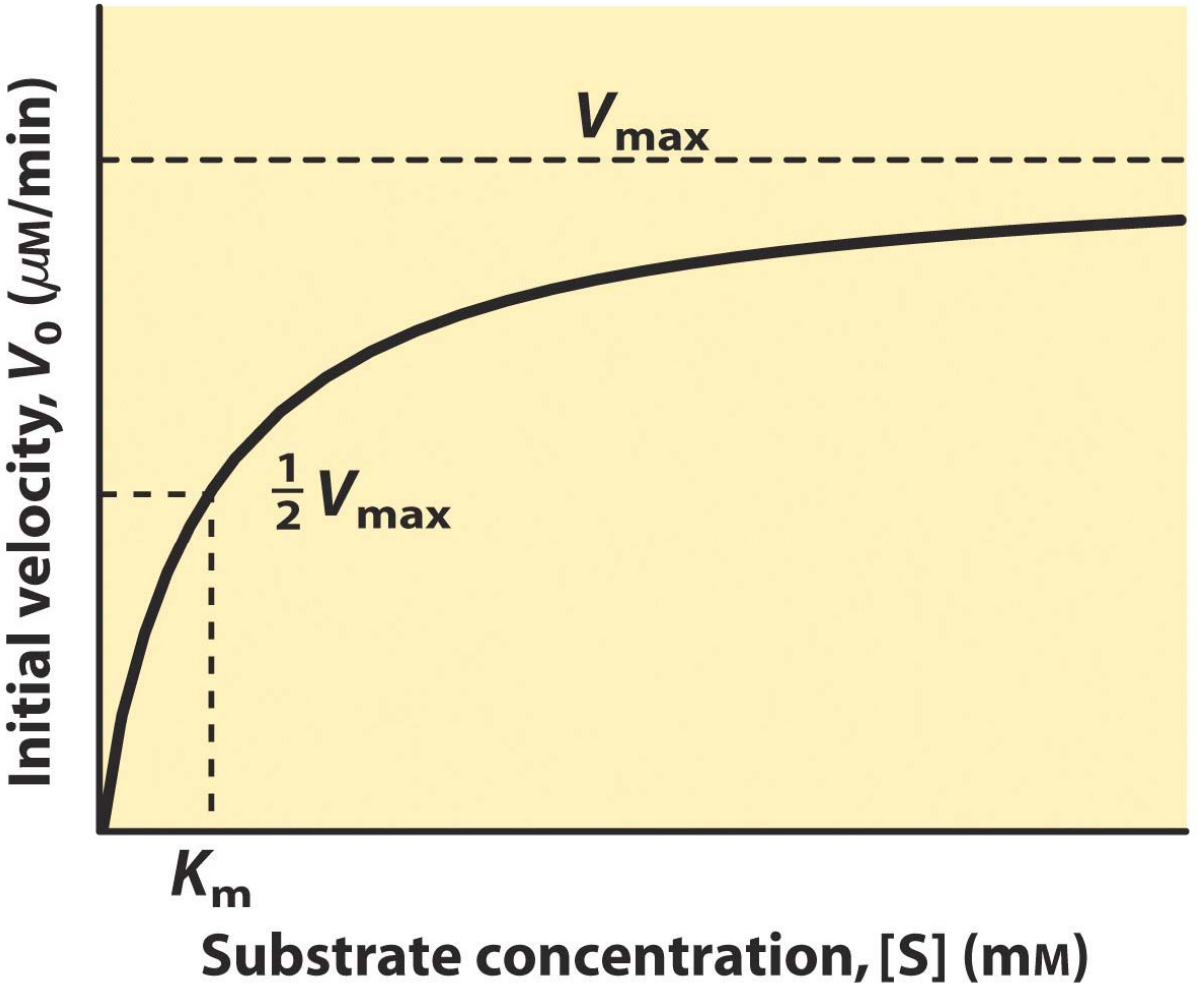


動力學實驗操作

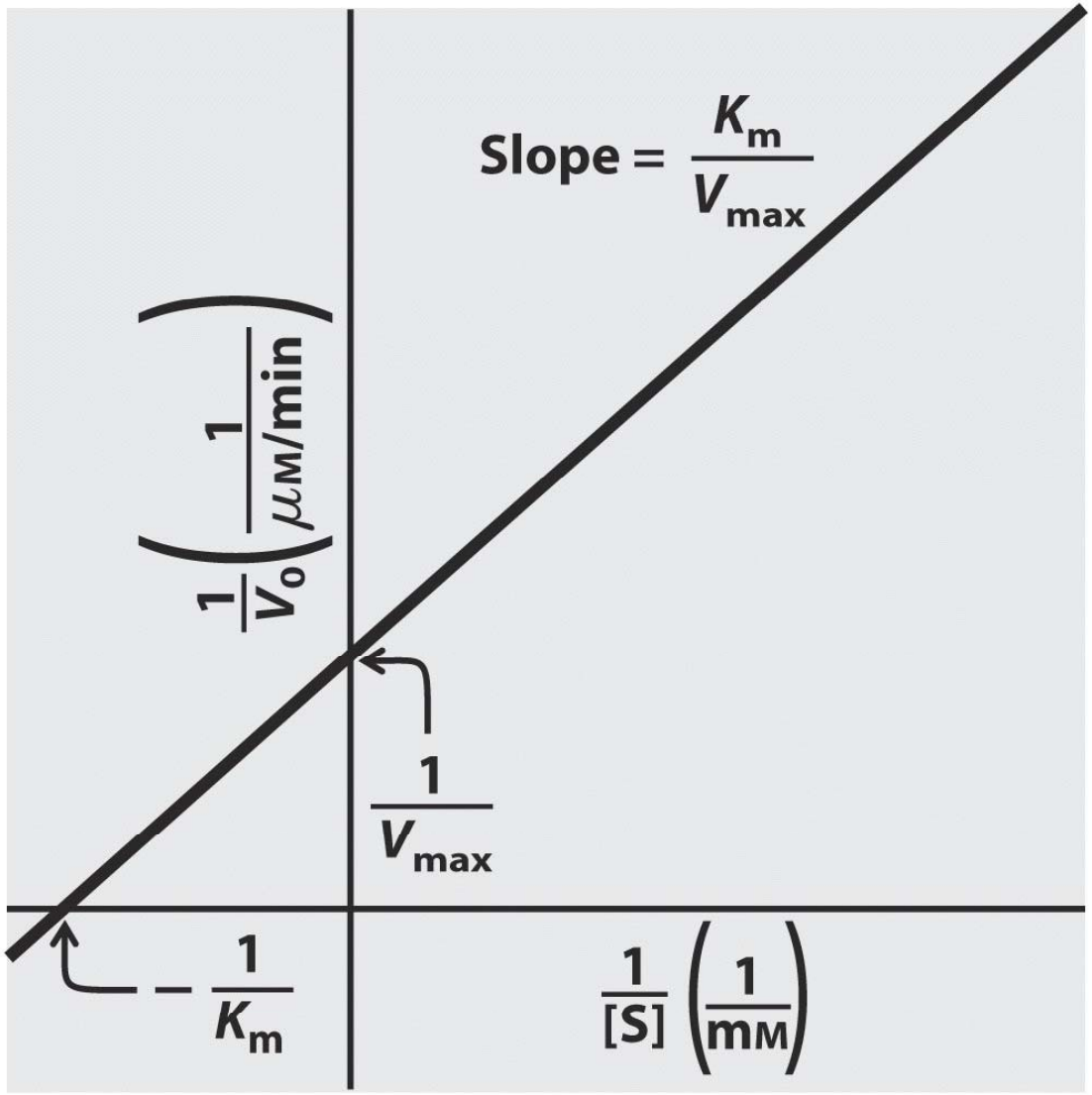
1) 先取固定量的 酵素	→ E
2) 加入各種不同濃度的 基質	→ S (x 軸
3) 在一定 時間 內測 生成物 量 (P/t)	→ v_o (y 軸)
4) (x, y) 作圖得 雙曲線 之一股推 漸近點	→ V_{max}
5) 當 y = 1/2 V_{max} 時求其 x (即 [S]) 即得	→ K_m



