بسم الله الرحمن الرحيم

BioChemistry | FINAL 12

# Enzymes pt.4

Written by : Jannat Nasri
Ismail Abu Shaqra

Reviewed by : NST

# Kinetics of enzymatic reactions

Kinetics is the study of the rate of chemical reactions and the factors that affect how fast a reaction proceeds, such as activation energy, temperature, concentration, and catalysts.

When we measure the rate of a reaction, we can look at the change in the concentration of either the reactants or the products. These changes are equal in magnitude but opposite in sign, since reactants are consumed while products are formed. If they weren't equal, it would imply that material is disappearing, which doesn't happen in real-life reactions.

# Kinetics

- **Biochemical Kinetics: the science that studies rates of chemical reactions**
- **An example is the reaction (A → P), The velocity, *v*, or rate, of the reaction A → P is the amount of P formed or the amount of A consumed per unit time, *t*. That is,**

$$v = \frac{d[\mathrm{P}]}{dt} \quad \text{or} \quad v = \frac{-d[\mathrm{A}]}{dt}$$

In enzymatically catalyzed reactions, the process differs from uncatalyzed reactions. In an uncatalyzed reaction, the reactant molecules must collide with sufficient energy, and only a few collisions will lead to a reaction, especially if the reactant concentration is low. As a result, the reaction rate increases proportionally with reactant concentration, showing a linear relationship.
In contrast, when an enzyme is present, the situation changes. The enzyme's active sites become the limiting factor. As the substrate concentration increases, the reaction rate initially rises, but eventually it reaches a maximum velocity, known as Vmax. At this point, all the enzyme's active sites are fully saturated with substrate, and the reaction rate no longer increases with additional substrate. This leads to a plateau on the graph of reaction rate versus substrate concentration.

When we measure the reaction rate, we can use integration (التكامل)

# Reaction Rate Law


- **The rate is a term of change over time**
- **The rate will be proportional to the conc. of the reactants**
- **It is the mathematical relationship between reaction rate and concentration of reactant(s)**
- **For the reaction (A + B → P), the rate law is**

$$\text{Rate} = \frac{-\Delta[\text{A}]}{\Delta t} = \frac{-\Delta[\text{B}]}{\Delta t} = \frac{\Delta[\text{P}]}{\Delta t} \qquad v = \frac{-d[\text{A}]}{dt} = k[\text{A}]$$

They should be equal to each other. We use a negative sign for the reactants because their concentration decreases over time, and a positive sign for the products because their concentration increases over time.

- **From this expression, the rate is proportional to the concentration of A, and *k* is the rate constant**

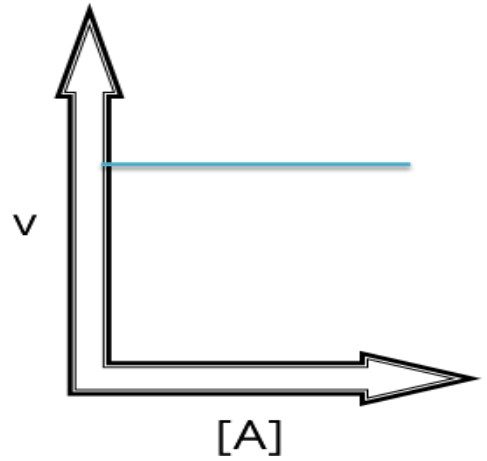
Imagine that we have ten empty chairs and one student. The rate at which that student sits down would be, for example, one student per second. With two students, it would be two per second, and so on, up to ten students per second. However, once we go beyond ten students, the rate will initially still increase a little, but eventually it will plateau. This happens because the chairs become fully saturated or even over-saturated, and at that point, the concentration of students trying to sit no longer increases the rate.

The same thing for enzymes.When you increase the concentration of the reactants, you also increase the likelihood of the enzyme and substrate binding, which leads to a higher rate of reaction. However, once the enzyme's active sites are fully saturated, further increases in reactant concentration no longer raise the reaction rate. At that point, we no longer see a direct relationship between reactant concentration and reaction rate.

