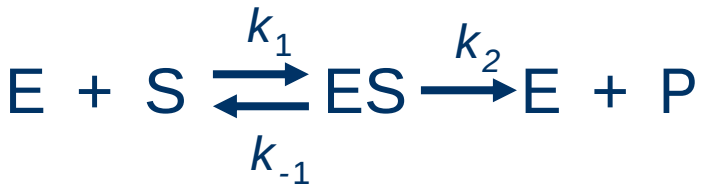
Enzyme kinetics

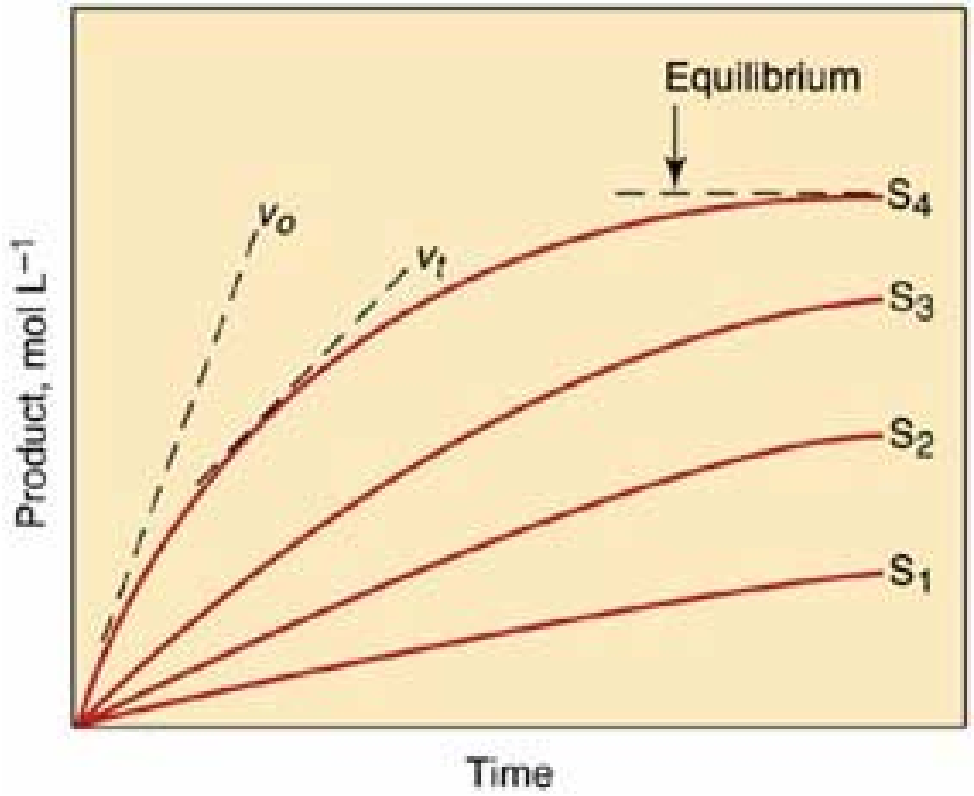
- Study of the rates of enzyme-catalyzed reactions
- Provides information on enzyme specificities and mechanisms