Michaelis–Menten plot

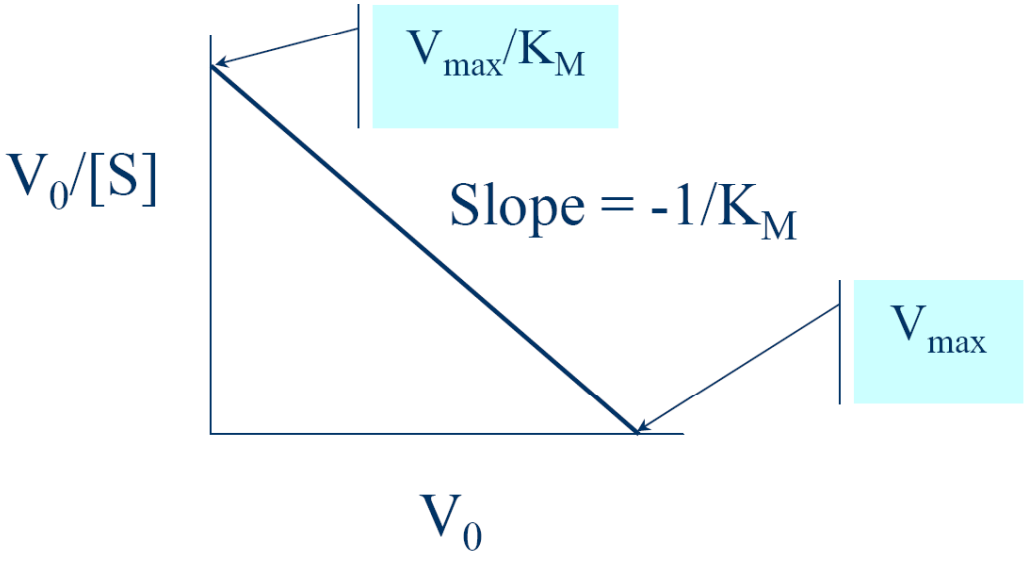


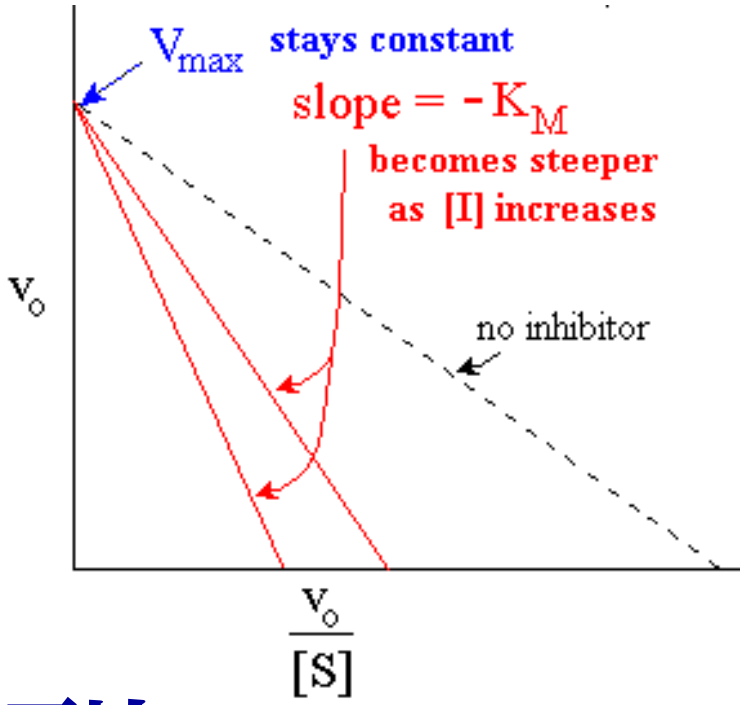
Lineweaver–Burk plot (double reciprocal plot)



