LECTURE 8: ENZYMES KINETICS

- Kinetics of single substrate enzyme catalysed reactions
- Michaelis- Menten (M-M) equation and its transformations
- Mechanism of Bi-substrate and Multi-substrate
- Mechanism Co-operativity and the Hill Plot

Why enzyme kinetics is important?

1. Substrate binding constants can be measured as well as inhibitor strengths and maximum catalytic rates

2. Kinetics alone will not give a chemical mechanism but combined with chemical and structural data mechanisms can be elucidated

3. Kinetics help understand the enzymes role in metabolic pathways

4. Under "proper" conditions rates are proportional to enzyme concentrations and these can be used to determine " metabolic problems"

Chemical kinetics and Elementary Reactions

A simple reaction like $A \rightarrow B$ may proceed through several elementary reactions like $A \rightarrow I_1 \rightarrow I_2 \rightarrow B$ Where I_1 and I_2 are intermediates

The characterization of elementary reactions comprising an overall reaction process constitutes its mechanistic description

Rate Equations

Consider aA + bB + • • • + zZ. The rate of a reaction is proportional to the frequency with which the reacting molecules simultaneously bump into each other

$$\text{Rate} = k[A]^a[B]^b \bullet \bullet \bullet [Z]^z$$

The order of a reaction = the sum of exponents

The order means how many molecules have to bump into each other at one time for a reaction to occur

A first order reaction one molecule changes to another

$$A \rightarrow B$$

A second order reaction two molecules react

$$A + B \rightarrow P + Q$$

or

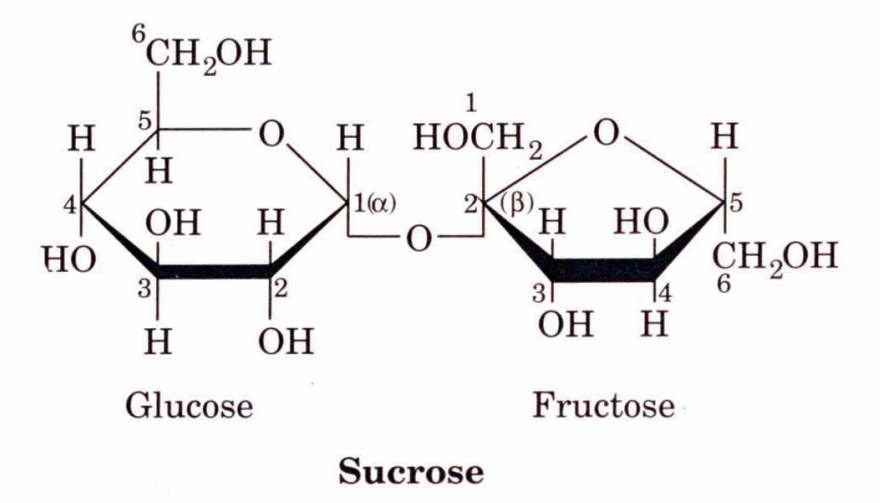
$$2A \rightarrow P$$

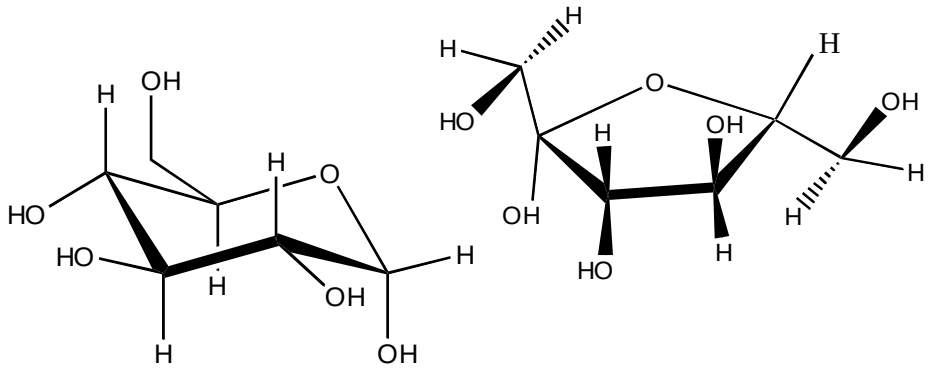
Kinetics of Enzymes

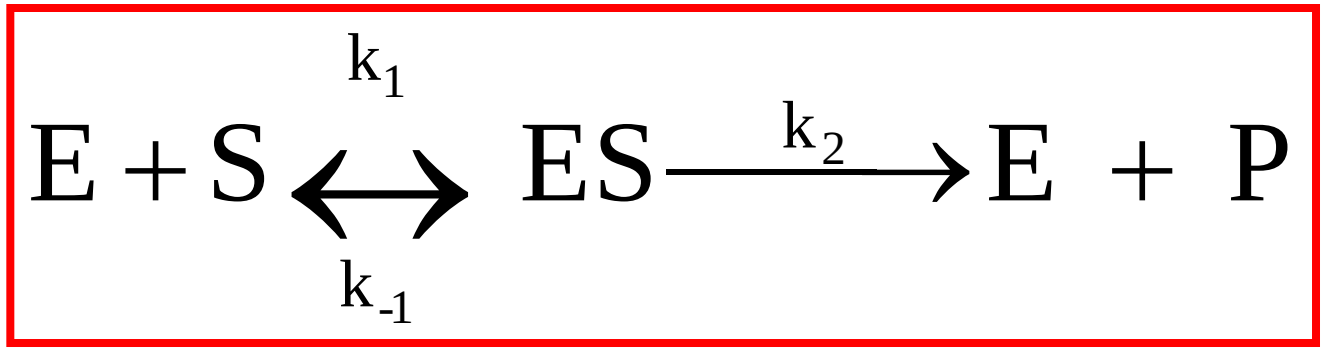
Enzymes follow zero order kinetics when substrate concentrations are high. Zero order means there is no increase in the rate of the reaction when more substrate is added

Given the following breakdown of sucrose to glucose and fructose

Sucrose + H_2O ⟶ Glucose + Fructose







E = Enzyme S = Substrate P = Product

ES = Enzyme-Substrate complex

k_1 rate constant for the forward reaction

k_{-1} = rate constant for the breakdown of the ES to substrate

k_2 = rate constant for the formation of the products

Enzymes kinetics…..

- The **VELOCITY** (reaction rate) of an enzyme catalysed reaction is dependent upon the substrate concentration **[S]**

- Varying amounts of Substrate added to fixed amount of Enzyme
- **E** becomes saturated with **S**