# The order of the reaction & the rate constant (*k*)

- **A multistep reaction can go no faster than the slowest step**

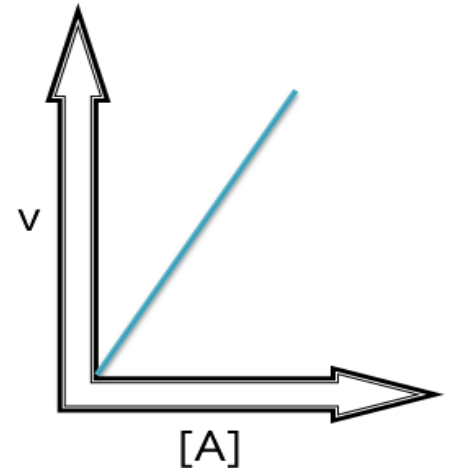
A multistep reaction means the reaction happens in several smaller steps, not all at once. Among these steps, one is the slowest. That slow step controls the speed of the whole reaction – this is called the rate-determining step.

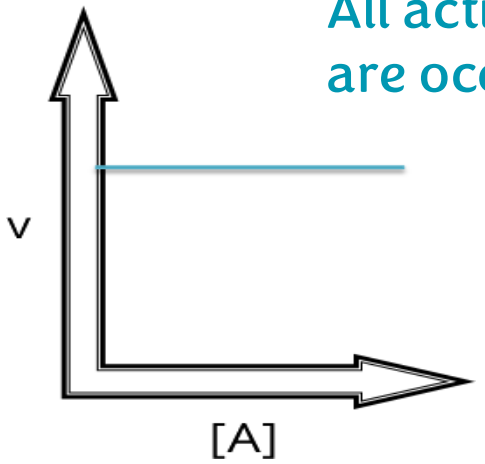



$$v = k(A)^{n1}(B)^{n2}(C)^{n3}$$

- ***k*** **is the rate constant: the higher the activation energy (energy barrier), the smaller the value of k**
- **(n1+n2+n3) is the overall order of the reaction**
- **Dimensions of k**

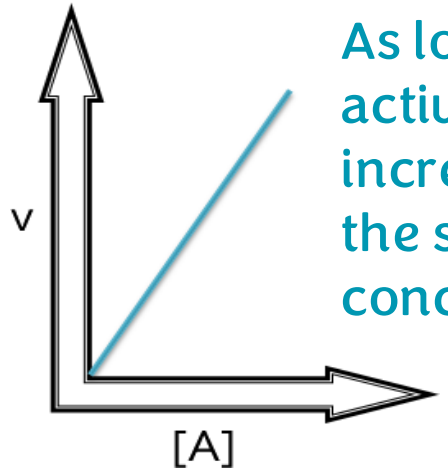
| Overall order | V= | Dimentions of k |
|---|---|---|
| Zero | *k* | $(conc.)(time)^{-1}$ |
| First | *k*(A) | $(time)^{-1}$ |






All active sites are occupied

Velocity independent: In a zero-order reaction, the rate is constant and does not change with [A].




As long as I have available active sites, the rate increases by increasing the substrate's concentration

Velocity dependent: In a first-order reaction, the rate increases as [A] increases.