Eadie-Hofstee Plot

$V_0/[S] = V_{max}/K_M - V_0/K_M$





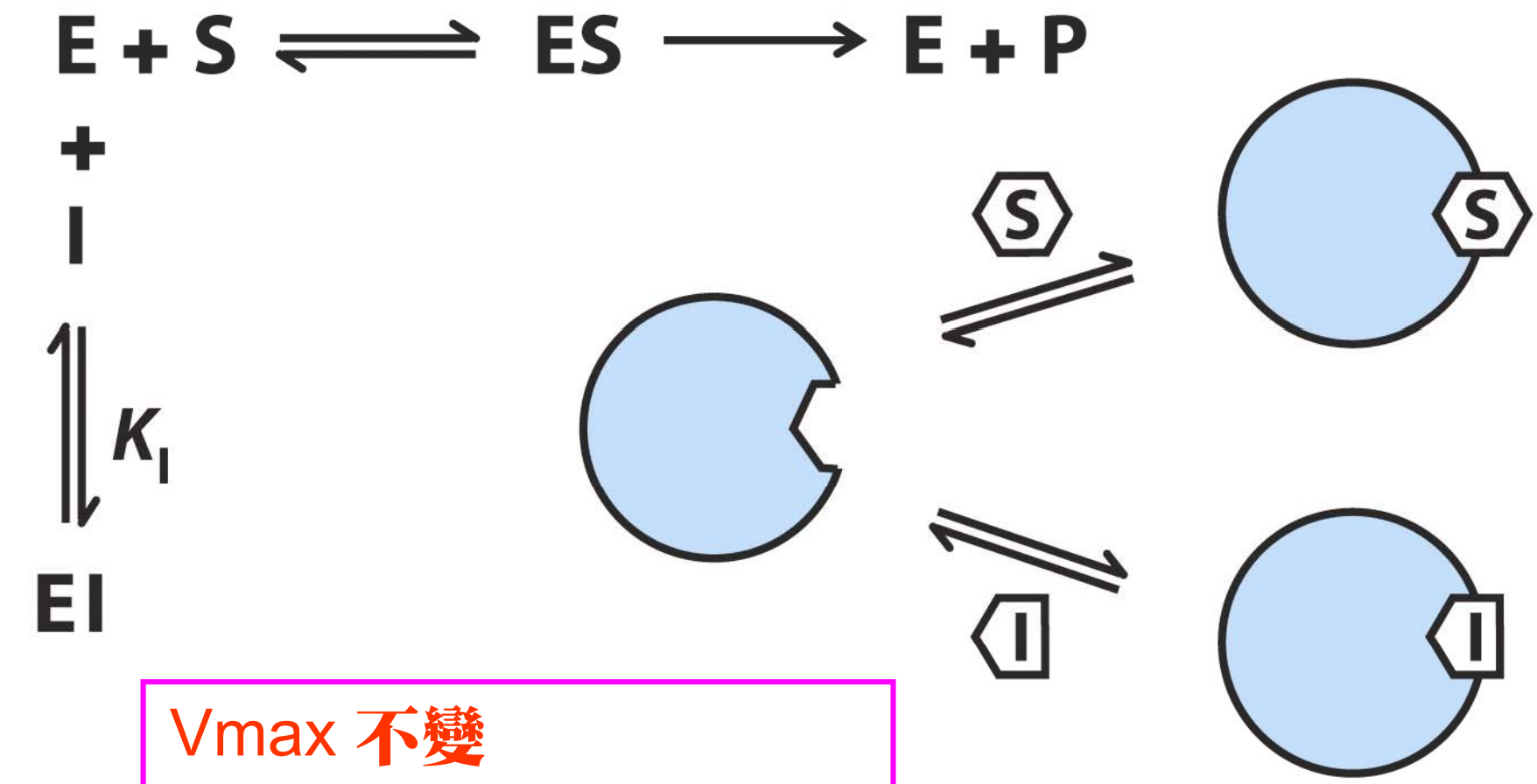
兩種畫法都可以

An advantage of an **Eadie-Hofstee plot** over a Lineweaver Burk plot is that the **Eadie-Hofstee plot** does not require a long extrapolation to calculate Km