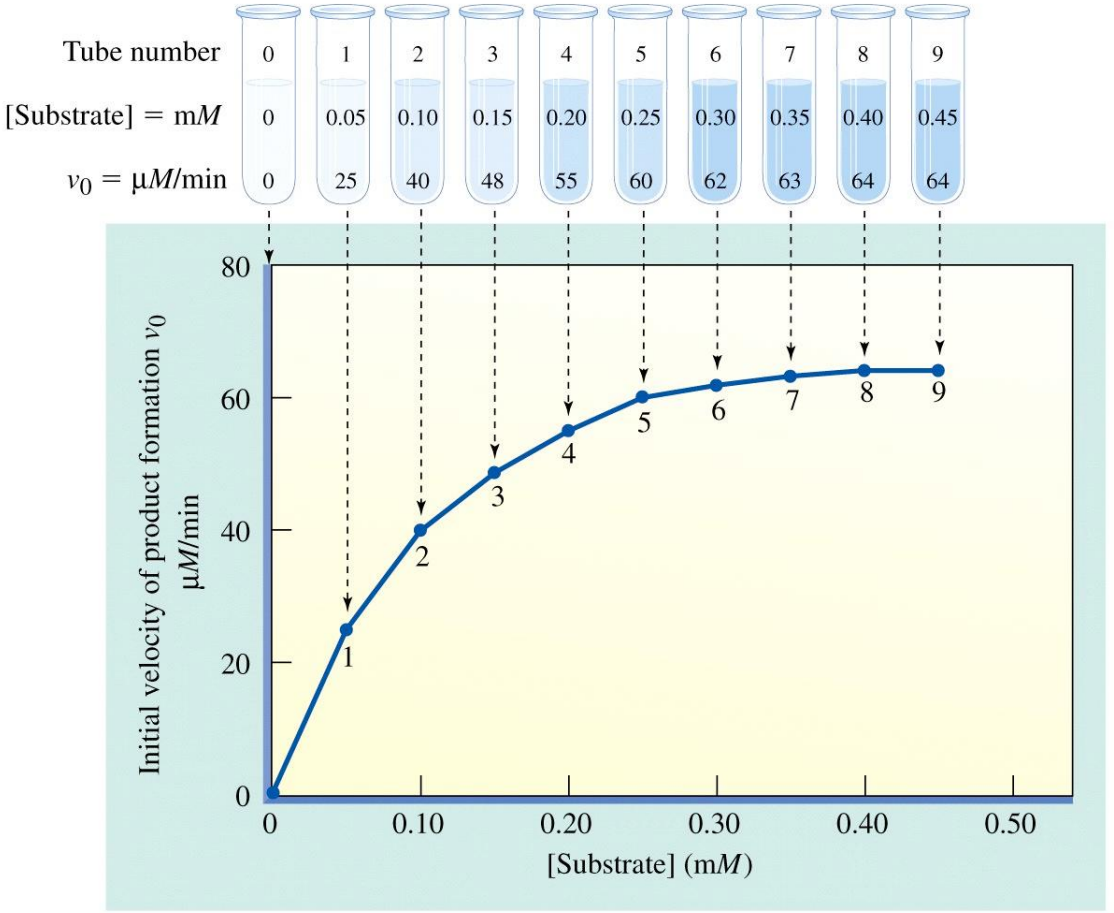
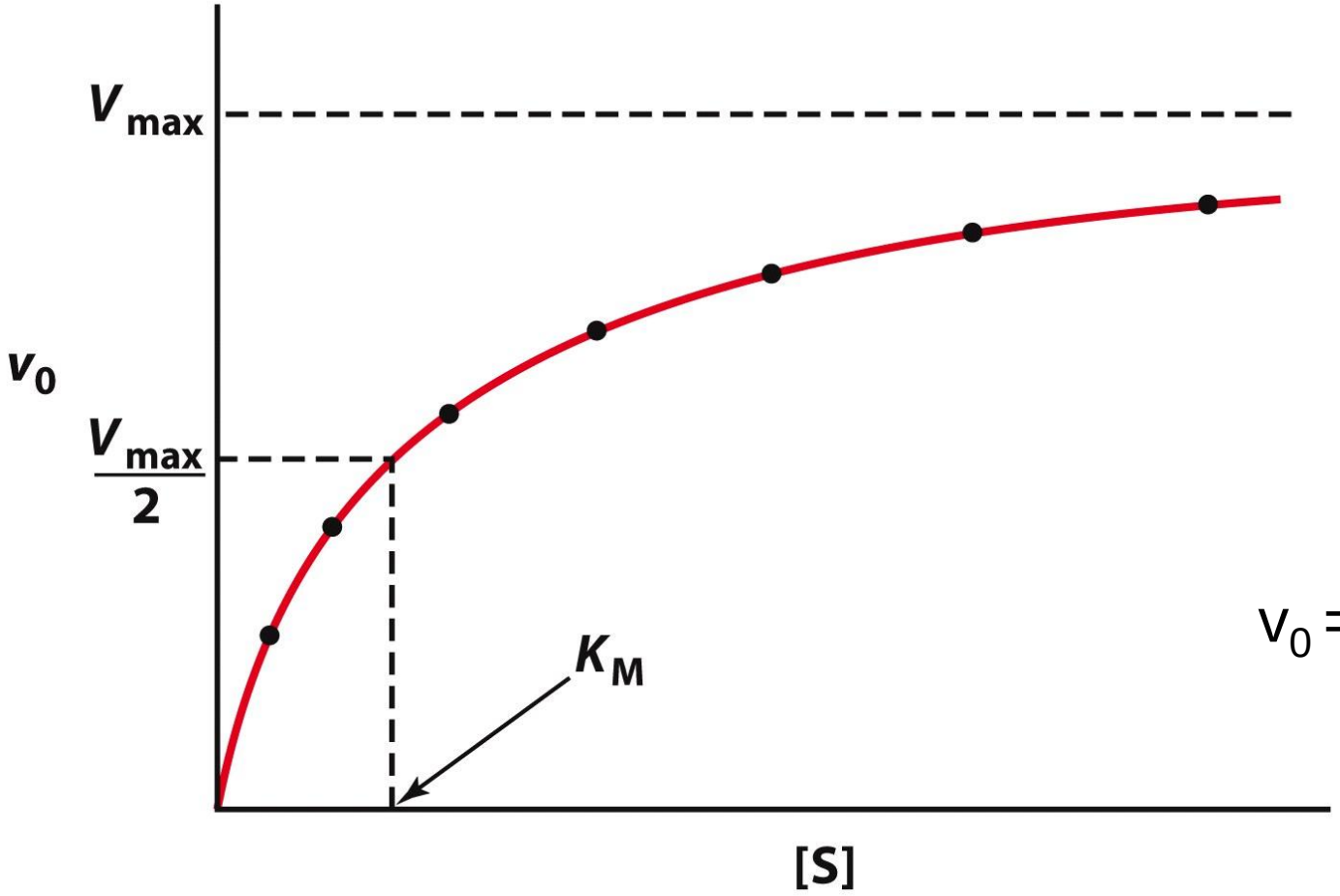


Figure 5-3 Concepts in Biochemistry, 3/e

Enzymes kinetics: Michaelis- Menten equation



$$v_0 = \frac{V_{max}[S]}{K_M + [S]}$$

Significance of Michaelis- Menten equation

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

- K_M - substrate concentration where v_o equals one-half V_{max}
- Used to evaluate the specificity of an enzyme for a substrate
- K_M – is usually a little higher than the physiological [S]

Small K_M → tight binding

High K_M → weak binding

Hexose Kinase

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

Glucose	$K_M = 8 \times 10^{-6}$
Allose	$K_M = 8 \times 10^{-3}$
Mannose	$K_M = 5 \times 10^{-6}$

Turnover number

Kcat – enzyme turn over number defined as the number of reactions a molecule of enzyme can catalyze per second under optimal condition

$$k_{cat} = \frac{V_{max}}{[E]_T} = k_2$$

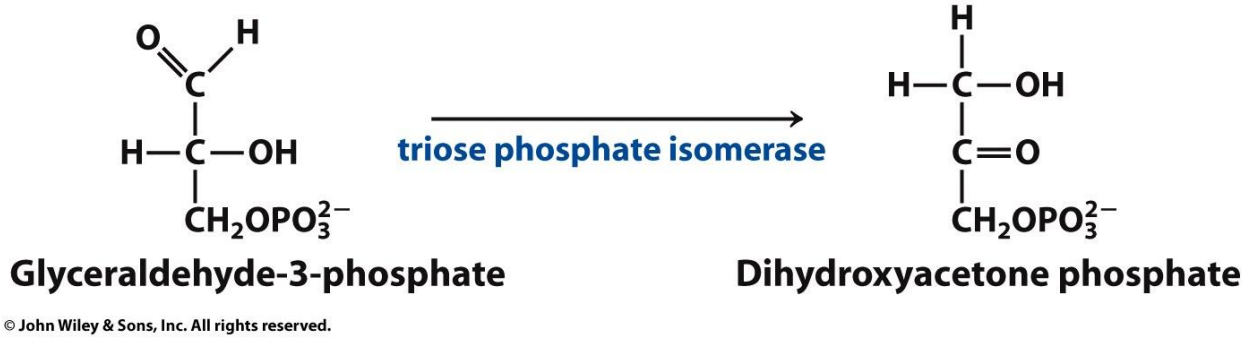
TABLE 7-1 | Catalytic Constants of Some Enzymes

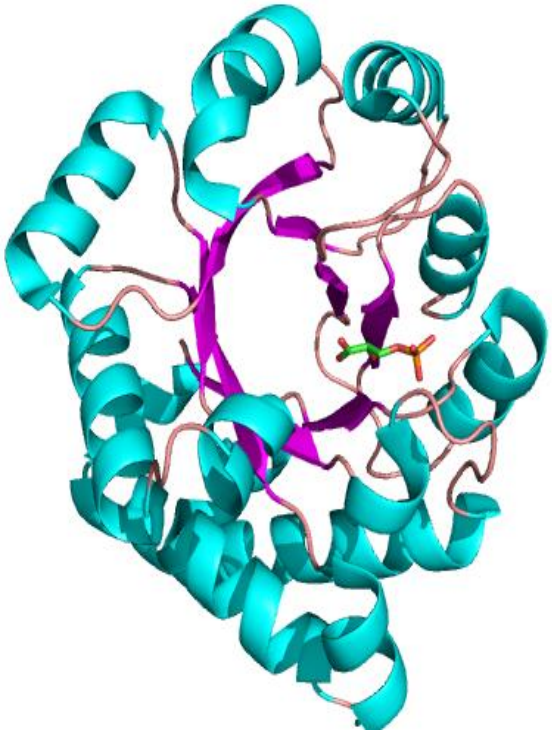
Enzyme	k_{cat} (s^{-1})
Staphylococcal nuclease	95
Cytidine deaminase	299
Triose phosphate isomerase	4300
Cyclophilin	13,000
Ketosteroid isomerase	66,000
Carbonic anhydrase	1,000,000

Catalytic efficiency

- Defined as:

$$\frac{k_{cat}}{K_M}$$





Triose Phosphate Isomerase

$K_{cat}/K_M = 2.4 \times 10^8\ M^{-1}s^{-1}$

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

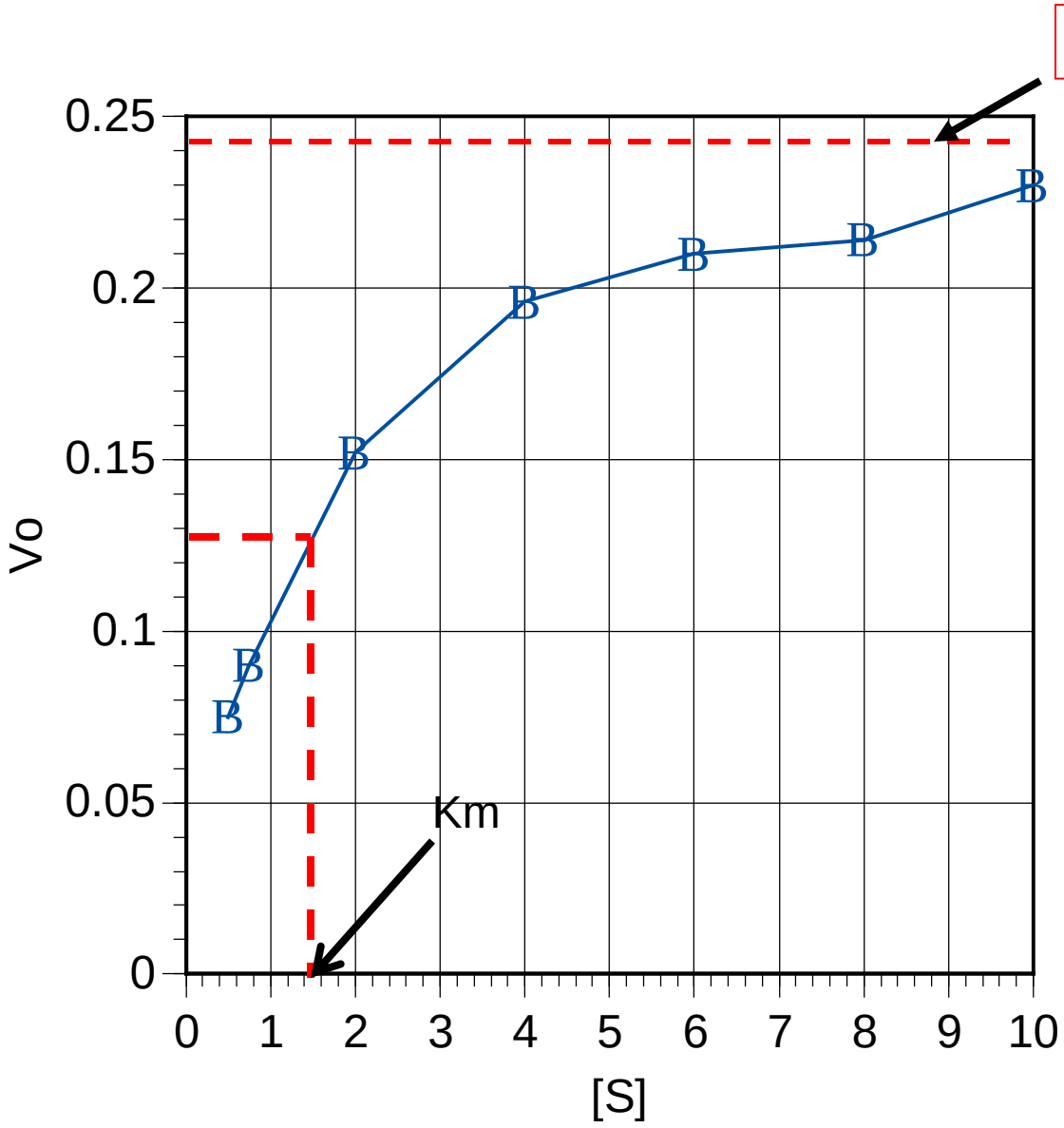
3. Assumes $k_{-2} = 0$

4. Assume steady state conditions (see next slide)

Steady State Assumption

- The M-M equation was derived in part by making several assumptions. An important one was: **the concentration of substrate must be much greater than the enzyme concentration.**
- In the situation where [S] >> [E] and at initial velocity rates, it is assumed that the changes in the concentration of the intermediate ES complex are very small over time (v_o). This condition is termed a **steady-state rate,** and is referred to as **steady-state kinetics**. **Therefore, it follows that the rate of ES formation will be equal to the rate ES breakdown.**

Difficult to determine V_{max} experimentally



[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

K_m ~ 1.3 mM

V_{max} ~ 0.25

Lineweaver-Burke plot (double reciprocal plot)

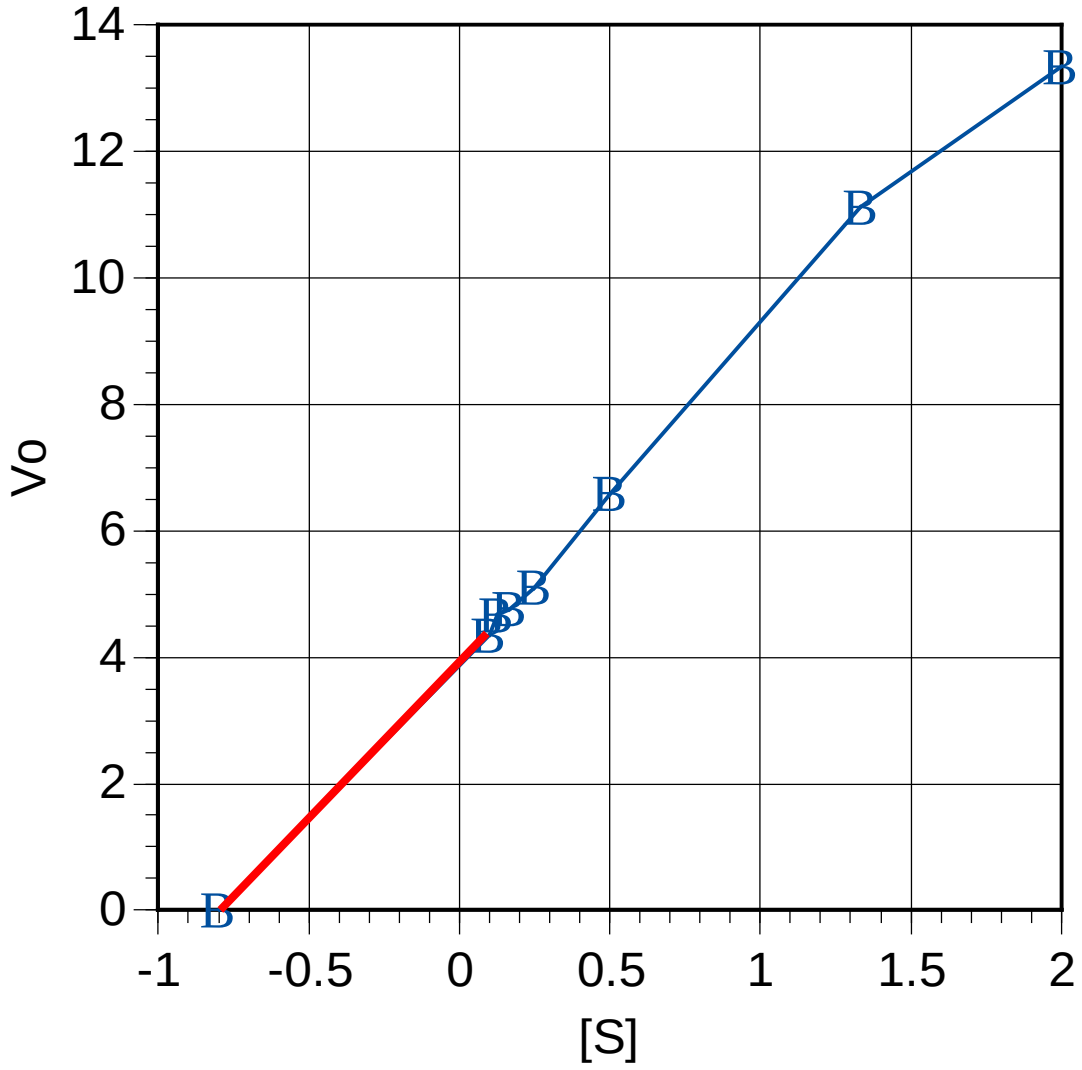
- it is difficult to determine V_{max} experimentally
- hyperbola equation can be transformed into the equation for a straight line by taking the reciprocal of each side
- the formula for a straight line is y = mx + b

$$\frac{1}{V} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

$$y = m \cdot x + b$$

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

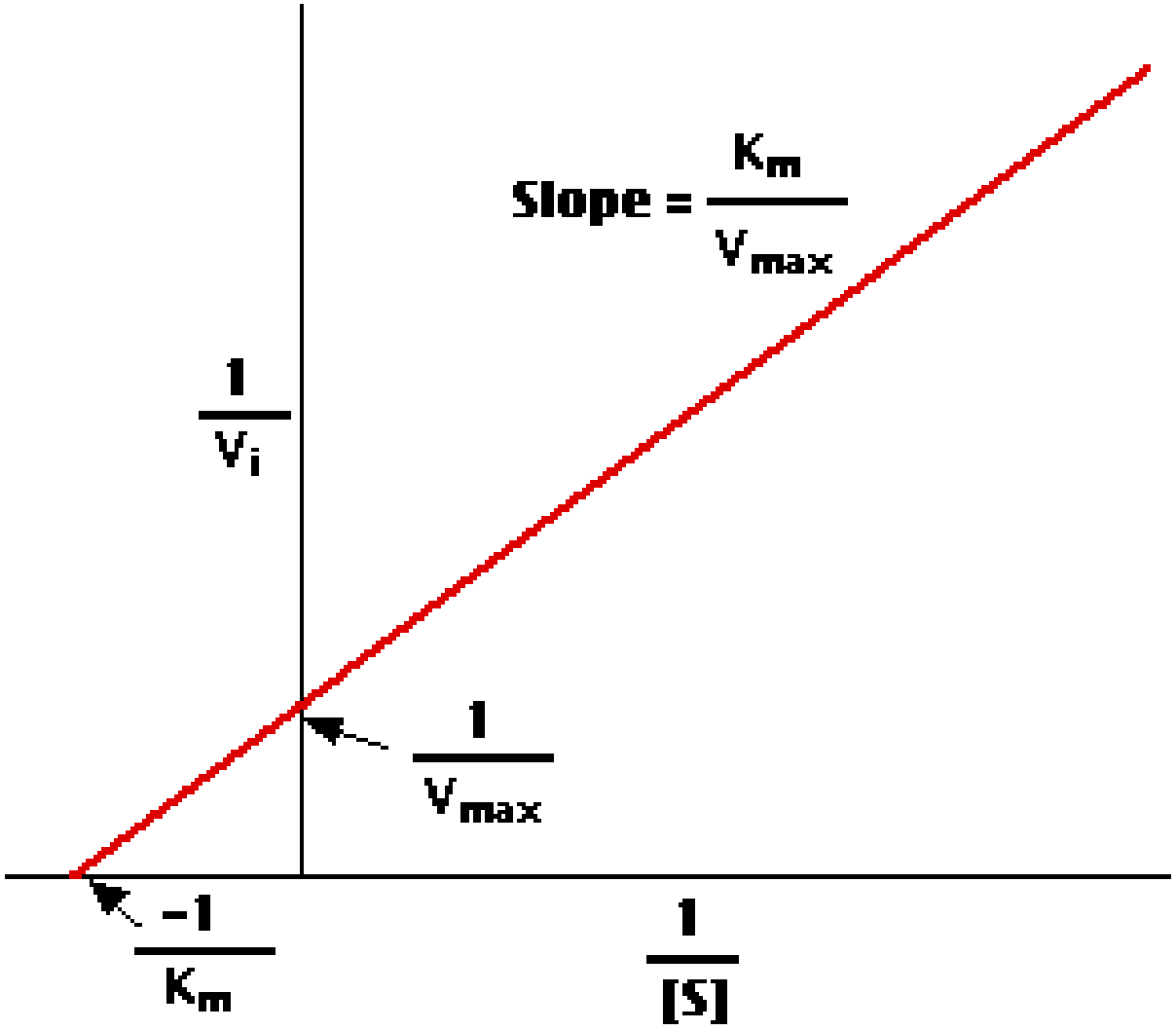
Difficult to determine V_{max} experimentally



