BIOCHEMISTRY 2

2ND CLASS

UNIVERSITY OF ANBAR

COLLOGE OF SCIENCE

BIOLOGY DEPARTMENT

2021-2022

Enzyme Kinetics

Lecture Three (3)

Hameed Hussein Ali
Chemistry Department
College of Science

References:

Harper's Illustrated Biochemistry

Lippincott Biochemistry

Lehninger Principles of Biochemistry

Stryer Biochemistry

SYLABUSE

1- Enzymes
2- Vitamins and Coenzymes.
3- Nucleotides and Nucleic acids.
4- Carbohydrate Metabolism.
5- Lipids Metabolism.
6- Amino acids and Proteins Metabolism.
7- Nucleic acids metabolism.

Enzymes

Major Concepts

study of reaction rate and Kinetics of Enzymes. .A

B. What does Km mean?

C. Know the Inhibition of enzymes.

Rate constants and reaction order

Rate constant (k) measures how rapidly a rxn occurs

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B + C$$

Rate (v, velocity) = (rate constant) (concentration of reactants)

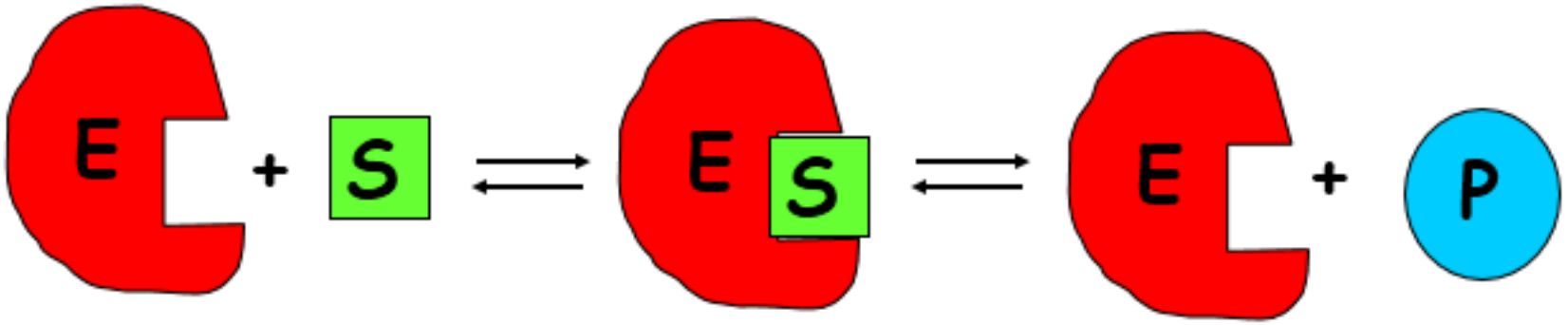
$$v = k_1 [A]$$

1st order rxn (rate dependent on concentration of 1 reactant)

$$v = k_{-1}[B][C]$$

2nd order rxn (rate dependent on concentration of 2 reactants)

Zero order rxn (rate is independent of reactant concentration)