酵素的抑制

可逆性抑制	不可逆抑制
抑制劑與酵素非共價結合	抑制劑與酵素行共價性修飾
Competitive Non-competitive (Mixed inhibition) Uncompetitive	Penicillin 青黴素 重金屬 (Hg, Pb) DFP, TPCK Sarin (-Ser) PCMB (-Cys)

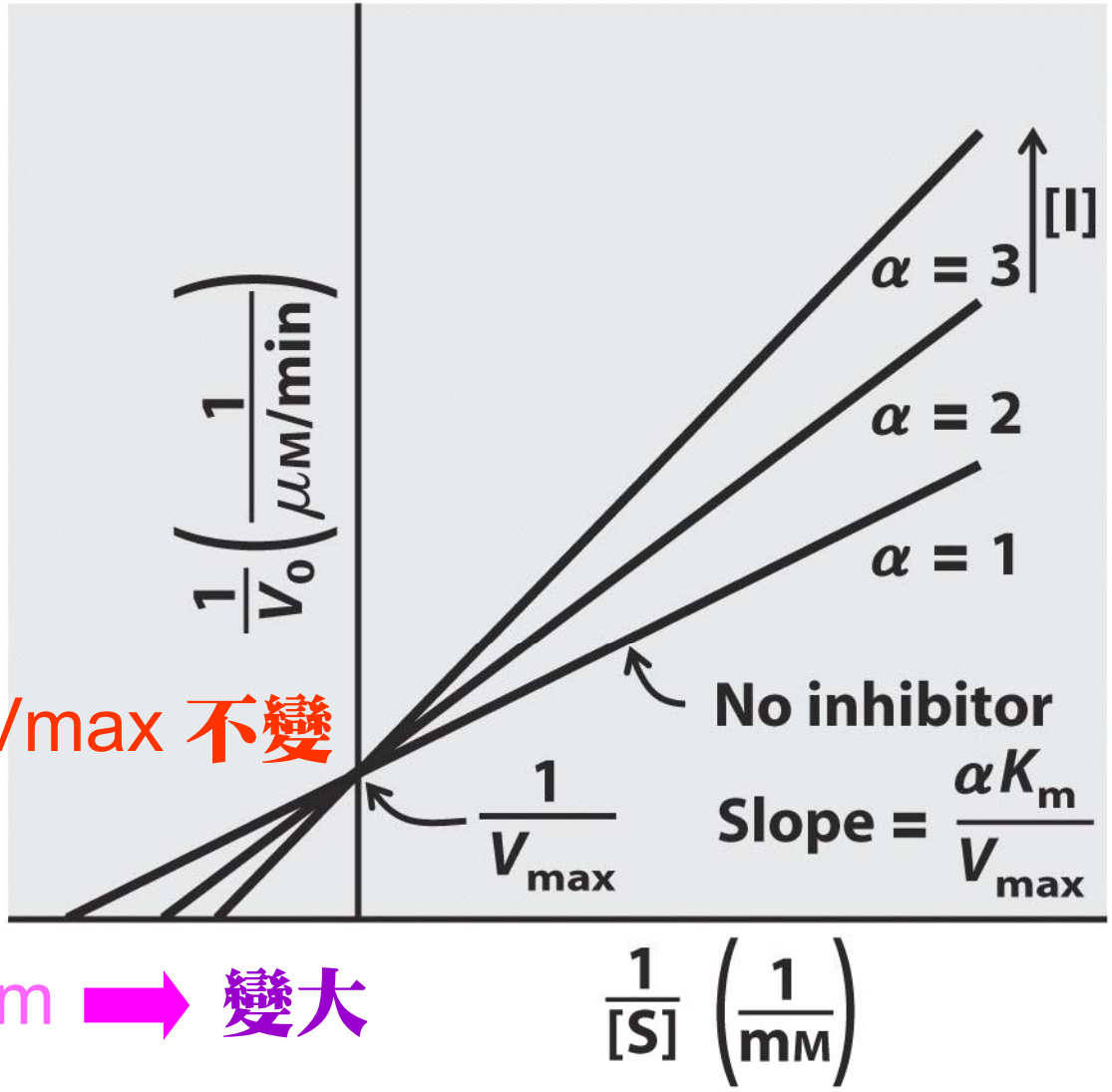
(a) Competitive inhibition



Vmax 不變

Km 變大 (親和力變小)