Formula for a simple enzyme-catalyzed reaction



- E = free enzyme
- S = substrate
- ES = enzyme-substrate complex
- P = product

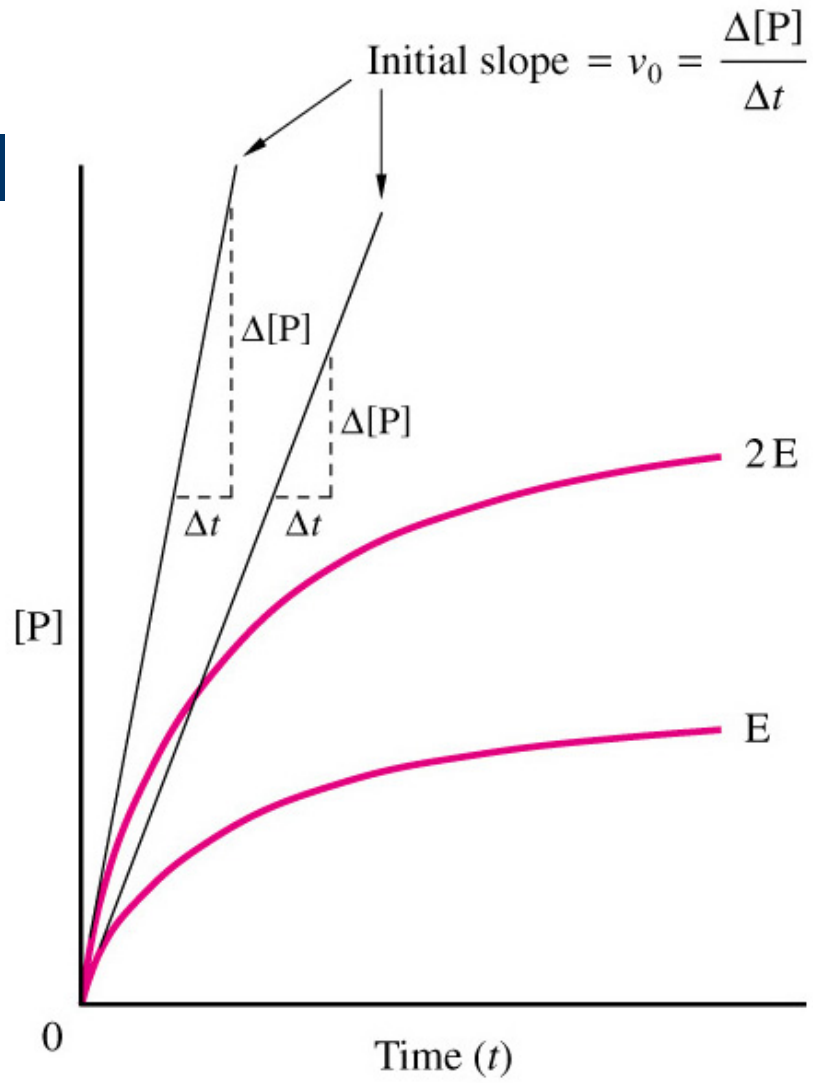
What are we measuring?



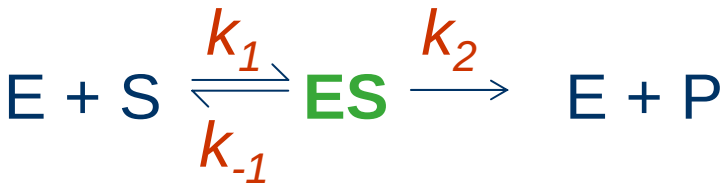
Increasing [S]

Initial velocity

- Measured at the very beginning of a reaction when very little P has been made.

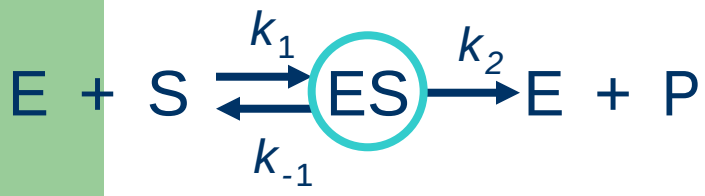


For enzyme-catalyzed reactions

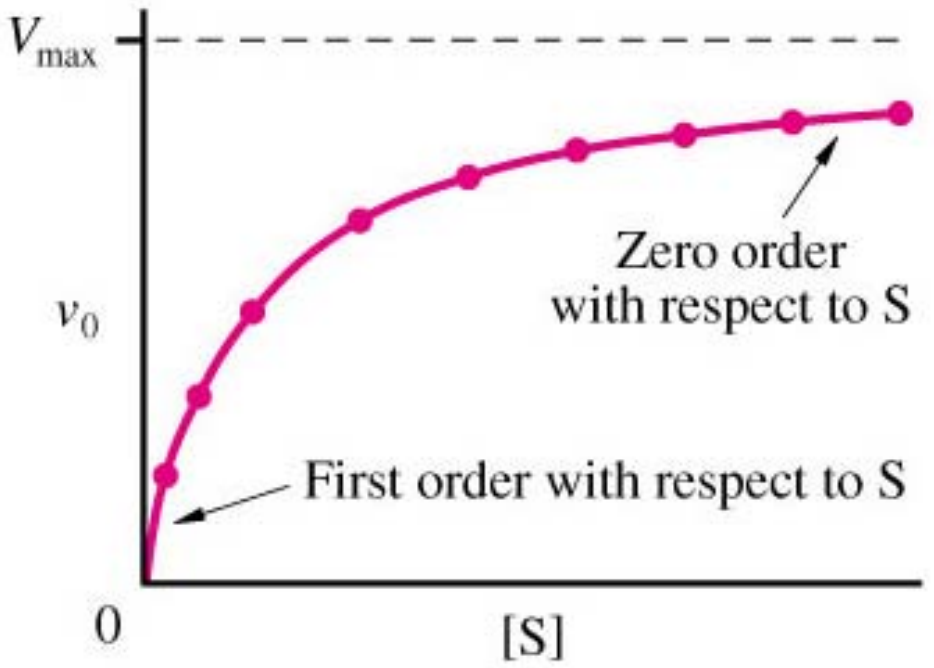


- k_1 is rate constant for formation of ES
- k_{-1} is rate constant for conversion of ES to E+S
- k_2 is rate constant for product formation. For this reaction, $k_2 = kcat$
- Initial velocity assumption: measure activity before appreciable P accumulates: $v_0 = k_2$ [ES]