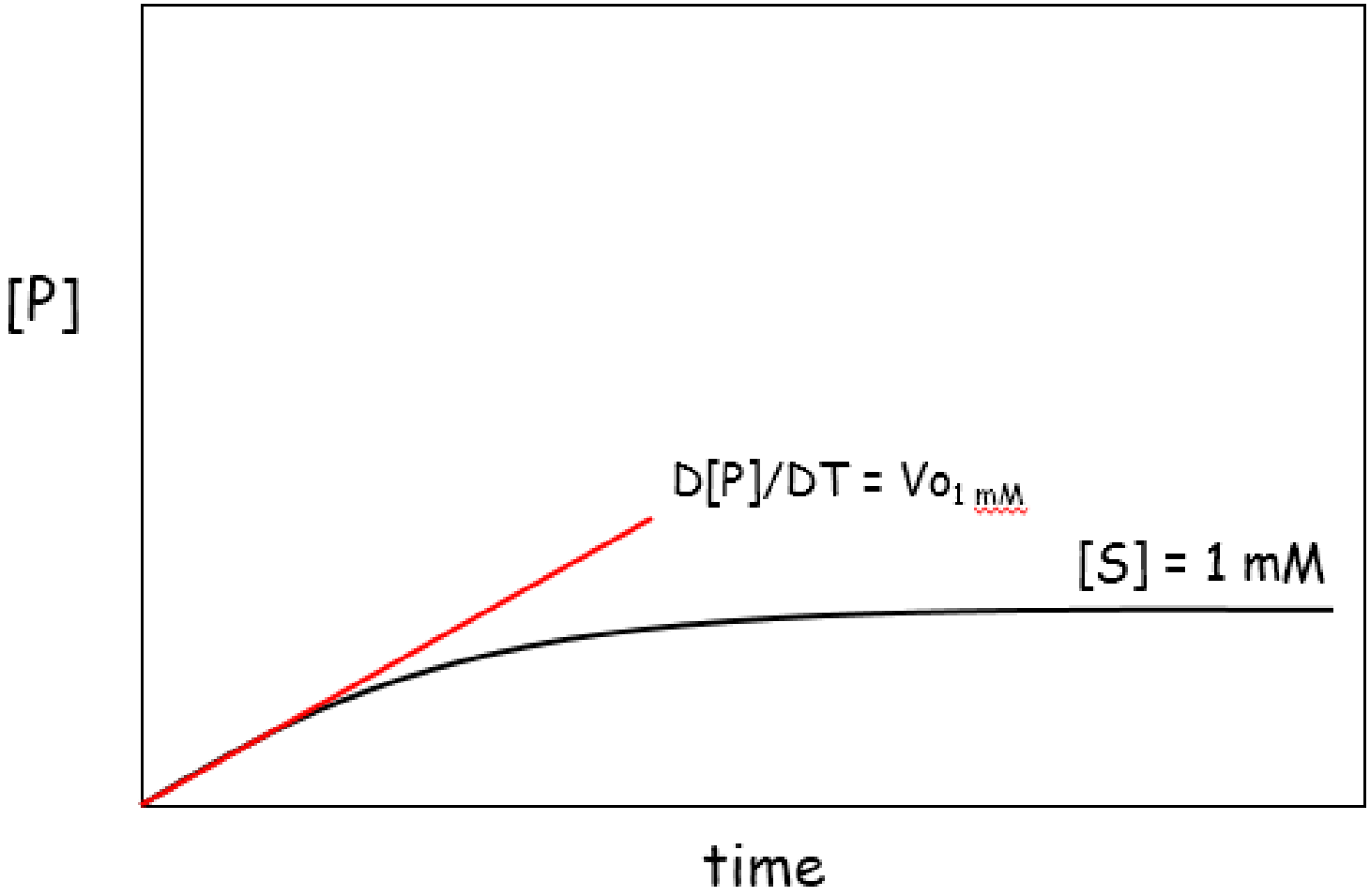


$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

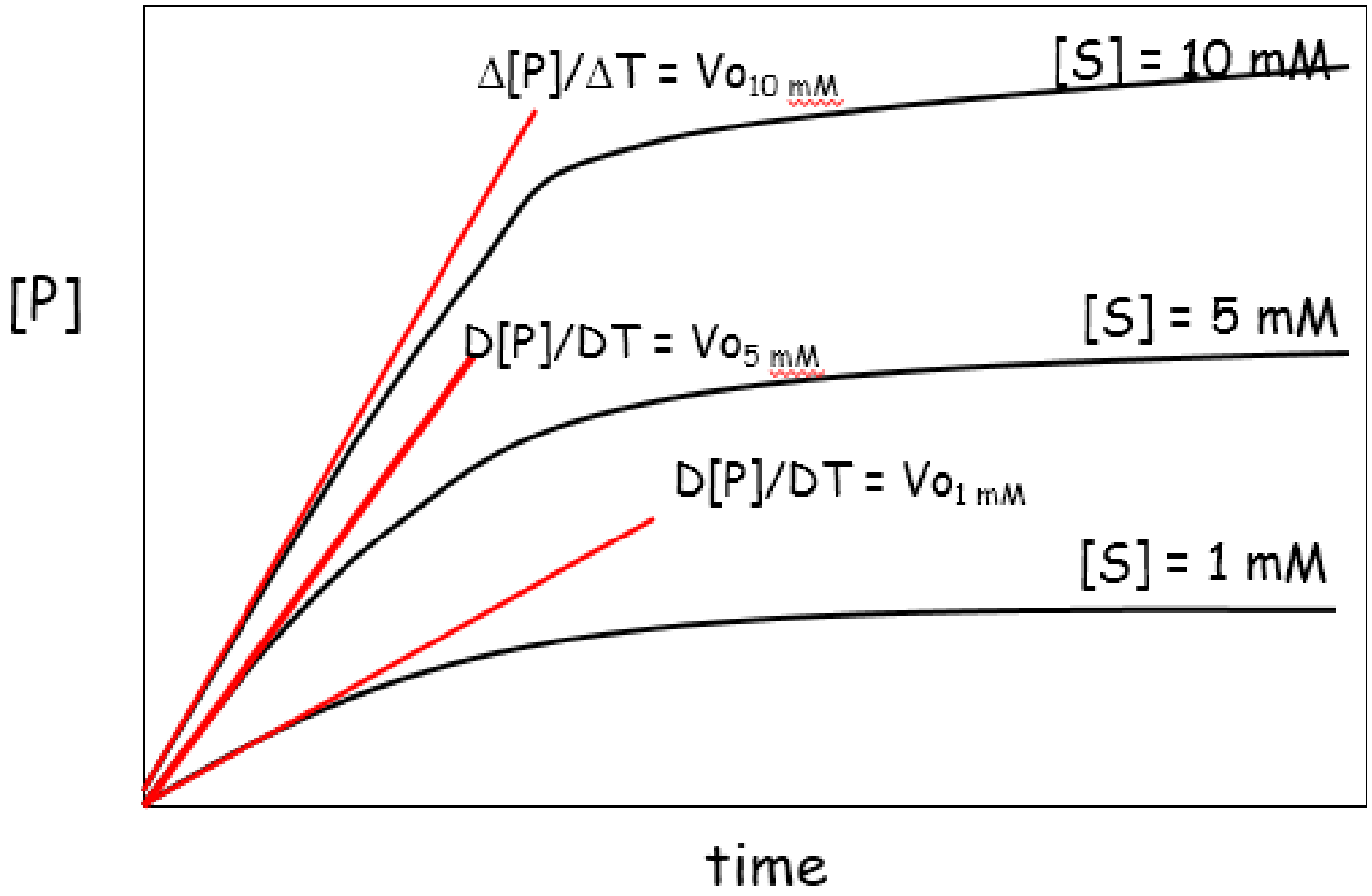
Initial Velocities

Hold [E] constant

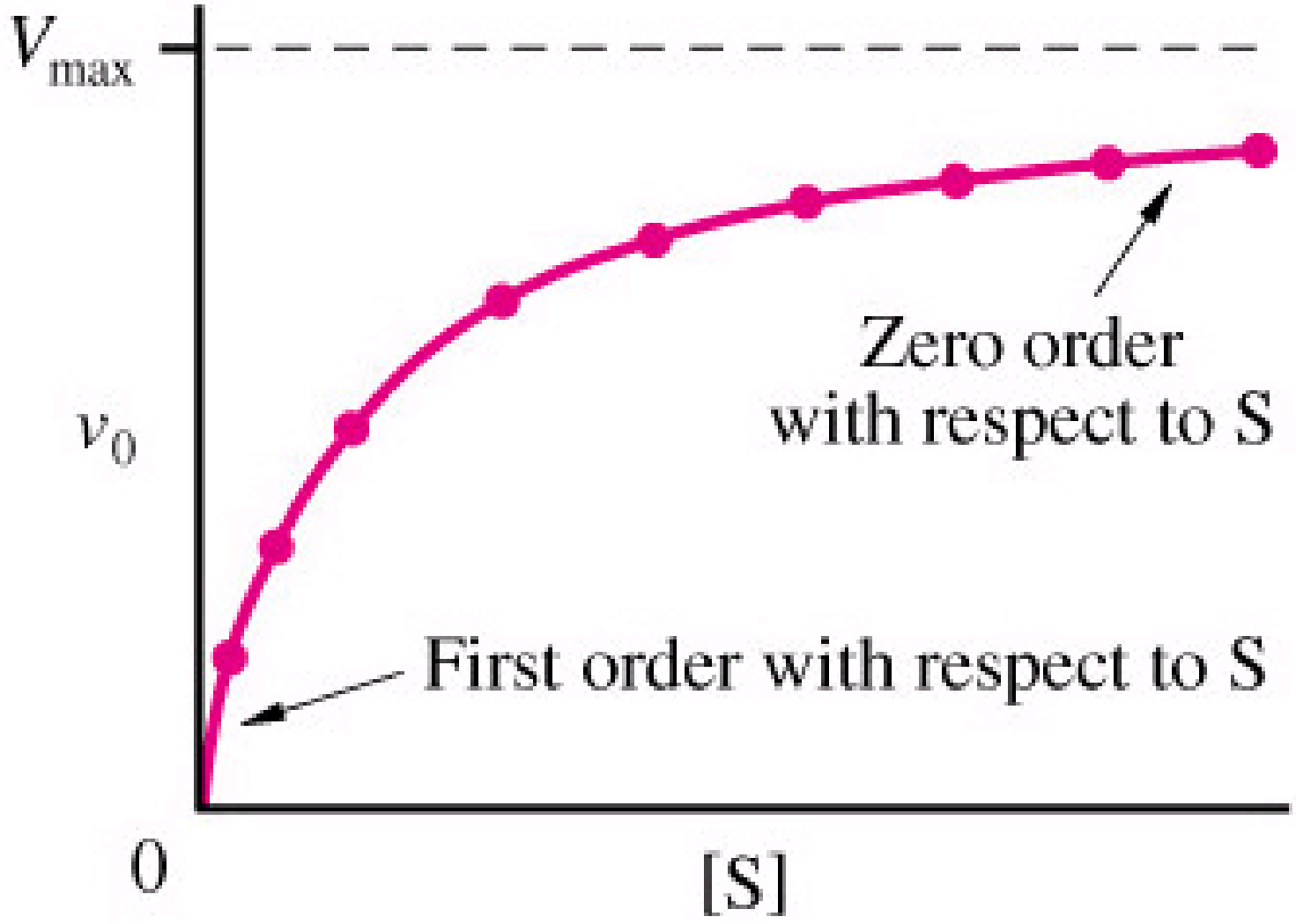
[E]<<<<<[S]



Initial Velocities



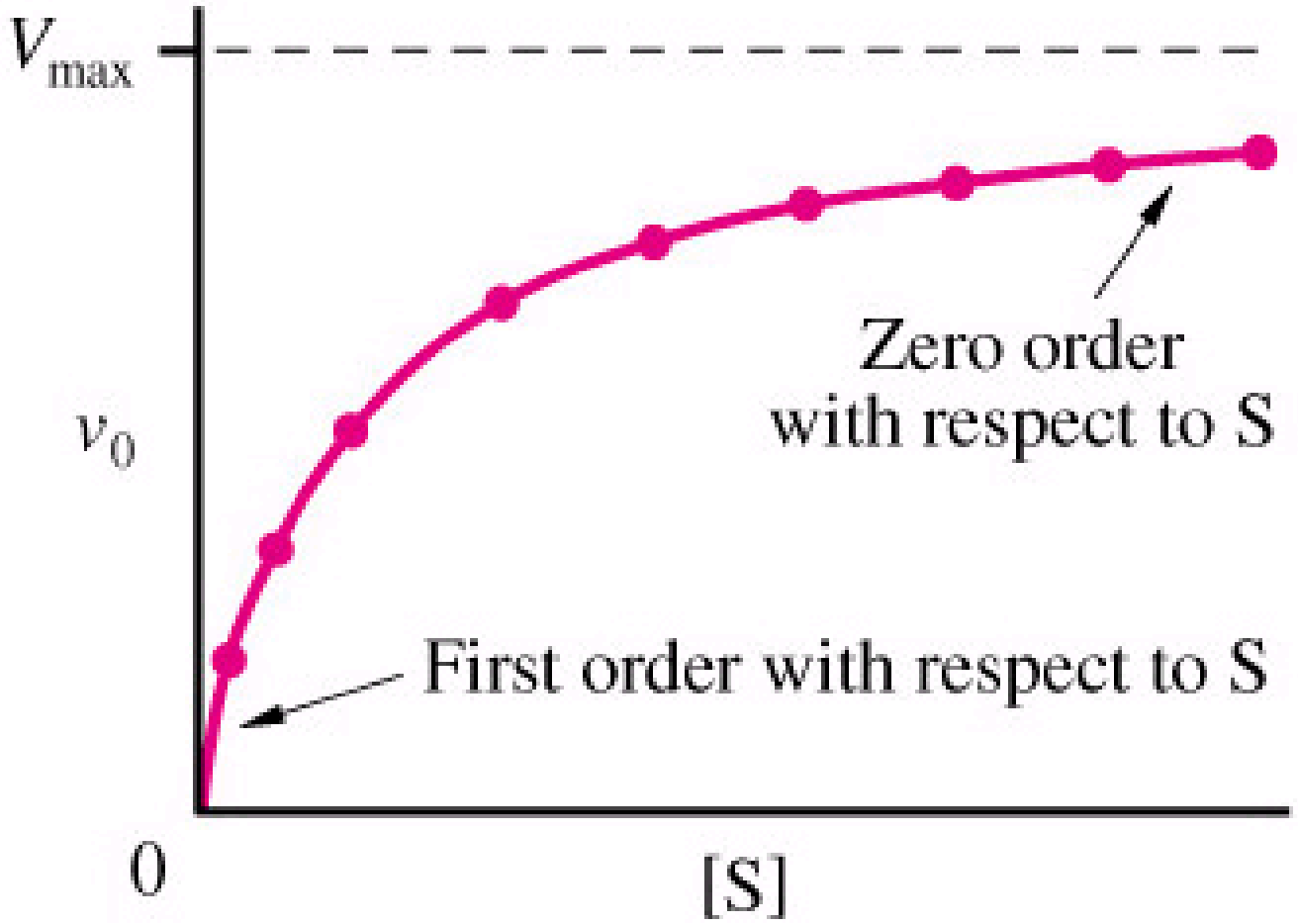
Plot Vo vs. [S]


V_{max}
v_0
0
[S]
Zero order
with respect to S
First order with respect to S

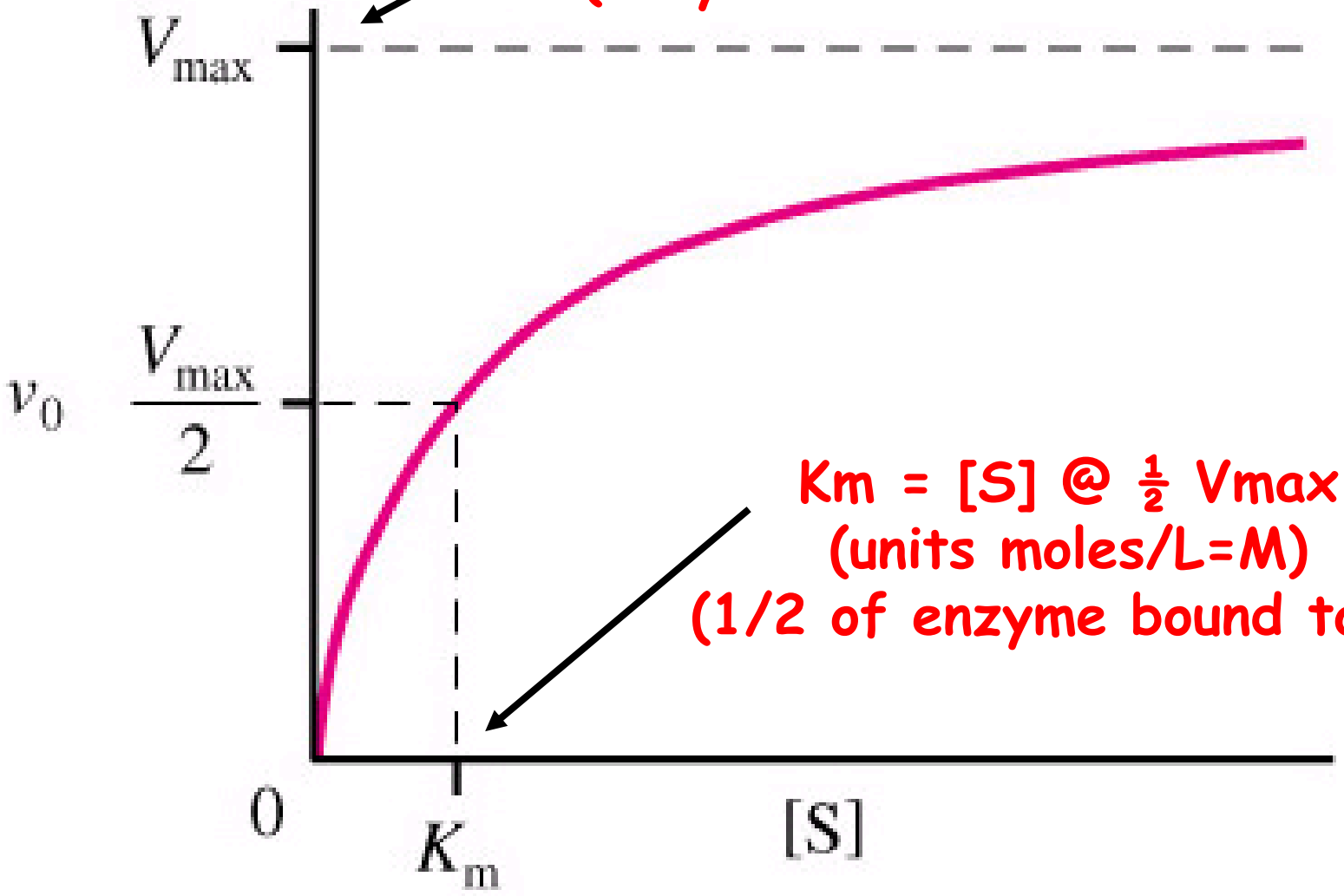
Leonor Michaelis, 1875–1949

Maud Menten, 1879–1960

- **Michaelis-Menton Equation**
- **Describes rectangular hyperbolic plot**
- $V_0 = \frac{V_{max}[S]}{K_m + [S]}$



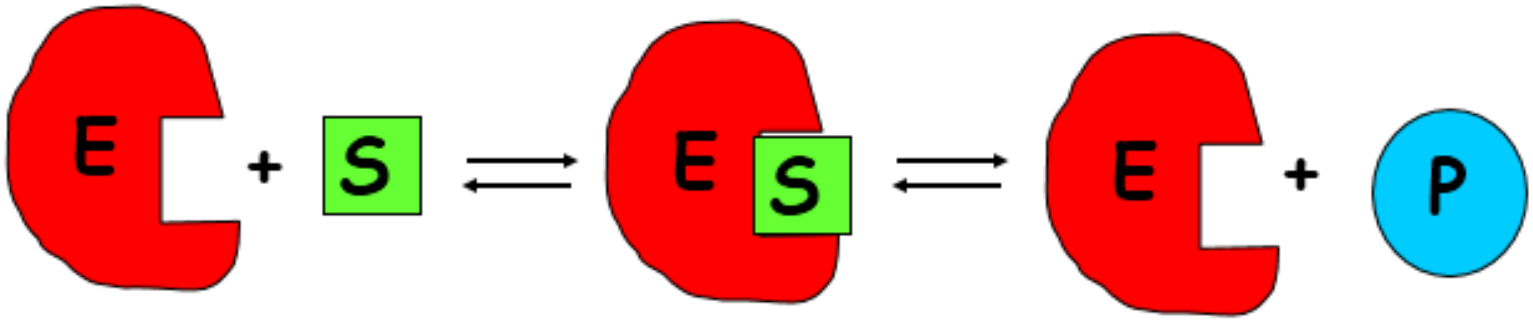
V_{max} = velocity where all of the enzyme is bound to substrate (enzyme is saturated with S)