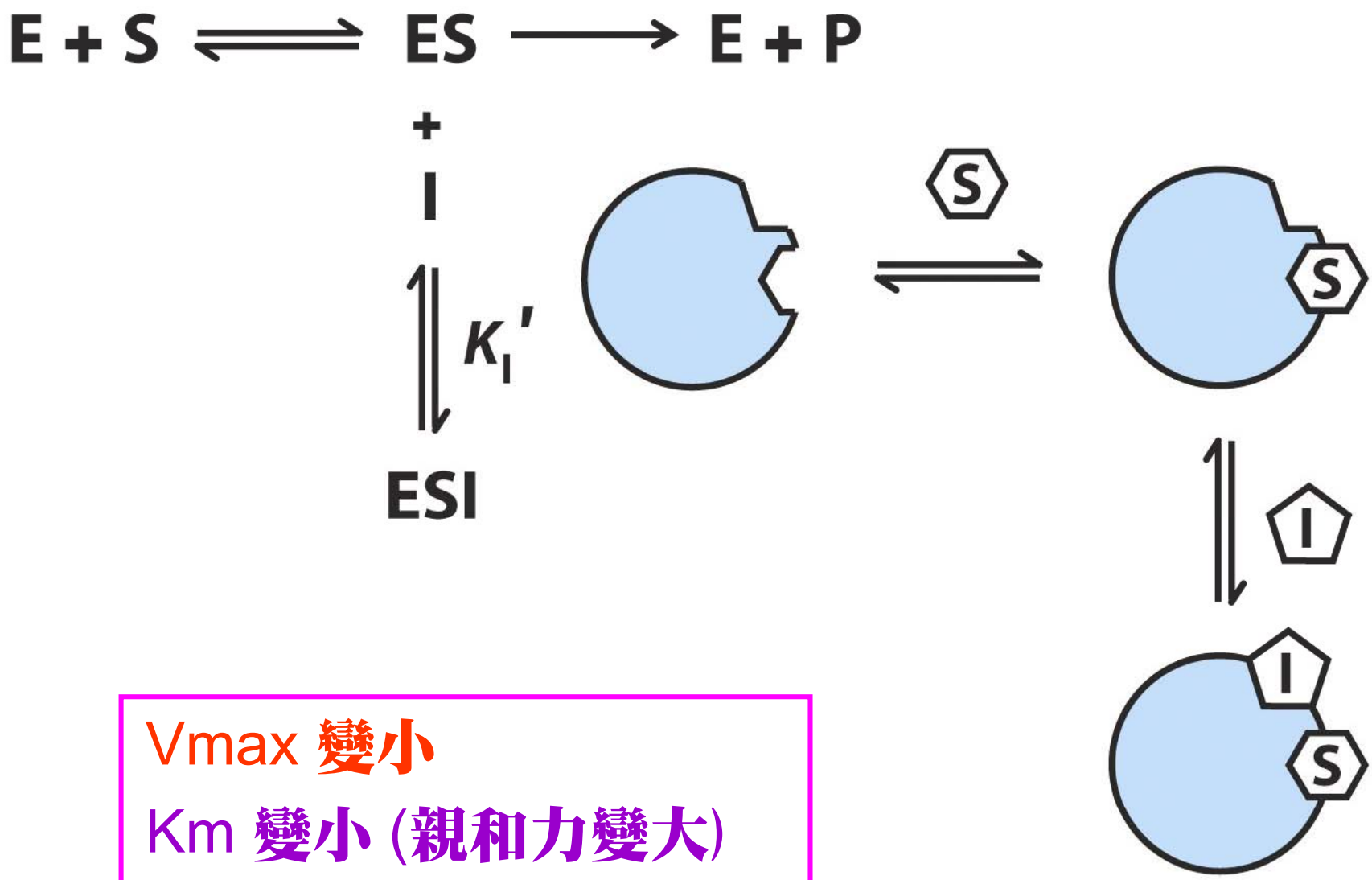
$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$