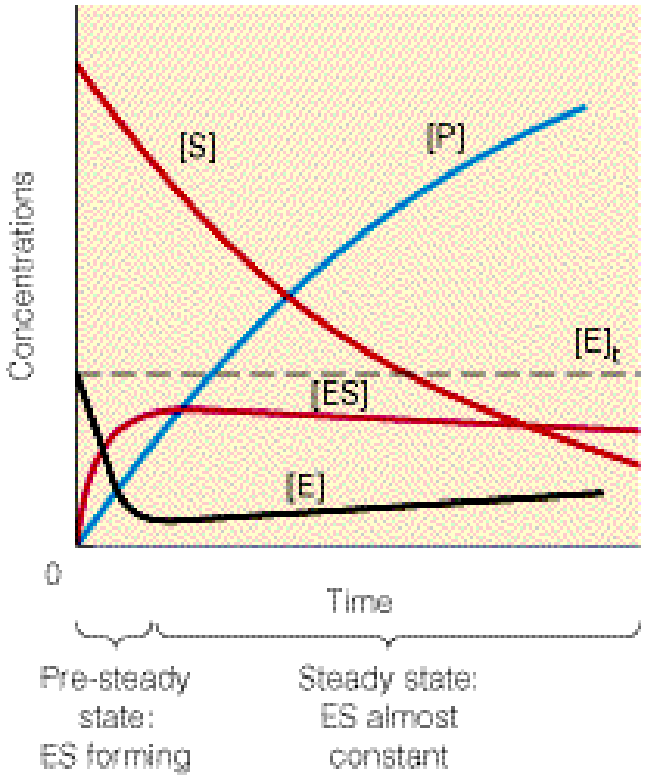
Enzyme-catalyzed reactions exhibit saturation kinetics



At high [S], the enzyme is said to be saturated with respect to substrate



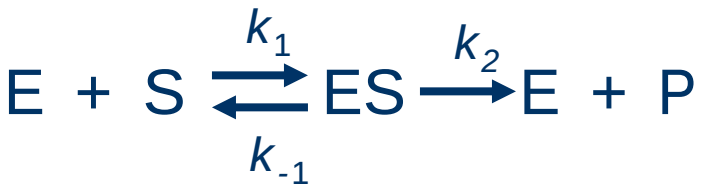
Steady State



The more ES present, the faster ES will dissociate into E + P or E + S. Therefore, when the reaction is started by mixing enzymes and substrates, the [ES] builds up at first, but quickly reaches a STEADY STATE, in which [ES] remains constant. This steady state will persist until almost all of the substrate has been consumed.

Michaelis-Menten equation

- If you assume that the formation of ES equals its breakdown, making [ES] constant (steady state), then:



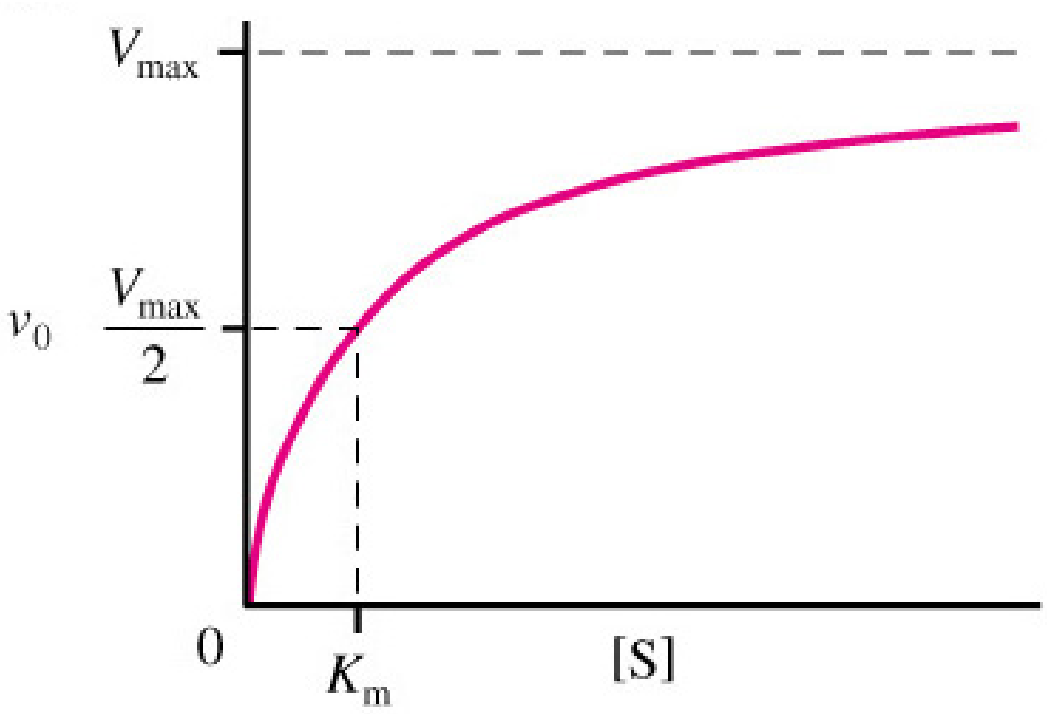
k_1 [E][S] = k_{-1} [**ES**] + k_2 [**ES**]