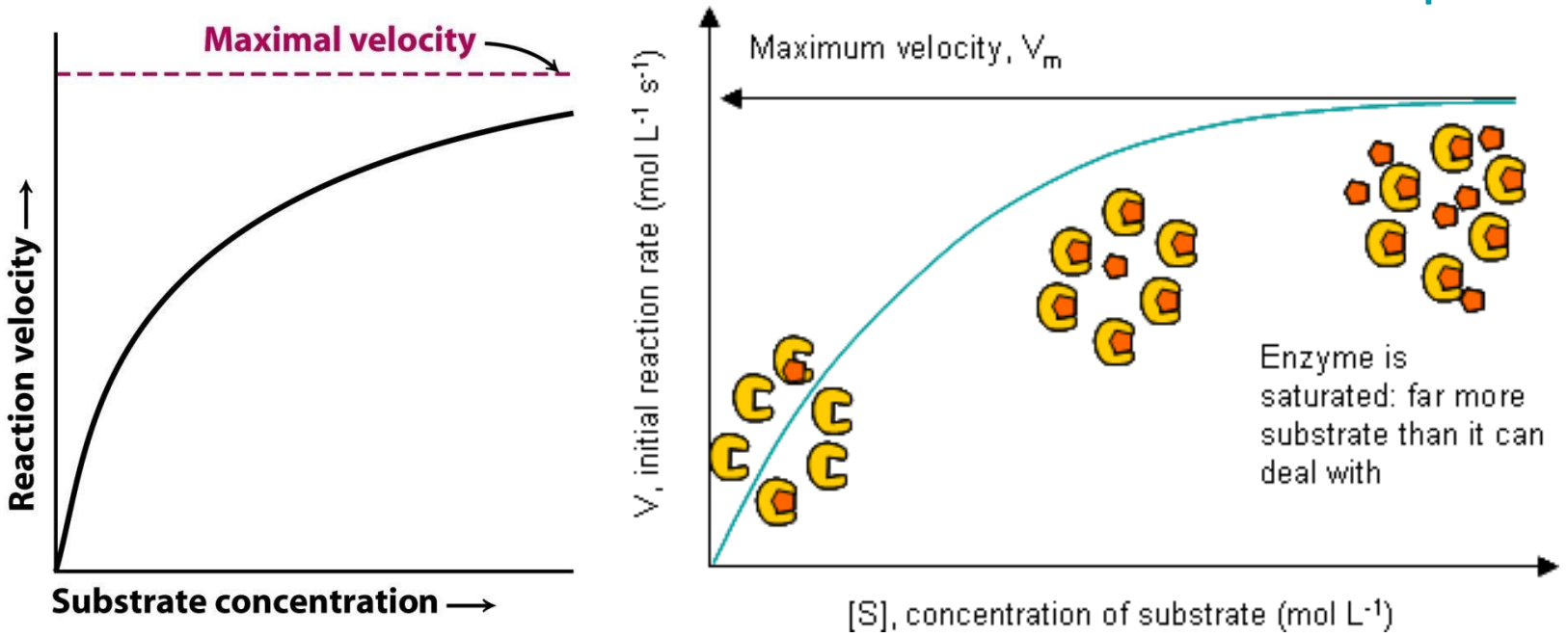
**What about reactions with orders higher than 1 ?**