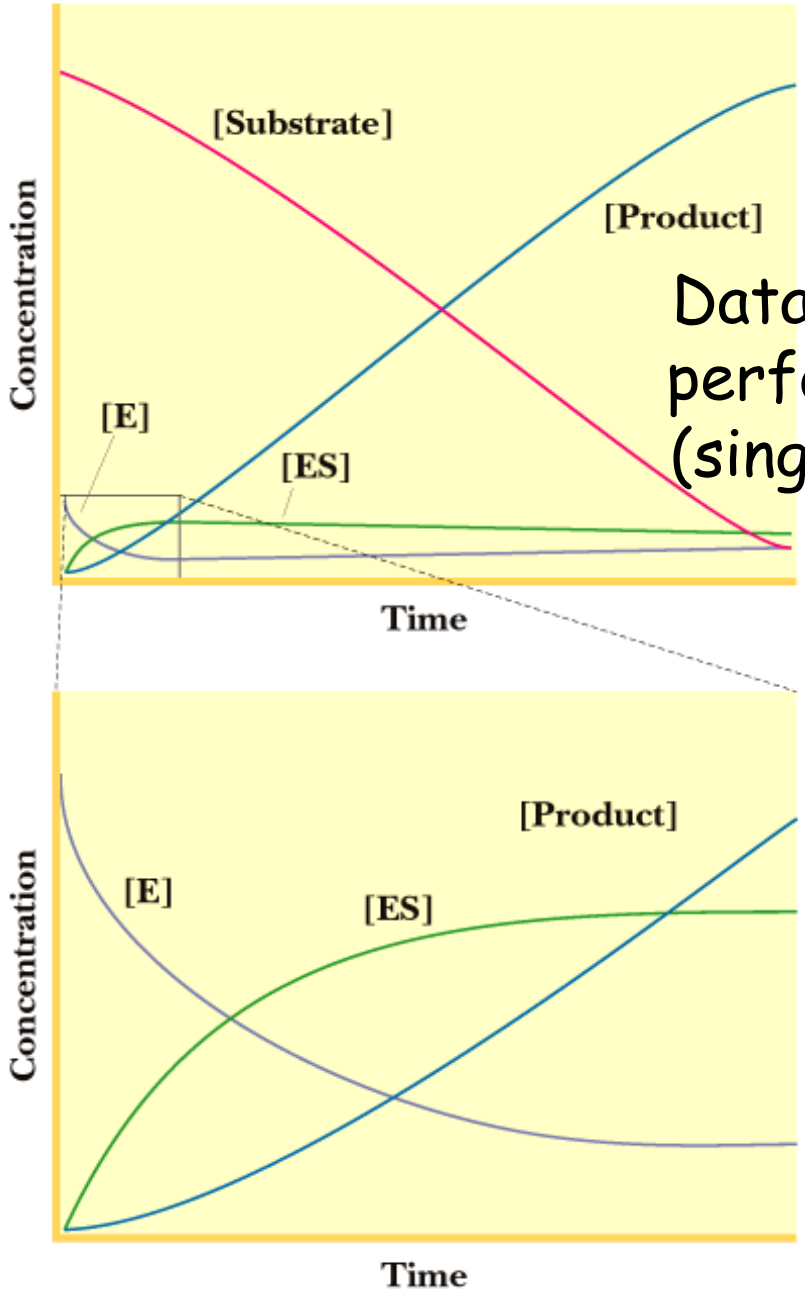
Km = [S] @ ½ Vmax
(units moles/L=M)
(1/2 of enzyme bound to S)

Initial Velocity Assumption

1) Measurements made to measure initial velocity (v_o). At v_o very little product formed. Therefore, the rate at which E + P react to form ES is negligible and k_{-2} is 0. Therefore

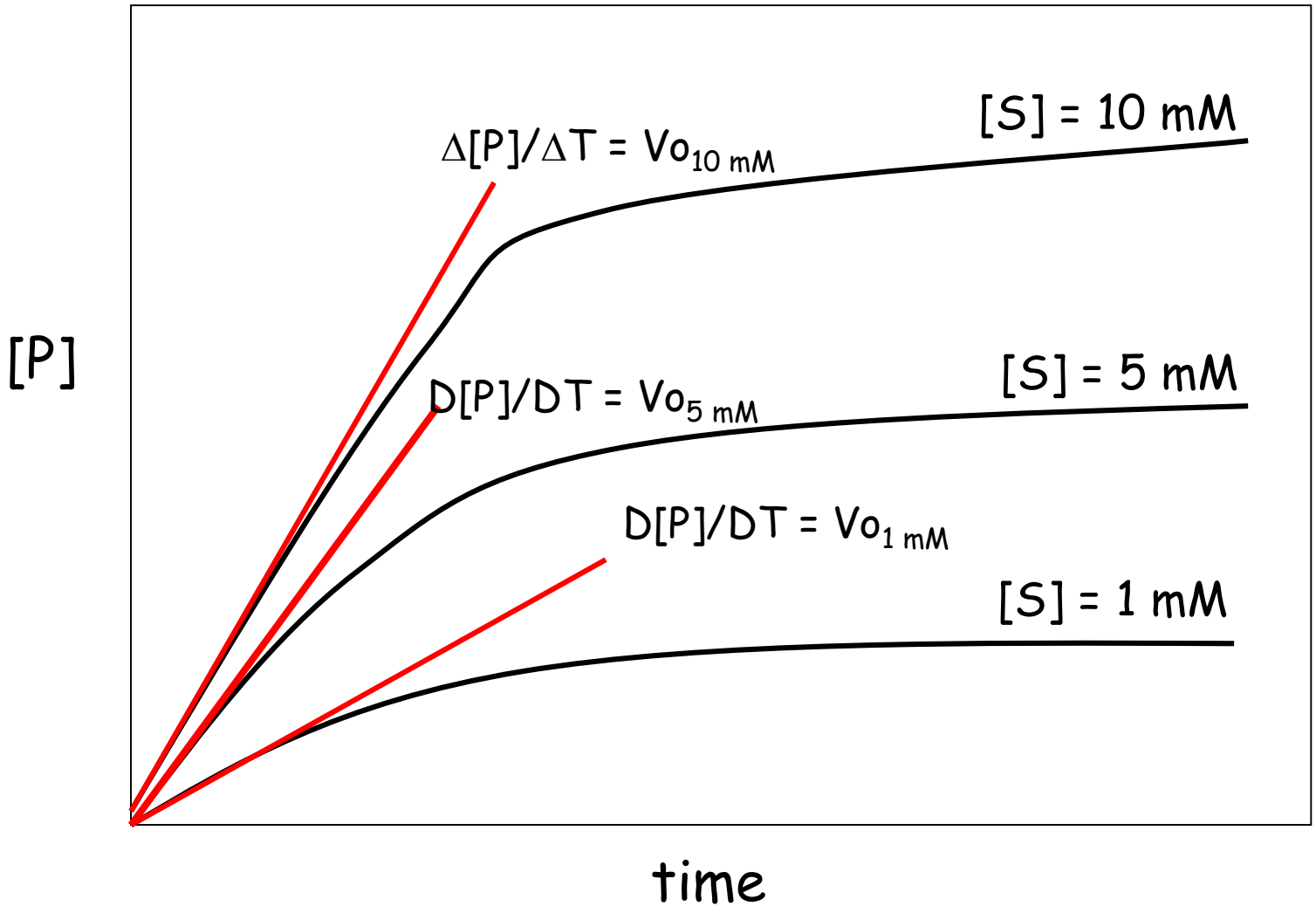


$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$



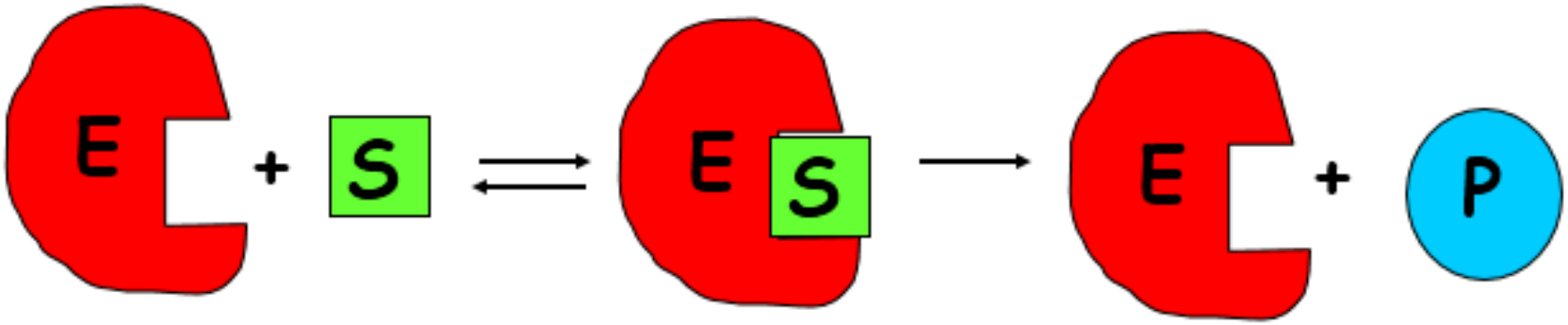
Data from a single experiment performed with at a single [S]. (single point on Vo vs. [S] plot)

Initial Velocities



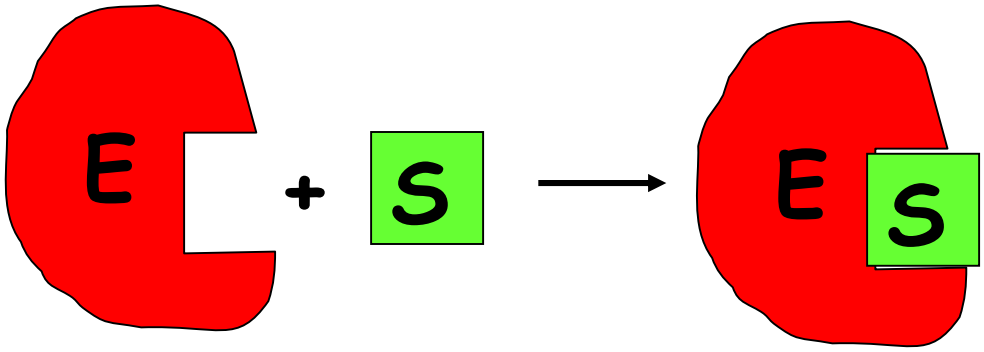
Steady State Assumption

Steady state Assumption = [ES] is constant.
The rate of ES formation equals the rate of ES breakdown



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

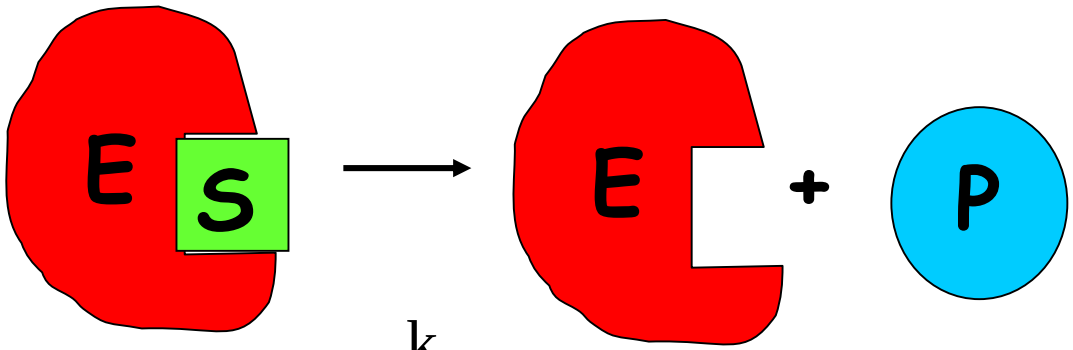
Rate of ES formation



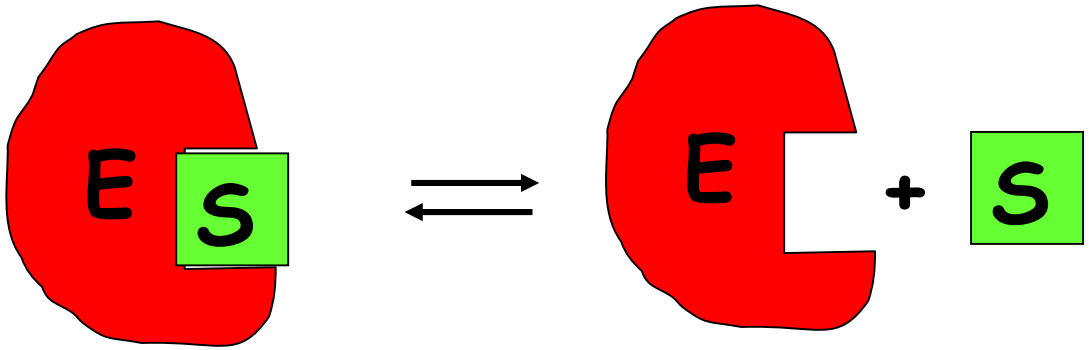
$$E + S \xrightarrow{k_1} ES$$

$$\text{Rate} = k_1 [E] [S]$$

Rate of ES breakdown



$$ES \xrightarrow{k_2} E + P$$



$$ES \xrightarrow{k_{-1}} E + S$$

Rate = (k_2 [ES]) + (k_{-1}[ES])