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

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

K_m

- K_m is the [S] at 1/2 V_{max}
- K_m is a constant for a given enzyme
- K_m is an estimate of the equilibrium constant for S binding to E
- Small K_m means tight binding; high K_m means weak binding



K_M is a measure of [S] required for effective catalysis to occur

Understanding V_{max}

The theoretical maximal velocity

- V_{max} is a constant for a given enzyme
- V_{max} is the theoretical maximal rate of the reaction - but it is NEVER achieved
- To reach V_{max} would require that ALL enzyme molecules have tightly bound substrate

The turnover number

A measure of catalytic activity

- The k_{cat} is a direct measure of the catalytic production of product under saturating substrate conditions.
- k_{cat}, the turnover number, is the maximum number of substrate molecules converted to product per enzyme molecule per unit of time.
- According to M-M model, $k_{cat} = V_{max}/E_t$
- Values of k_{cat} range from less than 1/sec to many millions per sec

The catalytic efficiency

- It shows what the enzyme can accomplish when abundant enzyme sites are available.
- It is the k_{cat}/K_M value that allows direct comparison of the effectiveness of an enzyme toward different substrates.

Values of k_{cat} (Turnover Number) for Some Enzymes

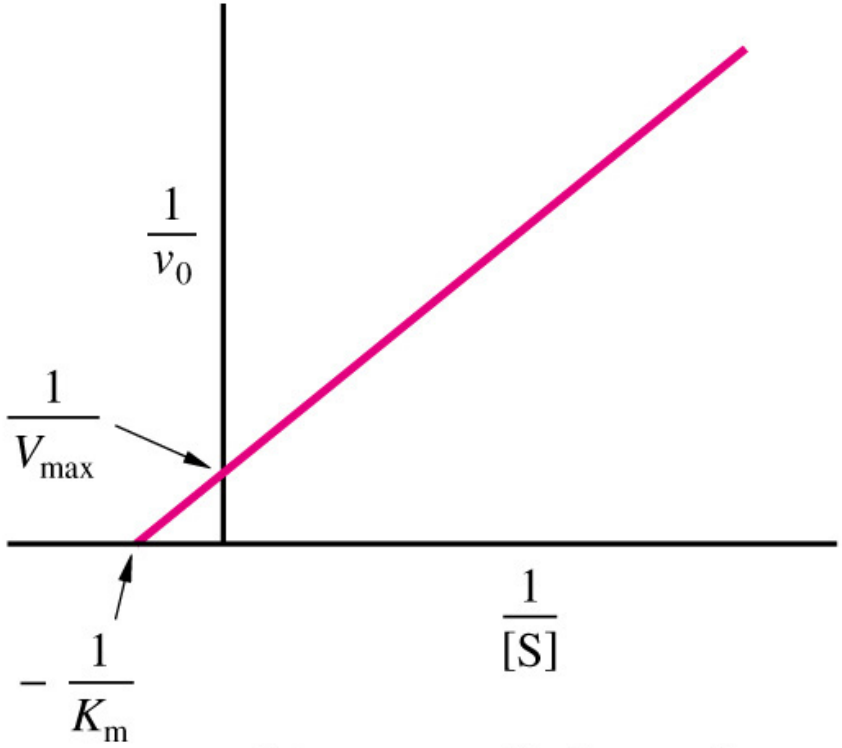
Enzyme	k_{cat} (sec^{-1})
Catalase	40,000,000
Carbonic anhydrase	1,000,000
Acetylcholinesterase	14,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Lysozyme	0.5

Enzymes Whose k_{cat}/K_m Approaches the Diffusion-Controlled Rate of Association with Substrate