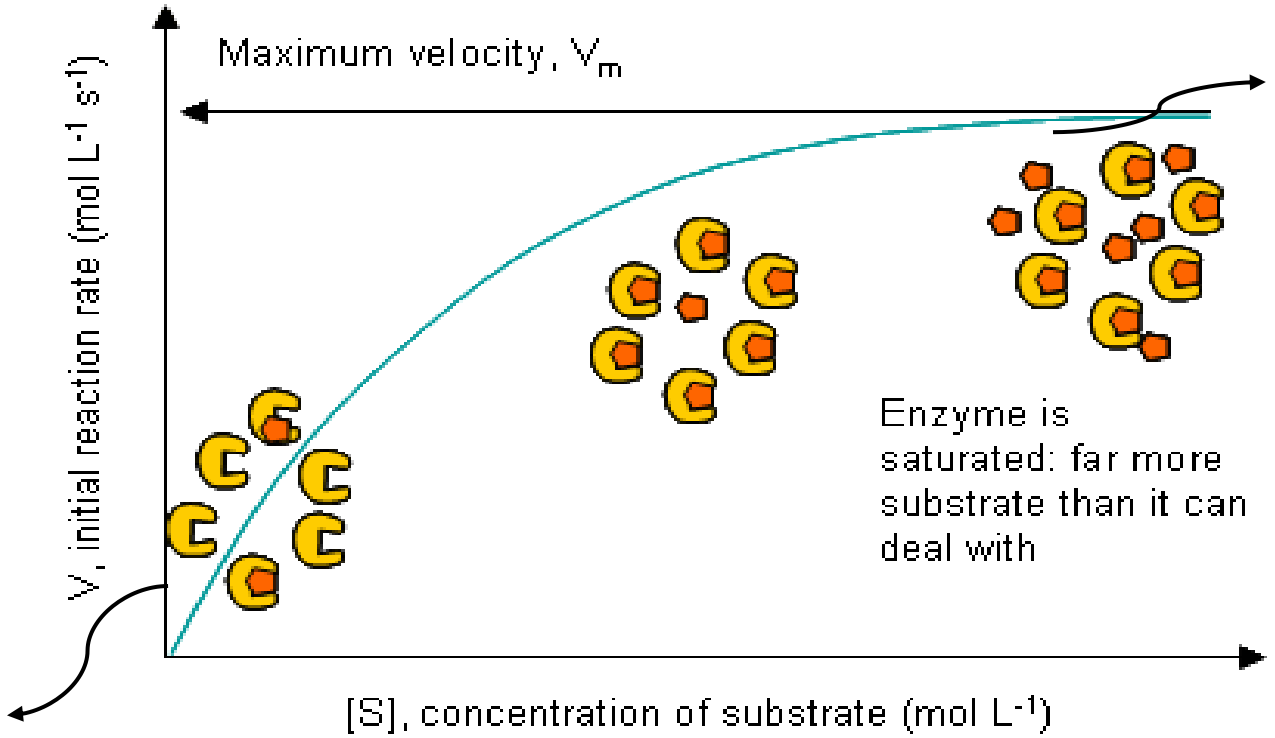
When we want to measure the rate constant k for a reaction that depends on more than one reactant, we convert it to first order reaction . How? We make the concentration of one reactant (in case of second order rxn) very high, so it stays almost constant. Then the reaction behaves like a first-order reaction with respect to the other reactant. This is what we call a pseudo-first order reaction.

# Enzyme kinetics

- Enzymatic reactions may either have a simple behavior or complex (allosteric) behavior
- Simple behavior of enzymes: as the concentration of the substrate rises, the velocity rises until it reaches a limit
- Thus; enzyme-catalyzed reactions have hyperbolic (saturation) plots

Some enzymes adopt a sigmoidal plot






At high concentrations, the reaction acts as a zero-order reaction

At low concentrations, the reaction acts as a first-order reaction

This means that the reaction is a combination of zero and first order.

# Enzyme kinetics

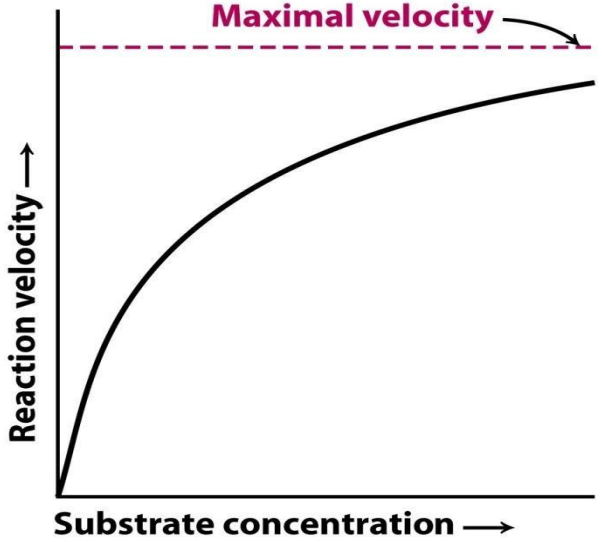
- The maximal rate, $V_{max}$, is achieved when the catalytic sites on the enzyme are saturated with substrate