Vmax 不變

Km ➡ 變大

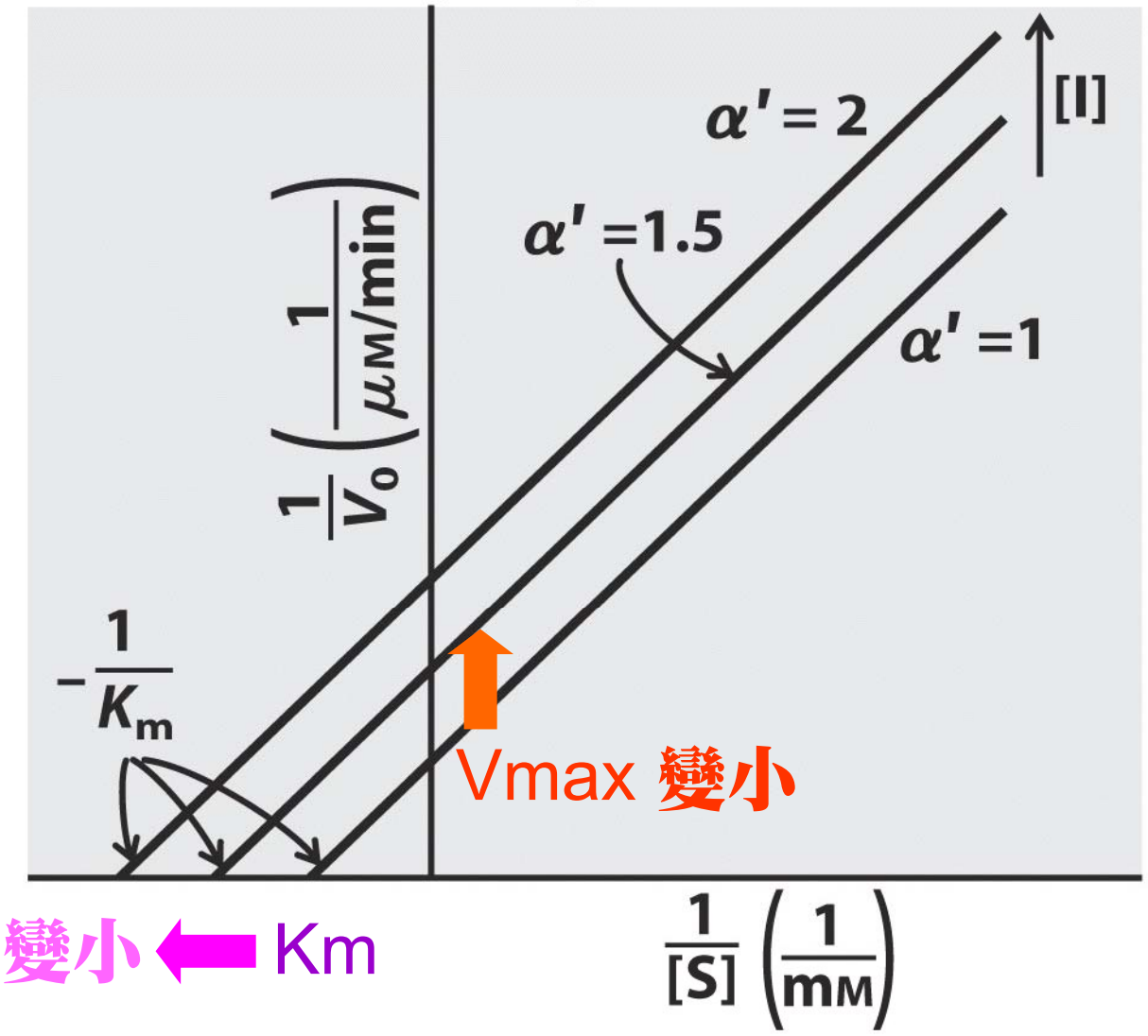
(b) Uncompetitive inhibition



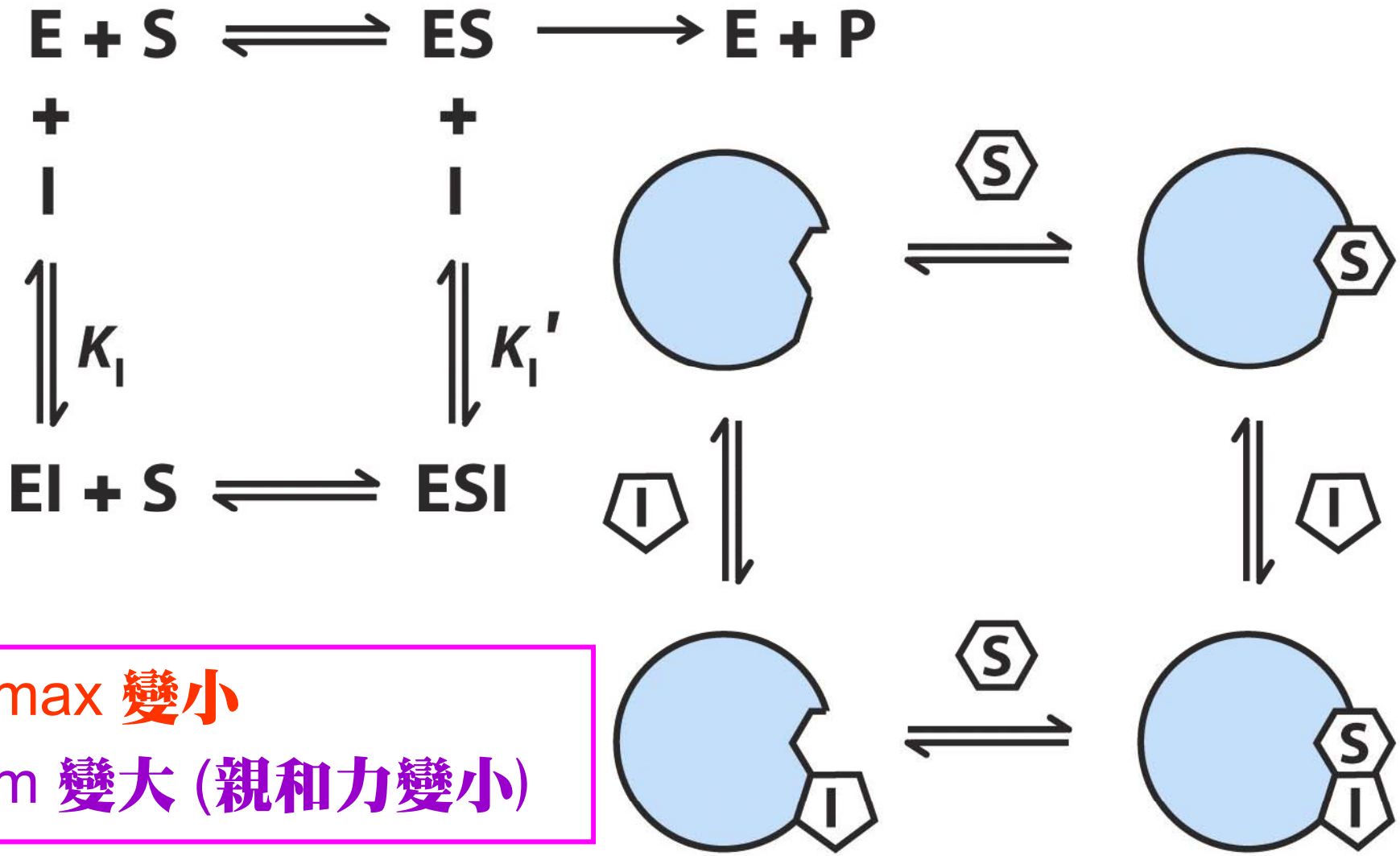
Vmax 變小

Km 變小 (親和力變大)

$$\frac{1}{V_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$



(c) Mixed inhibition (Noncompetitive)



Vmax 變小

Km 變大 (親和力變小)

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$