Enzyme	Substrate	k_{cat} (sec^{-1})	K_m (M)	k_{cat}/K_m ($sec^{-1}\ M^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	0.012	8.3×10^7
	HCO_3^-	4×10^5	0.026	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	800	5×10^{-6}	1.6×10^8
	Malate	900	2.5×10^{-5}	3.6×10^7
Triosephosphate isomerase	Glyceraldehyde-3-phosphate*	4.3×10^3	1.8×10^{-5}	2.4×10^8
β-Lactamase	Benzylpenicillin	2×10^3	2×10^{-5}	1×10^8

Measuring K_m and V_{max}

- Curve-fitting algorithms can be used to determine K_m and V_{max} from v vs. [S] plots
- Michaelis-Menton equation can be rearranged to the "double reciprocal" plot and K_m and V_{max} can be graphically determined

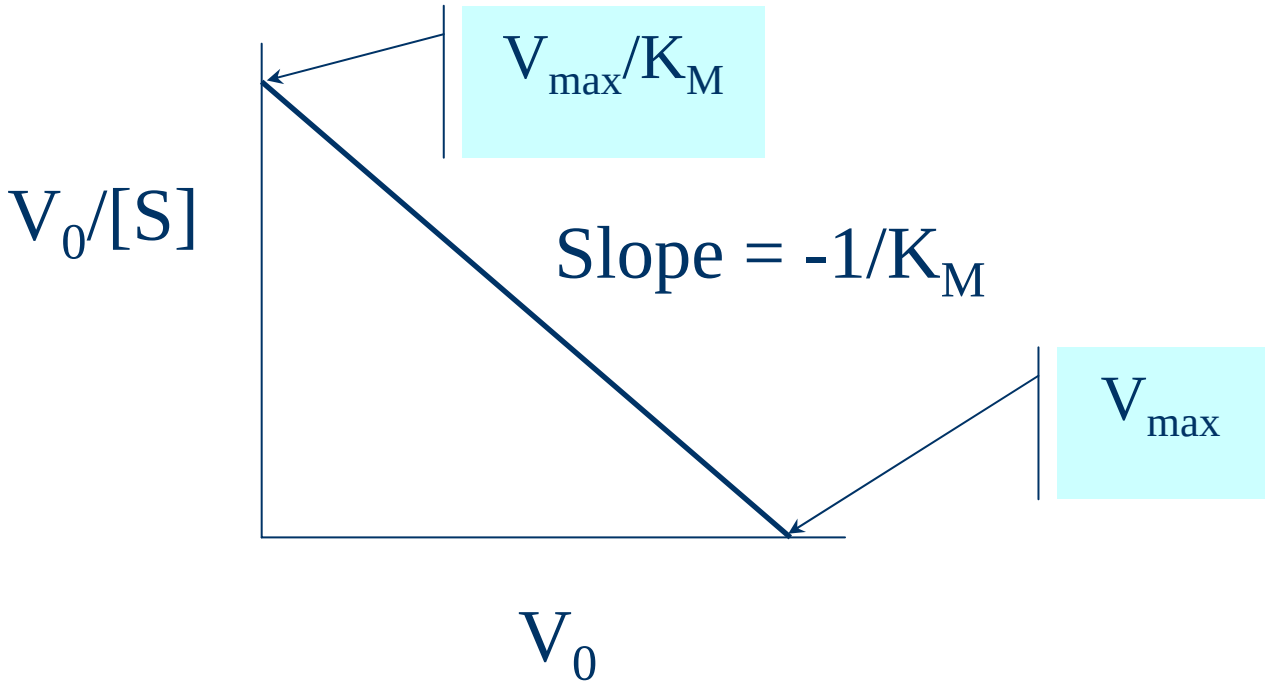


Lineweaver-Burk equation:

$$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

Eadie-Hofstee Plot

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



Enzyme Activity

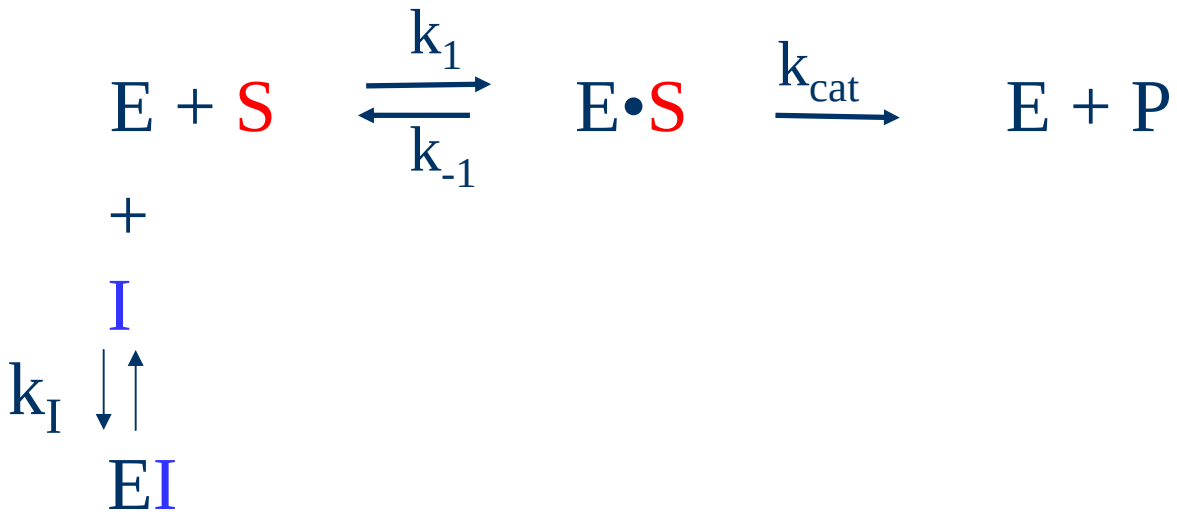
- Amount of reaction that a certain amount of enzyme will produce in a specified period of time
- Activity determined by measuring the amount of product formed or substrate that disappeared
- IU of enzyme activity is
  - The amount of enzyme necessary to produce 1 µmole of product (or the loss of 1 µmol of substrate) per minute under specified conditions of substrate concentration, pH and Temperature

Enzyme Inhibition

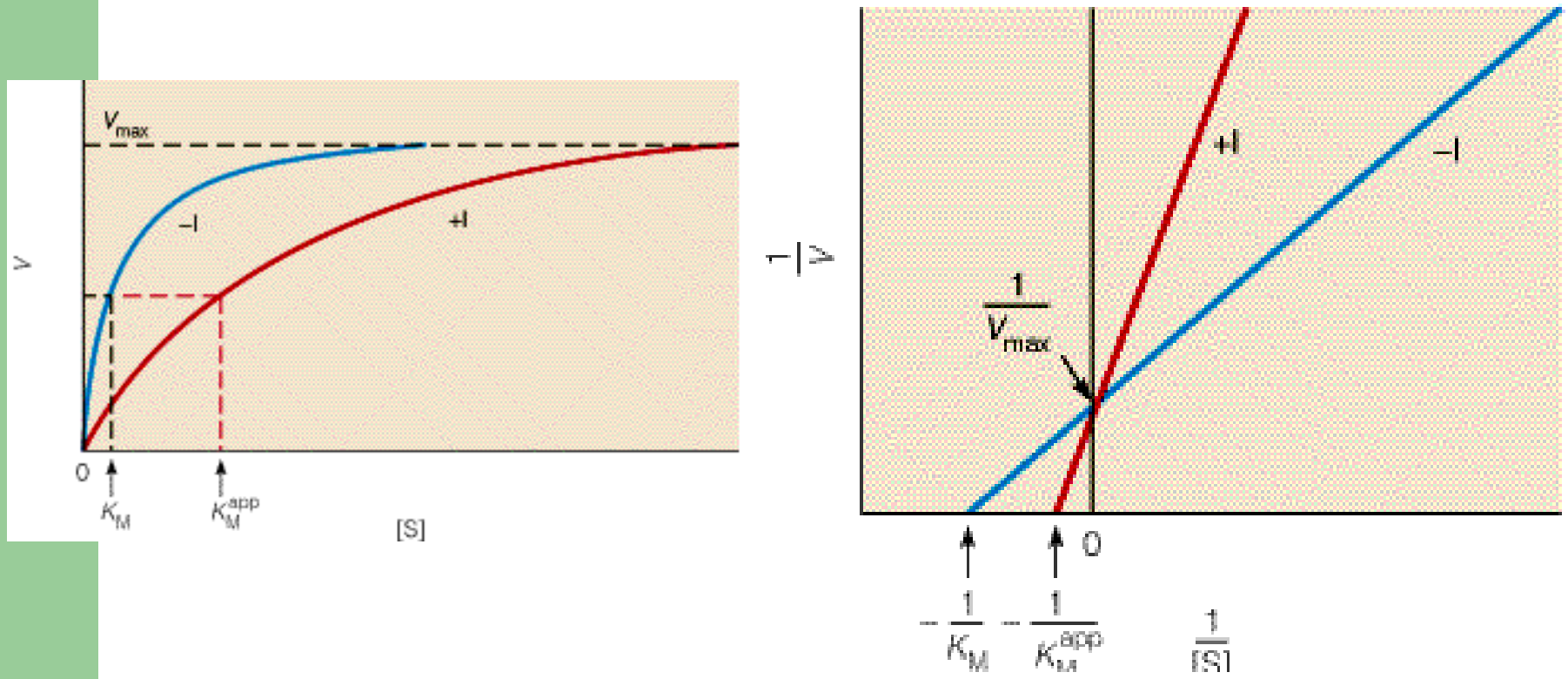
- Many different kinds of molecules inhibit enzymes and act in a variety of ways.
- One major distinction is whether the inhibition is
  - Reversible
    - Competitive with the substrate or
    - Non-competitive with the substrate
  - Irreversible (I is covalently bound, incapacitating the enzyme)

Reversible Inhibition: Competitive

- Inhibitor resembles substrate but can't undergo the catalytic step, so it wastes the enzyme's time by preventing S binding. i.e. Inhibitor COMPETES with substrate for binding.

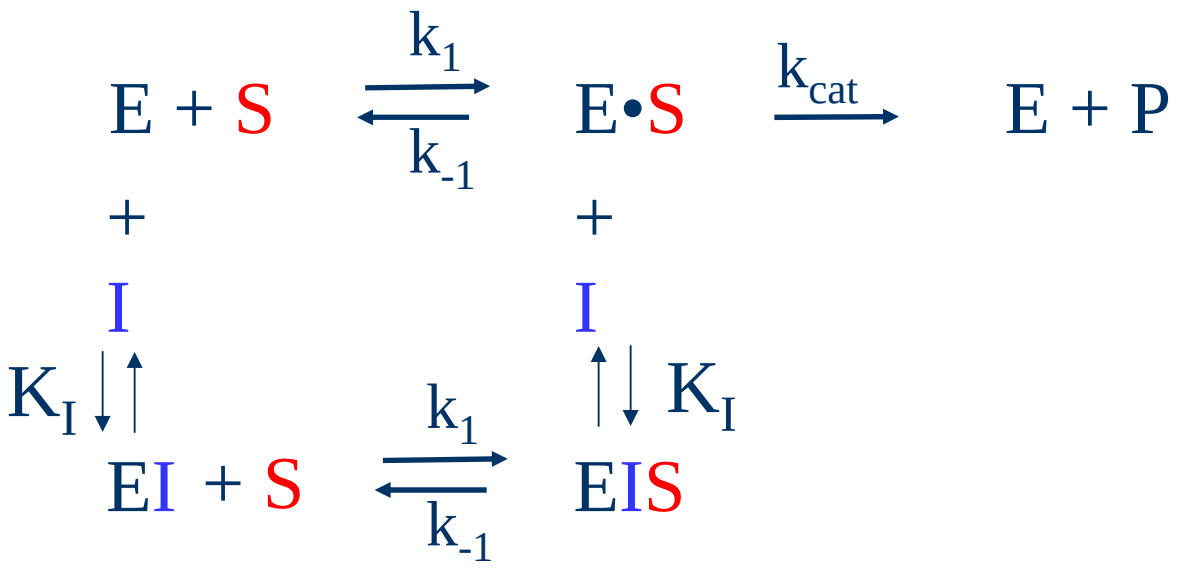


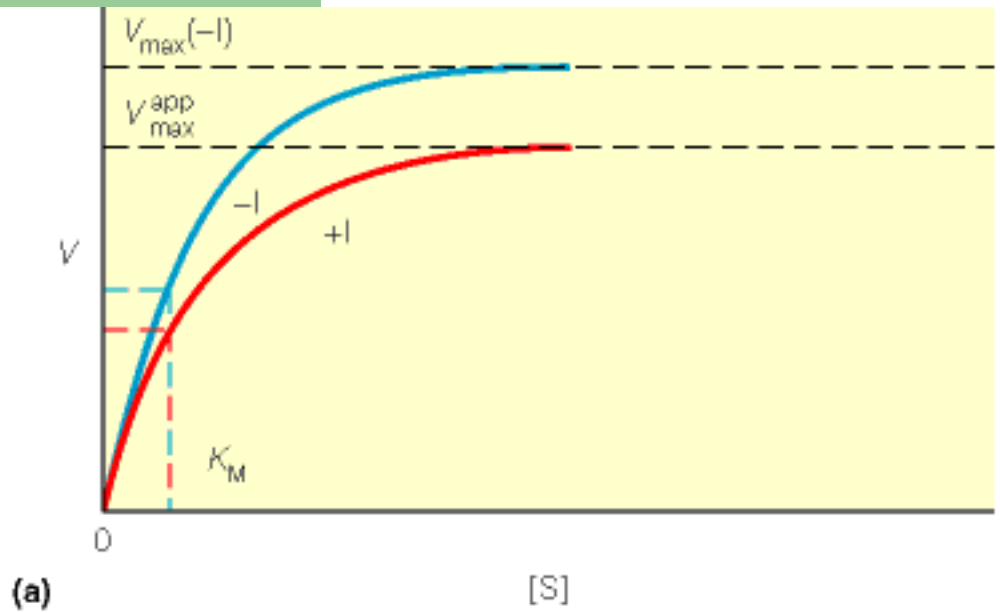
Reversible Inhibition: Competitive



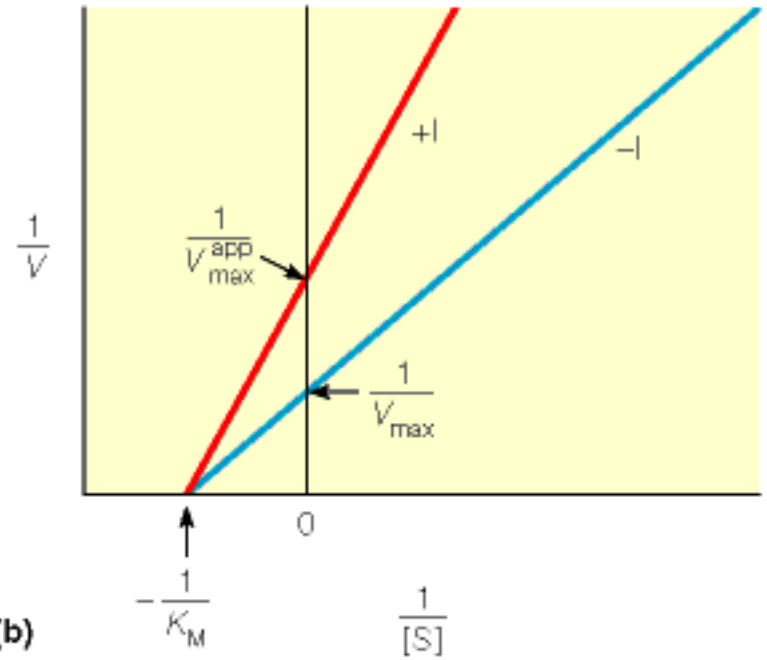
Reversible Inhibition: Non-Competitive

- A molecule or ion binds at a remote site on the enzyme in such a way that it affects k_{cat}.

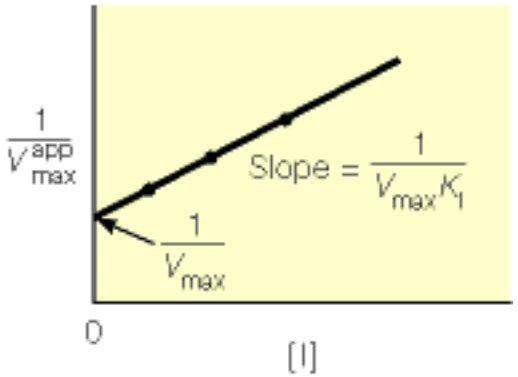



$V_{max}(-I)$
V^{app}_{max}
$-I$
$+I$
V
K_M
0
[S]

(a)


$+I$
$-I$
$\frac{1}{V}$
$\frac{1}{V^{app}_{max}}$
$\frac{1}{V_{max}}$
0
$-\frac{1}{K_M}$
$\frac{1}{[S]}$

(b)


$\frac{1}{V^{app}_{max}}$
Slope = $\frac{1}{V_{max}K_I}$
$\frac{1}{V_{max}}$
0
[I]

(c)

Enzyme Inhibition

Inhibitor Type	Binding Site on Enzyme	Kinetic effect
Competitive Inhibitor	Specifically at the catalytic site, where it competes with substrate for binding in a dynamic equilibrium- like process. Inhibition is reversible by substrate.	V_{max} is unchanged; K_m, is increased.
Noncompetitive Inhibitor	Binds E or ES complex other than at the catalytic site. Substrate binding unaltered, but ESI complex cannot form products. Inhibition cannot be reversed by substrate.	K_m appears unaltered; V_{max} is decreased proportionately to inhibitor concentration.
Uncompetitive Inhibitor	Binds only to ES complexes at locations other than the catalytic site. Substrate binding modifies enzyme structure, making inhibitor- binding site available. Inhibition cannot be reversed by substrate.	Apparent V_{max} decreased; K_m, is decreased.