[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



Provides a more precise way to determine V_{max} and K_M

Other enzymatic kinetics plots

The Hanes plot

Makes use of the Lineweaver-Burk equation by multiplying with [S] on both sides

Plot $[S]/V_0$ vs $[S]$

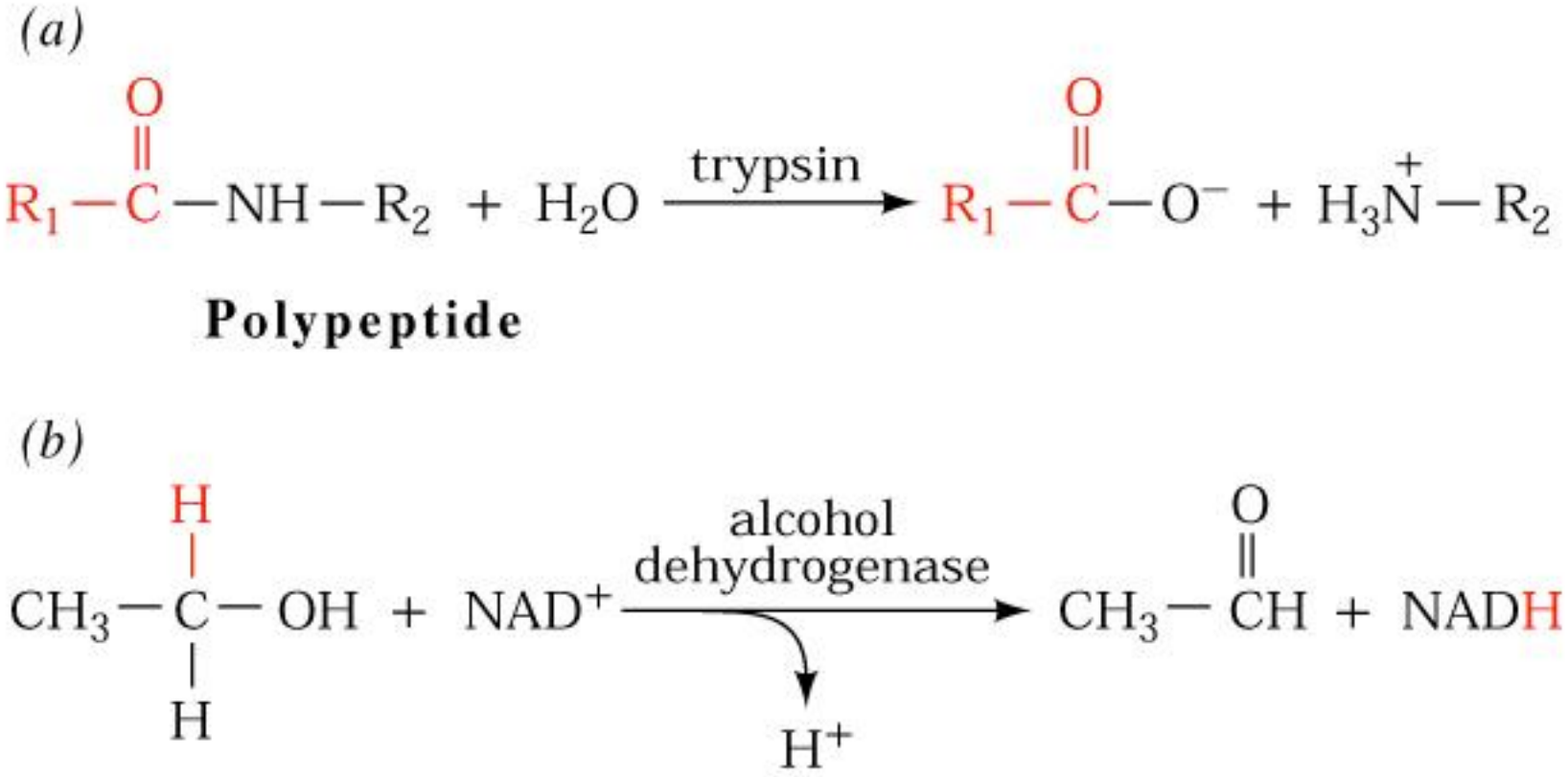
The Eadie-Hofstee plot

Multiply both sides of the Lineweaver-Burk equation by the factor V_0V_{max}

Plot V_0 vs $V_0/[S]$

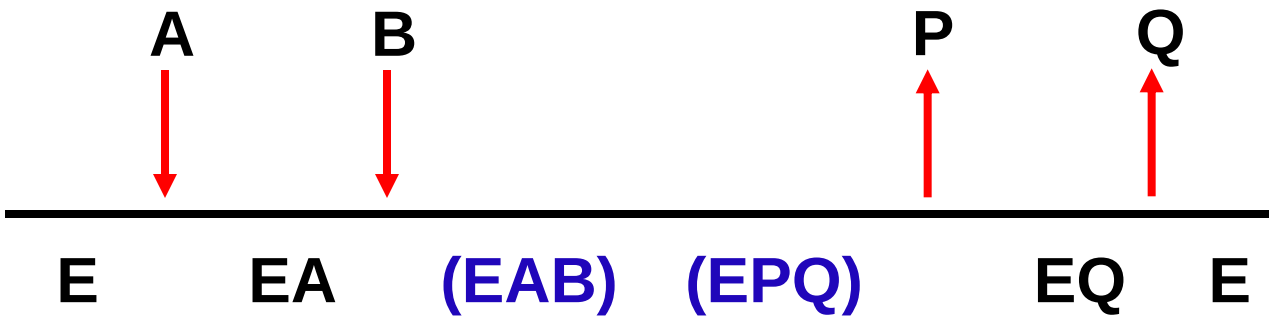
What about bisubstrate reactions?

