Enzymes : Kinetics

Dr.Sulieman Al-Khalil

- The rate of a reaction is a direct measure of the free energy difference between the ground state and the transition state
- Steady-state enzyme reactions can be described by the parameters k_{cat} and K_M
- Enzyme inhibitors can be powerful tools

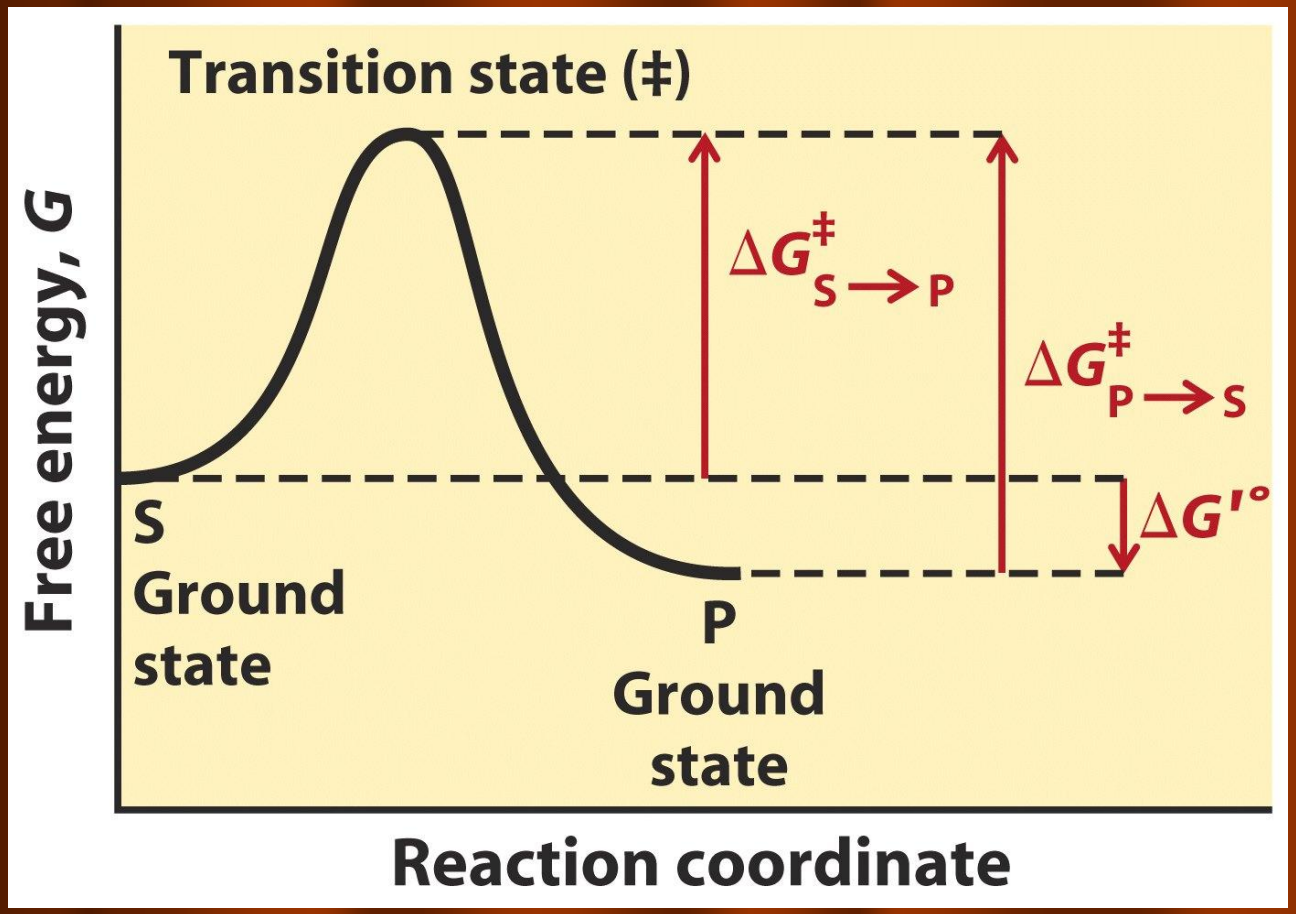
Transition state theory

- Remember from thermodynamics that there is a simple relationship between the free energy difference between two states and the equilibrium constant

  $\Delta G = -RT \ln K_{eq}$

- A transition state can be thought of as an equilibrium with its corresponding ground state.
- The larger the fraction of molecules in the transition state, the faster the reaction rate.
- Because of this relationship, the rate of a reaction is a measure of the free energy difference, DG, between the ground state and the transition state.

Transition state theory



$$\Delta G = -RT\ln K_{eq}$$

$$\Delta G^{\ddagger} = -RT\ln k \frac{h}{k_b T}$$

$$k = \frac{k_b T}{h} e^{-\Delta G^{\ddagger}/RT}$$

(eq. 6-6 in text)

Rates and Reaction Order

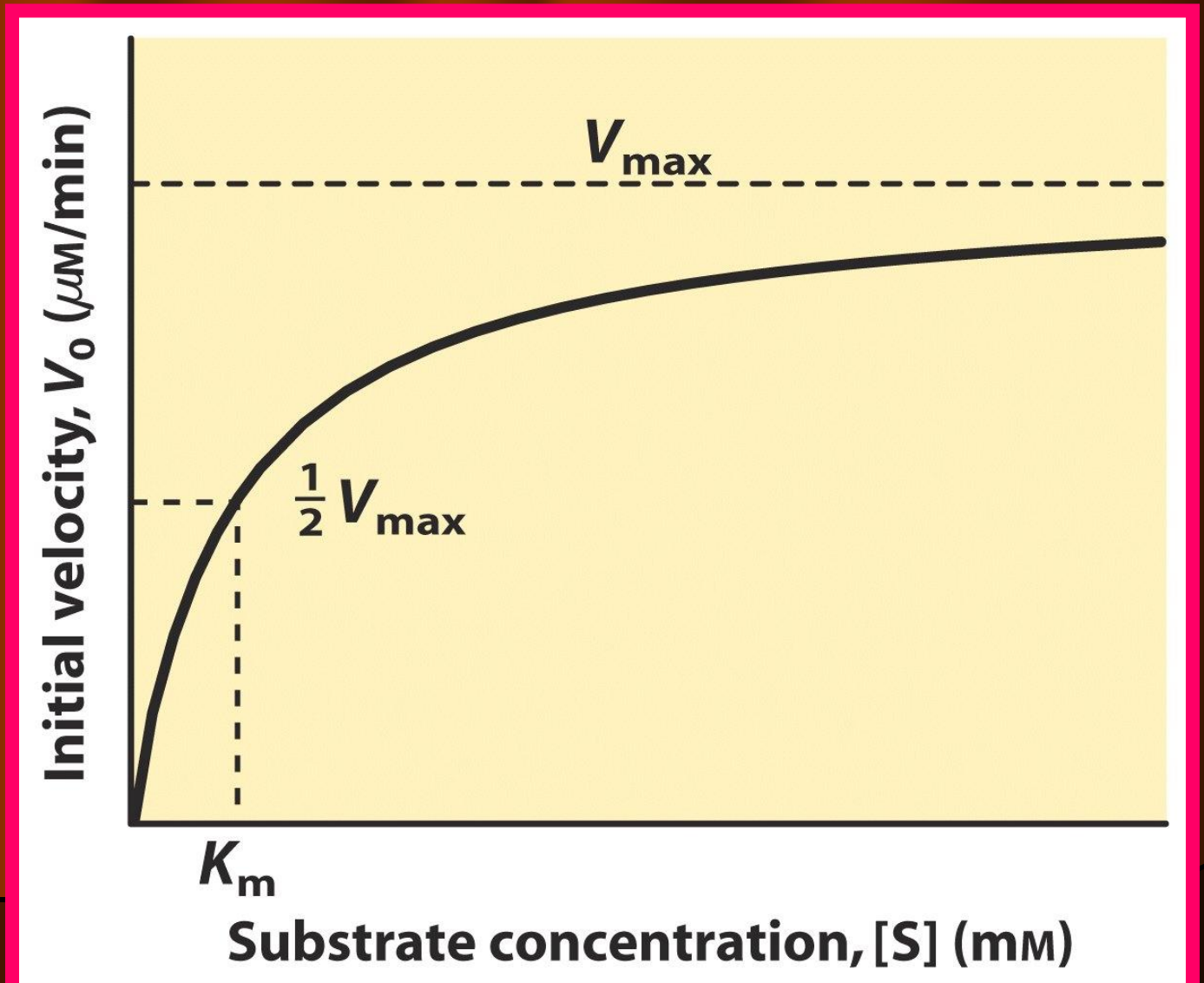
$V = k[A]$

Reaction is first-order: rate depends linearly on [A]

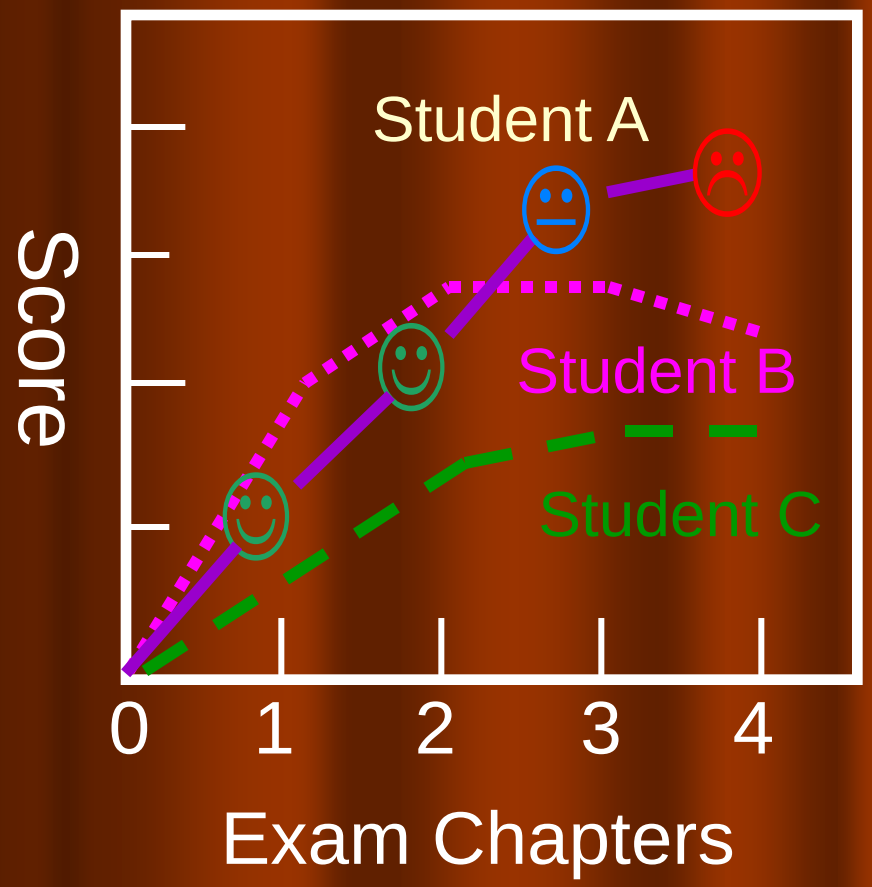
$V = k[E][S]$

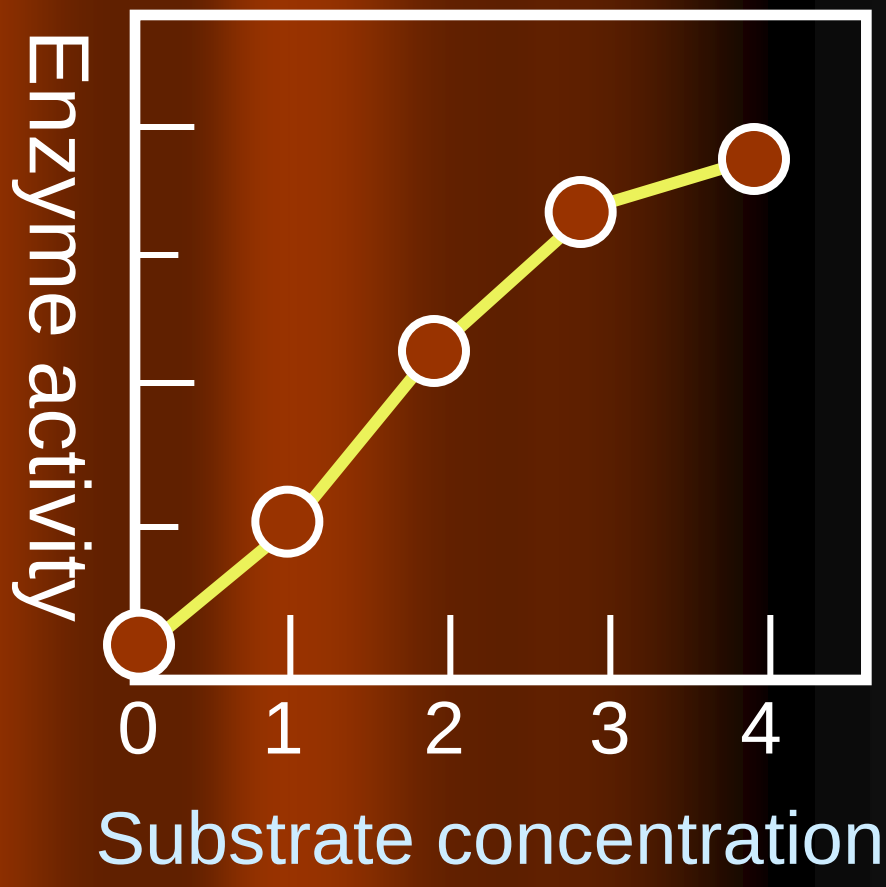
Reaction is second-order overall: first-order in [E] and first-order in [S]

Enzymes Display 'Saturation' Behavior With Increased Substrate Concentration



Enzyme Kinetics





Increase the substrate concentration,
observe the change of enzyme activity

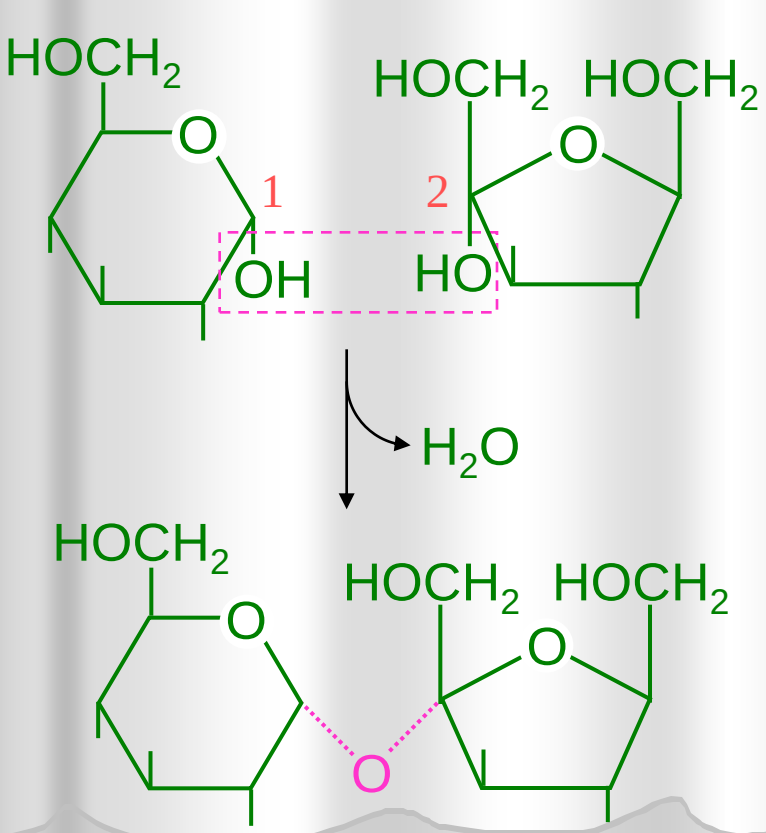
Juang RH (2004) BCbasics

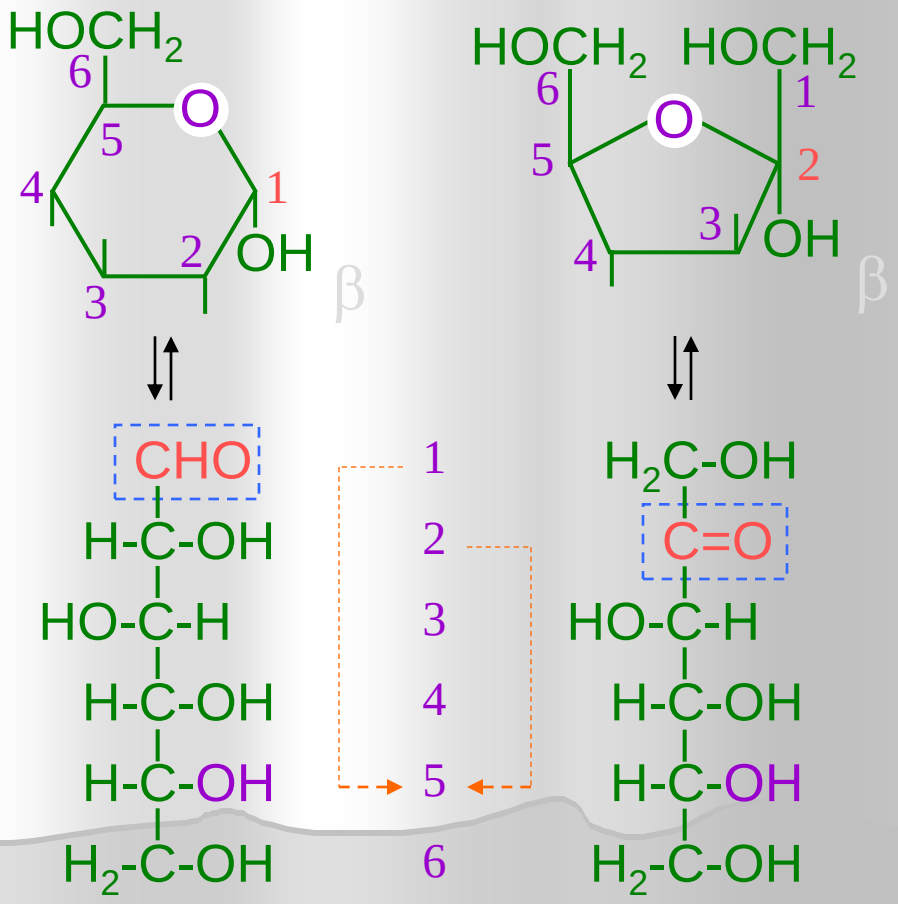
Invertase (IT)