Rate = [ES](k_2 + k_{-1})

Therefore……..if the rate of ES formation equals the rate of ES breakdown

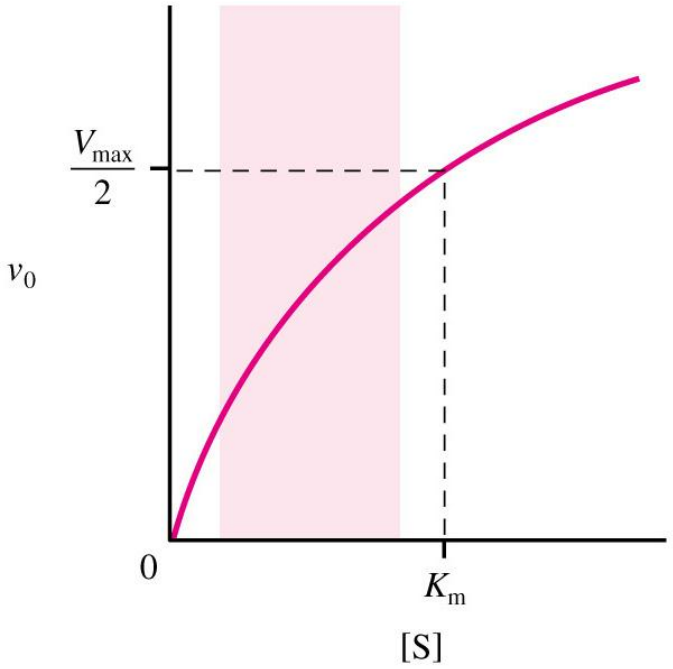
1) $k_1[E][S] = [ES](k_{-1} + k_2)$

2) $(k_{-1} + k_2) / k_1 = [E][S] / [ES]$

3) $(k_{-1} + k_2) / k_1 = K_m$ (Michaelis constant)

What does Km mean?

1. Km = [S] at $\frac{1}{2}$ V_{max}
2. Km is a combination of rate constants describing the formation and breakdown of the ES complex
3. Km is usually a little higher than the physiological [S]



What does Km mean?

4. Km represents the amount of substrate required to bind ½ of the available enzyme (binding constant of the enzyme for substrate)
5. Km can be used to evaluate the specificity of an enzyme for a substrate (if obeys M-M)
6. Small K_m means tight binding; high K_m means weak binding

Hexose Kinase

Glucose + ATP <-> Glucose-6-P + ADP

Glucose	Km = 8 X 10^{-6}
Allose	Km = 8 X 10^{-3}
Mannose	Km = 5 X 10^{-6}

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

What does k_{cat} mean?

1. k_{cat} is the 1st order rate constant describing ES → E+P
2. Also known as the turnover # because it describes the number of rxns a molecule of enzyme can catalyze per second under optimal condition.
3. Most enzyme have k_{cat} values between 10^2 and 10^3 s^{-1}
4. For simple reactions $k_2 = k_{cat}$, for multistep rxns k_{cat} = rate limiting step

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

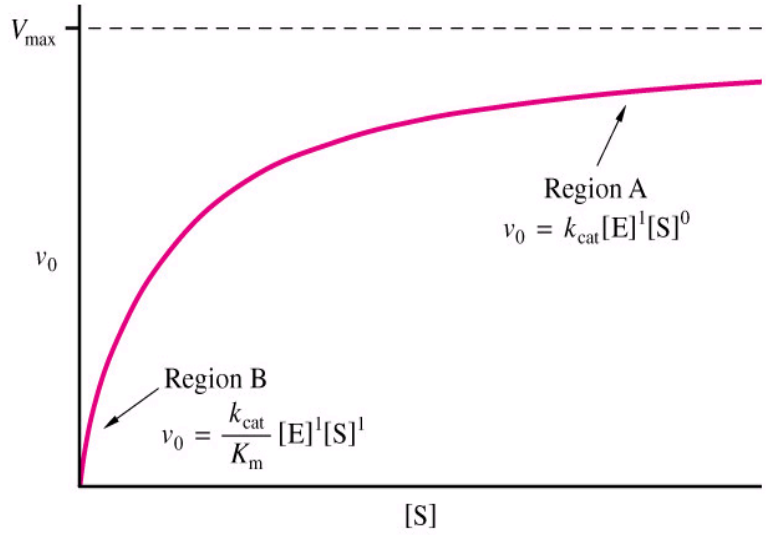
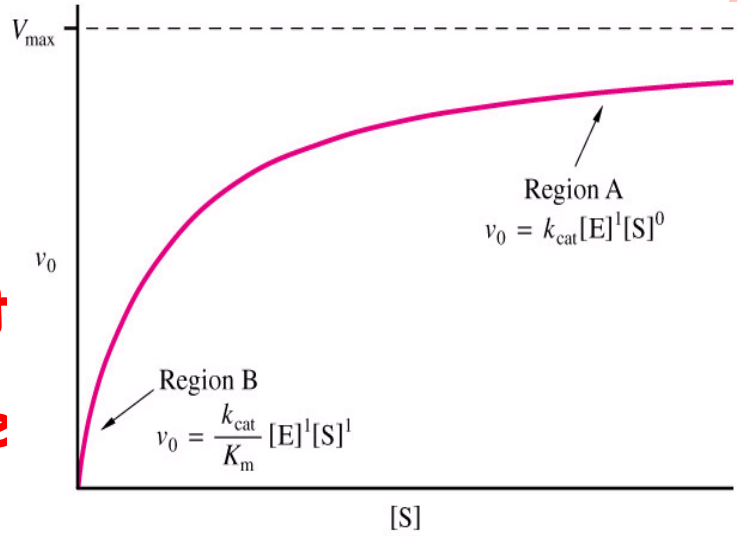


TABLE 6–7 Turnover Number, k_{cat}, of Some Enzymes

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	0.5

What does k_{cat}/K_m mean?

- It measures how the enzyme performs when S is low
- k_{cat}/K_m describes an enzymes preference for different substrates = specificity constant
- The upper limit for k_{cat}/K_m is the diffusion limit - the rate at which E and S diffuse together (10^8 to 10^9 m^{-1} s^{-1})
- Catalytic perfection when k_{cat}/K_m = diffusion rate
- More physiological than kcat



Region B: $E + S \xrightarrow{\frac{k_{cat}}{K_m}} E + P$

Enzyme	Reaction Catalyzed	K_M (mol/L)	$k_{cat}(s^{-1})$	k_{cat}/K_M $[(mol/L)^{-1}\,s^{-1}]$
Chymotrypsin	Ac–Phe–Ala $\xrightarrow{H_2O}$ Ac–Phe + Ala	1.5×10^{-2}	0.14	9.3
Pepsin	Phe–Gly $\xrightarrow{H_2O}$ Phe + Gly	3×10^{-4}	0.5	1.7×10^{3}
Tyrosyl-tRNA synthetase	Tyrosine + tRNA $\longrightarrow$ tyrosyl-tRNA	9×10^{-4}	7.6	8.4×10^{3}
Ribonuclease	Cytidine 2′, 3′ cyclic phosphate $\xrightarrow{H_2O}$ cytidine 3′-phosphate	7.9×10^{-3}	7.9×10^{2}	1.0×10^{5}
Carbonic anhydrase	$HCO_3^- + H^+ \longrightarrow H_2O + CO_2$	2.6×10^{-2}	4×10^{5}	1.5×10^{7}
Fumarase	Fumarate $\xrightarrow{H_2O}$ malate	5×10^{-6}	8×10^{2}	1.6×10^{8}

Limitations of M-M

1. Some enzyme catalyzed rxns show more complex behavior

   $$E + S <-> ES <-> EZ <-> EP <-> E + P$$

   With M-M can look only at rate limiting step

2. Often more than one substrate

   $$E+S_1 <-> ES_1+S_2 <-> ES_1S_2 <-> EP_1P_2 <-> EP_2+P_1 <-> E+P_2$$

   Must optimize one substrate then calculate kinetic parameters for the other

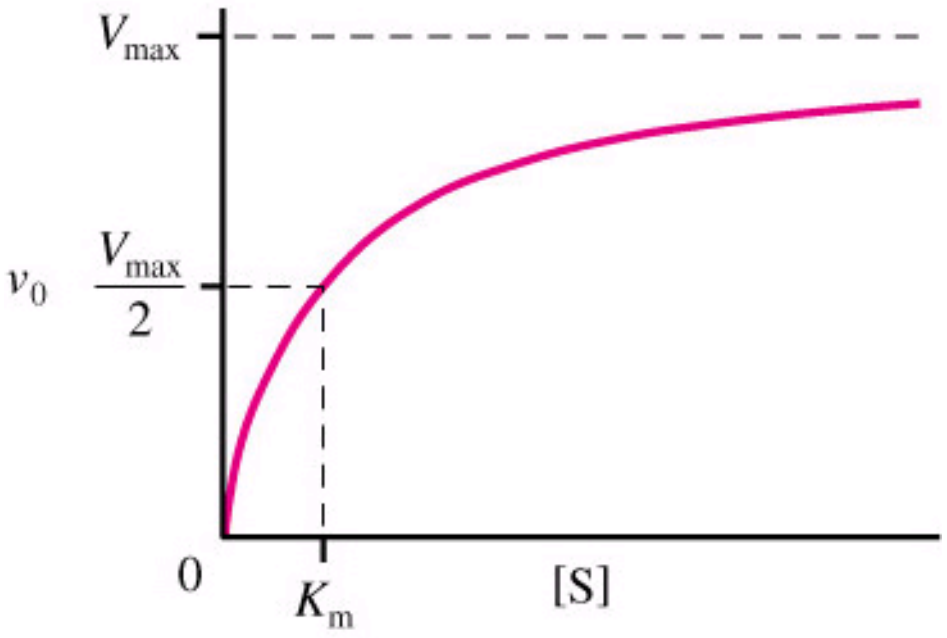
3. Assumes $k_{-2} = 0$

4. Assume steady state conditions

Michaelis-Menton

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

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

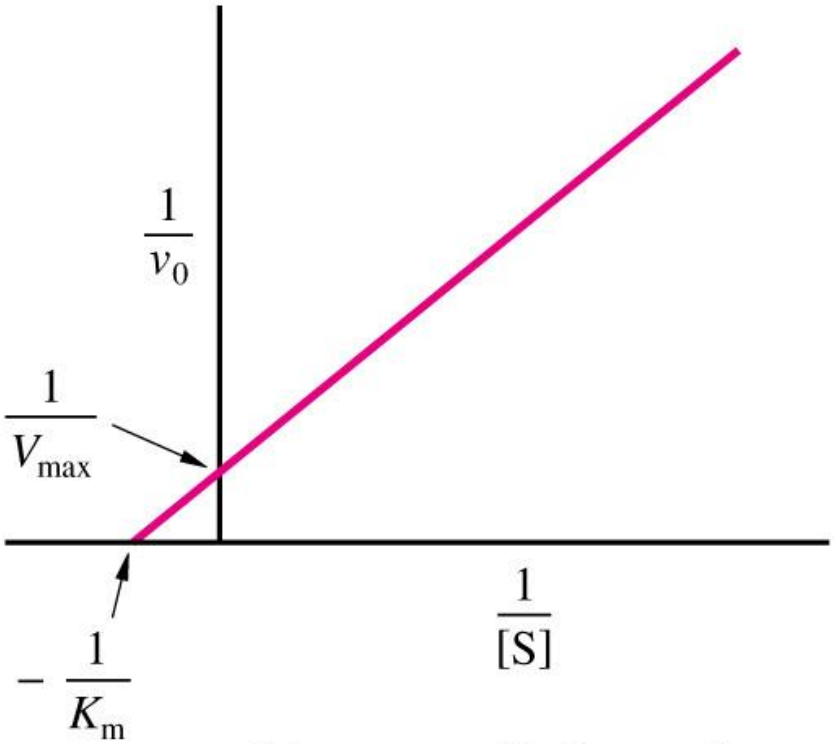


- V_{max}
- K_m
- k_{cat}
- k_{cat}/K_m

How do you get values for V_{max}, K_m and k_{cat}?