Bisubstrate reactions

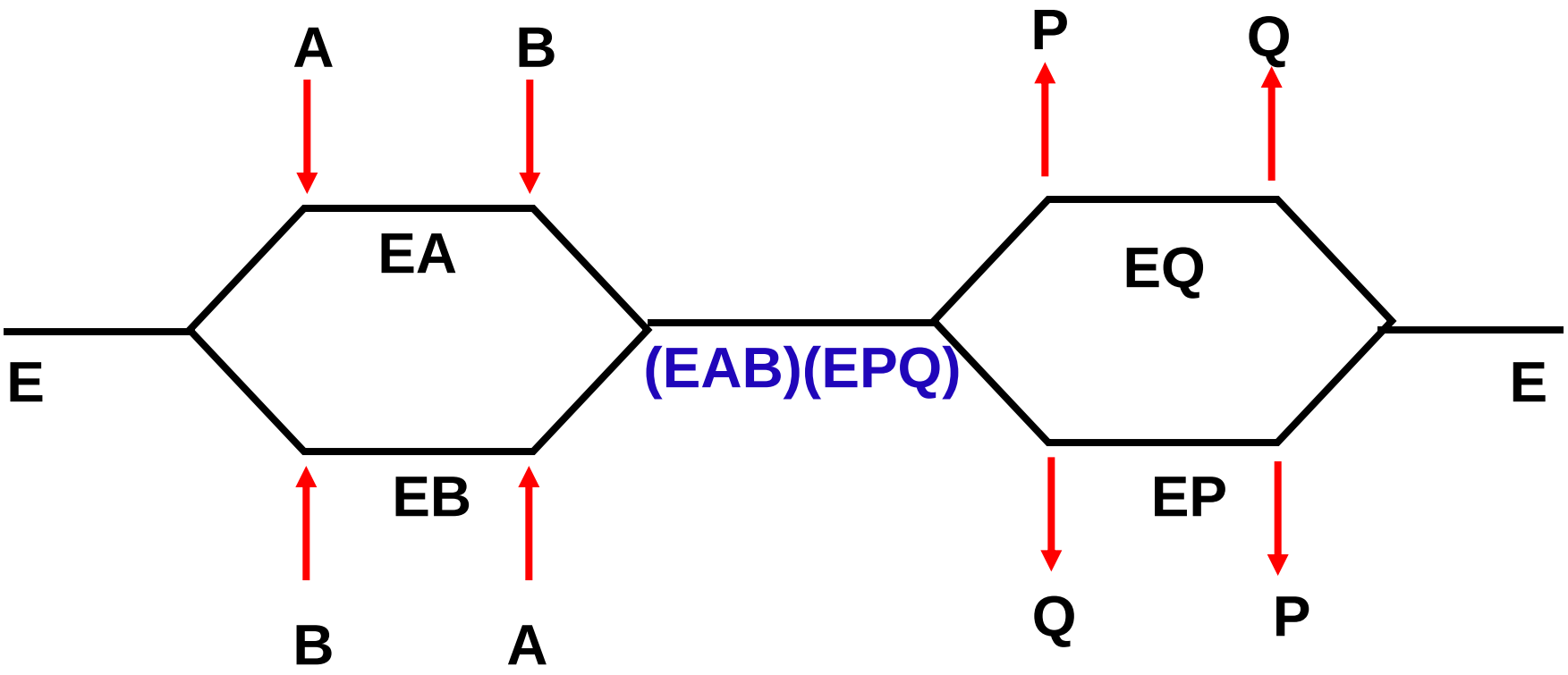


a) Sequential Reactions

- **All substrates must combine with enzyme before reaction can occur**

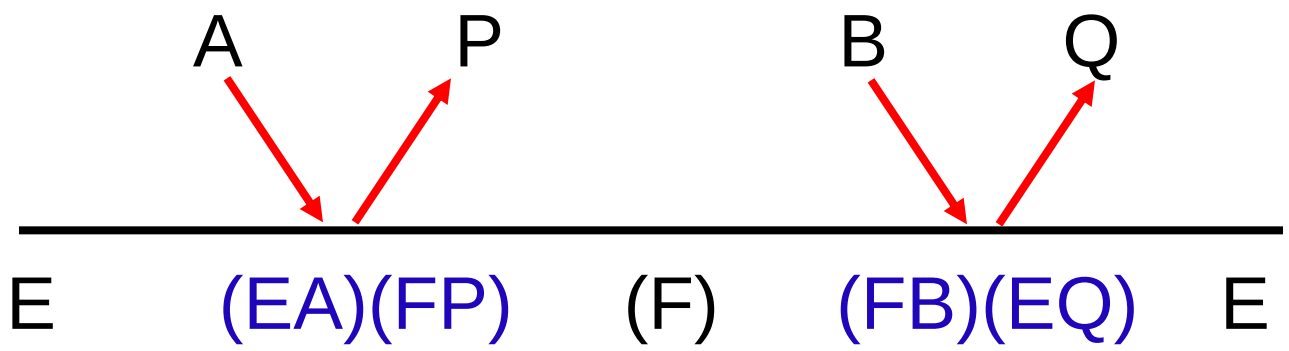


b) Random bisubstrate reactions



c) Ping-Pong Reactions

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



Types of enzyme inhibition

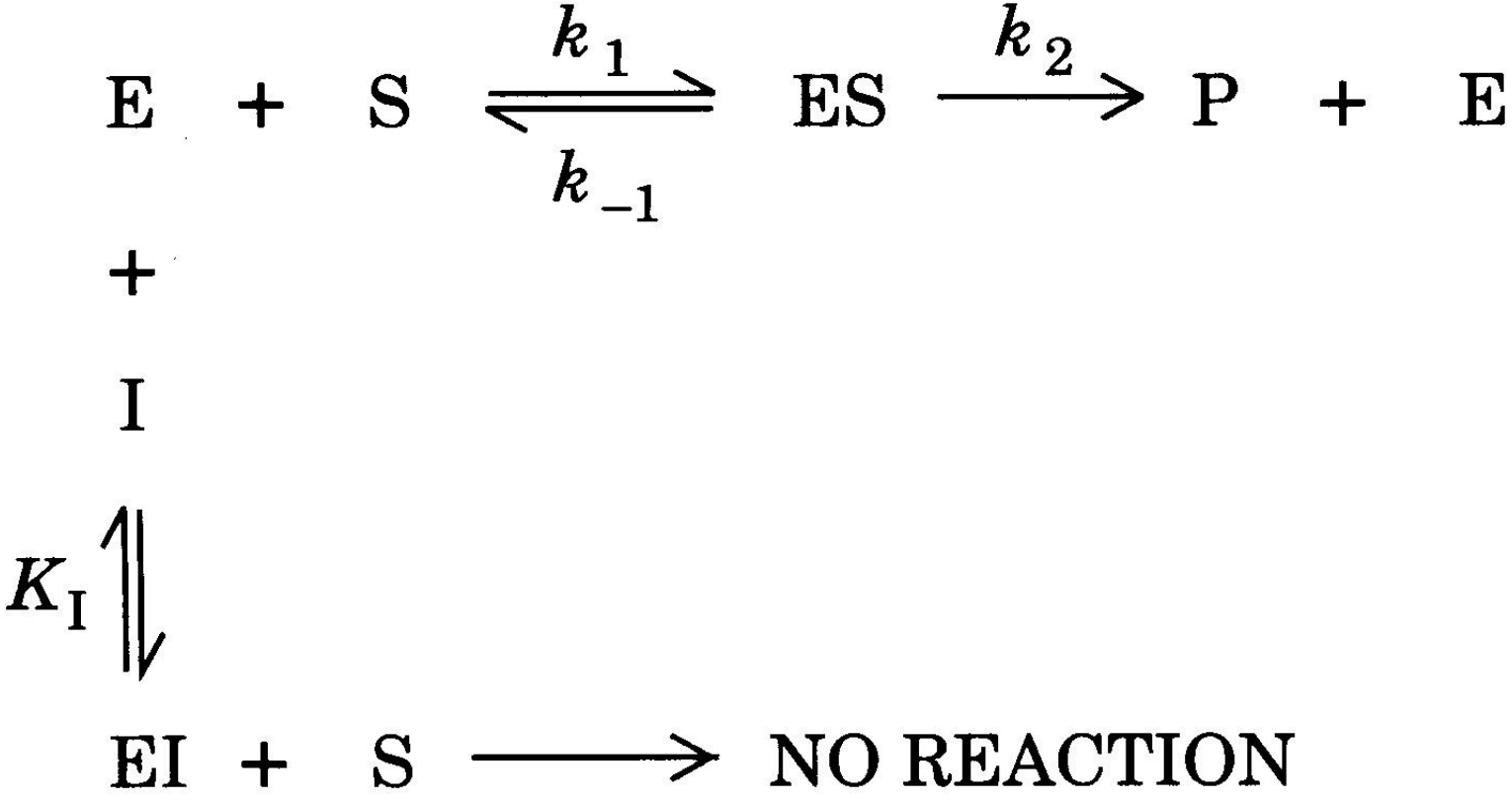
- **Inhibitor**- substance that binds to an enzyme and interferes with its activity

- Inhibitor can prevent **ES** complex formation or prevent the **ES** complex breakdown to **E** + **P**

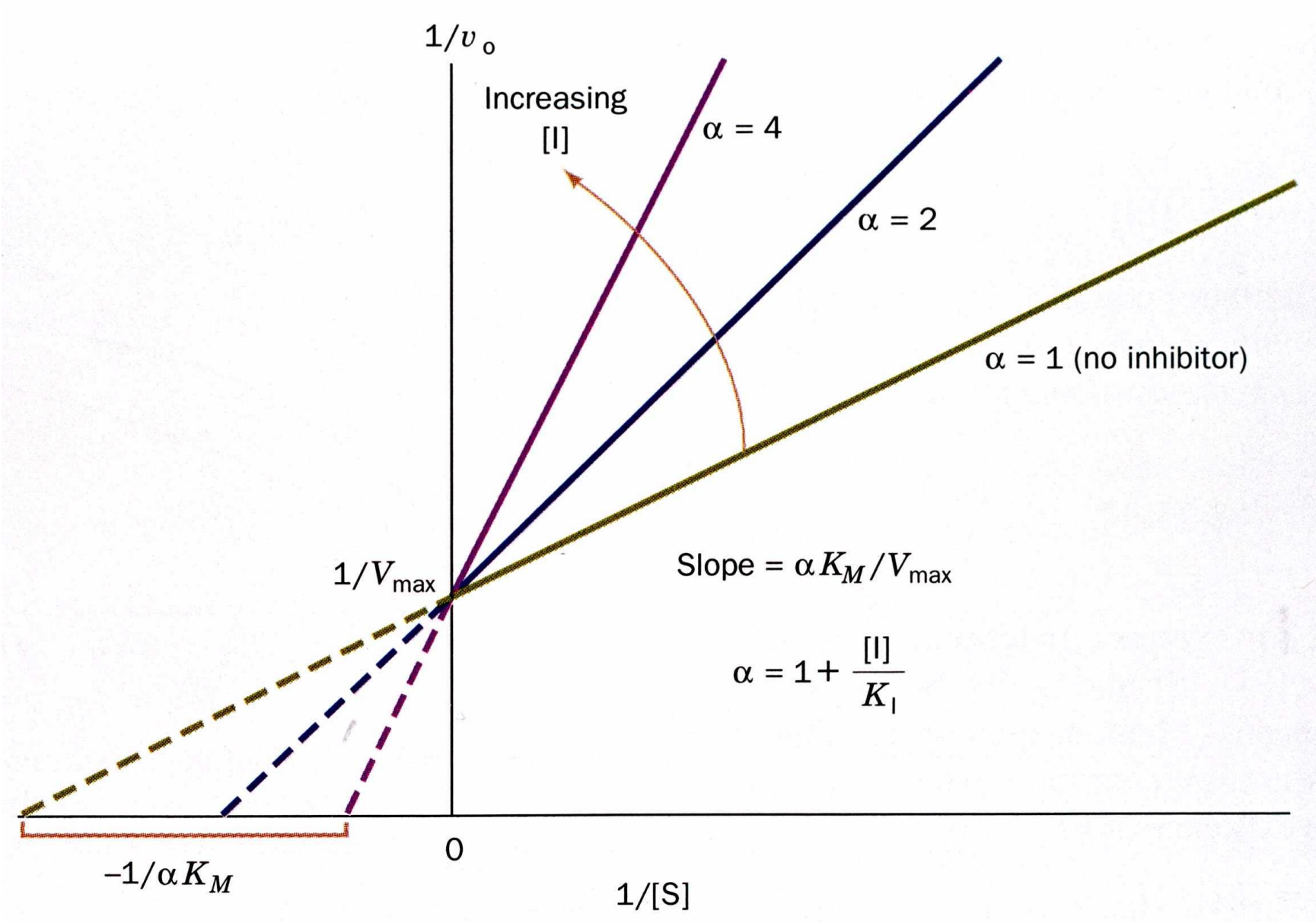
A. Irreversible – covalent
B. Reversible – non-covalent binding
    i. Competitive
    ii. Uncompetitive
    iii. Non-competitive