Non-reducing sugar

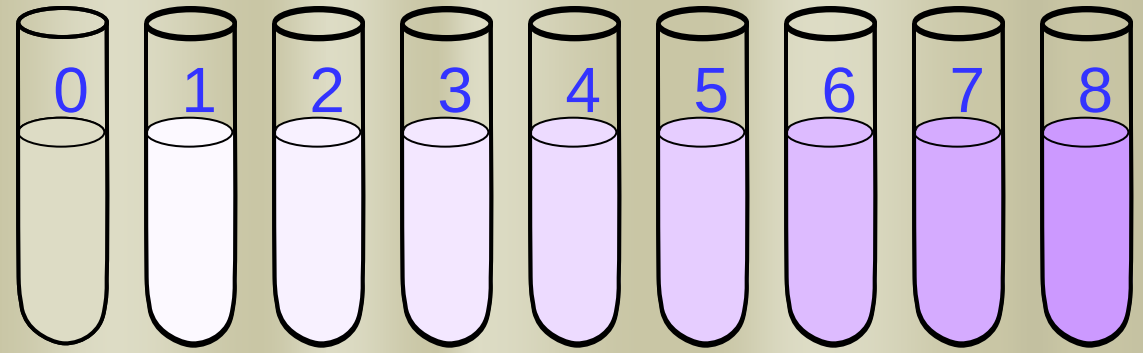
Reducing sugars --> Reducing Power

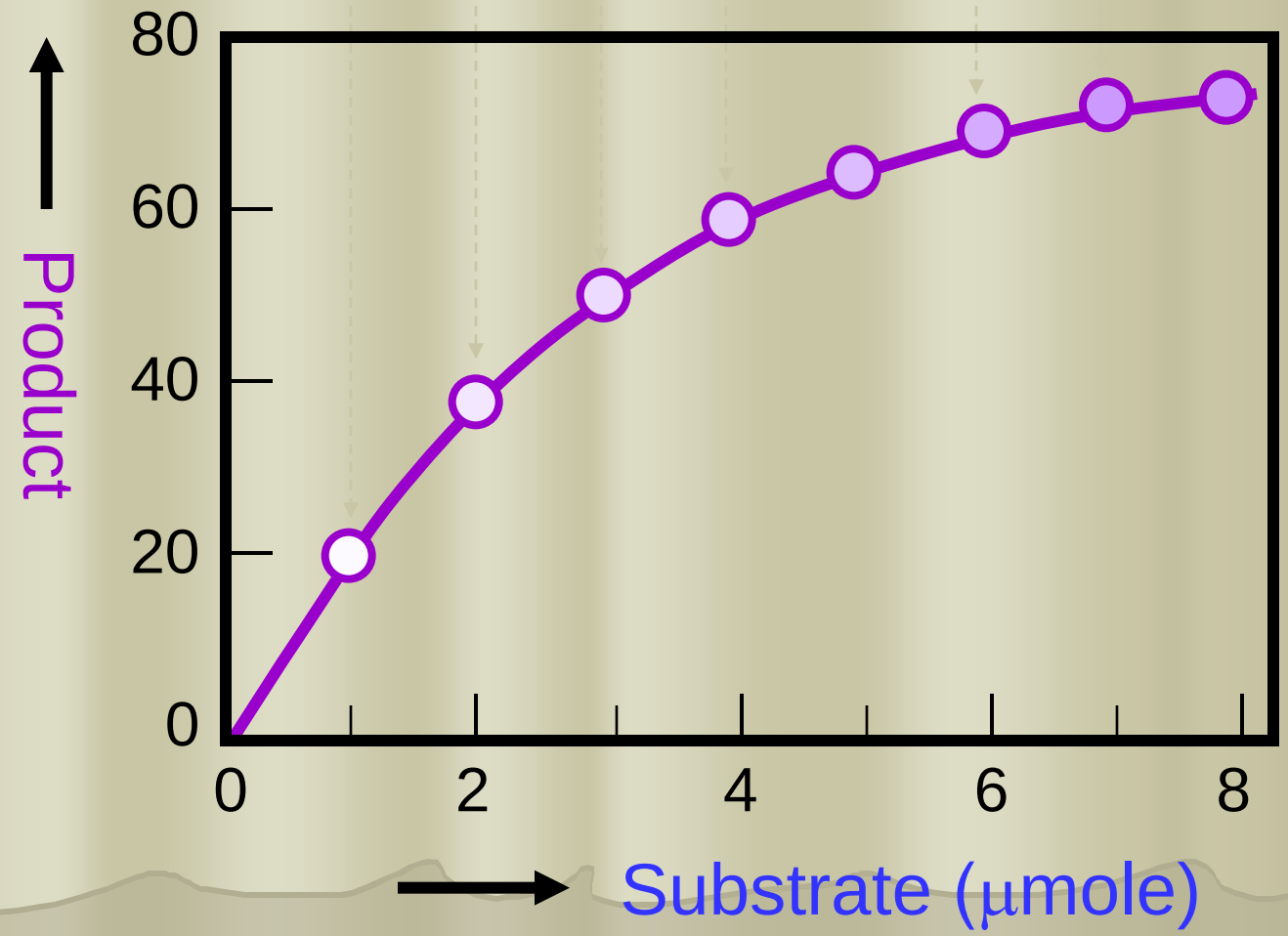
Sucrose **IT** Glucose + Fructose





Increase Substrate Concentration





S + E → P (in a fixed period of time)

Kinetics

- rate of a reaction and how the rate changes in response to experimental parameters.
- The general purpose of studying enzyme kinetics is to learn about how an enzyme catalyzes its reaction by examining how the reaction rate, or the free energy change for reaction, depends on experimental conditions – *i.e.* solution conditions, temperature, mutation of important amino acids
- For A → B, the rate, $v = k[A]$

- For an irreversible 1st order reaction the rate is proportional to [A]; *k* is a **rate constant**, a proportionality constant that reflects the probability of reaction under a given set of conditions (pH, temperature, ionic conditions).
- The value of *k* changes with conditions, but it does not change with changes in the concentration of A.
- Now examine the effect of substrate concentration on the initial rate of an enzyme-catalyzed reaction .

- At low [S], the initial rate of reaction increases with increasing [S]
- As [S] continues to increase, the rate increases by less and less
- Finally a point is reached at which further increases in [S] do not increase the rate. This maximal rate is called ***V*max**
- Under *V*max conditions, the enzyme is **saturated** with substrate and can function no faster
- The overall shape of the rate curve is hyperbolic, similar to the oxygen saturation curve for binding to myoglobin

- Saturation effect is exhibited by nearly all enzymes.
- This finding led to the conclusion that enzymes combine with their substrates to form a complex as a necessary first step in catalysis.
- This was expanded into a general theory by Michaelis and Menton.

1. Enzyme (E) first combines with substrate (S) to form ES complex in a fast, reversible reaction.

   $E + S \leftrightarrow ES$

2. ES goes on to react, forming product (P), which is released from E to regenerate free E

   $ES \leftrightarrow P + E$

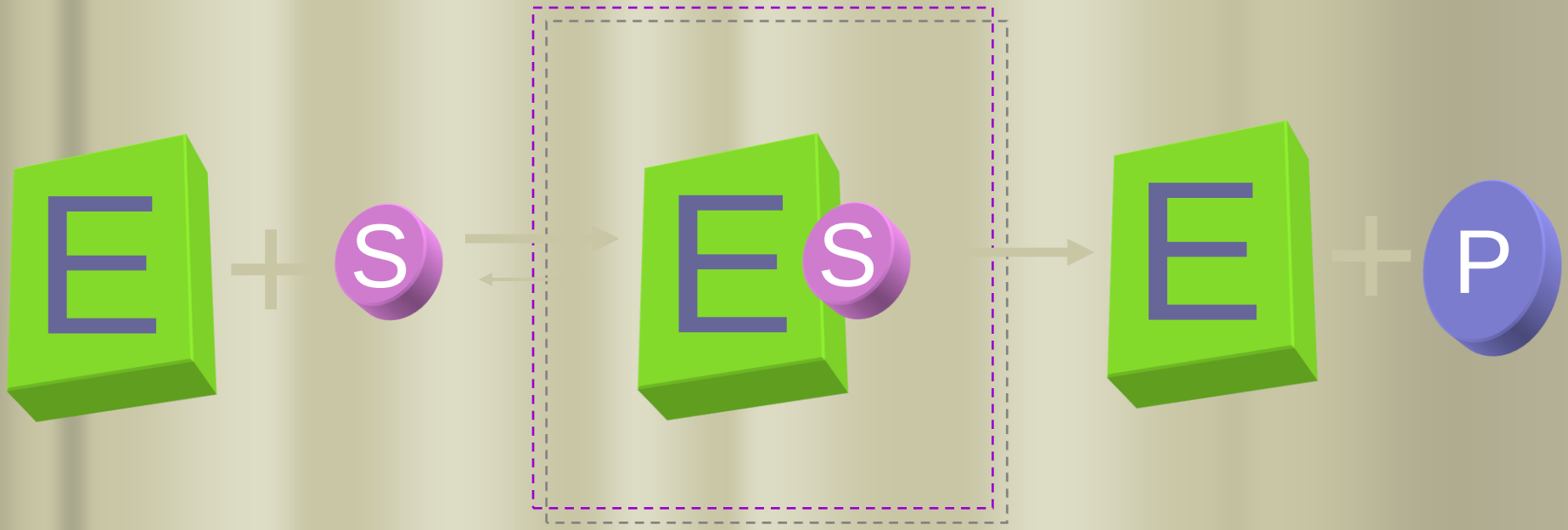
3. The second step, the reaction, is **rate-limiting** (slower) – the overall rate of the reaction is limited by this step and is therefore proportional to the concentration of ES

3. At any given instant, the enzyme exists in two forms: free (E) and in substrate complex (ES). *The rate will be maximal when essentially all the enzyme is in the ES form*. This condition exists at high [S] due to mass action

   $E + S \rightarrow ES$ The equilibrium is shifted to the right

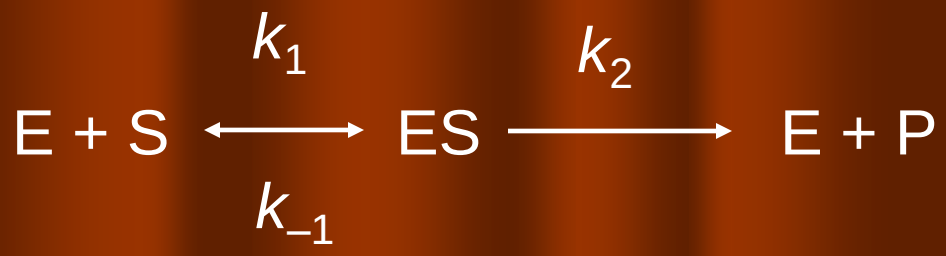
5. At high enough [S], essentially all the E will be in the ES form. As ES reacts and regenerates free E, it will immediately be bound to another molecule of S. Under these conditions the enzyme is saturated with substrate so that $V0 = V\text{max}$.

Steady State Theory



In steady state, the production and consumption of the transition state proceed at the same rate. So the concentration of transition state keeps a constant.

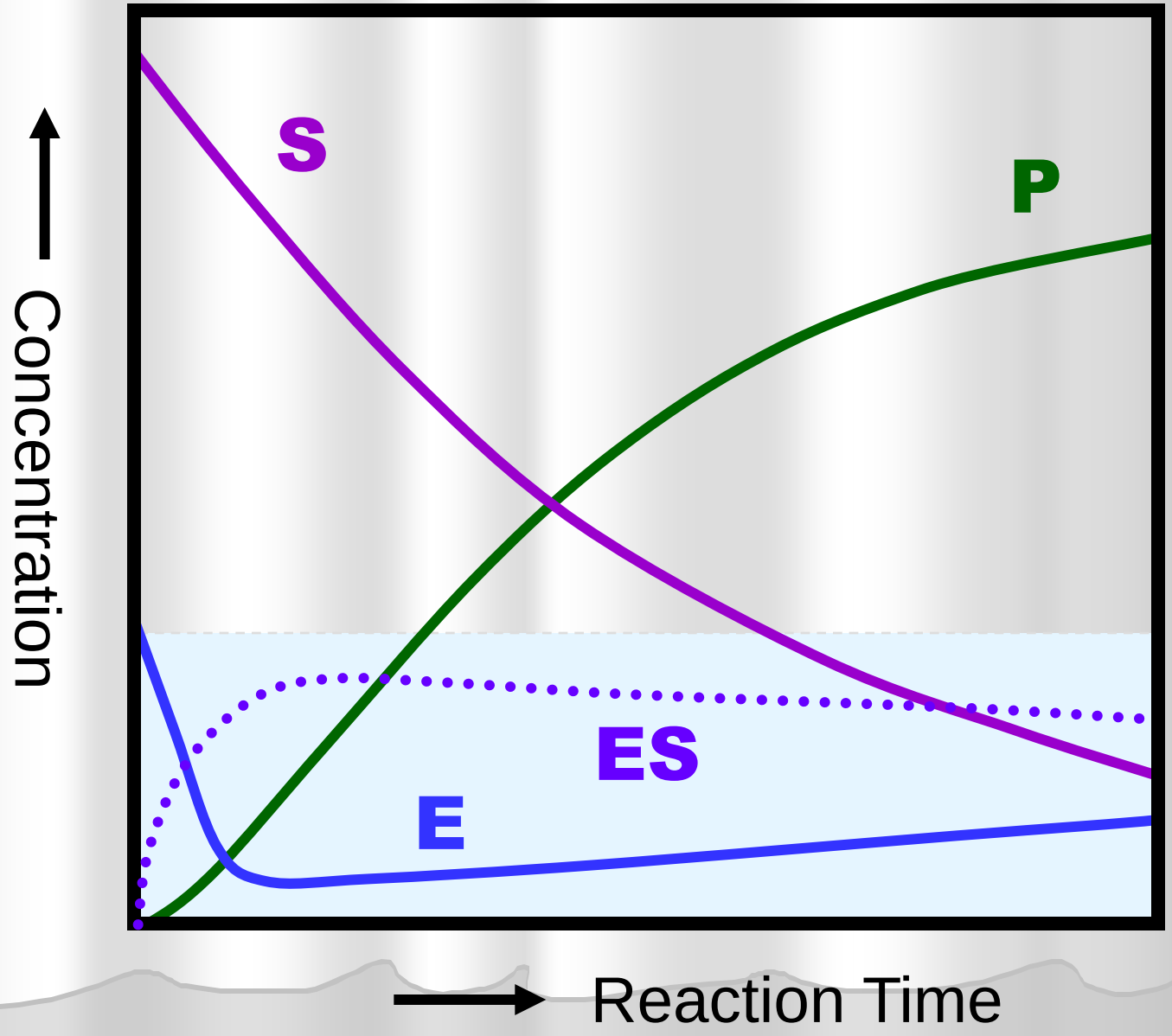
Steady-state kinetics



Initial rate measurement:

- **[ES] remains essentially constant**
- **[S] does not change significantly and is approximately $[S]_t$**

Constant ES Concentration at Steady State



Juang RH (2004) BCbasics

- The quantitative relationship between *V0* and [S] is described by the **Michaelis-Menton equation.**

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

- We will derive this relationship in class.
- Significance of *K*M: a useful numerical relationship exists when the initial rate is half the maximal rate – *V0*= 1/2 *V*max

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

- Divide by *V*max

$$\frac{1}{2} = \frac{[S]}{K_M + [S]}$$

- Solve for *K*M by inverting

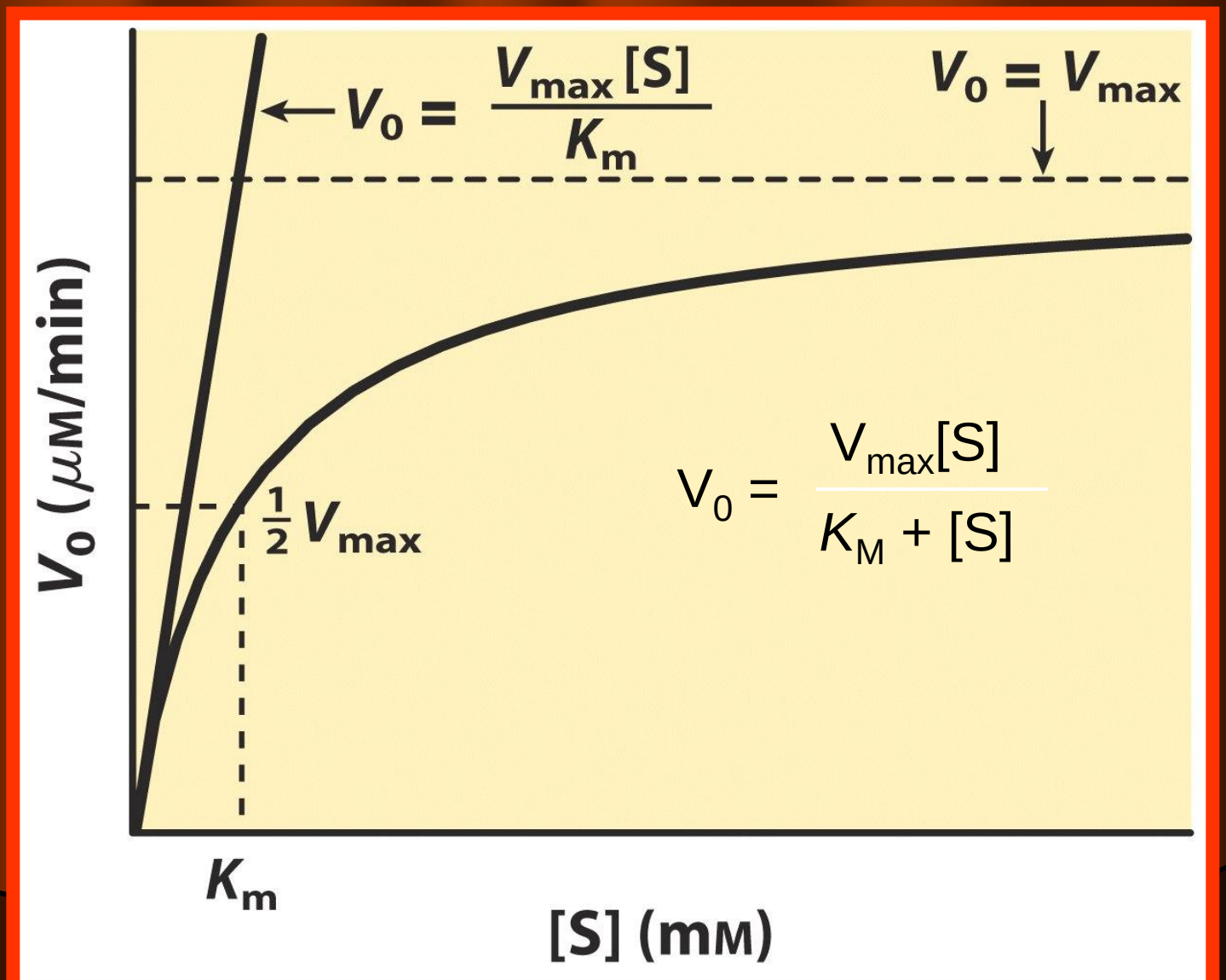
$$2 = \frac{K_M + [S]}{[S]} = \frac{K_M}{[S]} + 1$$

$$\frac{K_M}{[S]} = 1$$

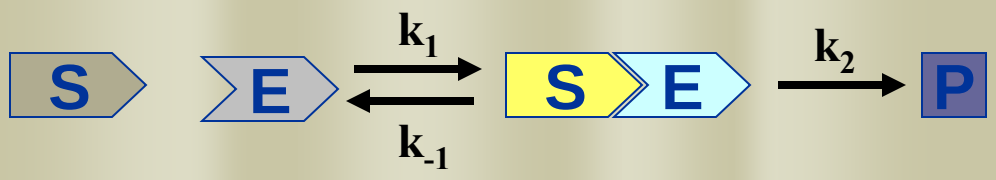
- $K_M = [S]$ when *V0* = 1/2 *V*max
- note that therefore *K*M has units of concentration

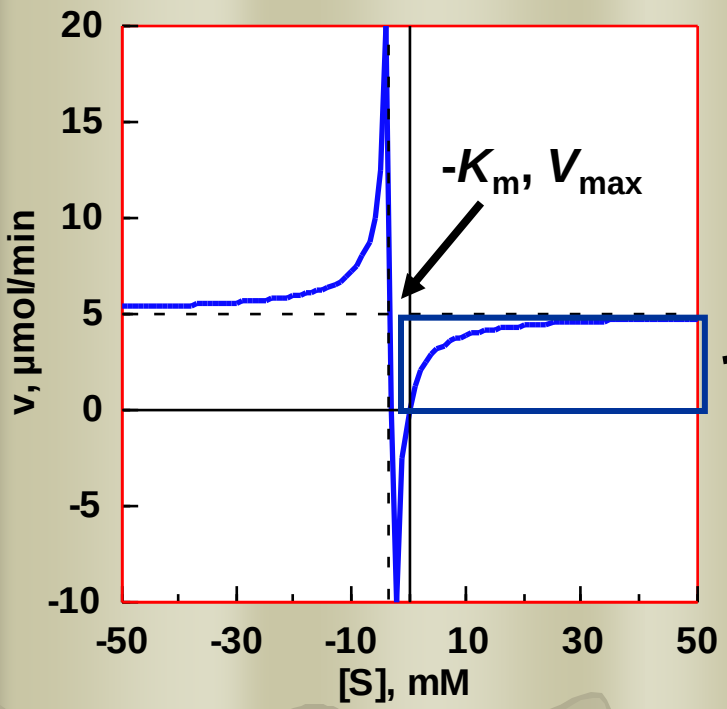
$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Parameters Obtained From Steady-state Analysis