- Can determine K_m and V_{max} experimentally
- Km can be determined without an absolutely pure enzyme
- K_{cat} values can be determined if V_{max} is known and the absolute concentration of enzyme is known ($V_{max} = k_{cat}[E_{total}]$

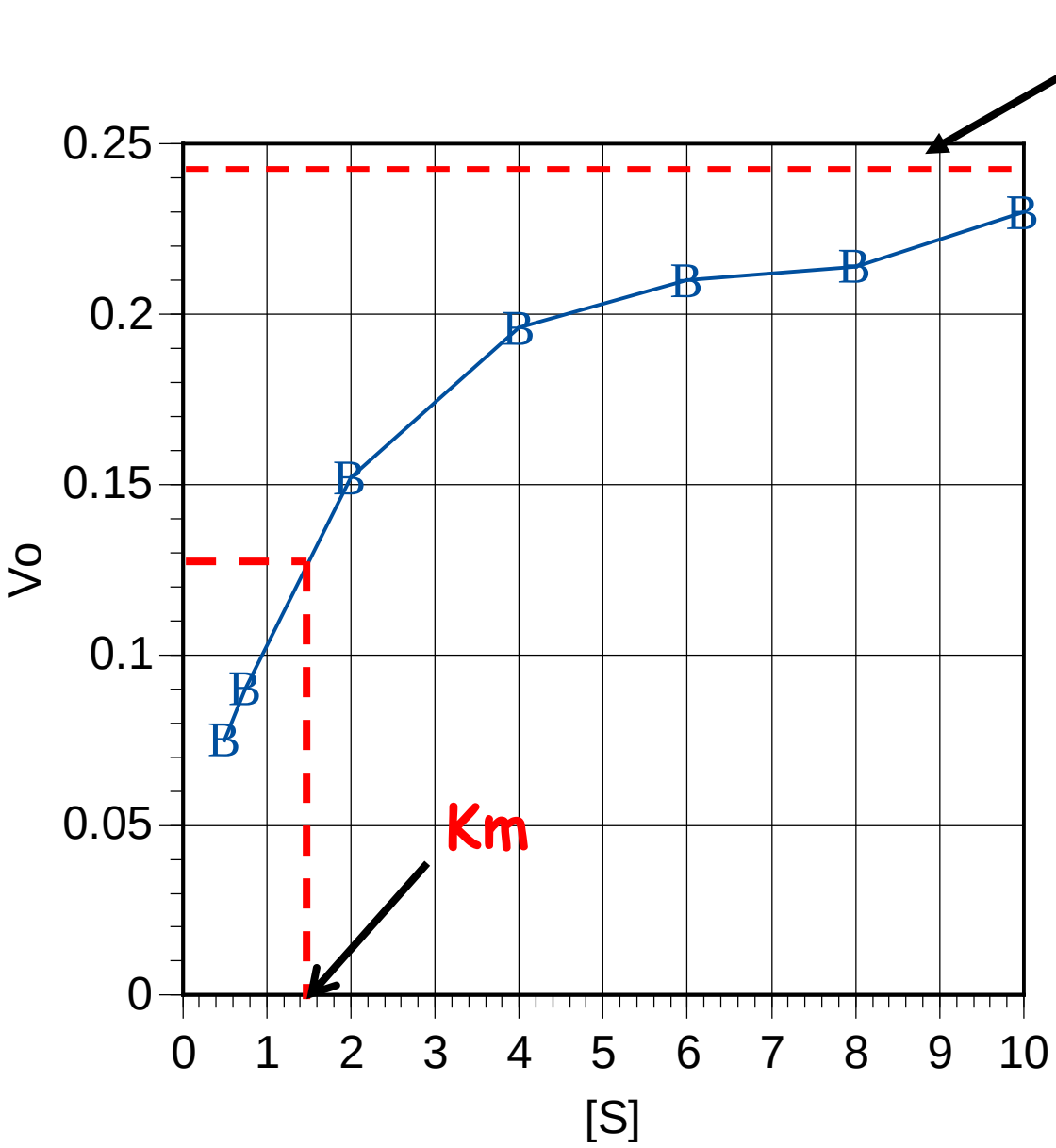
Lineweaver-Burke Plots
(double reciprocal plots)



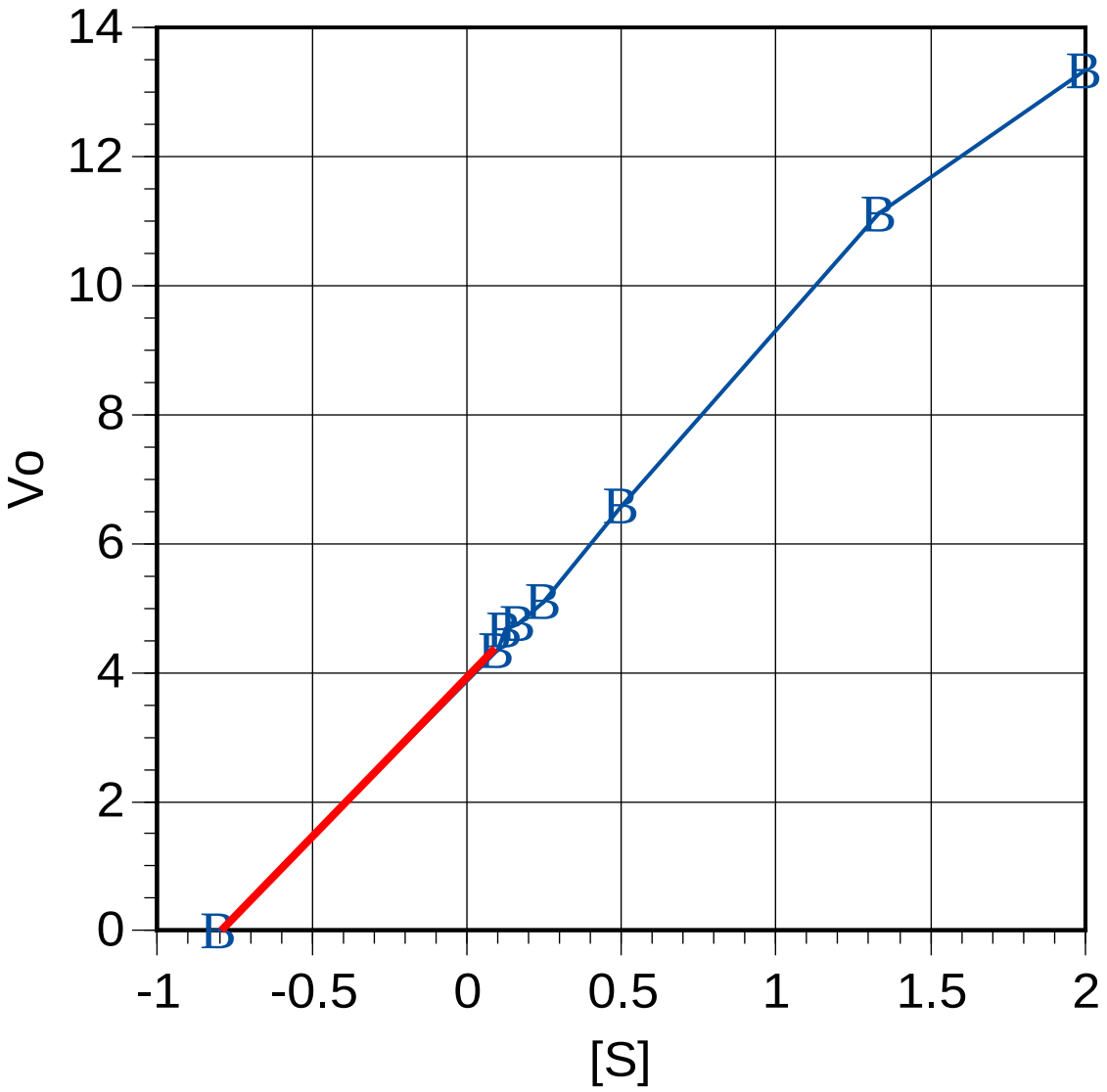
Lineweaver-Burk equation:

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

- Plot $1/[S]$ vs $1/V_o$
- L-B equation for straight line
- X-intercept = $-1/Km$
- Y-intercept = $1/Vmax$
- Easier to extrapolate values w/ straight line vs hyperbolic curve



[S]	Vo
0.5	0.075
0.75	0.09
2	0.152
4	0.196
6	0.21
8	0.214
10	0.23



[S]	Vo
2.000	13.333
1.333	11.111
0.500	6.579
0.250	5.102
0.167	4.762
0.125	4.673
0.100	4.348

-1/Km = -0.8

Km = 1.23 mM

1/Vmax = 4.0

Vmax = 0.25

Enzyme Inhibition

- **Inhibitor** – substance that binds to an enzyme and interferes with its activity
- Can prevent formation of **ES** complex or prevent **ES** breakdown to **E + P**.
- Irreversible and Reversible Inhibitors
- **Irreversible inhibitor** binds to enzyme through covalent bonds (**binds irreversibly**)
- **Reversible Inhibitors** bind through non-covalent interactions (disassociates from enzyme)
- Why important?

Reversible Inhibitors

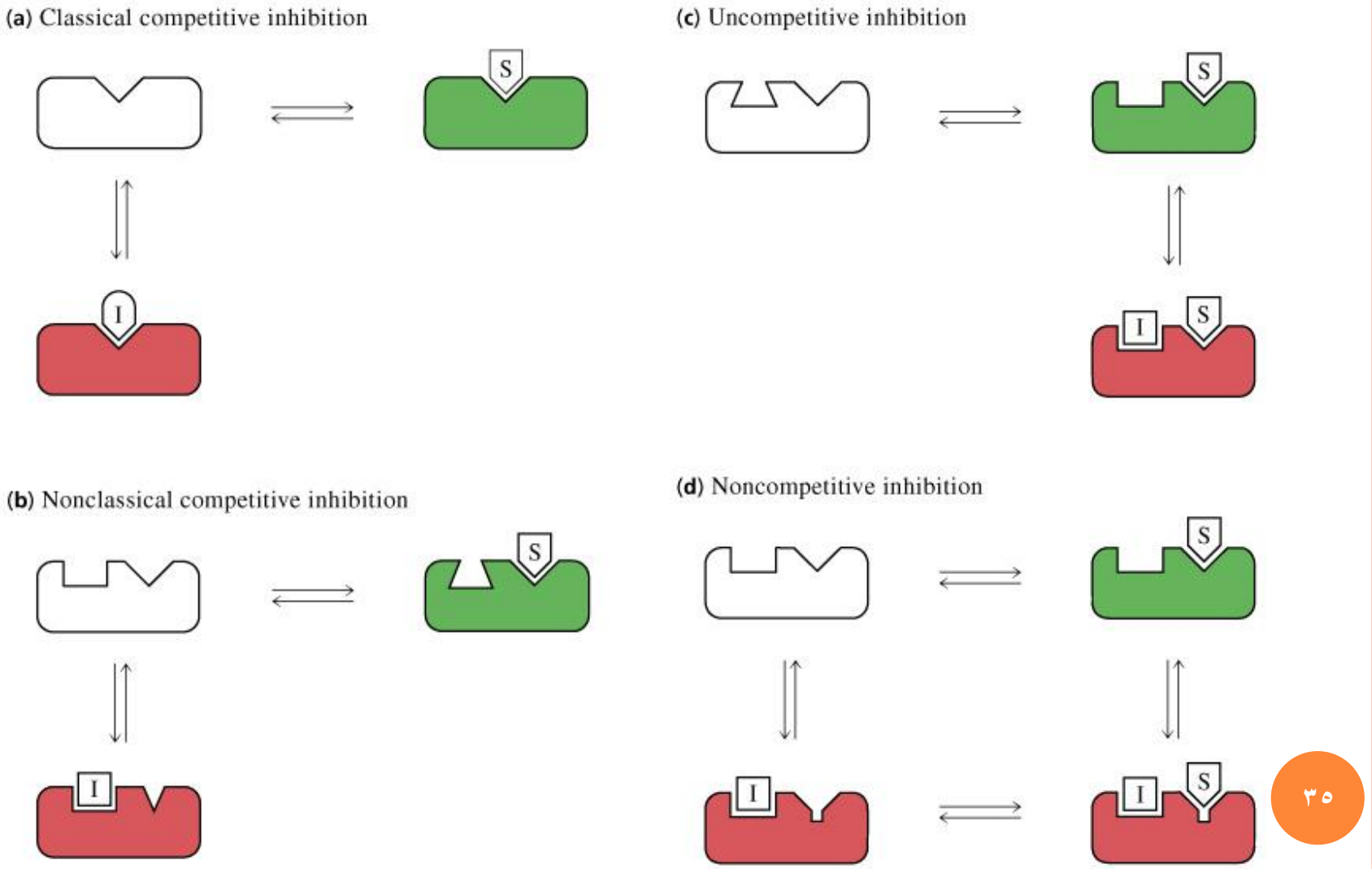
$$E + S \leftrightarrow ES \rightarrow E + P$$

$$E + I \leftrightarrow EI$$

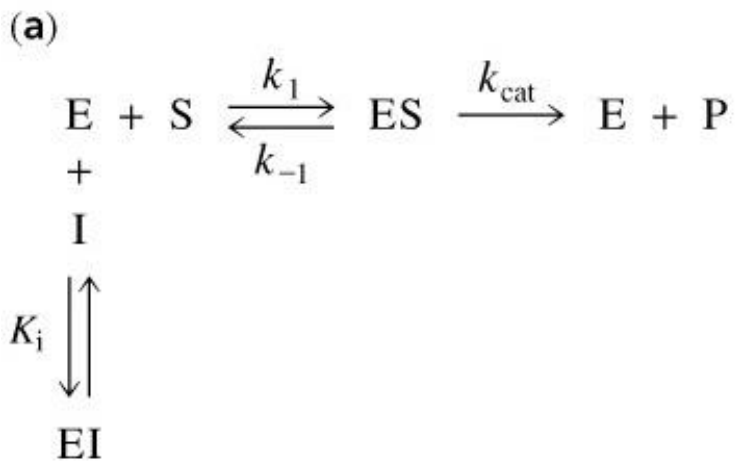
$$K_i = [E][I]/[EI]$$

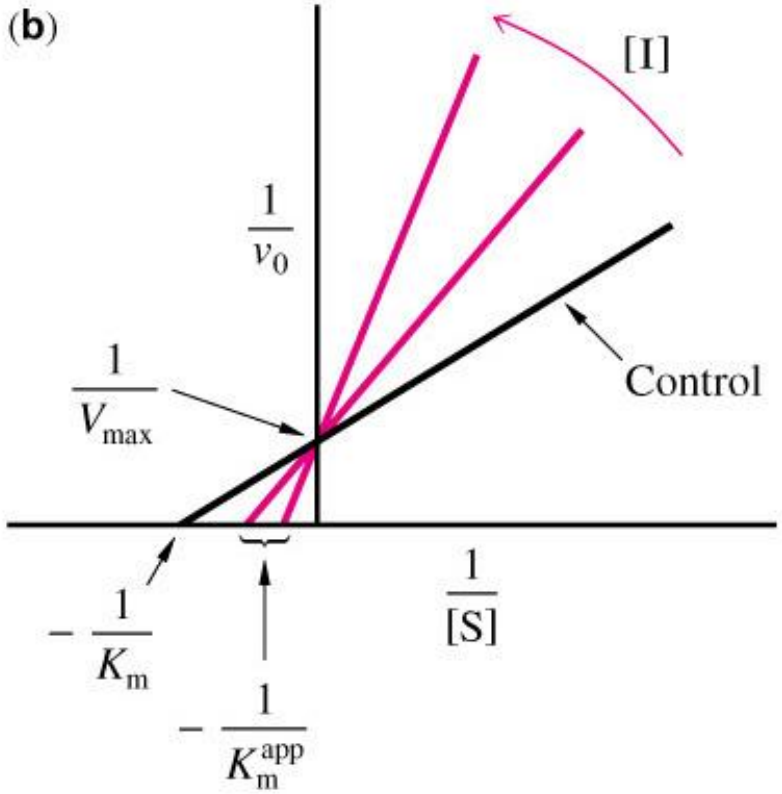
- Competitive
- Uncompetitive
- Non-competitive

Types of Reversible Enzyme Inhibitors



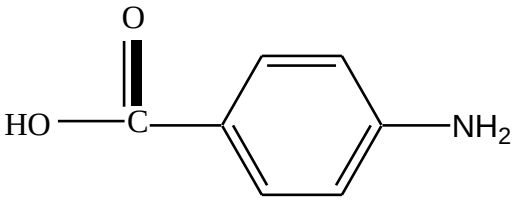
Competitive Inhibitor (CI)



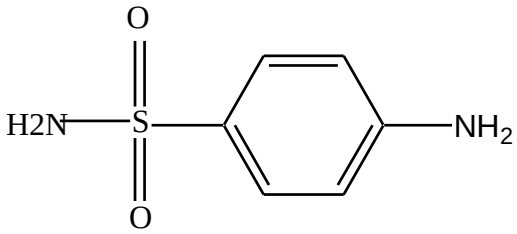


- CI binds free enzyme
- Competes with substrate for enzyme binding.
- Raises Km without effecting Vmax
- Can relieve inhibition with more S

Competitive Inhibitors look like substrate



PABA



Sulfanilamide

PABA precursor to folic acid in bacteria

$O_2C\text{-}CH_2\text{-}CH_2\text{-}CO_2$ -------> $O_2C\text{-}CH{=}CH\text{-}CO_2$

succinate fumarate

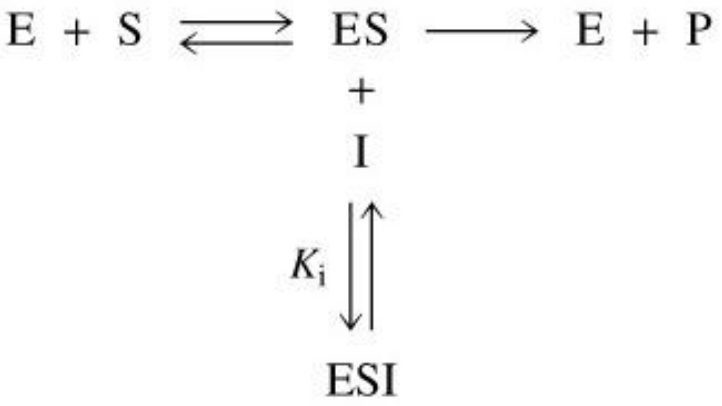
Succinate dehydrogenase

$O_2C\text{-}CH_2\text{-}CO_2$

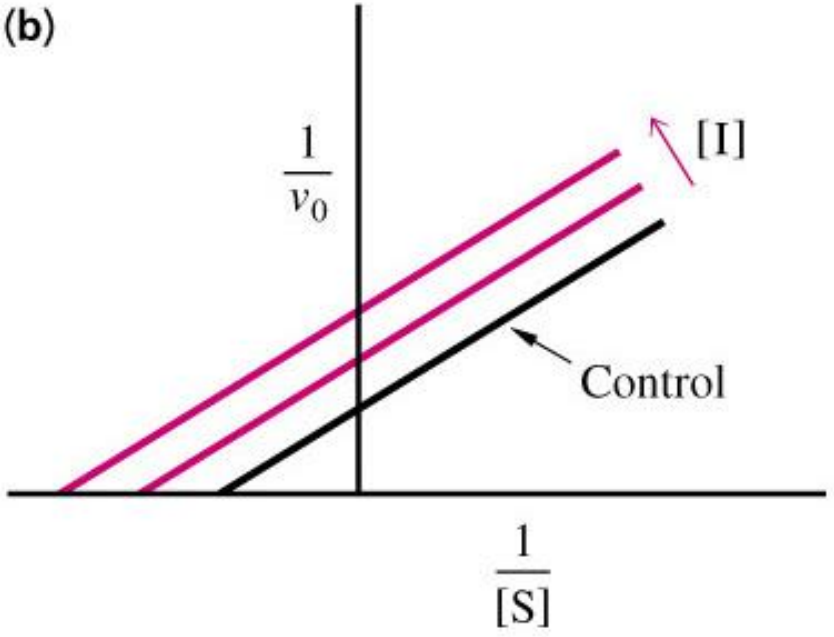
Malonate

Uncompetitive Inhibitor (UI)

(a)

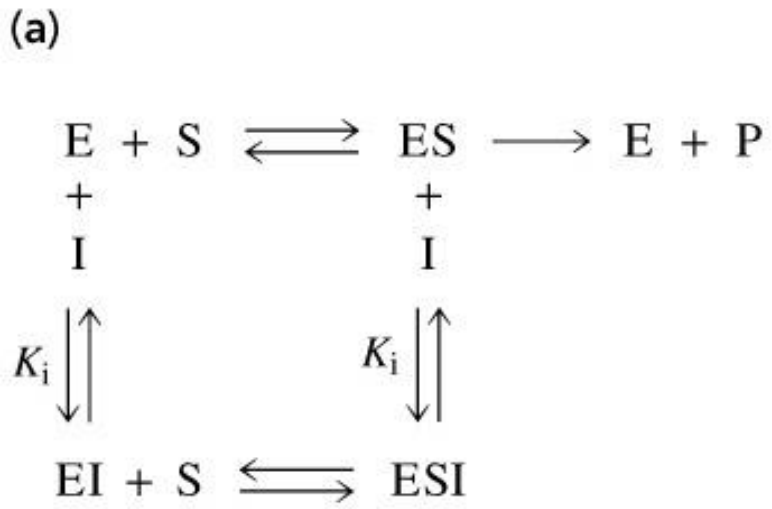


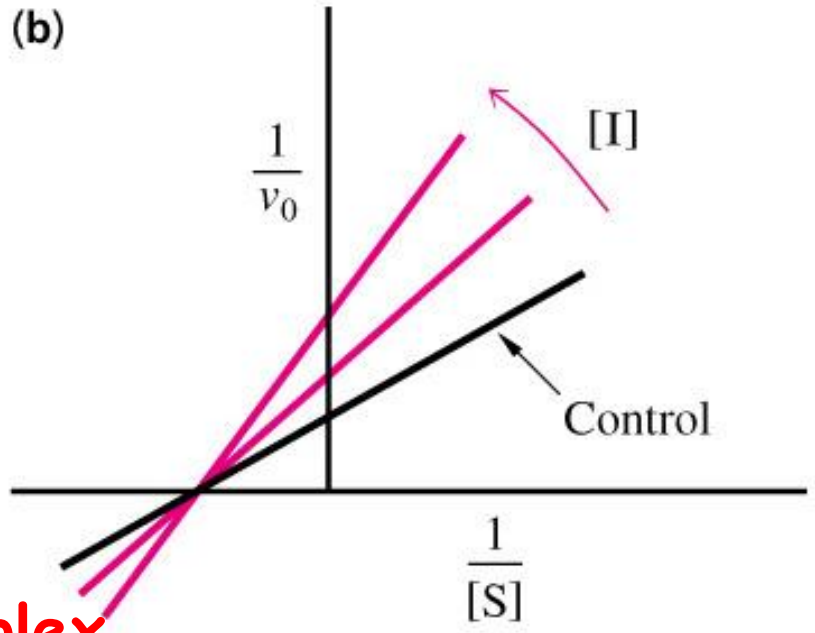
(b)



- UI binds ES complex
- Prevents ES from proceeding to E + P or back to E + S.
- Lowers Km & Vmax, but ratio of Km/Vmax remains the same
- Occurs with multisubstrate enzymes

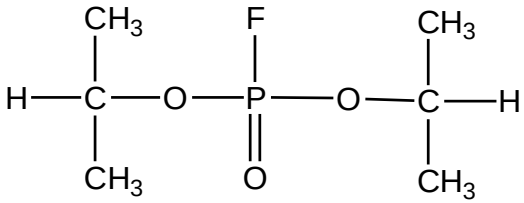
Non-competitive Inhibitor (NI)



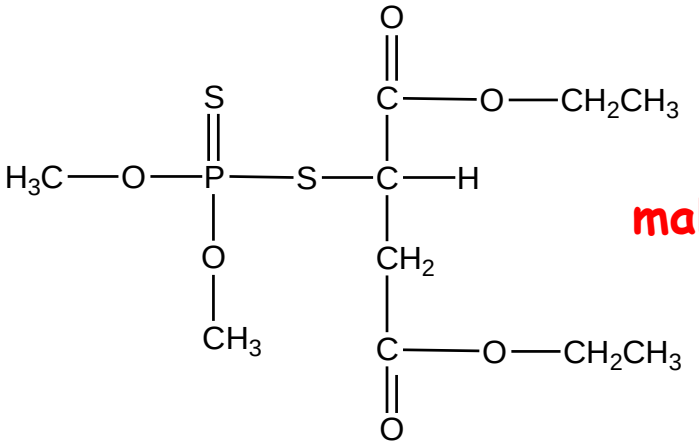


- NI can bind free E or ES complex
- Lowers Vmax, but Km remains the same
- NI's don't bind to S binding site therefore don't effect Km
- Alters conformation of enzyme to effect catalysis but not substrate binding

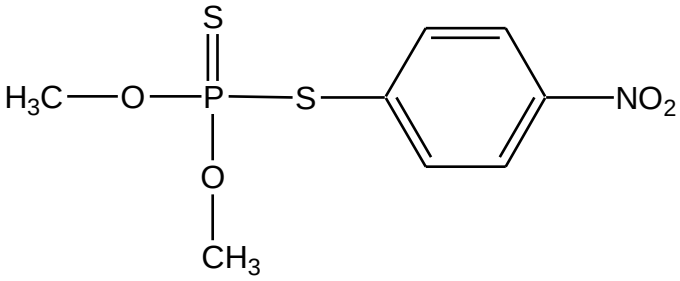
Irreversible Inhibitors



Diisopropyl fluorophosphate
(nerve gas)

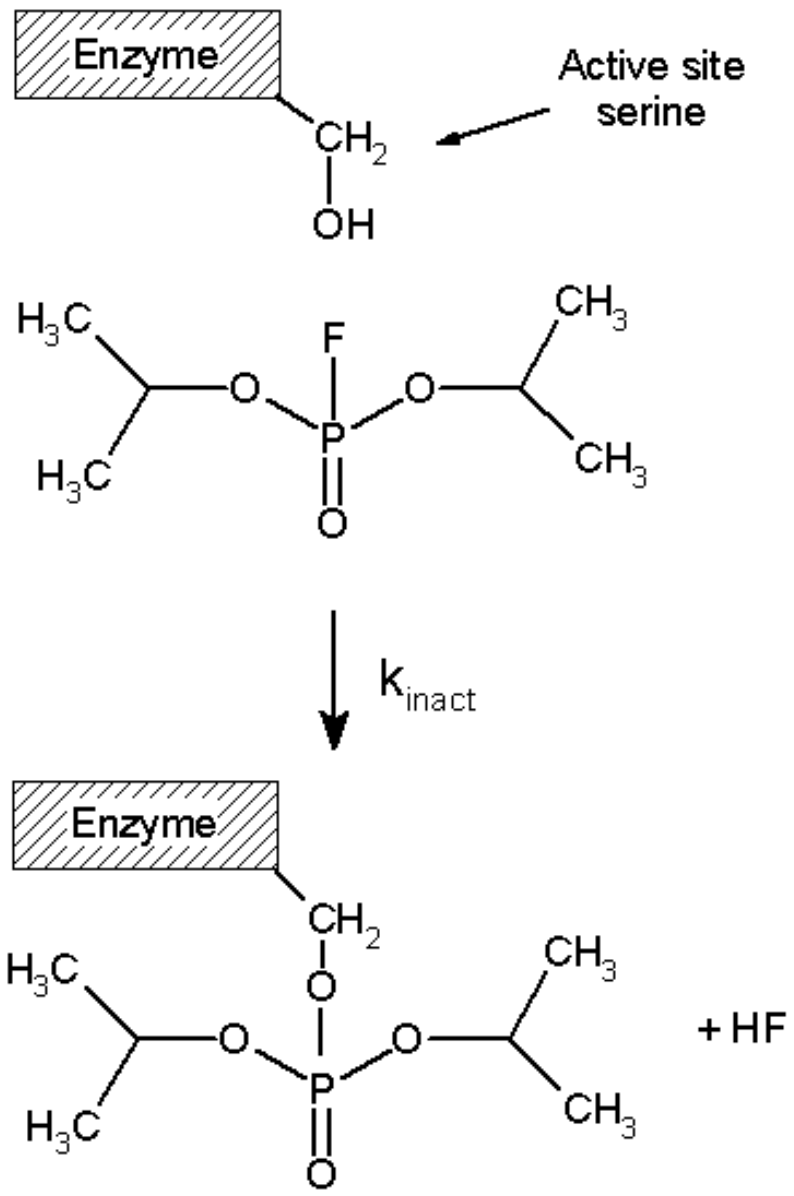


malathion



parathion

- Organophosphates
- Inhibit serine hydrolases
- Acetylcholinesterase inhibitors


Enzyme
Active site serine
CH2
OH
H3C
F
CH3
O
O
P
H3C
CH3
O
kinact
Enzyme
CH2
O
H3C
CH3
O
O
P
H3C
CH3
O
+ HF

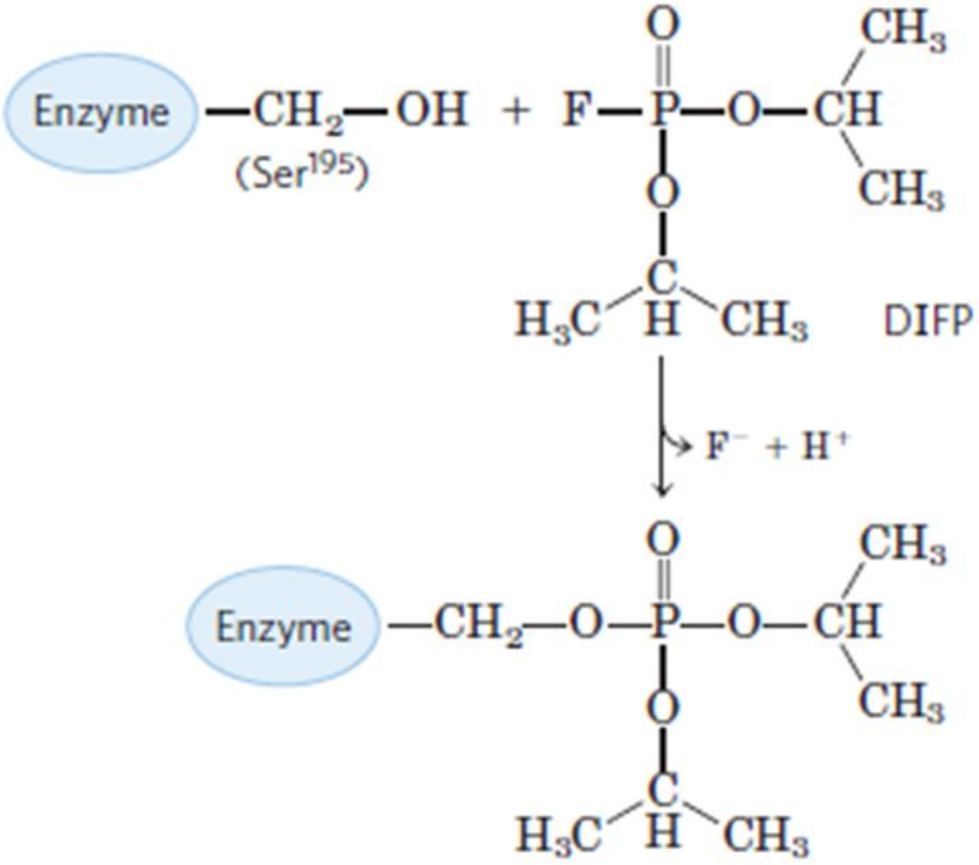


FIGURE 6–16 Irreversible inhibition. Reaction of chymotrypsin with diisopropylfluorophosphate (DIFP), which modifies Ser^{195}, irreversibly inhibits the enzyme. This has led to the conclusion that Ser^{195} is the key active-site Ser residue in chymotrypsin.

Kinetics of Multisubstrate Reactions

$$E + A + B \leftrightarrow E + P + Q$$

- Sequential Reactions

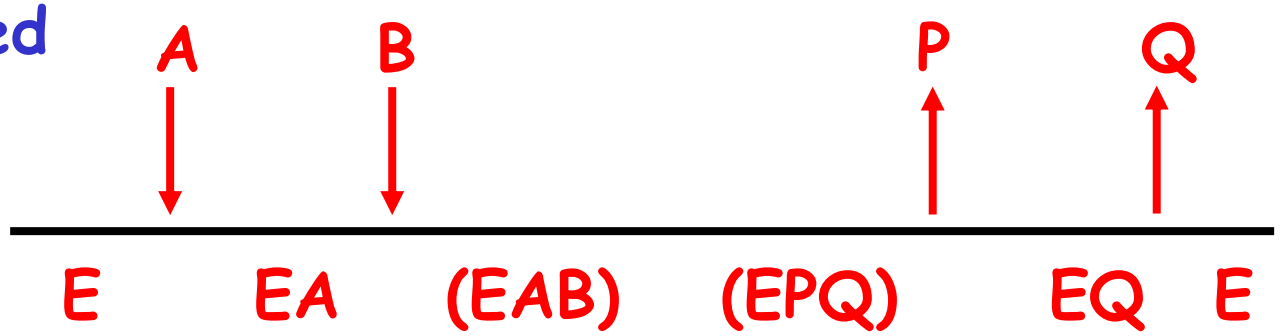
a) ordered

b) random

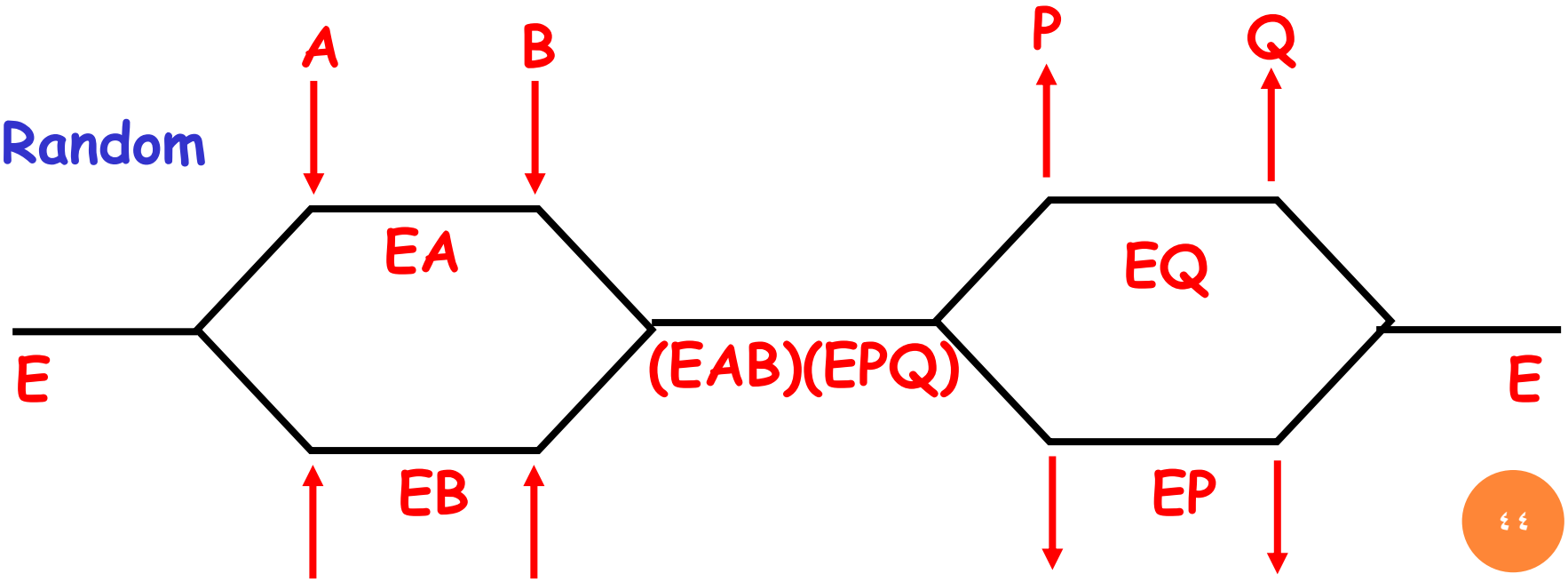
- Ping-pong Reactions
- Cleland Notation

Sequential Reactions

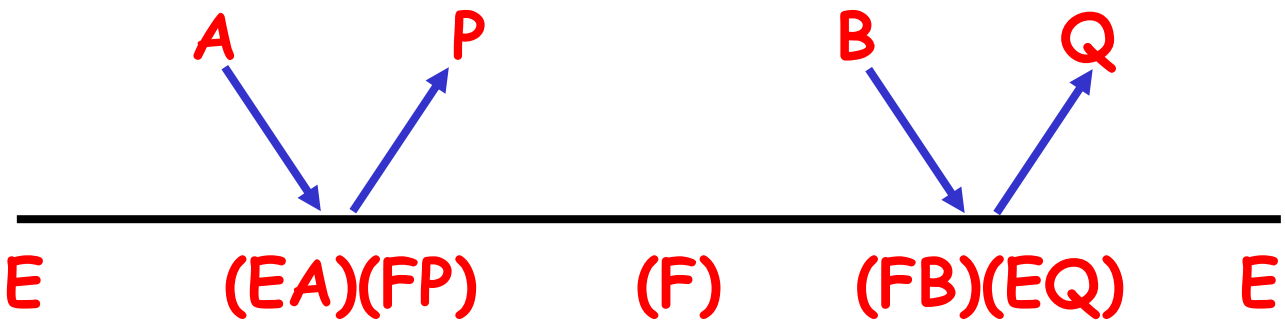
Ordered



Random



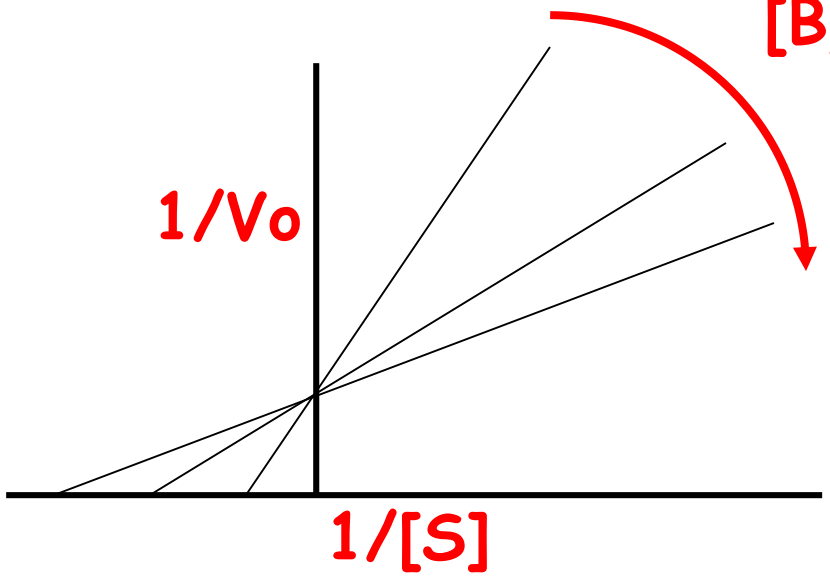
Ping-Pong Reactions



- In Ping-Pong rxns first product released before second substrate binds
- When E binds A, E changes to F
- When F binds B, F changes back to E

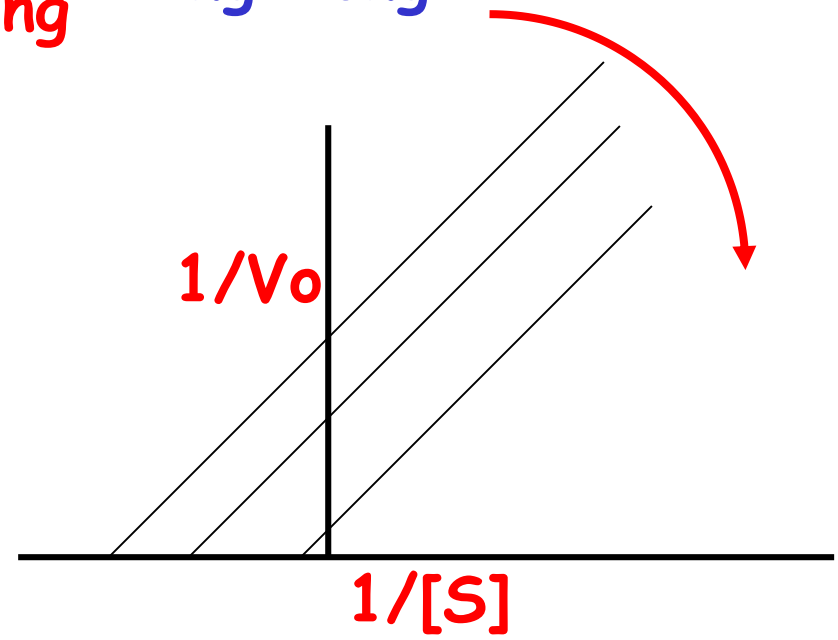
Lineweaver-Burke Plot of Multisubstrate Reactions

Sequential



Vmax doesn't change
Km changes

Ping-Pong



Both Vmax & Km change

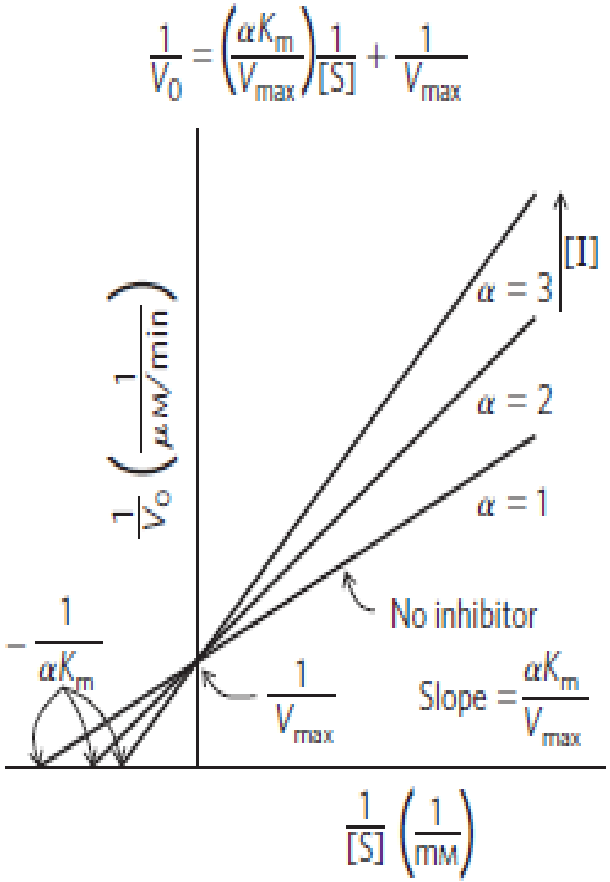


FIGURE 1 Competitive inhibition.

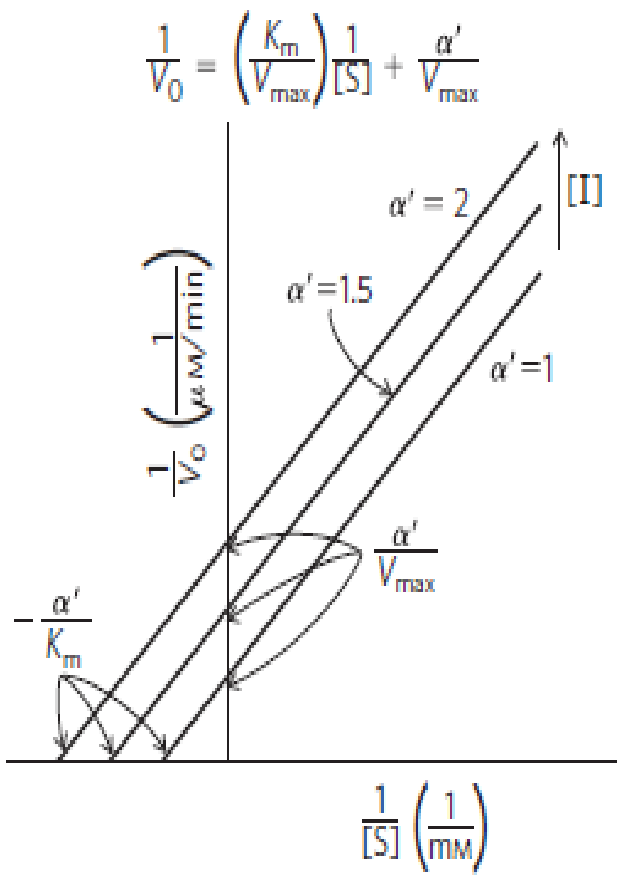


FIGURE 2 Uncompetitive inhibition.

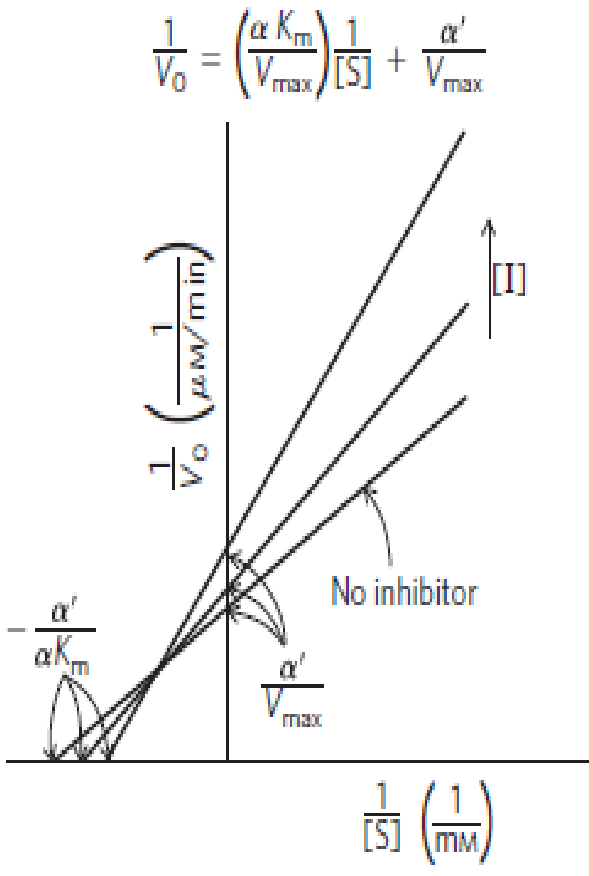


FIGURE 3 Mixed inhibition.

Thank you very much for your attention!