Competitive Inhibition

Inhibitor molecule binds to the same site on the enzyme as the substrate preventing the substrate from binding

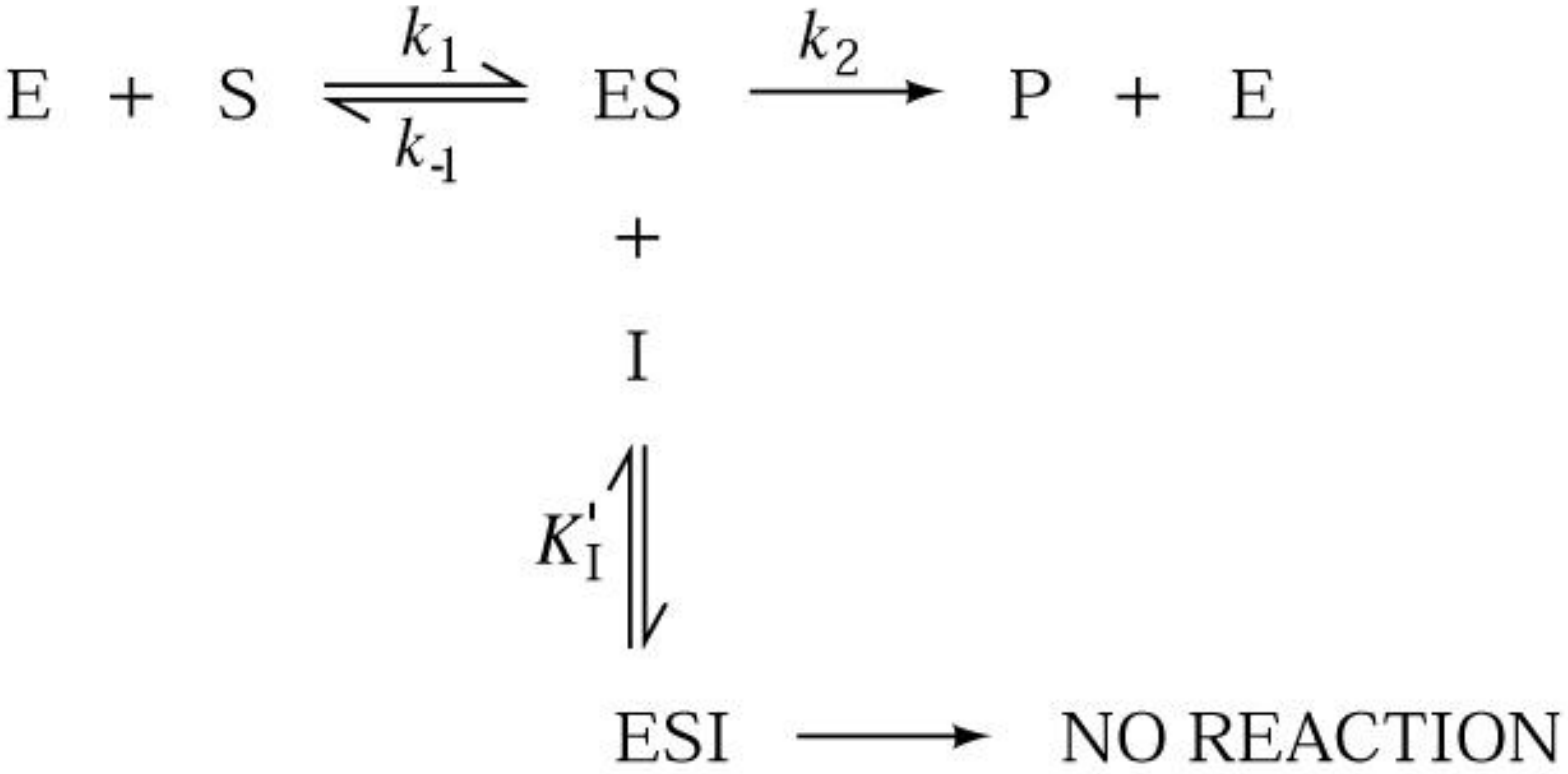


Competitive Inhibition: Lineweaver-Burk Plot

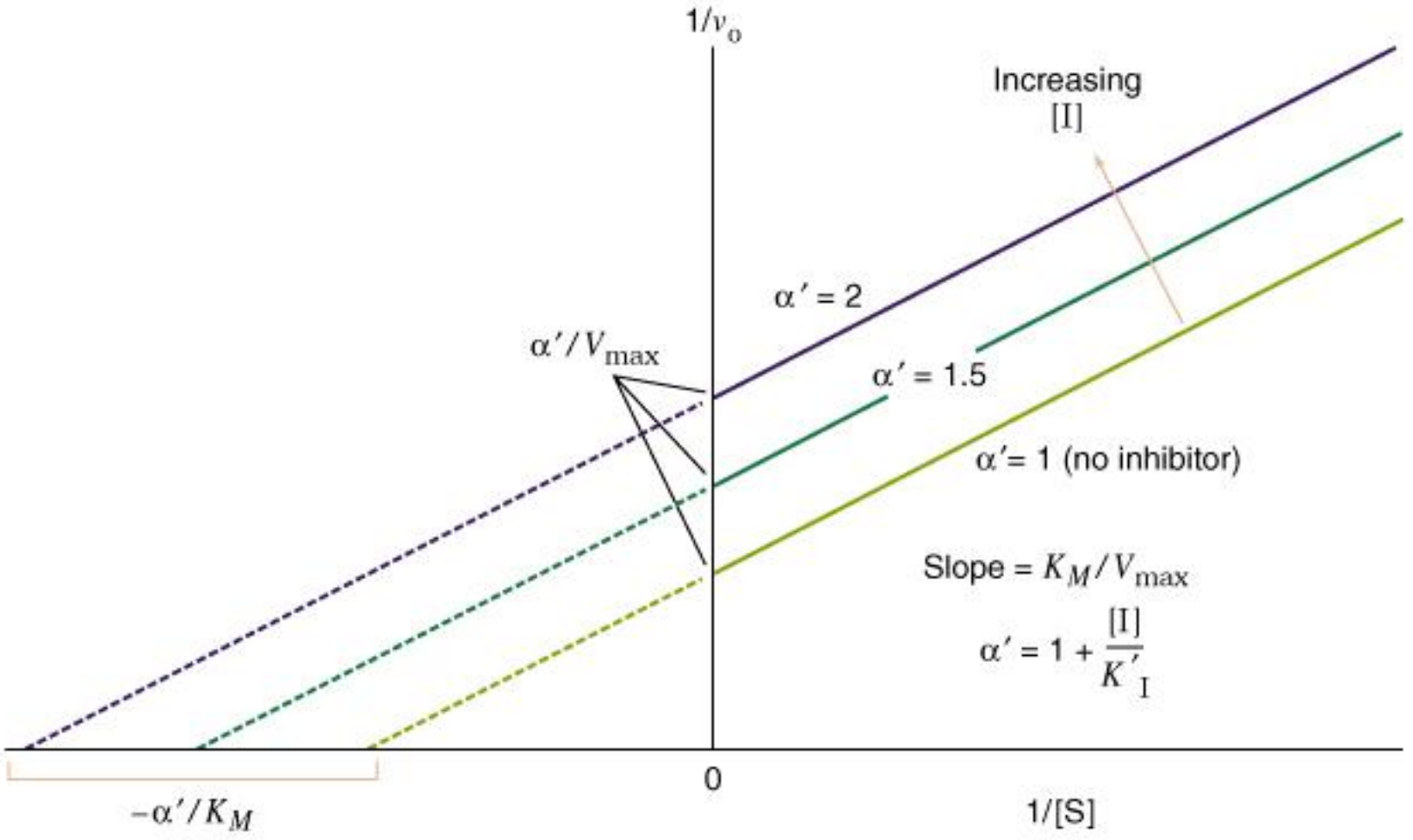


Uncompetitive Inhibition

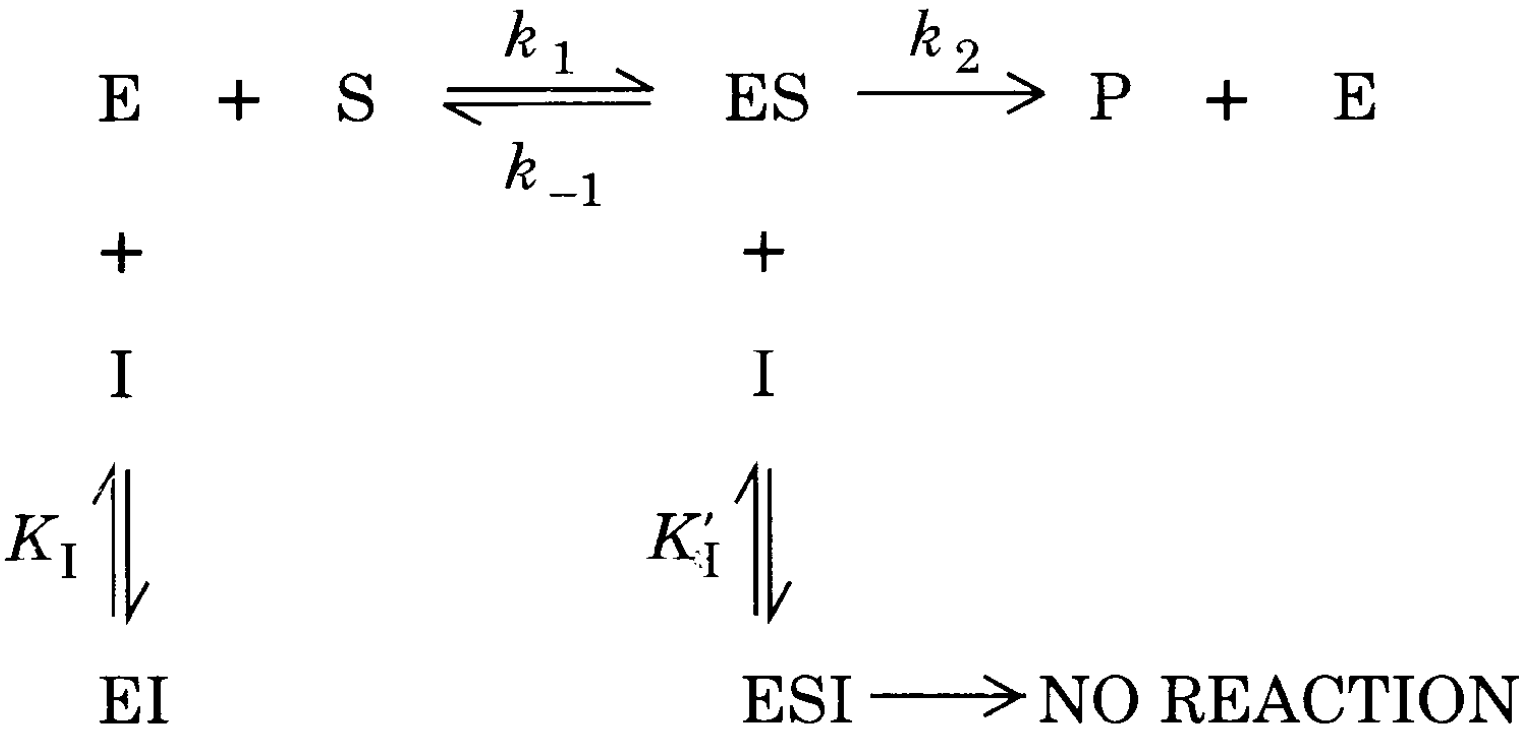
Inhibitor molecule bind to some other site on the enzyme other than the active site reducing its catalytic power