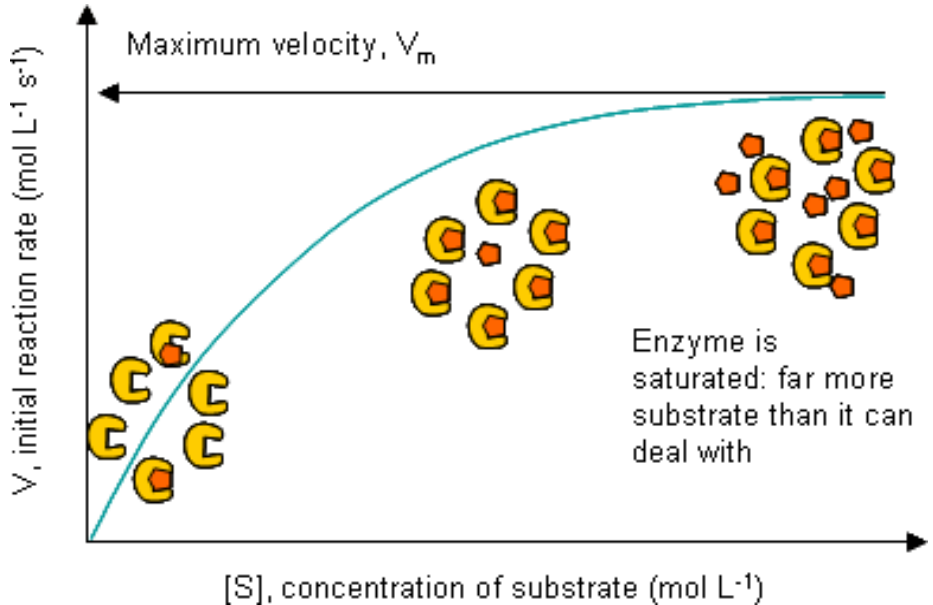
- $V_{max}$ reveals the turnover number of an enzyme

  - The number of substrate molecules converted into product by an enzyme molecule in a unit of time when the enzyme is fully saturated with substrate

- At $V_{max}$, the reaction is in zero-order rate since the substrate has no influence on the rate of the reaction

At the beginning, there is low substrate concentration, which means there are more vacancies (active sites) for it, so it goes linear, when substrate concentration is much higher compared to the number of active sites, plateau is reached, But when substrate concentration is near to the number of active sites, it increases but not linearly. This gives us a hyperbolic plot (like myoglobin). Vmax is the highest possible velocity that can be reached when concentration is very high compared to the concentration of the active site. The graph could be sigmoid. Some mathematical equations have been made in order to calculate Vmax



# Expression of enzyme kinetic reactions "Steady State Assumption"

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

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

$$v = k_2\, ES$$

$$\frac{dES}{dt} = k_1\, E\, .\, S - k_{-1}\, ES - k_2\, ES$$

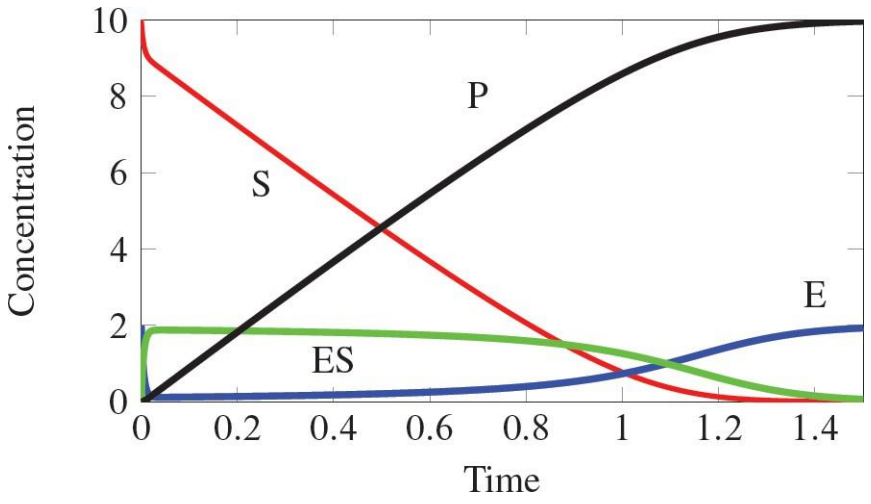
$$0 = k_1\, E\, .\, S - k_{-1}\, ES - k_2\, ES$$