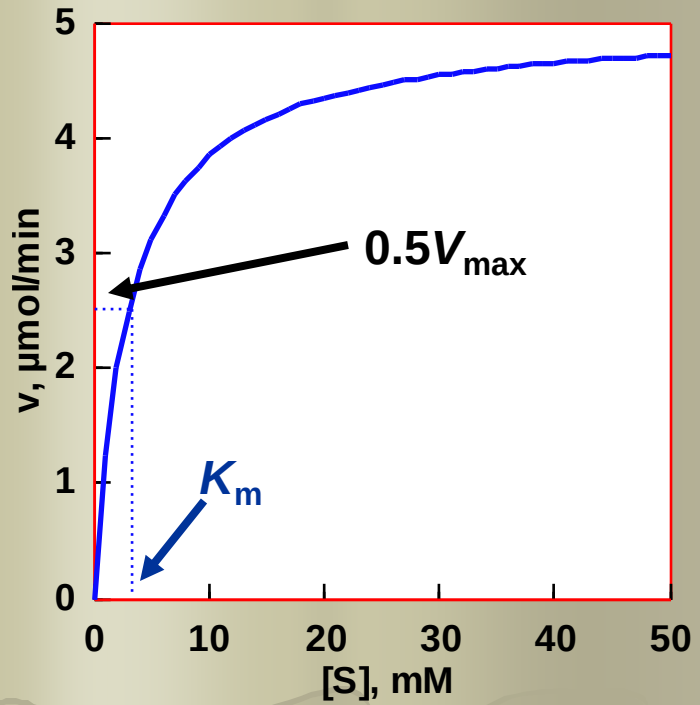


Enzymes



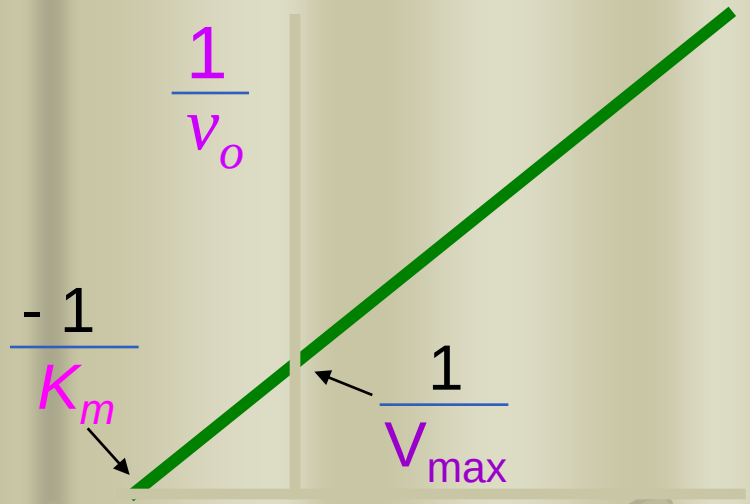


$$v = \frac{V_{max}[S]}{K_m + [S]}$$

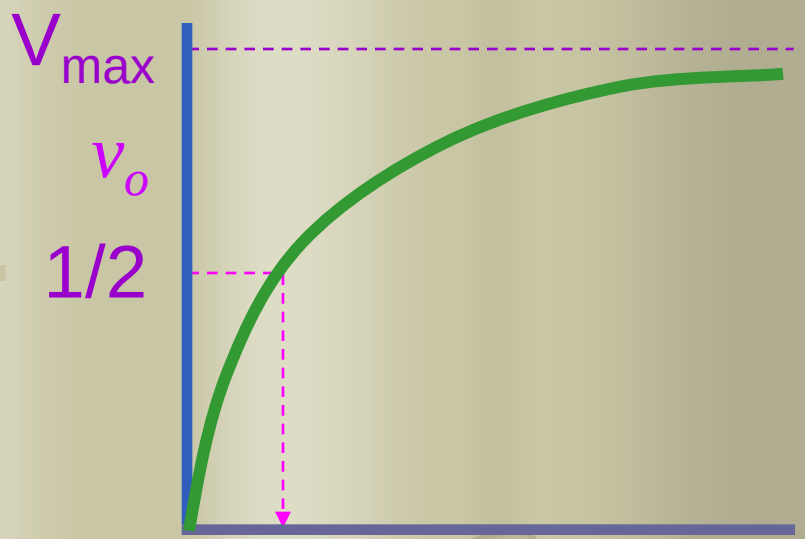


An Example for Enzyme Kinetics (Invertase)

1) Use predefined amount of Enzyme → E
2) Add substrate in various concentrations → S (x 軸)
3) Measure Product in fixed Time (P/t)→ v_o (y 軸)
4) (x, y) plot get hyperbolic curve, estimate → V_{max}
5) When y = 1/2 V_{max} calculate x ([S]) → K_m



Double reciprocal 1/S

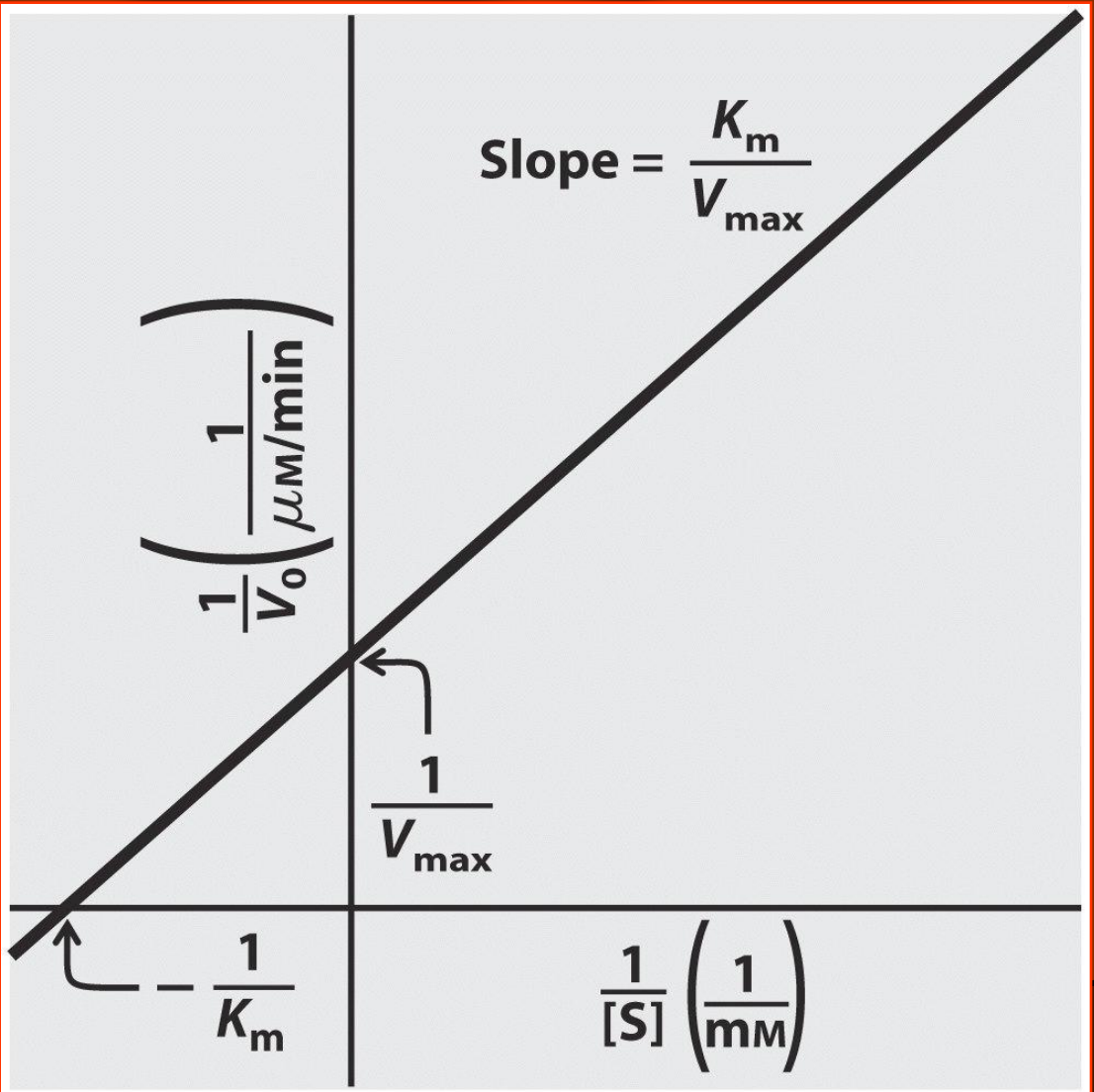


Direct plot S

- Each enzyme has a characteristic *K*M for a given substrate. Approximate value can be determined just from looking at a plot of rate vs [S] .
- More accurate values can be obtained by using non-linear regression program to fit the equation to the data.
- Historically, another method has been to transform the data to a linear form.
- The most famous of these transformations is the **Lineweaver-Burk** plot, 1/*V0* vs 1/[S].

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

Lineweaver-Burk plot: Linear transformation of data



Don't do this to your data!

- There are other linear transformations also, none are as good as just fitting the equation directly to the data using non-linear regression.
- The Lineweaver-Burk is particularly bad because it weights the data at low [S] most heavily – and these are often the least accurate experimentally.

A Real Example for Enzyme Kinetics

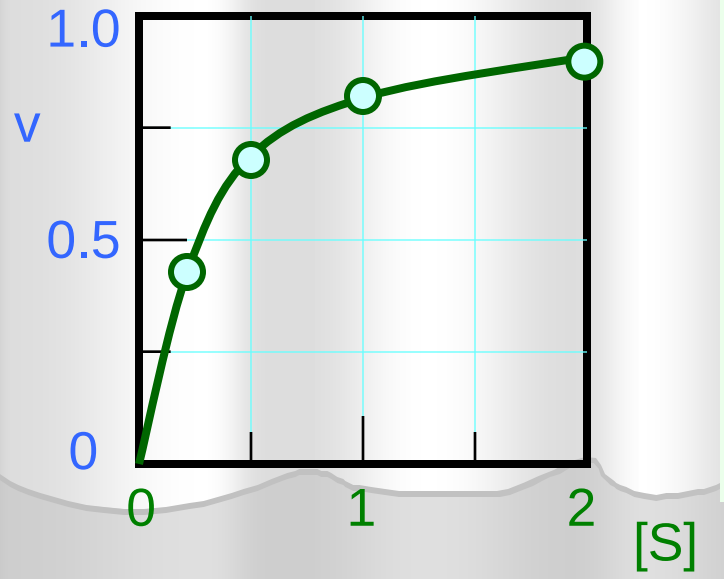
Data

	Substrate	Product		Velocity	Double reciprocal	
no	[S]	Absorbance		v (μmole/min)	1/S	1/v
1	0.25	0.21	→	0.42	4	2.08
2	0.50	0.36	→	0.72	2	1.56
3	1.0	0.40	→	0.80	1	1.35
4	2.0	0.46	→	0.92	0.5	1.16

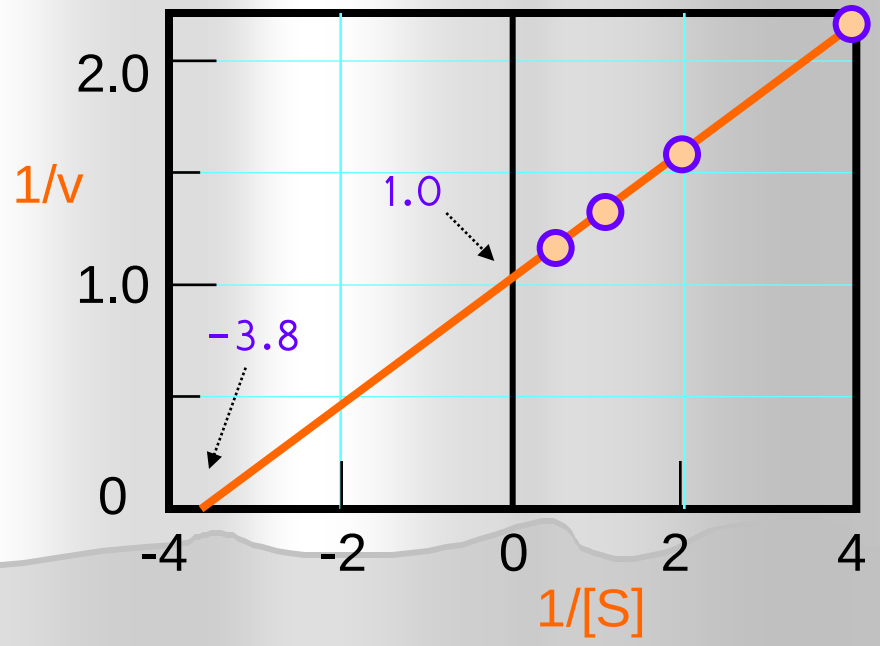
(1) The product was measured by spectroscopy at 600 nm for 0.05 per μmole

(2) Reaction time was 10 min

Direct plot



Double reciprocal



What does *K*M mean?

- If it is high (*e.g.* catalase has a *K*M for H2O2 of 25 mM) the enzyme requires a high concentration of substrate to reach its half-maximal rate.
- If *K*M is low (hexokinase has a *K*M for glucose of 0.05 mM) the enzyme reaches its half-maximal rate at low substrate concentration.

- *K*M is sometimes thought of as a measure of the affinity of an enzyme for a particular substrate – the higher the *K*M, the lower the affinity.
- Under certain circumstances, *K*M really does equal *K*d, the equilibrium dissociation constant for the substrate.
- But remember, *K*M is a combination of forward and reverse rate constant, and not necessarily a simple equilibrium constant.

K_m: Affinity with Substrate

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

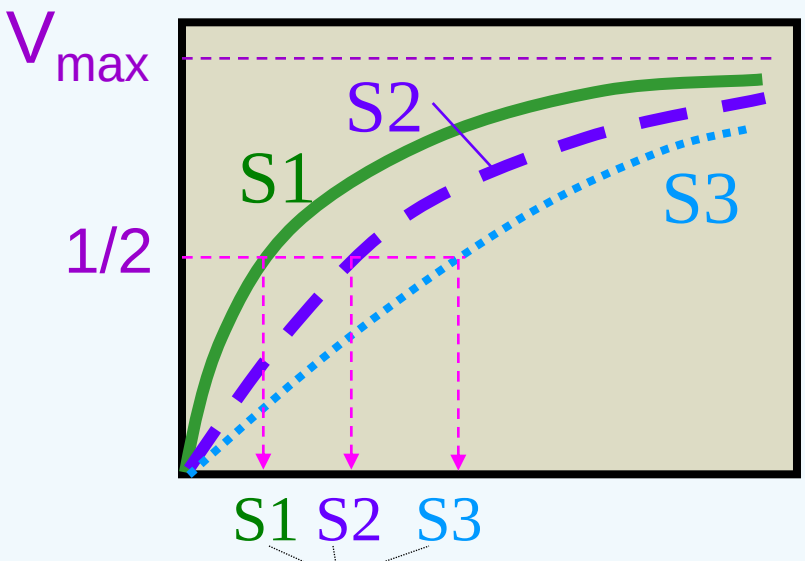
If $v_o = \frac{V_{max}}{2}$

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

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

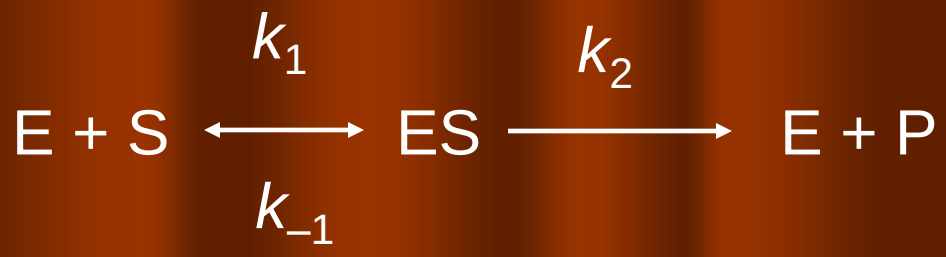
$$K_m = [S]$$

When using different substrate



Affinity changes

What Does K_M Mean?



- Concentration of S that gives half-maximal rate

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

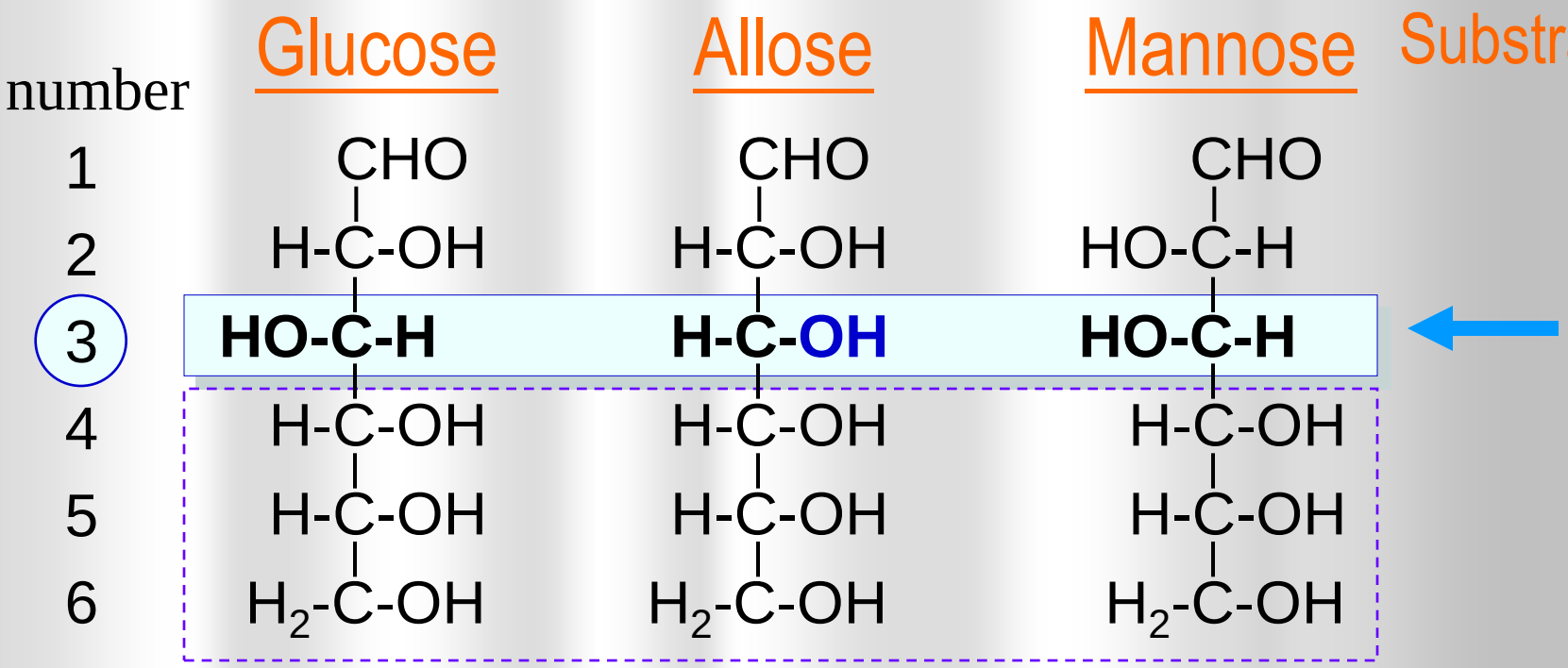
- If $k_{-1} >> k_2$ then $K_M = k_{-1} = \frac{K_D}{k_1}$

TABLE 6–6 K_m for Some Enzymes and Substrates

Enzyme	*Substrate*	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

K_m: Hexokinase Example

Glucose + ATP → Glc-6-P + ADP

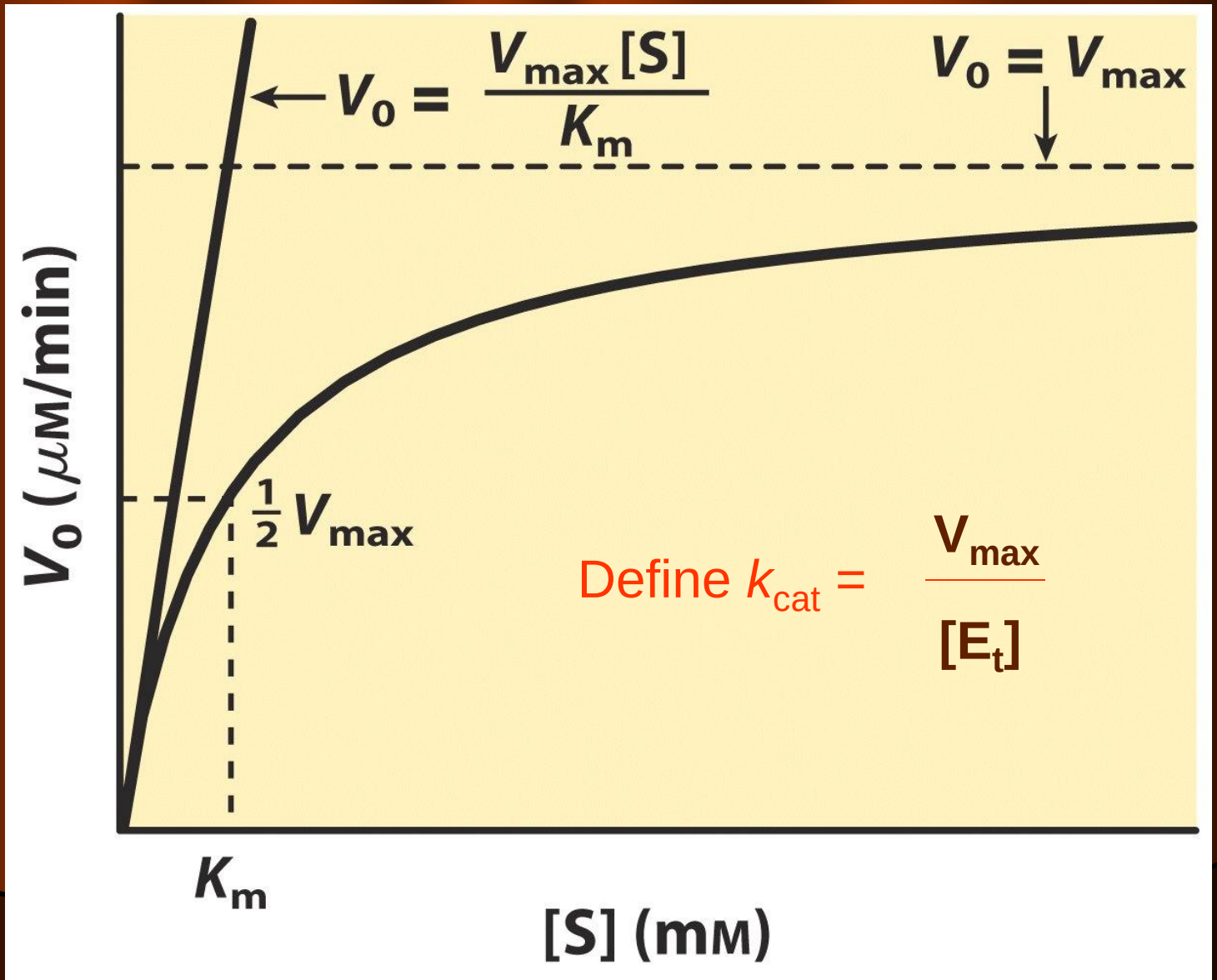


K_m = 8 | 8,000 | 5 μM

What does *V*max mean?

- Maximal velocity…but this is not so useful because the velocity, or rate, is in units of amount/time and is dependent on the enzyme concentration, which changes from experiment to experiment.
- A more useful parameter is *k*cat, which is equal to *V*max/[Et].

What is V_{max}?


$V_0 = \frac{V_{max}[S]}{K_m}$
$V_0 = V_{max}$
V_0 (μM/min)
$\frac{1}{2} V_{max}$
K_m
[S] (mM)
Define k_{cat} = $\frac{V_{max}}{[E_t]}$

Enzyme Kinetics

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

Direct plot

Significance
zero order
1st order

Observe v_o change under various [S], resulted plots yield V_{max} and K_m

E3
E2
E1

k_{cat}/K_m

k_{cat}
Turn over number

$k_3[E_t]$

V_{max} & **K_m**

Double reciprocal

Competitive

Non-competitive

Uncompetitive

Inhibition

Activity Unit
$\frac{1\ \mu mole}{min}$

Specific Activity
$\frac{unit}{mg}$

Activity

Maximum velocity

Affinity with substrate

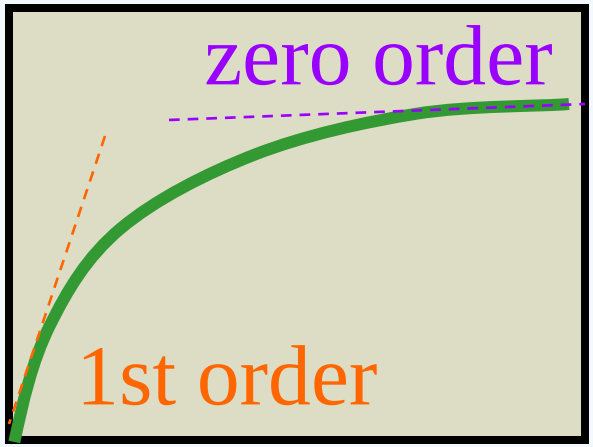
Bi-substrate reaction also follows M-M equation, but one of the substrate should be saturated when estimate the other

Significance of Enzyme Kinetics

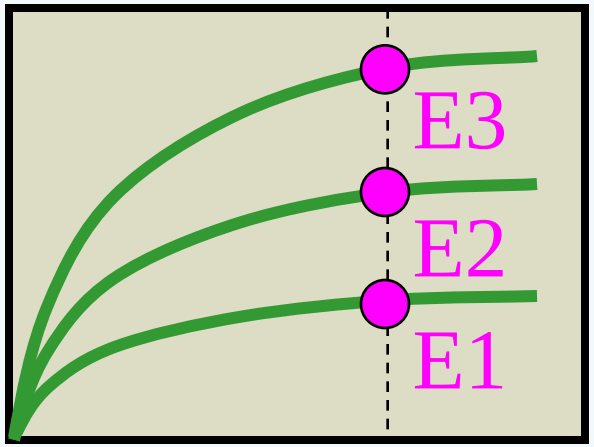
Obtain V_{max} and K_m

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

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



[S] = Low → High



[S] = Fixed concentration

- *k*cat is like the rate constant introduced in the beginning in the sense that an enzyme has a certain value of *k*cat for a certain substrate under a certain set of conditions – *k*cat does not depend on substrate concentration or on enzyme concentration.
- However, ***k*cat is the maximal rate constant for the overall reaction**, which may be a combination of rate constants for different reaction steps.
- If one step is the slowest, *k*cat is the rate constant for that step.

Turn Over Number, k_{cat}

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow[(v_o)]{k_3} E + P$$

When substrate excess, $k_3 = k_{cat}$, turn over number (t.o.n)

When [substrate] is low

$$v_o = \frac{V_{max}[S]}{K_m + [S]} = \frac{k_3[E][S]}{K_m + [S]} = \frac{k_3}{K_m}[E][S]$$

(IV)

Second order

Start from M-M eq. Omit the [substrate] Substrate specificity

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

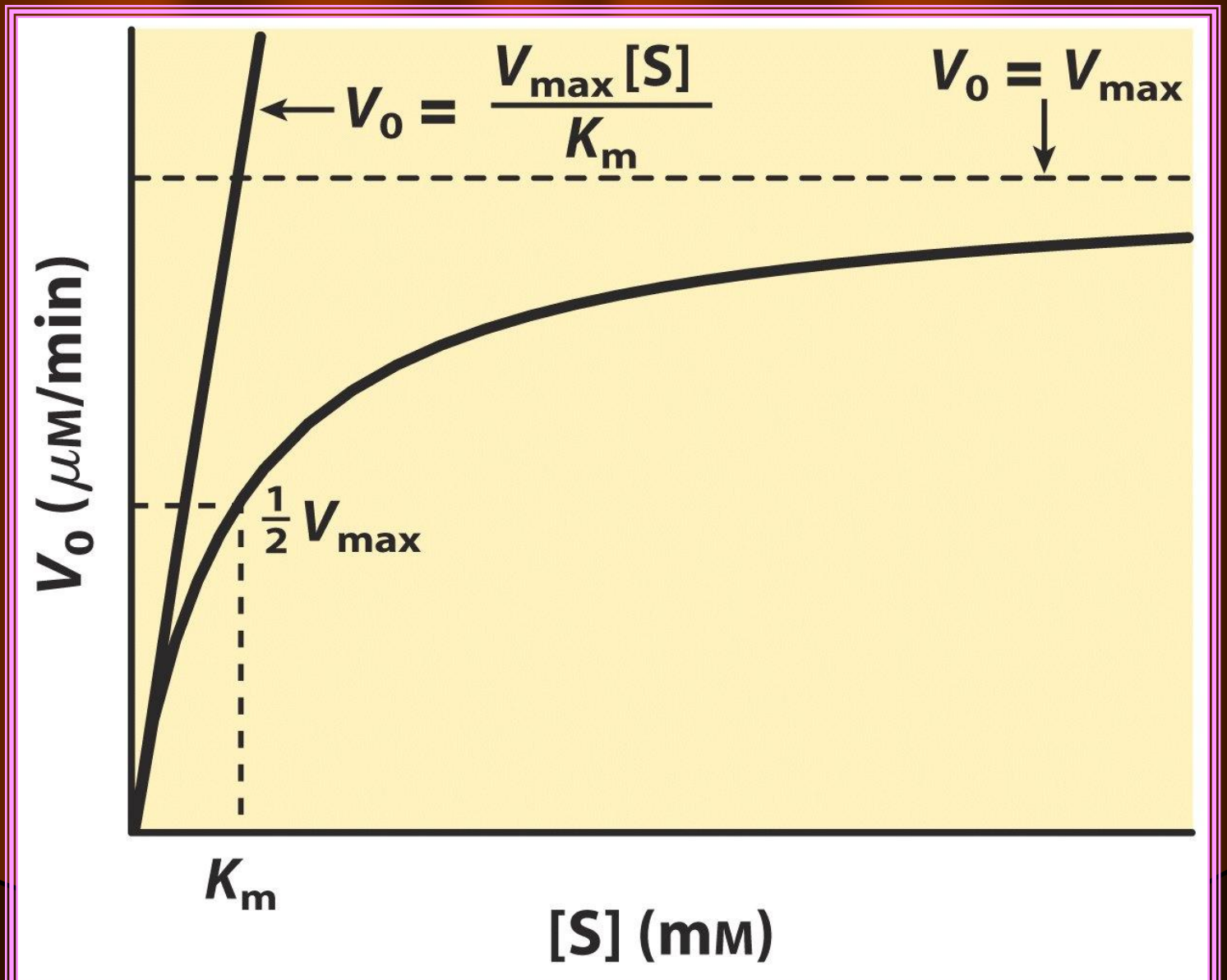
Enzyme	*Substrate*	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

Turn Over Numbers of Enzymes

Enzymes	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	140,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (ATPase)	ATP	0.4

The number of product transformed from substrate by one enzyme molecule in one second

What is (k_{cat}/K_M)?



- One more parameter – *k*cat/*K*M
- First we re-write the Michaelis-Menton equation
- Because *k*cat = *V*max/[Et]

$$V_0 = \frac{k_{cat}[E_t][S]}{K_M + [S]}$$

- When [S] << *K*M (as it usually is in the cell)

$$V_0 = \frac{k_{cat}}{K_M}[E_t][S]$$

- *V0* depends on the concentration of each of the two reactants: E and S. *k*cat/*K*M is a second-order rate constant with units of (concentration)–1(time)–1 for the reaction E + S → E + P

- The rate constant *k*cat/*K*M is affected by how often the enzyme and substrate form a complex when they interact by diffusion.
- Therefore, there is an upper limit for *k*cat/*K*M; it can never be larger than a value imposed by the diffusion rate, or in other words a reaction can't be faster than the time it takes for the to molecules to diffuse to each other.
- This upper limit is ~108–109 M–1 s–1. Some enzymes actually exhibit *k*cat/*K*M values near this diffusion-controlled limit.
- Such enzymes are said to have achieved catalytic perfection.

What is (k_{cat}/K_M)?

- First re-write Michaelis-Menten eq.

$$V_0 = \frac{k_{cat}[E_t][S]}{K_M + [S]}$$

Because $V_{max} = k_{cat}[E_t]$

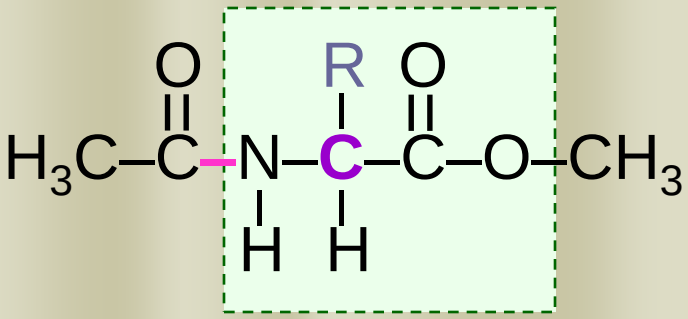
When $[S] << K_M$

$$V_0 = \frac{k_{cat}}{K_M}[E_t][S]$$

From the beginning of class, for the reaction of
E + S --> P, $V = k[E][S]$

k_{cat}/K_M is the rate constant for this rxn!

Chymotrypsin Has Distinct k_{cat}/K_m to Different Substrates



	R =	k_{cat} / K_m
Glycine	–H	1.3×10^{-1}
Norvaline	$–CH_2–CH_2–CH_3$	3.6×10^{2}
Norleucine	$–CH_2–CH_2–CH_2–CH_3$	3.0×10^{3}
Phenylalanine	$–CH_2–C_6H_5$	1.0×10^{5}

$(M^{-1} s^{-1})$

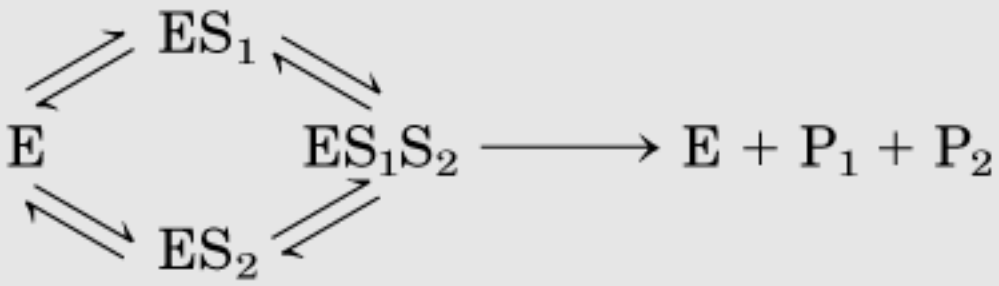
TABLE 6–8 Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

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.

(a) Enzyme reaction involving a ternary complex

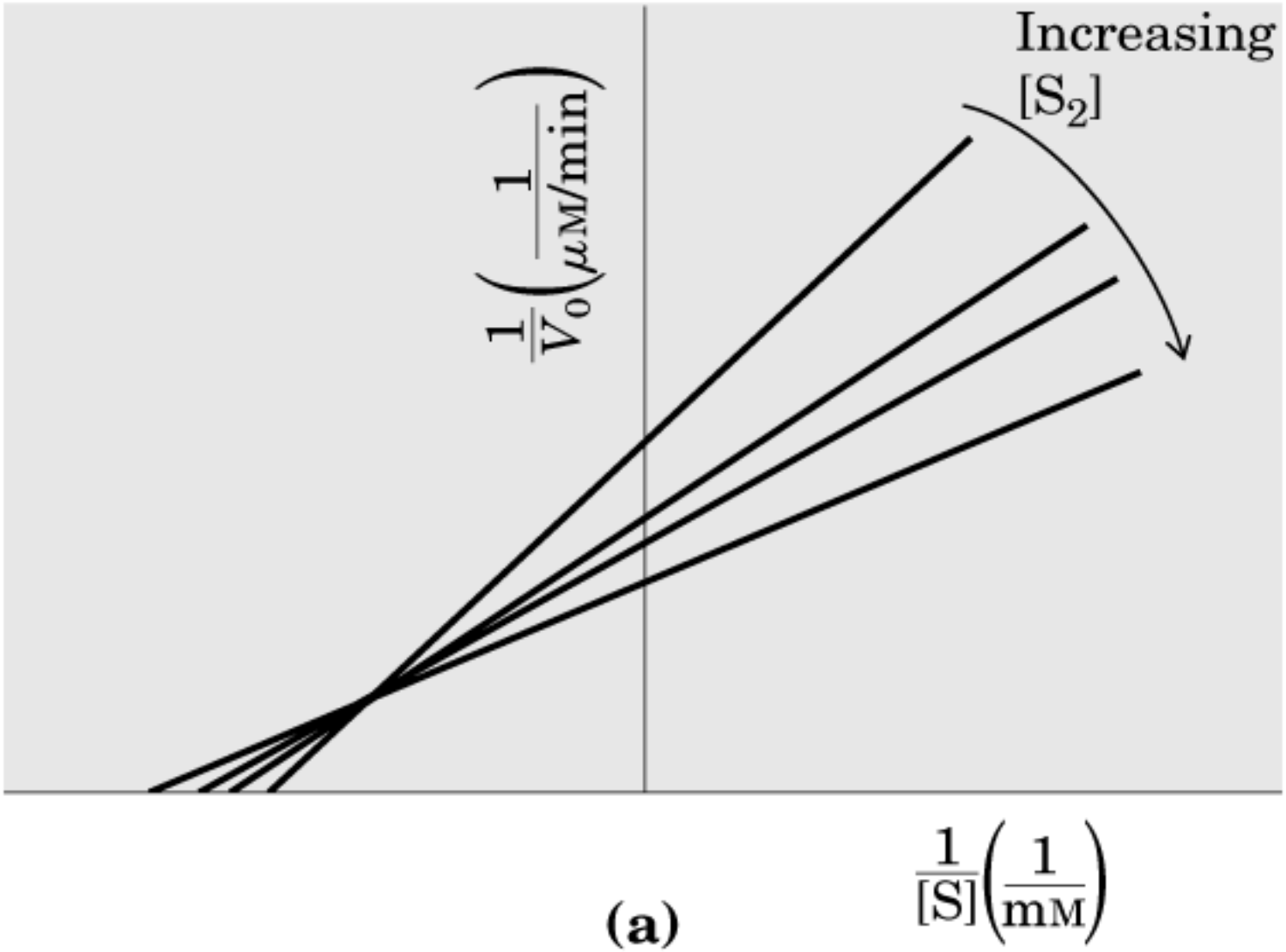
Random order



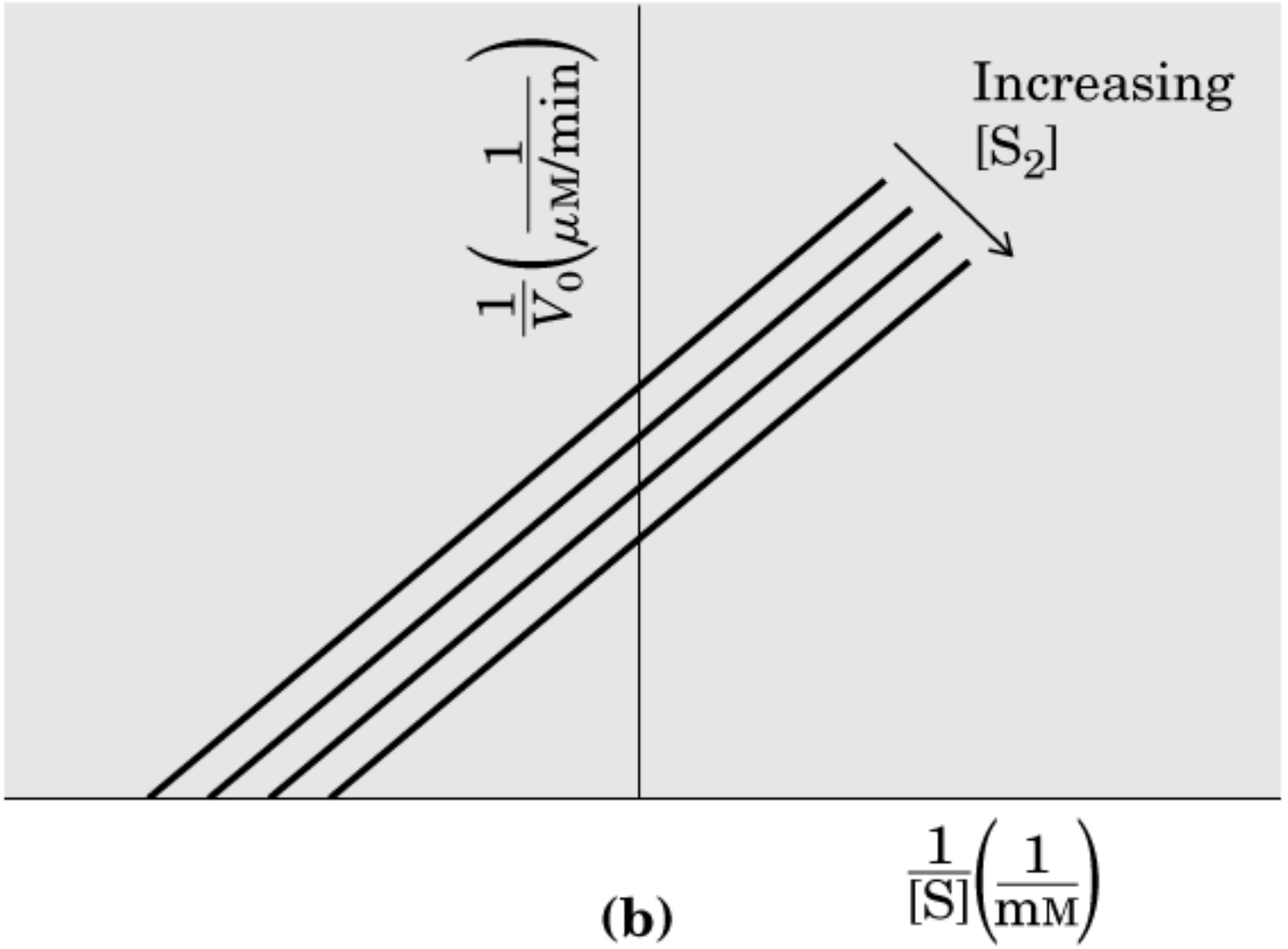
Ordered

(b) Enzyme reaction in which no ternary complex is formed

$$E + S_1 \rightleftharpoons ES_1 \rightleftharpoons E'P_1 \overset{P_1}{\rightleftharpoons} E' \overset{S_2}{\rightleftharpoons} E'S_2 \longrightarrow E + P_2$$



(a)


$\frac{1}{V_0}\left(\frac{1}{\mu M/min}\right)$
Increasing
$[S_2]$
$\frac{1}{[S]}\left(\frac{1}{mM}\right)$

(b)

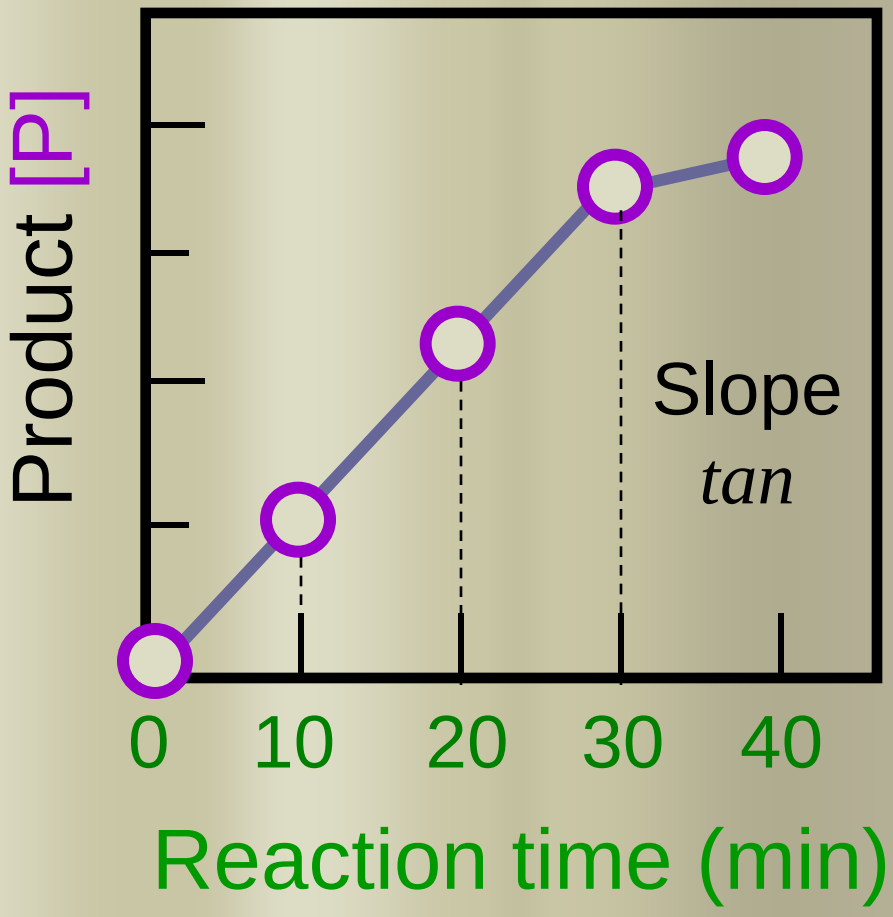
Enzyme Activity Unit

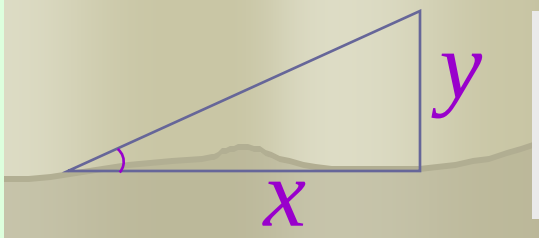
$$S \xrightarrow[t]{} P \ \mu mole$$

$$v_o = [P] / \min$$

$$\text{Unit} = \mu mole / \min$$

$$\text{Specific Activity} = \frac{\text{Activity Units}}{\text{Protein (mg)}}$$

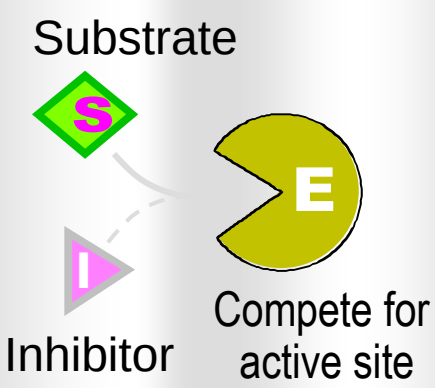
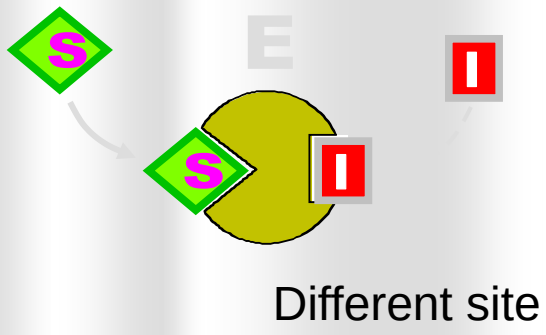
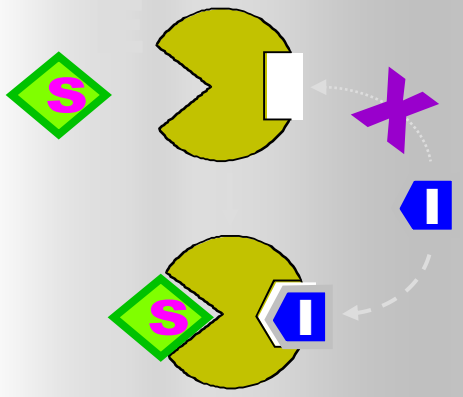




$$\frac{y}{x} = tan$$

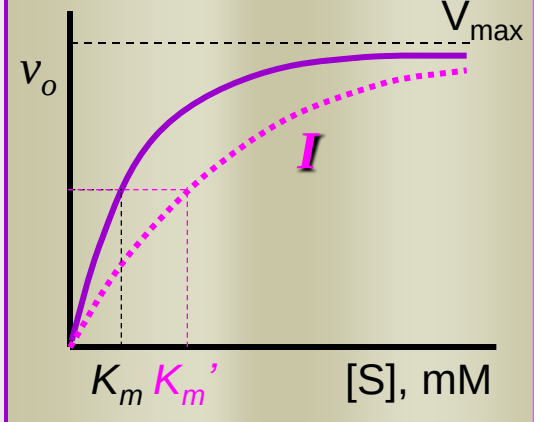
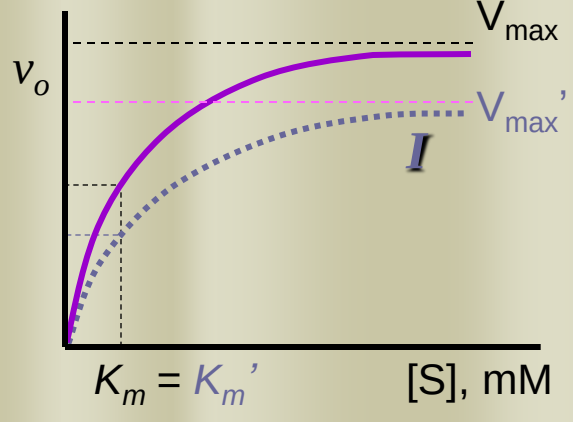
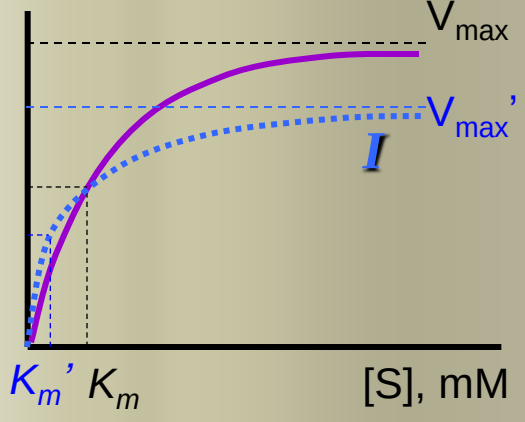
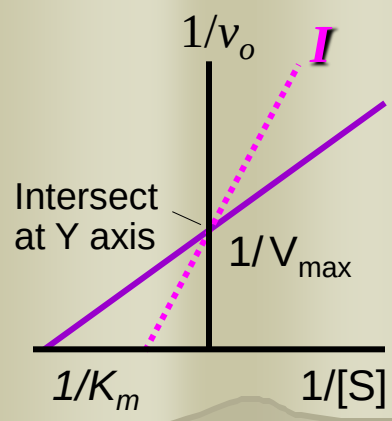
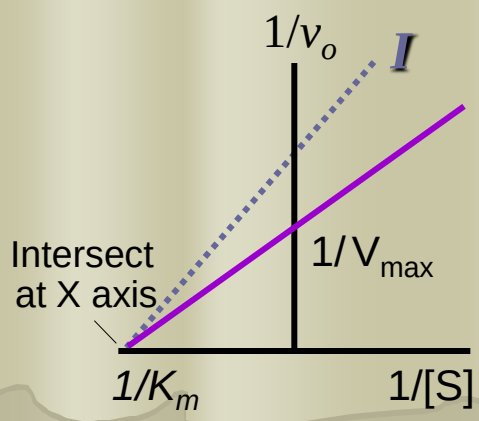
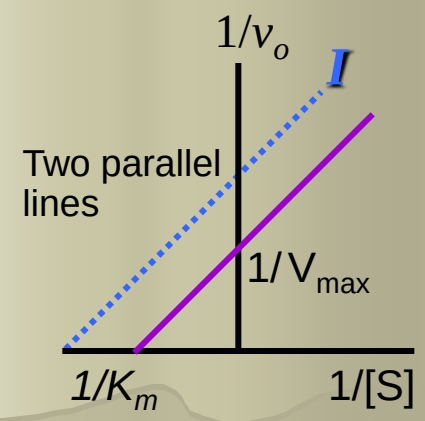
Enzyme inhibition

Enzyme Inhibition (Mechanism)

	Competitive	Non-competitive	Uncompetitive
Cartoon Guide			
Equation and Description	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\Updownarrow$ EI	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ $+$ I I $\Updownarrow$ $\downarrow\uparrow$ $EI + S \rightarrow EIS$	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\Updownarrow$ EIS
	[*I*] binds to free [E] only, and competes with [S]; increasing [S] overcomes Inhibition by [*I*].	[*I*] binds to free [E] or [ES] complex; Increasing [S] can not overcome [*I*] inhibition.	[*I*] binds to [ES] complex only, increasing [S] favors the inhibition by [*I*].

Juang RH (2004) BCbasics

Enzyme Inhibition (Plots)

	Competitive	Non-competitive	Uncompetitive
Direct Plots			
	V_{max} unchanged K_m increased	V_{max} decreased K_m unchanged	Both V_{max} & K_m decreased
Double Reciprocal			

Juang RH (2004) BCbasics

Enzyme inhibitors

- There are two general types of inhibition – reversible and irreversible
- Reversible – three types

1. **competitive** – inhibitor binds reversibly in the same or an overlapping site as the substrate binding site

   E + I ←→ EI

- The difference between S and I is that I is not converted into product, so EI is inactive. The rate equation becomes
- $V_0 = \frac{V_{max}[S]}{aK_M + [S]}$ where $a = \frac{[I]}{K_I}$

- *K*I is the dissociation constant for the enzyme-inhibitor complex

$$K_{\mathrm{I}} = \frac{[E][I]}{[EI]}$$

- Because formation of EI depends on [I] just as formation of ES depends on S, the actual rate of competitively inhibited reaction is dependent on the relative concentrations of I and S.
- Inhibition by a given competitive inhibitor can be overcome by increasing [S].

Competitive Inhibition

Product Substrate

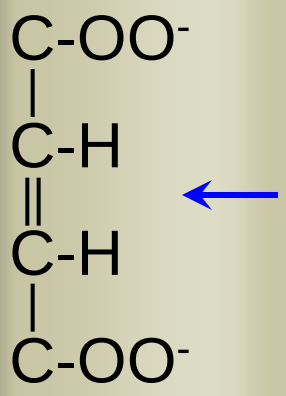
Competitive Inhibitor

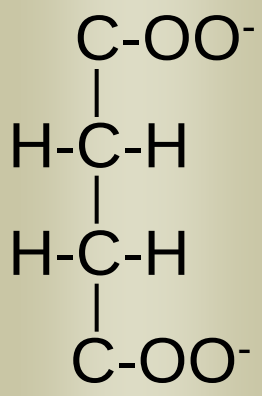
Succinate

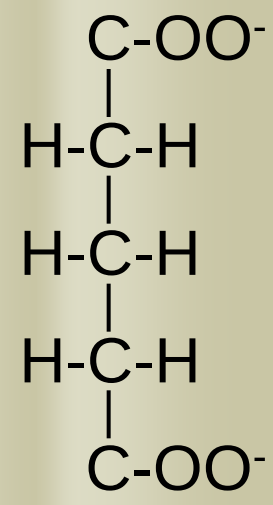
Glutarate

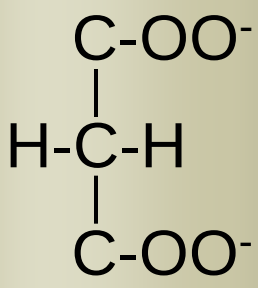
Malonate

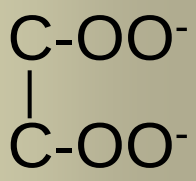
Oxalate









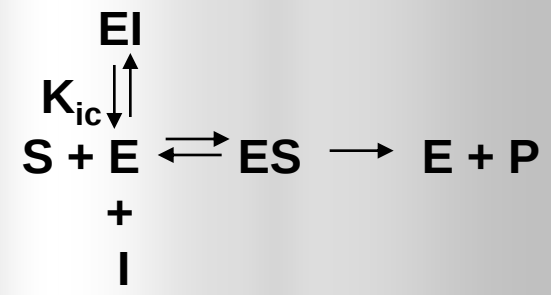


Succinate Dehydrogenase

Adapted from Kleinsmith & Kish (1995) Principles of Cell and Molecular Biology (2e) p.49

Competitive Inhibition

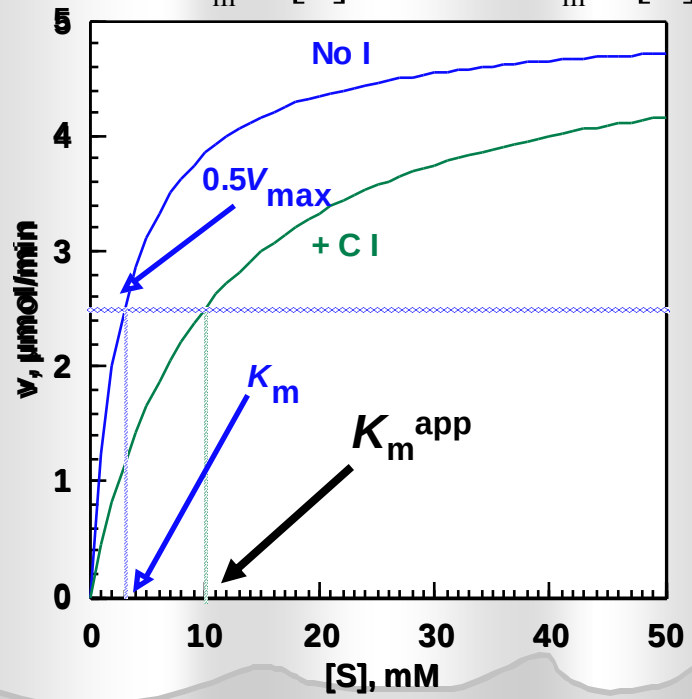
Competitive S CI E

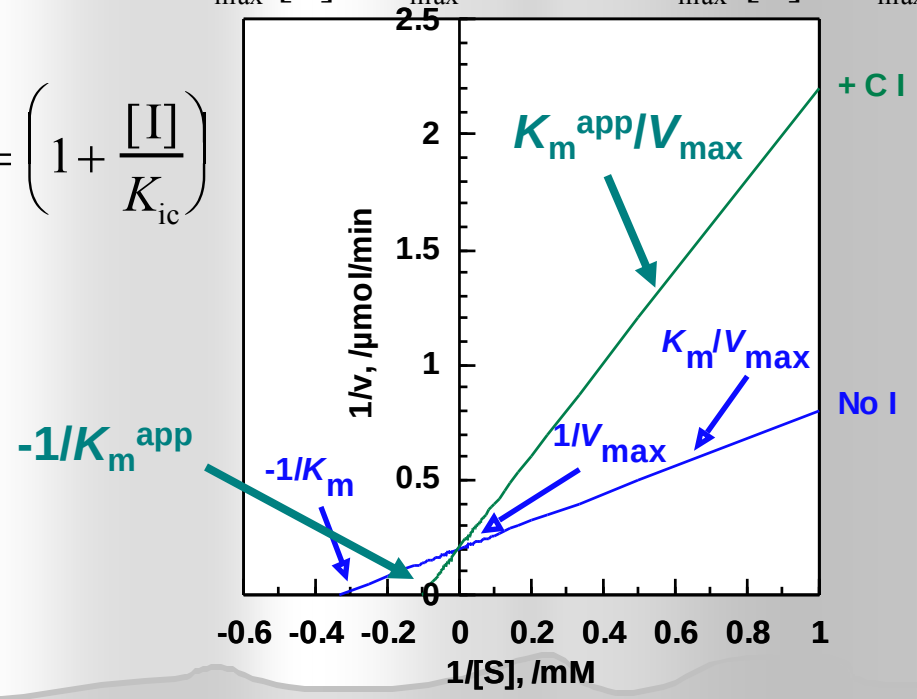


$$v = \frac{V_{max}[S]}{K_m + [S]} \qquad v = \frac{V_{max}[S]}{\alpha K_m + [S]}$$

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

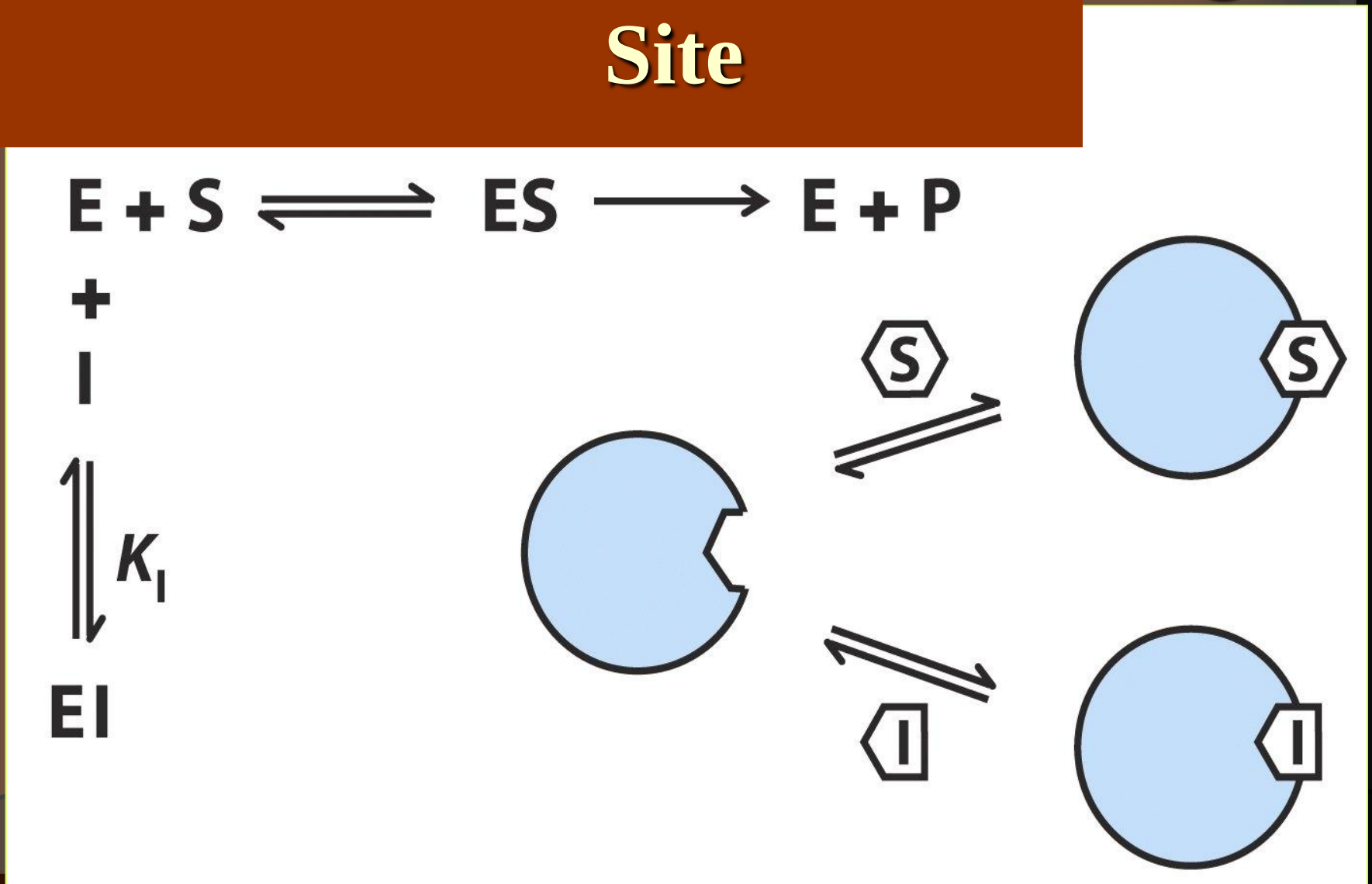
$$\alpha = \left(1 + \frac{[I]}{K_{ic}}\right)$$





- Thus *V*max is not affected by competitive inhibition – you can always get to the same maximal rate by adding S, but you need to add a higher concentration of S in the presence of inhibitor – in other words the effective *K*M is increased.
- Most competitive inhibitors resemble the substrates but are unreactive.

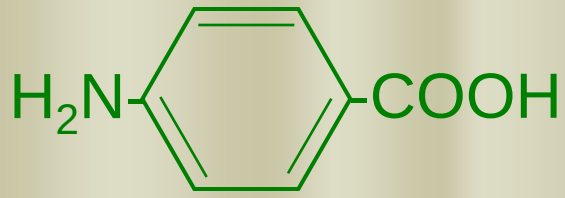
Competitive Inhibition: Inhibitor Binds in the Substrate Binding Site



Sulfa Drug Is Competitive Inhibitor

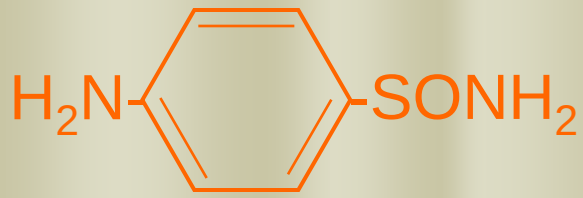
Domagk (1939)

Para-aminobenzoic acid (PABA)



Bacteria needs PABA for the biosynthesis of folic acid

Precursor → Folic acid → Tetrahydro-folic acid



Sulfa drugs has similar structure with PABA, and inhibit bacteria growth.

Sulfanilamide

Sulfa drug (anti-inflammation)

Adapted from Bohinski (1987) Modern Concepts in Biochemistry (5e) p.197

Enzyme Inhibitors Are Extensively Used

- Sulfa drug (anti-inflammation)

  Pseudo substrate competitive inhibitor

- Protease inhibitor **Alzheimer's disease**

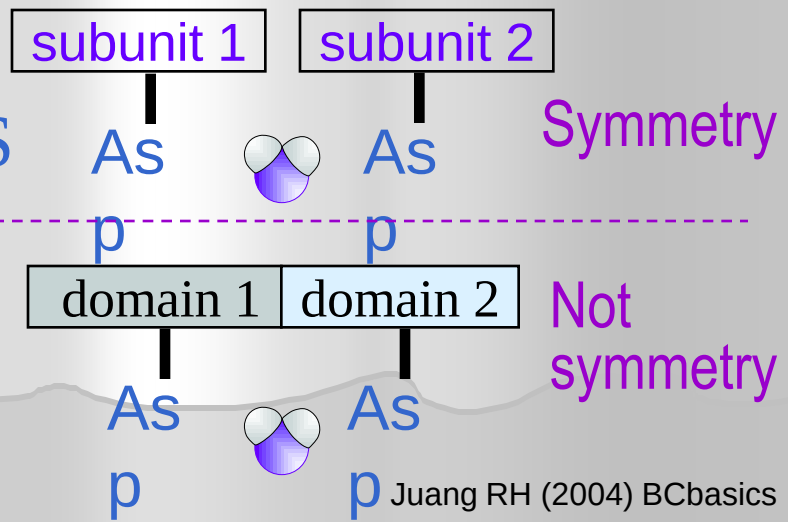
  Plaques in brain contains protein inhibitor

- HIV protease is critical to life cycle of HIV

  HIV protease (homodimer):

  ↑ **inhibitor** is used to treat AIDS

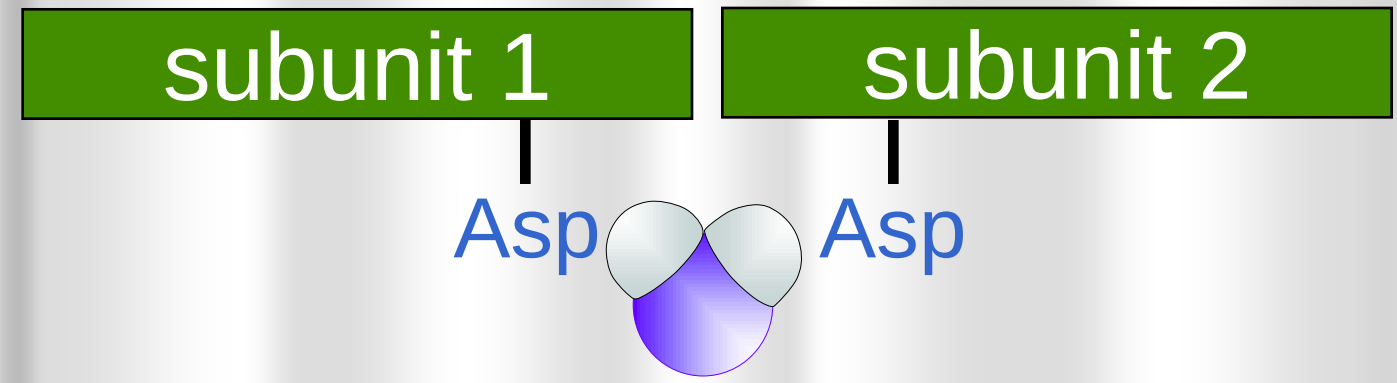
  → Human aspartyl protease: (monodimer)



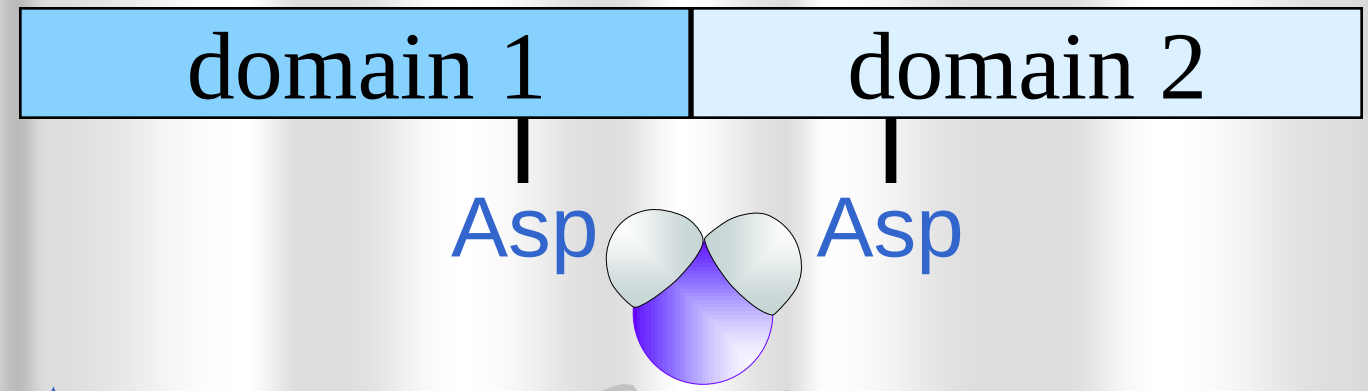
HIV protease vs Aspartyl protease

HIV Protease **inhibitor** is used in treating AIDS

↓ **HIV protease** (homodimer)



Symmetric dimer



Asymmetric monomer

↑ Aspartyl protease (monomer)

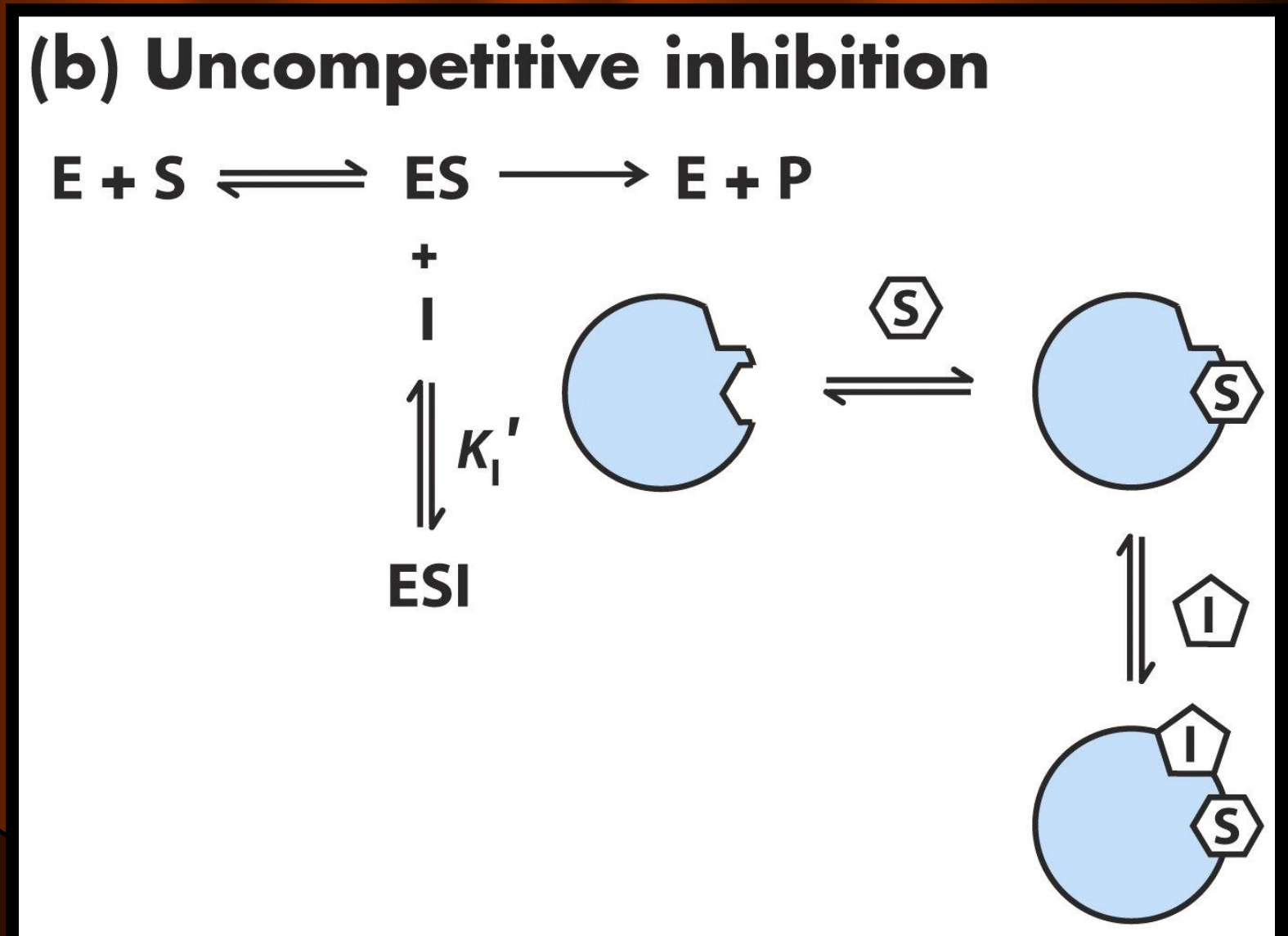
Juang RH (2004) BCbasics

2. Uncompetitive :

- the inhibitor binds only to the ES complex for form an ESI ternary complex, which then cannot go on to react.
- E + S ←→ ES
- ES + I ←→ ESI
- $$K'_{I} = \frac{[ES][I]}{[ESI]}$$
- $$V_0 = \frac{V_{max}[S]}{K_M + a'[S]}$$
- $$a' = 1 + \frac{[I]}{K'_{I}}$$

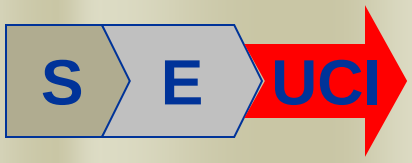
- Inhibition is not reversed by adding substrate; rate does not reach *V*max.
- Uncompetitive inhibition is usually observed in enzymes that bind two substrates.

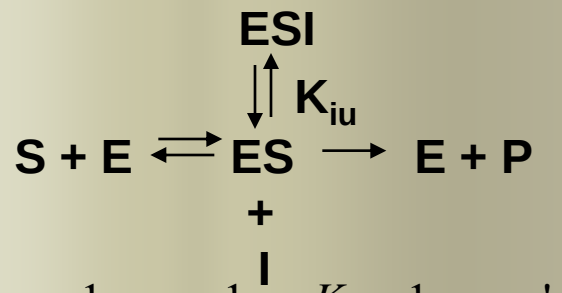
Inhibitor Only Binds to the ES Complex



Uncompetitive Inhibition

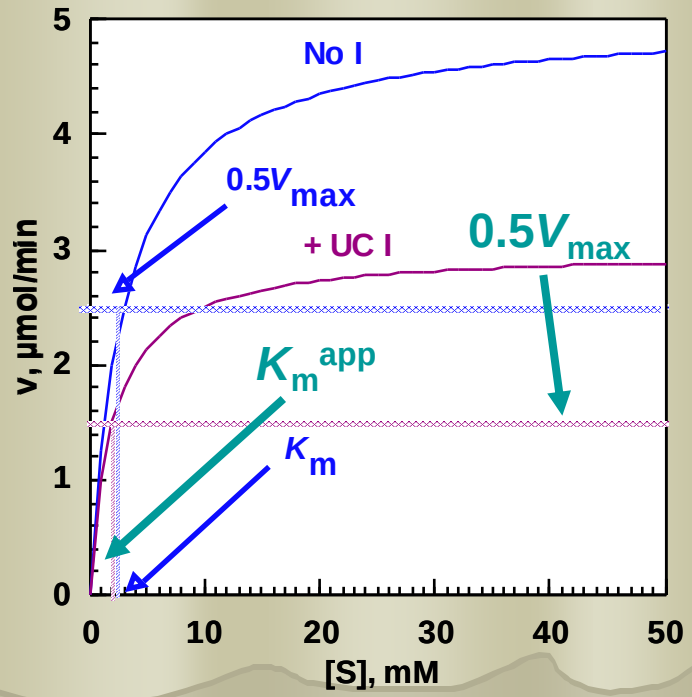
Uncompetitive (catalytic)



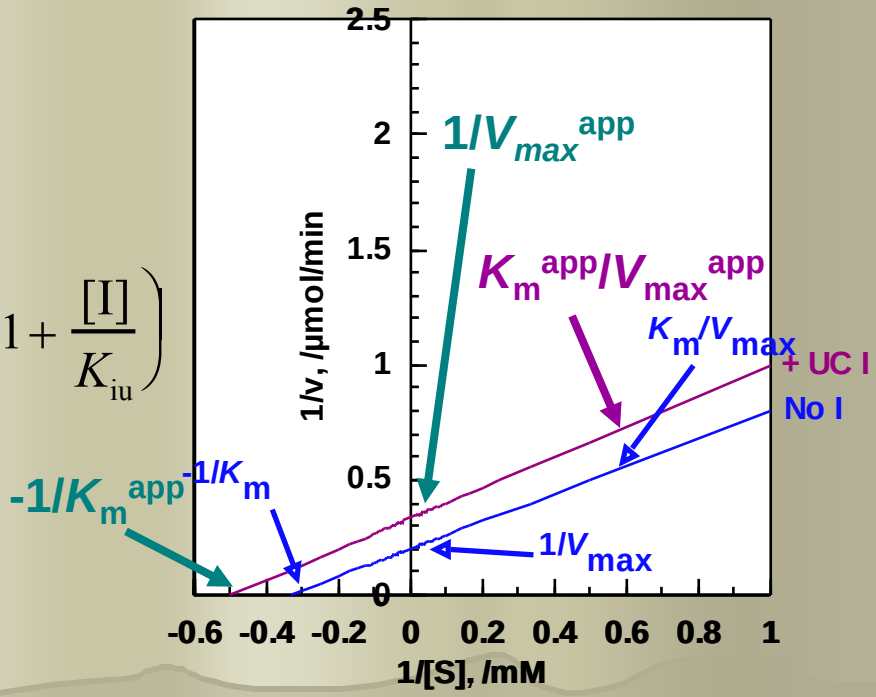


$$v = \frac{V_{max}[S]}{K_m + [S]} \qquad v = \frac{V_{max}[S]}{K_m + \alpha'[S]}$$

$$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}} \qquad \frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$



$$\alpha' = \left(1 + \frac{[I]}{K_{iu}}\right)$$



3. Mixed:

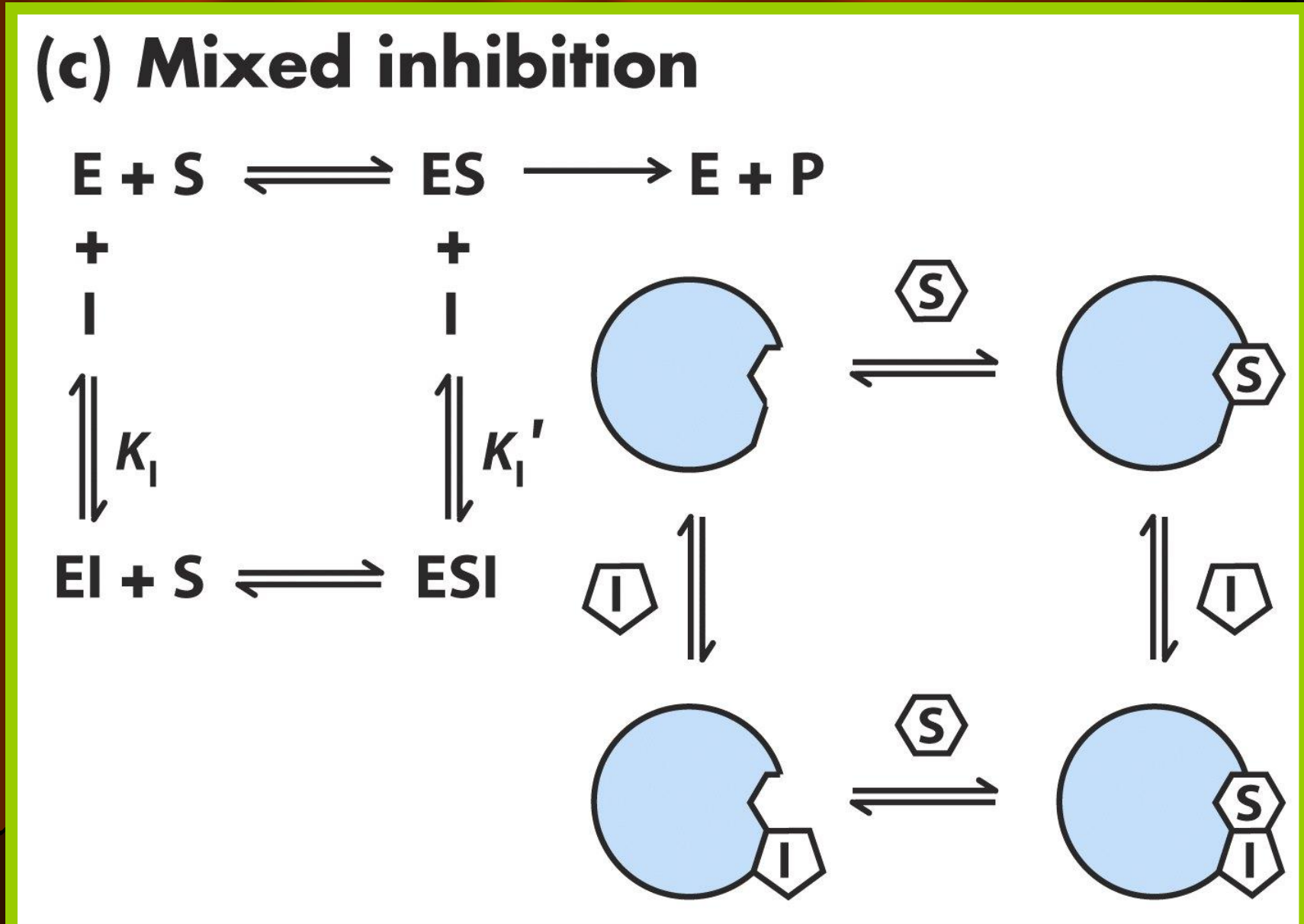
- the inhibitor can bind both to E and ES. The affinity can be the same – special case called **non-competitive** – or different.

$$V_0 = \frac{V_{max}[S]}{aK_M + a'[S]}$$

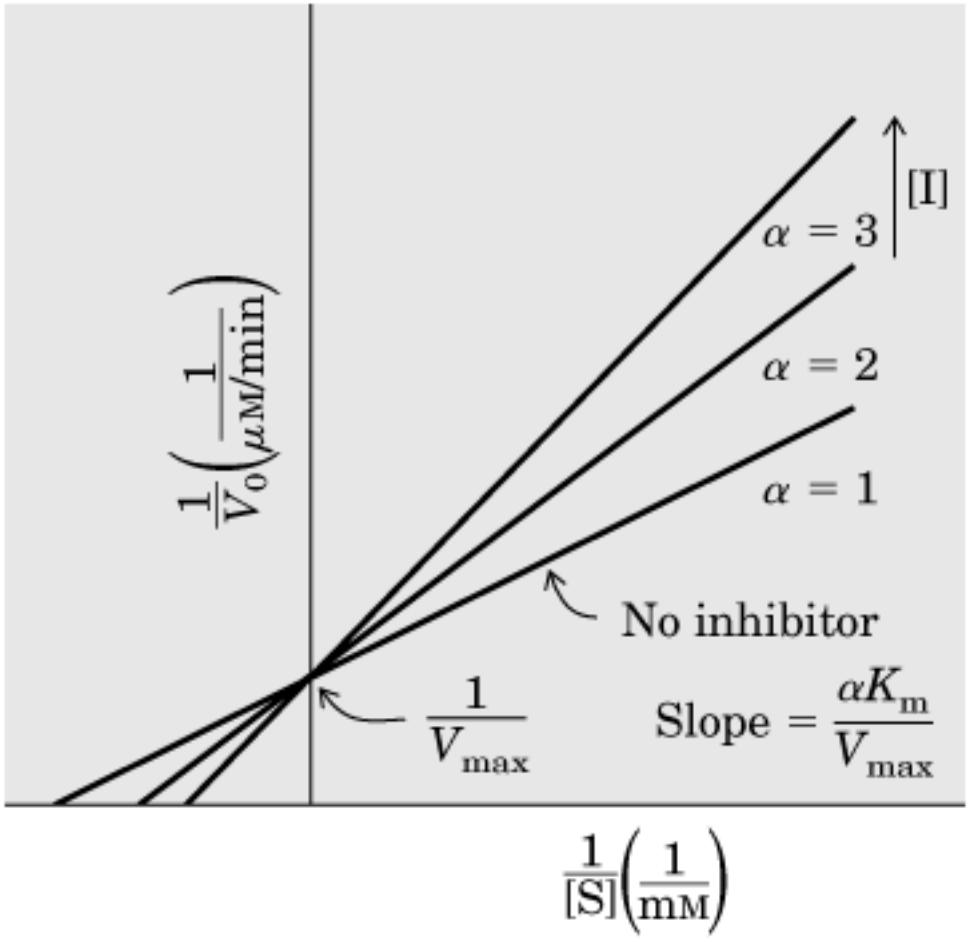
- If a and a' are the same value, it's noncompetitive inhibition.
- Mixed inhibition normally seen only in enzymes that bind two or more substrates.

- An inhibitor bound at the site of S1 may allow binding of S2 but not reaction (because S1 cannot bind).
- Such an inhibitor would be competitive for S1 but mixed for S2.

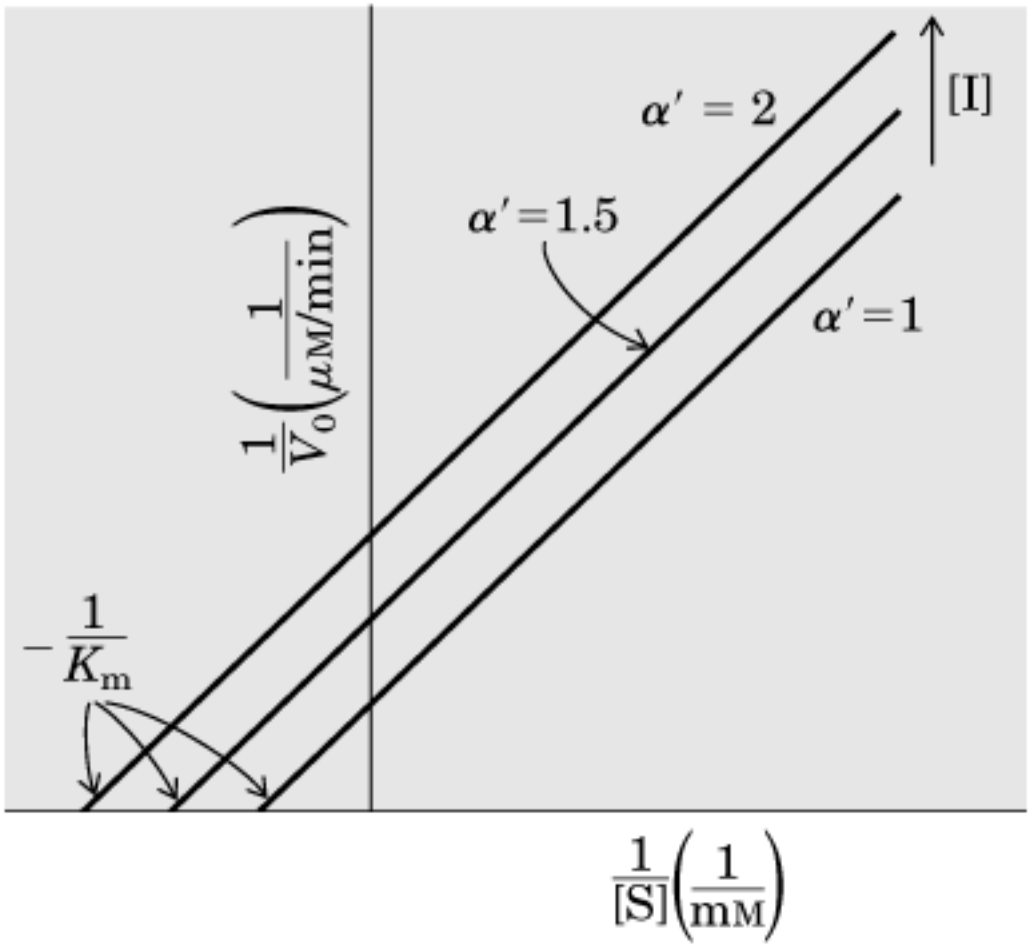
Inhibitor Binds Both E and ES



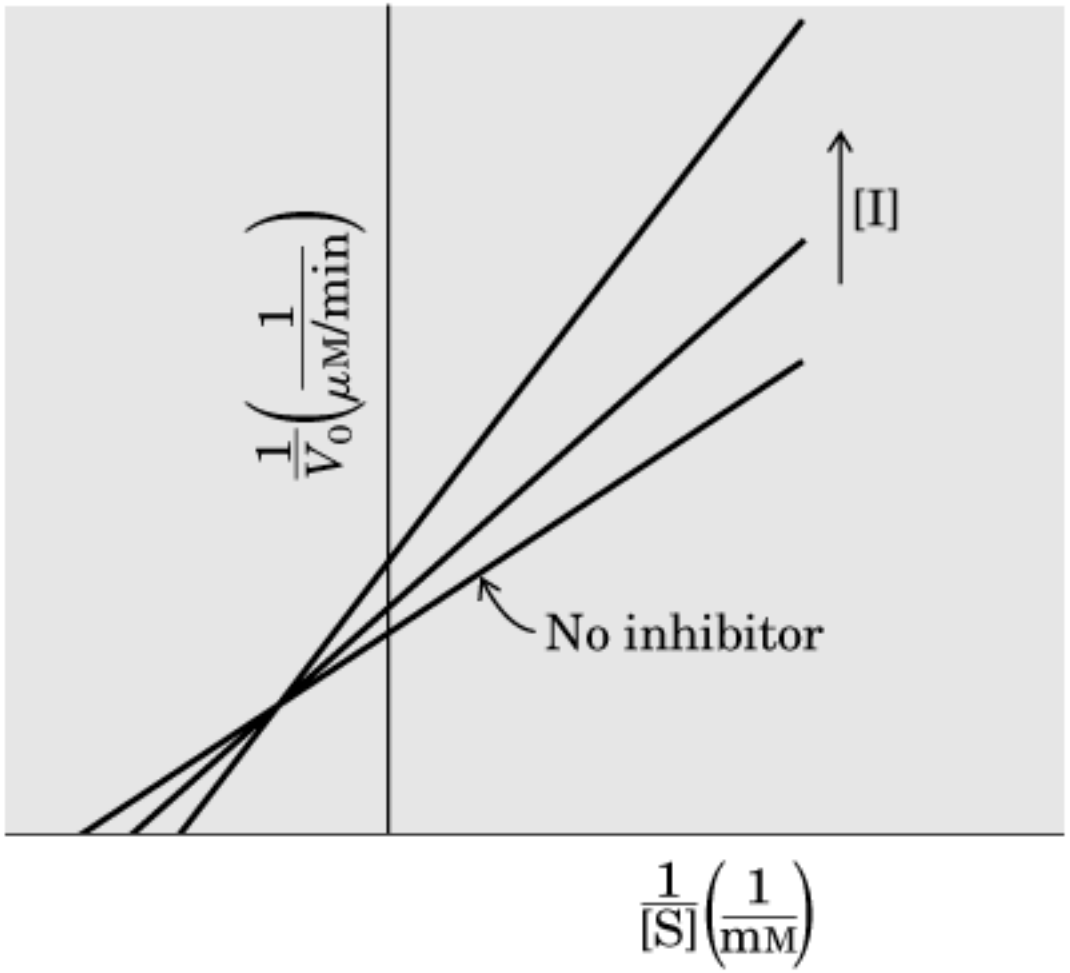
$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

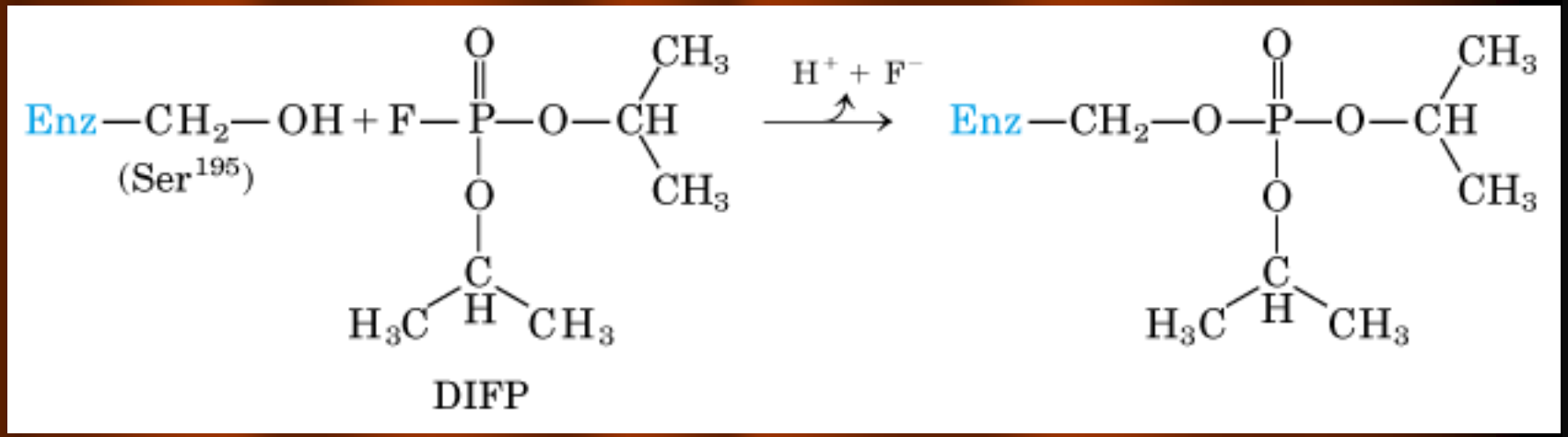


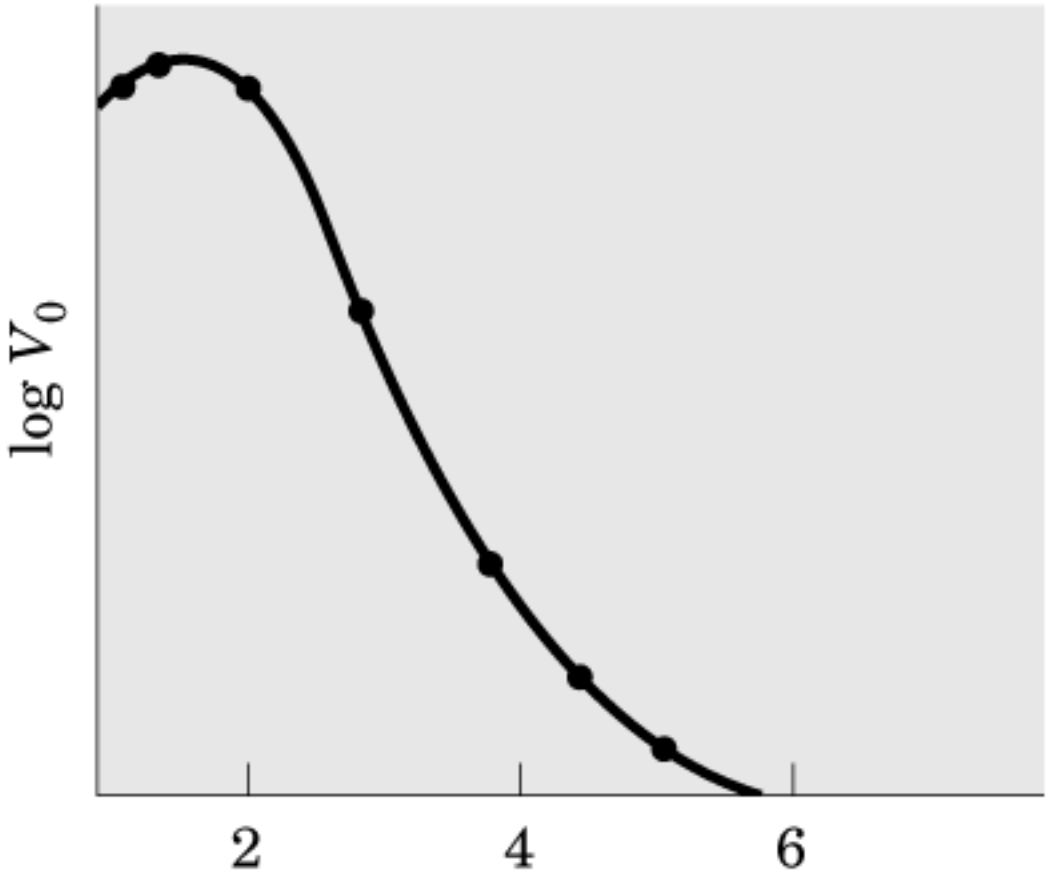
$$\frac{1}{V_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$



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

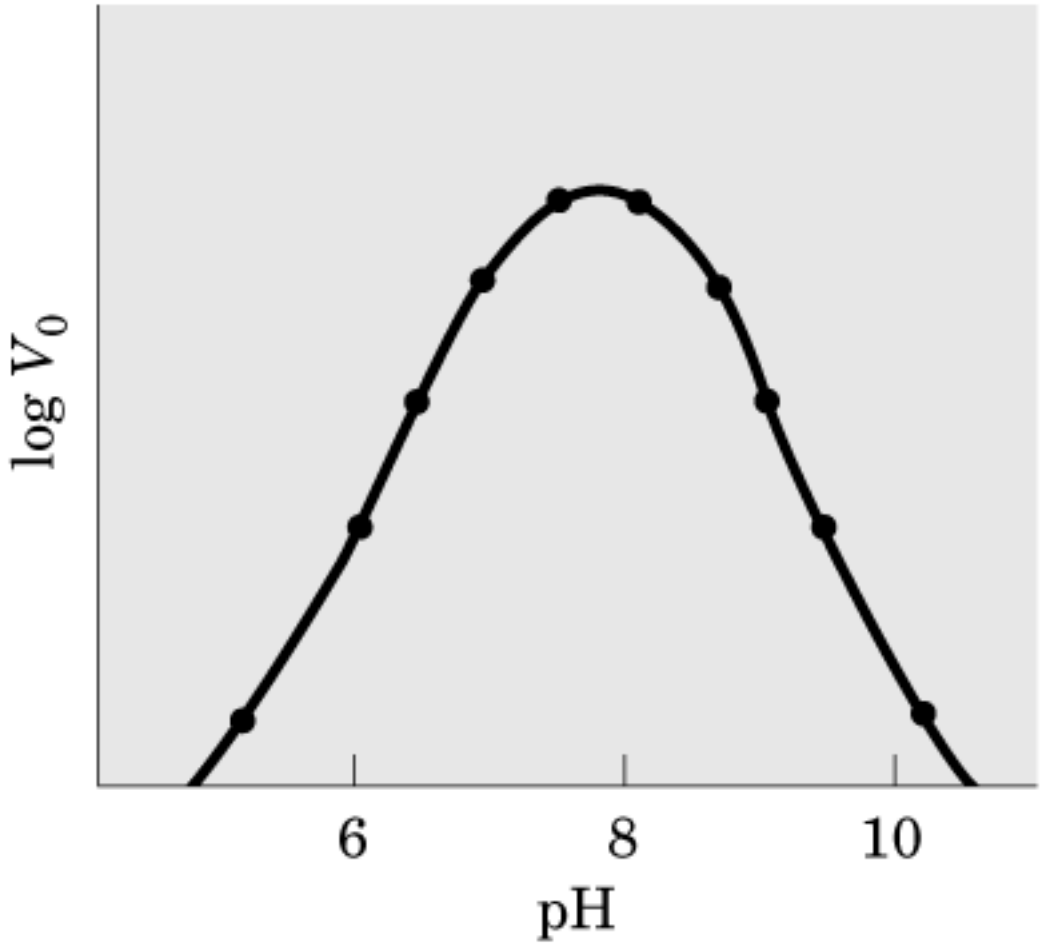



Enz—CH2—OH (Ser195) + F—P(=O)(—O—CH(CH3)2)—O—CH(CH3)2
H3C CH CH3
DIFP
H+ + F−
Enz—CH2—O—P(=O)(—O—CH(CH3)2)—O—CH(CH3)2
H3C CH CH3



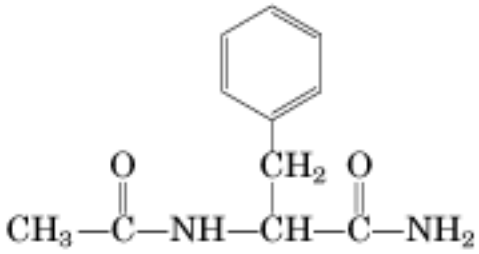
Pepsin

(a)

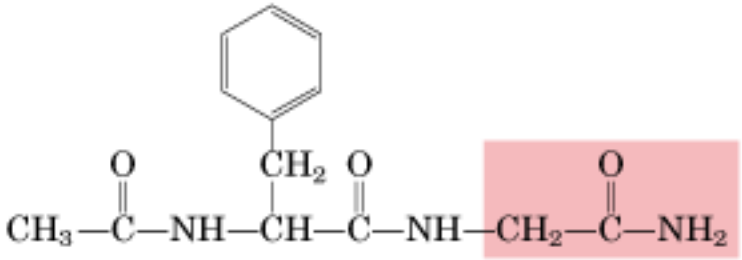


Glucose 6-phosphatase
(b)

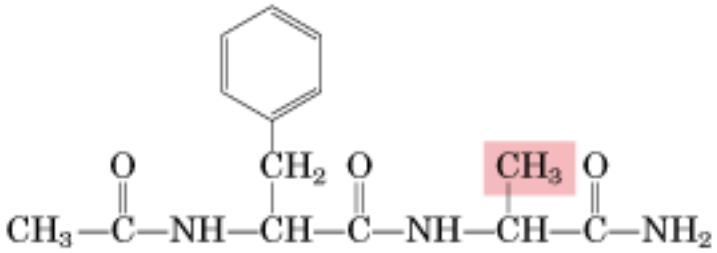
Substrate A



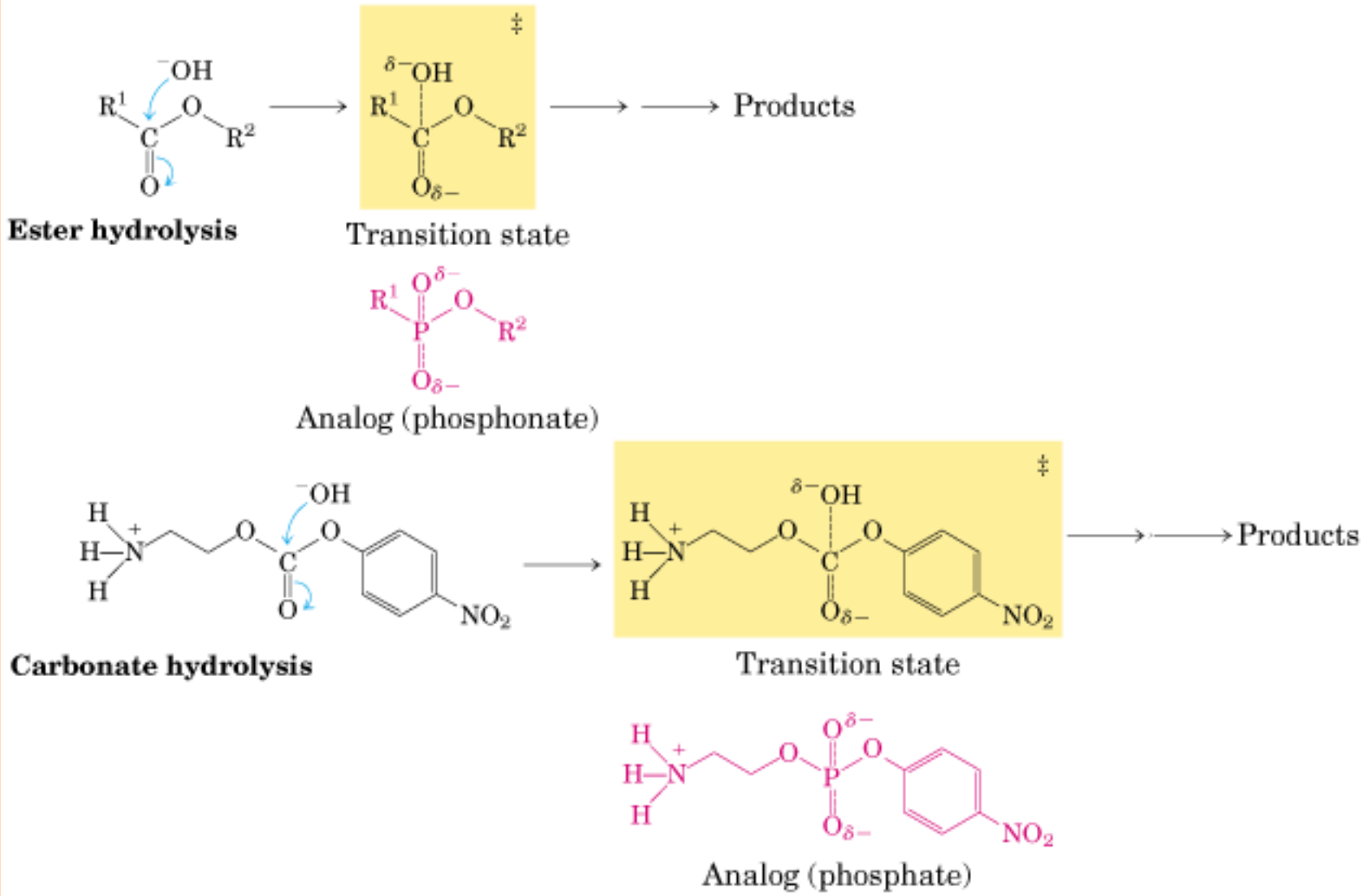
Substrate B



Substrate C

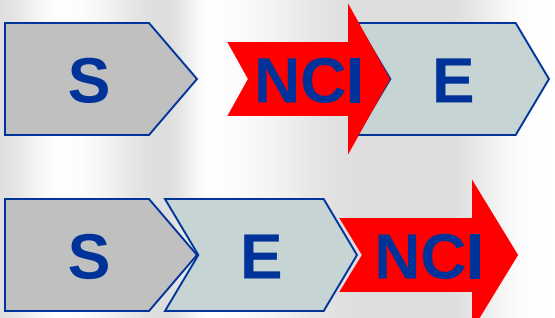


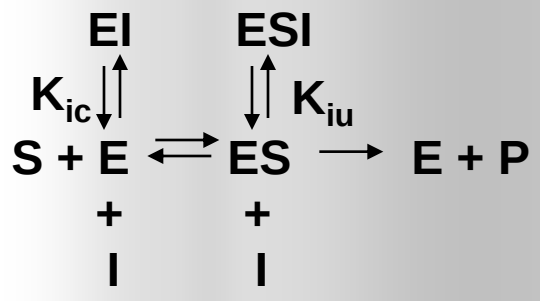
k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
0.06	31	2
0.14	15	10
2.8	25	114


Products
Ester hydrolysis
Transition state
Analog (phosphonate)
Products
Carbonate hydrolysis
Transition state
Analog (phosphate)

Noncompetitive Inhibition

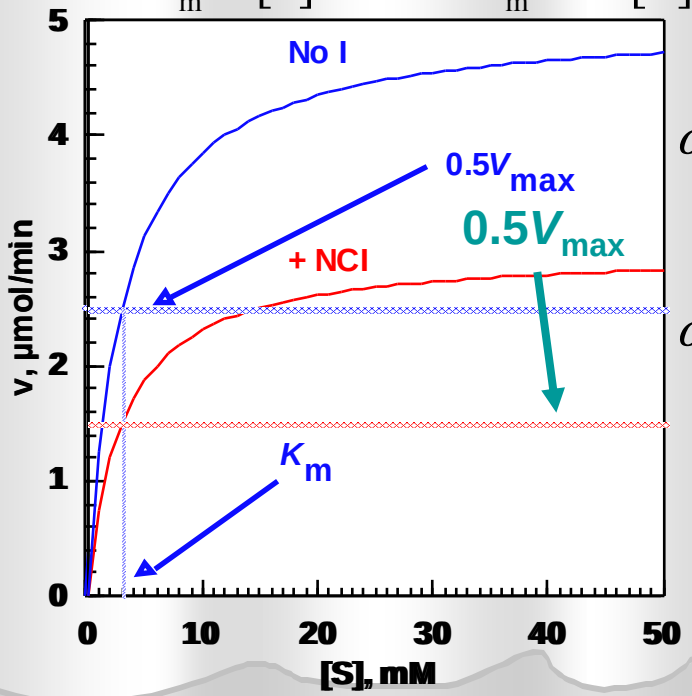
Noncompetitive (mixed-type)





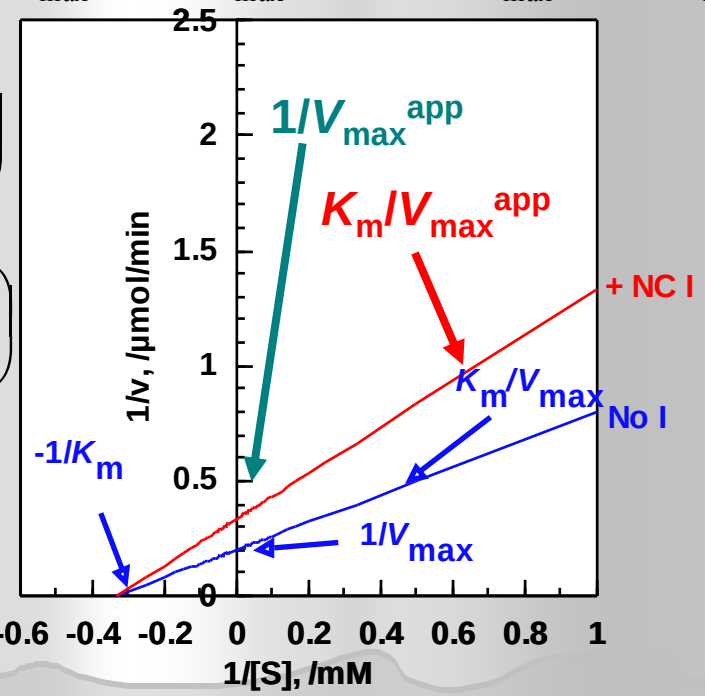
$$v = \frac{V_{max}[S]}{K_m + [S]} \qquad v = \frac{V_{max}[S]}{\alpha K_m + \alpha'[S]}$$

$$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}} \qquad \frac{1}{v} = \frac{\alpha K_m}{V_{max}}\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$



$$\alpha = \left(1 + \frac{[I]}{K_{ic}}\right)$$

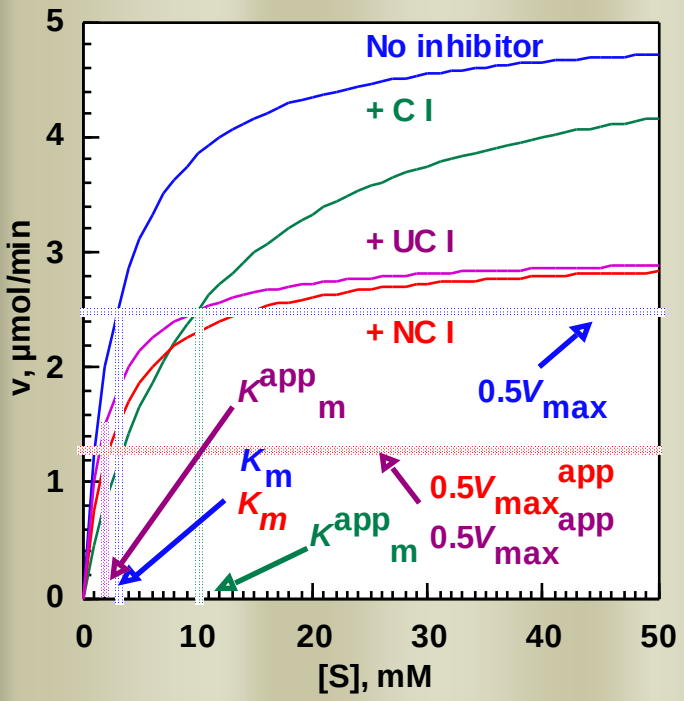
$$\alpha' = \left(1 + \frac{[I]}{K_{iu}}\right)$$



Enzyme Inhibition Summary

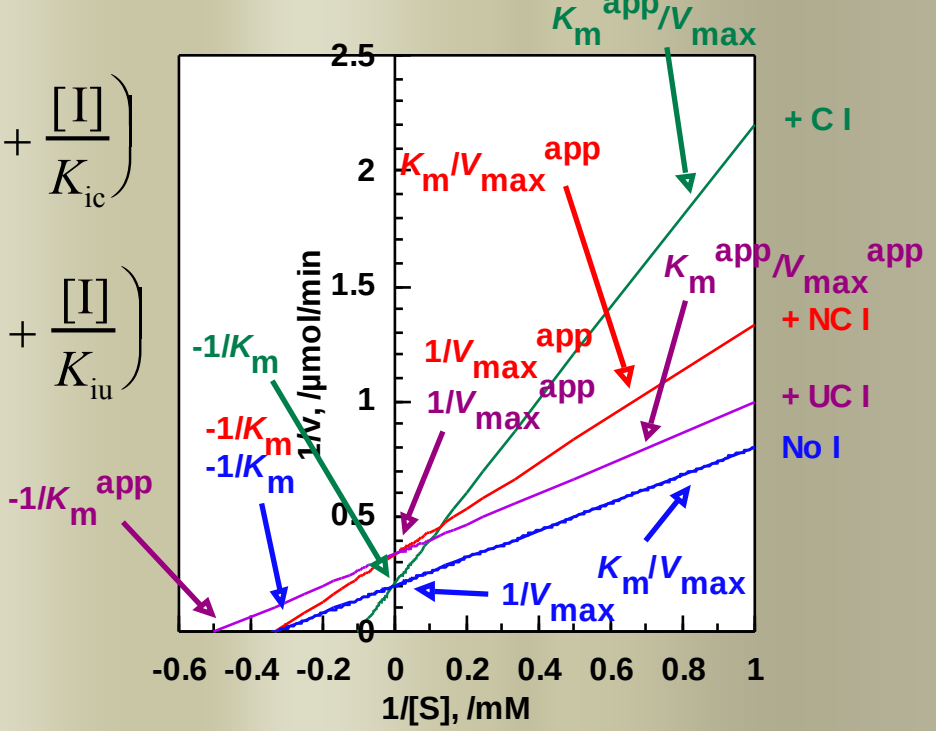
$$v = \frac{V_{max}[S]}{K_m + [S]} \qquad v = \frac{V_{max}[S]}{\alpha K_m + \alpha'[S]}$$

$$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}} \qquad \frac{1}{v} = \frac{\alpha K_m}{V_{max}}\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$



$$\alpha = \left(1 + \frac{[I]}{K_{ic}}\right)$$

$$\alpha' = \left(1 + \frac{[I]}{K_{iu}}\right)$$



Neat or what?

Irreversible inhibition

- the inhibitor combines with or destroys a functional group on the enzyme, thereby ‘killing’ the enzyme
- Typically the inhibitor undergoes partial reaction, but then forms a covalent bond with the enzyme.
- This can be useful for identifying important amino acids in an enzyme active site, because the amino acid that is linked to the inhibitor is sure to be in the vicinity of the active site.