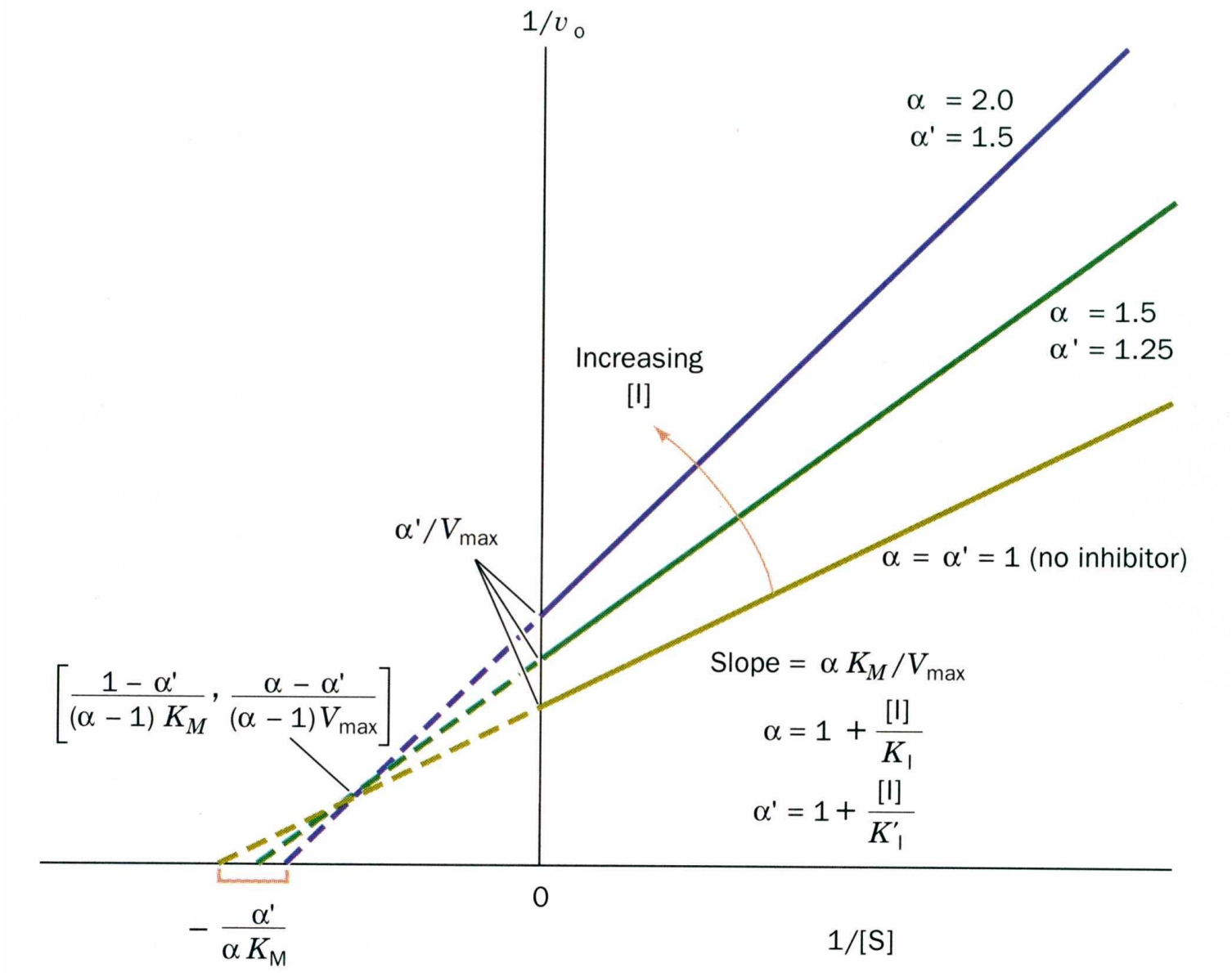
Uncompetitive Inhibition: Lineweaver-Burk Plot



Non-competitive inhibition (Mixed)



Non-competitive Inhibition: Lineweaver-Burk Plot



Enzyme Cooperativity

Unfortunately the kinetic constants that describe enzyme catalysis are very difficult to measure and as a result researchers do not tend to use the explicit mechanism, instead they use certain approximations

The two most popular approximations are:

1. Rapid Equilibrium
2. Steady State

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

The rapid equilibrium approximation assumes that the binding and unbinding of substrate to enzyme to much faster than the release of product. As a result, one can assume that the binding of substrate to enzyme is in equilibrium

That is, the following relation is true at all times (Kd= dissociation constant):

$$K_d = \frac{E.\ S}{ES}$$

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

Steady State Assumption

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

It is possible to relax the constraints that ES should be in equilibrium with E and S by assuming that ES has a relatively steady value of a wide range of substrate concentrations