$$E_t = E + ES$$

$$ES = \frac{E_t\, .\, S}{(k_{-1} + k_2)/k_1 + S}$$

$$v = \frac{E_t\, k_2\, S}{(k_{-1} + k_2)/k_1 + S}$$

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



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

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

When we calculate the rate of a reaction, we multiply the constant by the concentration.
Here:
k1: constant of producing enzyme-substrate complex from substrate
k2: constant of producing product
k-1: constant of producing substrate
k-2: constant of producing enzyme-substrate complex from product
There are 2 assumptions made here:
1-When products are formed, they cannot return to form enzyme-substrate complex. Hence the reaction is irreversible which is why in the second equation we there isn't an arrow from the products to the ES (theoretically all reactions are reversible, but essentially most reactions don't go back from products due to the energy difference
2- Steady state assumption: Enzyme-substrate complex has a fixed concentration which means:
rate of production of ES=rate of degradation of ES

Rate of formation of ES - rate of degradation of ES =0

Rate of formation of ES= k1×[S]×[E] → only possible way for formation of ES is from enzyme +substrate

Rate of degradation of ES = k2×[ES] +k-1x[ES] → degradation goes both ways (either towards enzyme +product or towards enzyme +substrate)

$$v = k_2\, ES$$

$$\frac{d\,ES}{dt} = k_1\, E\, .\, S - k_{-1}\, ES - k_2\, ES$$

$$0 = k_1\, E\, .\, S - k_{-1}\, ES - k_2\, ES$$

Enzyme has 2 possible states, either free or bound(ES).

So Et(total enzymes concentration) =E(free enzymes concentration)+ES(bound enzymes concentration)

$$E_t = E + ES$$

This formula can be derived from the formulas before

$$ES = \frac{E_t \,.\, S}{(k_{-1} + k_2)/k_1 + S}$$

Here we multiplied by k2 both sides

When vmax is reached, Et is going to be equal to ES.
Hence Vmax = [ES] ×k2 →here we put vmax since ES=ET

$$v = \frac{E_t\, k_2\, S}{(k_{-1} + k_2)/k_1 + S}$$

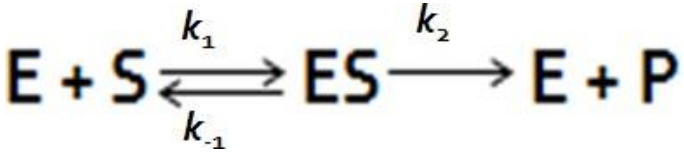
(k-1 + k2)/k1 can all be replaced into km (Michaelis constant)

Here we replaced [Et]xk2 by vmax and replaced (k-1+k2)/k1 by km

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

# The Michaelis constant ($K_m$)

- **For a reaction:**




**STEADY STATE APPROXIMATION**

$$\frac{d[ES]}{dt} = k_1[E][S] - K_{-1}[ES] - K_2[ES] = 0 \text{ (approx.)}$$

$$\frac{[E][S]}{[ES]} = \frac{K_{-1} + K_2}{k_1} = K_M \qquad \text{Equation 1}$$

- ***K*m, called the Michaelis constant is** $K_M = \frac{k_{-1} + k_2}{k_1}$

- Km has a unit of mol. It represents the rate of degradation of ES(k-1+k2)/rate of formation of ES (k1). This indicates the affinity of the enzyme to the substrate. So the higher the strength of binding(affinity), the higher the association, the lower the dissociation the lower the km value and vice versa. However km isn't a very accurate measurement of affinity since we are calculating the affinity of enzyme to substrate but we're including k2(constant of formation of product) in the formula, which isn't related

- **In other words, *K*m is related to the rate of dissociation of substrate from the enzyme to the enzyme-substrate complex**

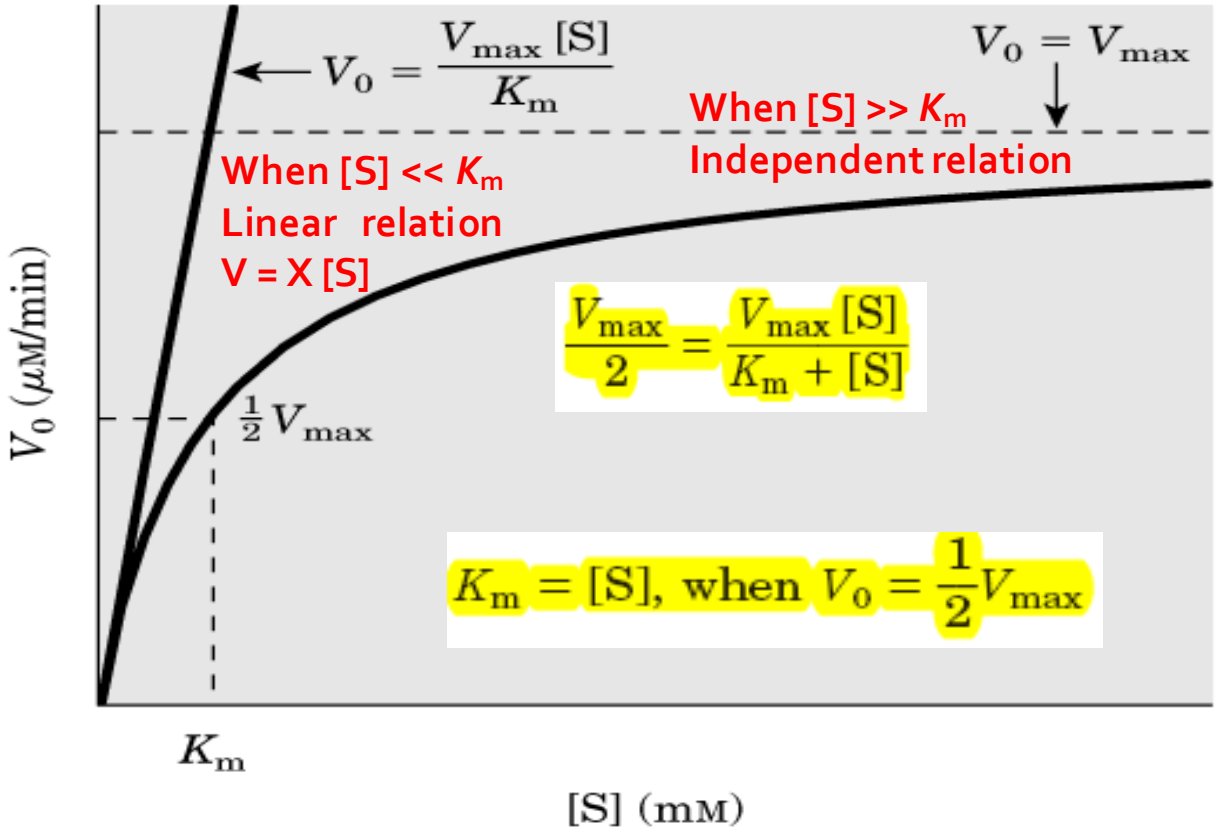
- ***K*m describes the affinity of enzyme for the substrate**