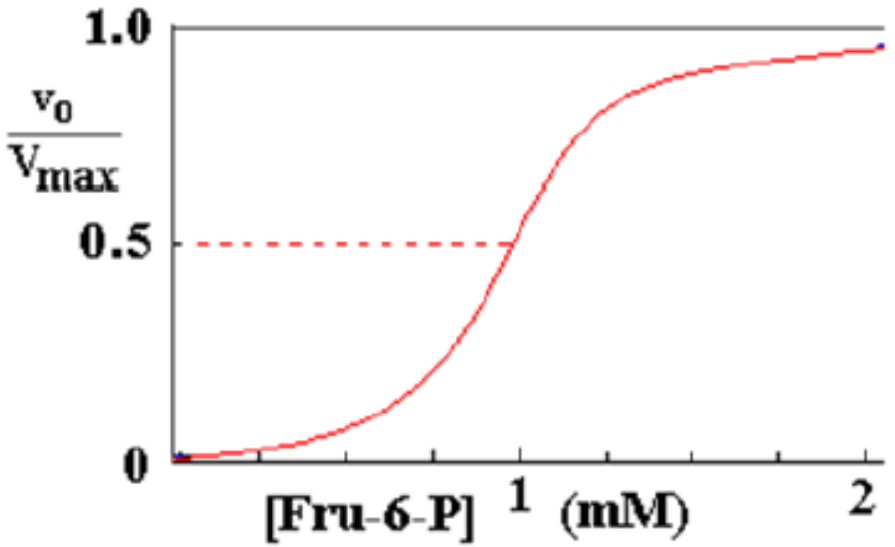
$$v = \frac{Vmax\ S}{K_m + S}$$

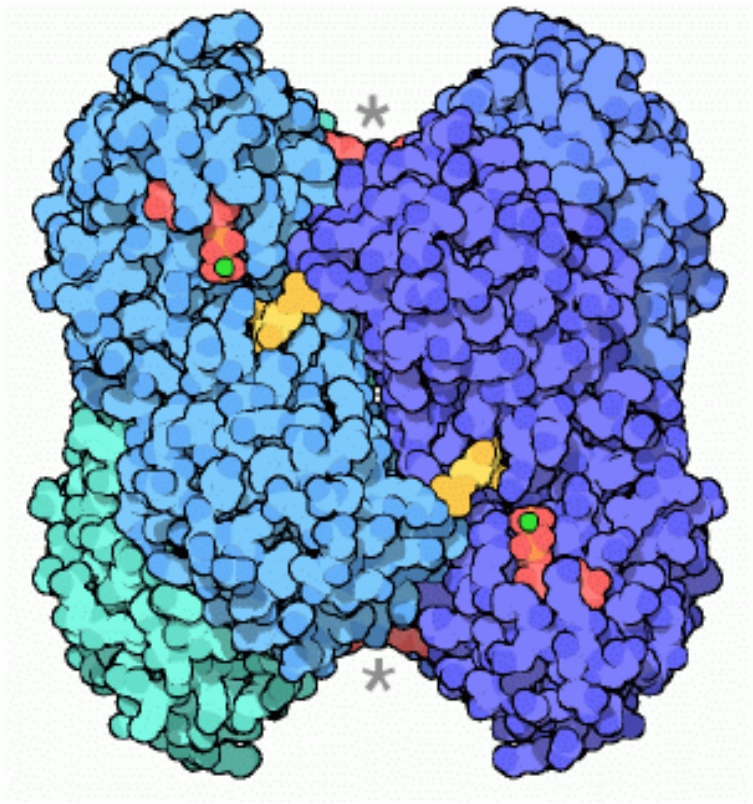
An example is the M-M equation

Sigmoid responses are generally seen in multimeric systems

Phosphofructokinase

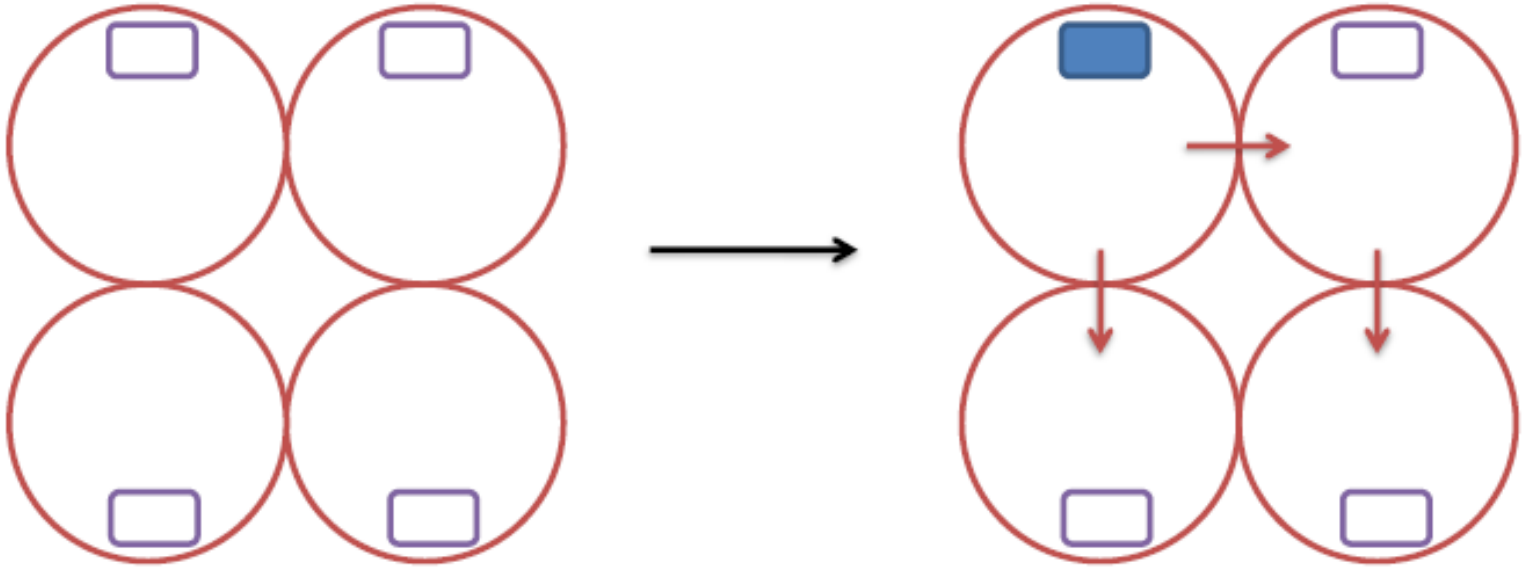
Tetramer of identical subunits





Sigmoid responses arise from cooperative interactions

Binding at one site results in changes in the binding affinities at the remaining sites.



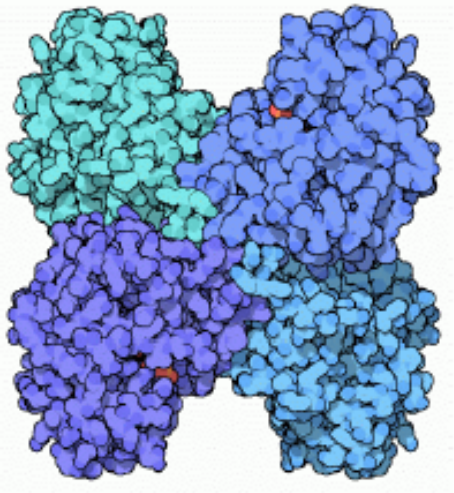
Hill Equation – Simplest Model

We assume that the ligands bind simultaneously (unrealistic!):

$$E + n\ S \longleftrightarrow ES$$

Assuming Rapid Equilibrium

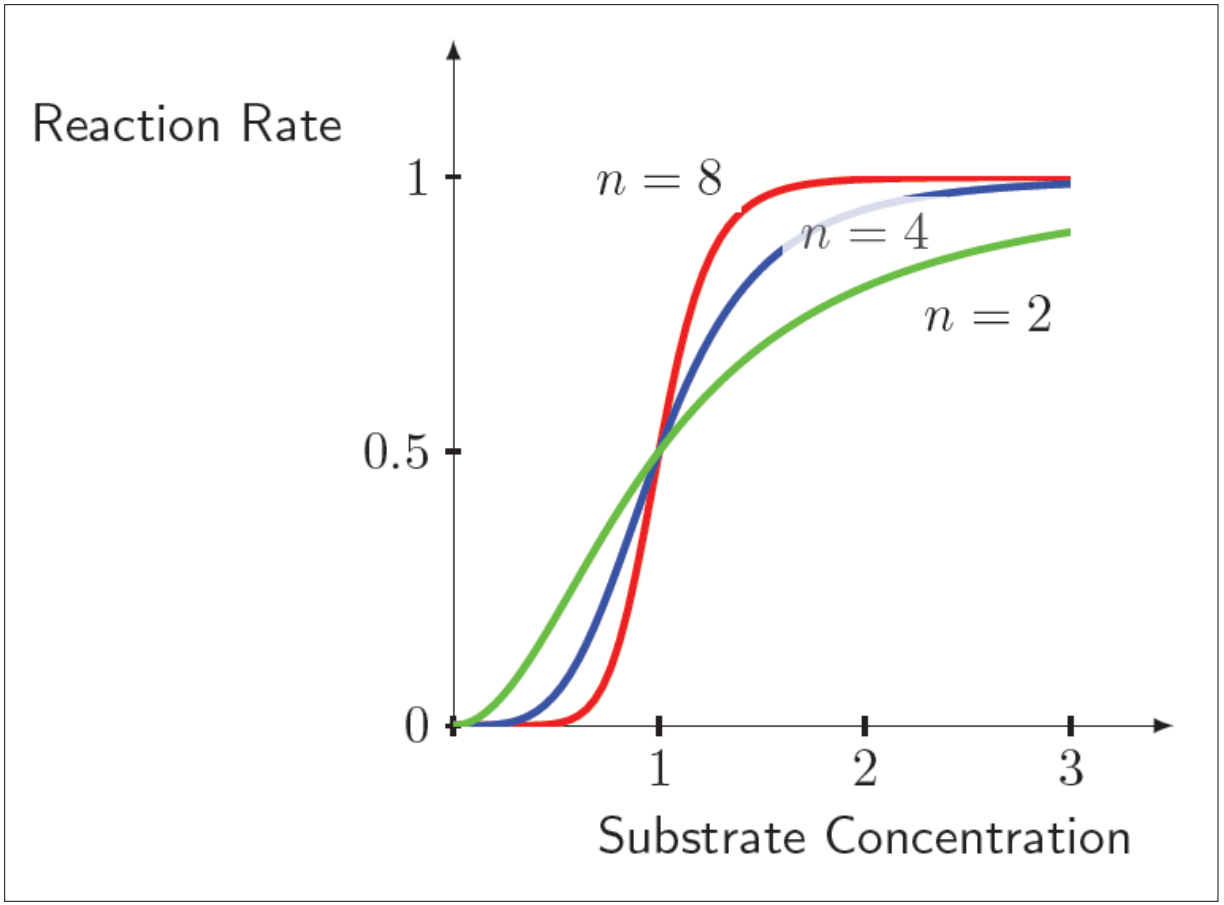
$$K = \frac{ES}{E\ .\ S^n}$$



$$E_t = E + ES$$

$$\frac{ES}{E_t} = \frac{S^n}{1/K + S^n} \Rightarrow v = \frac{Vmax\ S^h}{K_H + S^h}$$

Hill equation



The Hill Coefficient, **n**, describes the degree of cooperativity.

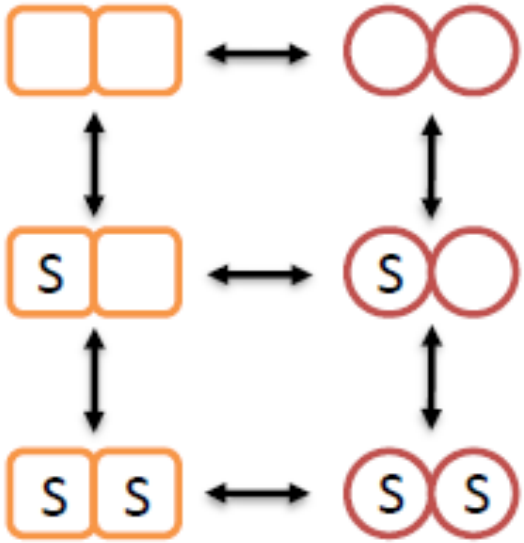
If n = 1, the equation reverts to a simple hyperbola

n > 1 : Positive Cooperativity
n = 1 : No Cooperativity
n < 1 : Negative Cooperativity

$$v = \frac{\mathrm{Vmax}\ S^{n}}{K + S^{n}}$$

← Hill coefficient

Other Models –MWC Model MWC Model or concerted model (Monod, Wyman, Changeux)



Is disallowed

1.Subunits exist in two conformations, relaxed (R) and taut (T)

2. One conformation has a higher binding affinity than the other (R)

3. Conformations within a multimer are the same

4. Conformations are shifted by binding of ligand

Taut (T) – less active

Relaxed (R) – more active

Next lecture

Applications of enzyme inhibition