# Expression of enzyme kinetic reactions
# Michaelis-Menten equation

- **A quantitative description of the relationship between the rate of an enzyme catalyzed reaction ($V_o$) & substrate concentration [S]**
  - ✓ **The rate constant ($K_m$) and maximal velocity ($V_{max}$)**

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

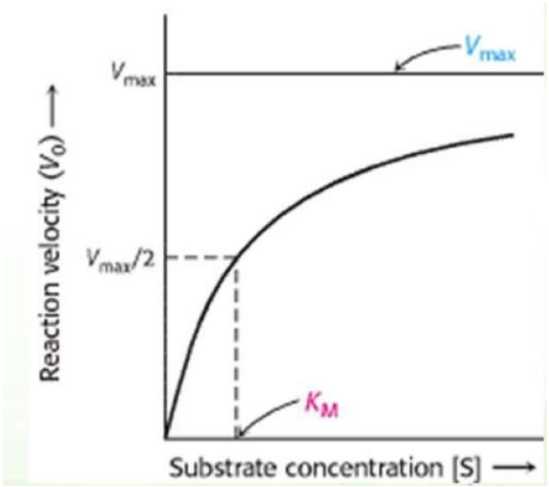
***The substrate concentration at which $V_o$ is half maximal is $K_m$***



# The Michaelis constant ($K_m$)

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

- The lower the $K_m$ of an enzyme towards its substrate, the higher the affinity
- When more than one substrate is involved? Each will have a unique $K_m$ & $V_{max}$
- $K_m$ values have a wide range. Mostly between ($10^{-1}$ & $10^{-7}$ M)



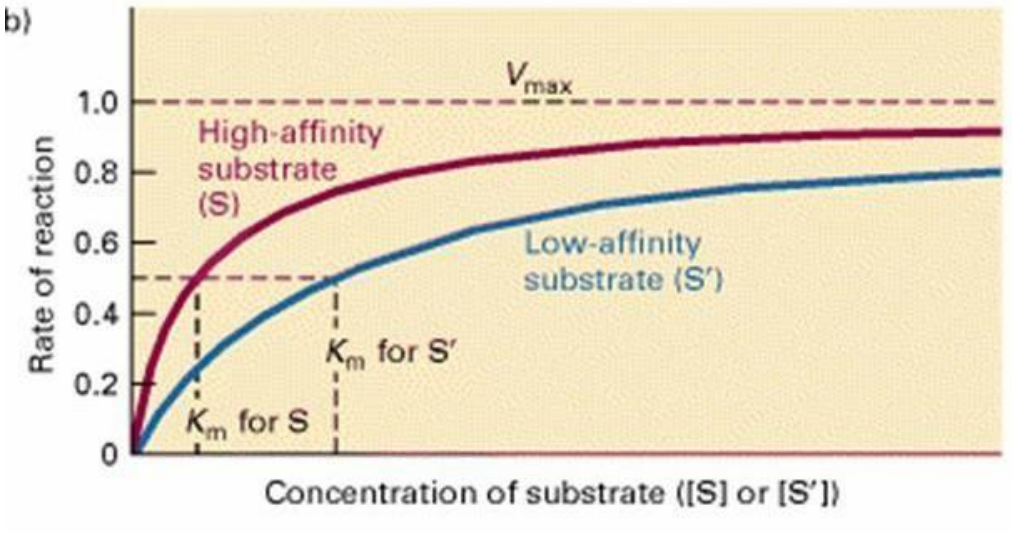




table 8–6

**$K_m$ for Some Enzymes and Substrates**

| Enzyme | Substrate | $K_m$ (mM) |
|---|---|---|
| Catalase | $H_2O_2$ | 25 |
| 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 |

# For any feedback, scan the code or click on it.

Corrections from previous versions:

| Versions | Slide # and Place of Error | Before Correction | After Correction |
|---|---|---|---|
| V0 → V1 | | | |
| V1 → V2 | | | |

# Additional Resources:

# رسالة من الفريق العلمي: