LESSON 7. ENZYME KINETICS AND REACTION RATE

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Adapted from a previous version by Dr. Herminia González Navarro

LESSON 7. ENZYME KINETICS AND REACTION RATE

1. Enzyme kinetics and reaction rate.

2. Measurement of the rate of an enzymatic reaction.

- **2.1.** The Michaelis-Menten Model.
- **2.2.** Concepts and meaning of the constants Vmax, Km and Kcat.
- **2.3.** Linearization of the Michaelis-Menten equation.

3. Phenomenon of cooperativity.

- **3.1.** Kinetics deviating from the Michaelis-Menten model: Enzymes with cooperativity
- **3.2.** Co-operative models
- **3.3.** Allosteric enzymes

4. Protein-ligand interaction.

What is enzyme kinetics?

Why do you think it is important to measure/understand enzyme kinetics?

https://www.menti.com/al33q1ms5uyi

1. Enzyme kinetics and reaction rate.

Enzyme kinetics is the study of the rate of biochemical reaction catalysed by an enzyme. It helps us to understand **enzymatic efficiency** and **to determine the concentration of an enzyme** in a solution or biological sample.

The **reaction rate** of an enzyme is determined by **the change in the amount of substrate or product** per unit time.

The **determination of the** rate of a biochemical reaction catalysed by an enzyme **can provide information about the presence of activators or inhibitors, tissue damage, etc.**

The study of how an enzyme responds to experimental parameters **can be used to**: design inhibitors, design activators or modulate activity.

1. Enzyme kinetics and reaction rate.

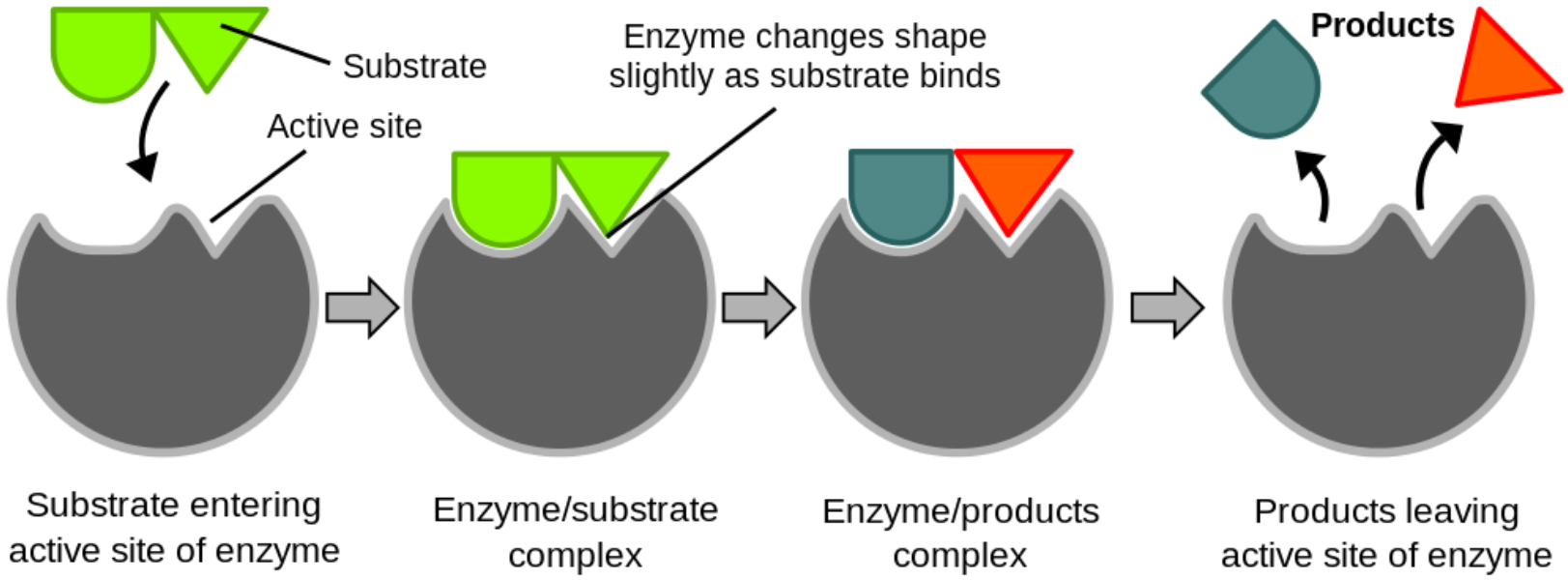
The best way to study the mechanism of action of an enzyme is to determine the reaction rate and how it changes in response to experimental parameters. This is studied in **enzyme kinetics**.

CHRISTENSEN, HALVOR N. / PALMER, G. A.

What is enzyme kinetics?

Determining the rate of an enzyme-controlled reaction and studying how it changes

1. Enzyme kinetics and reaction rate.

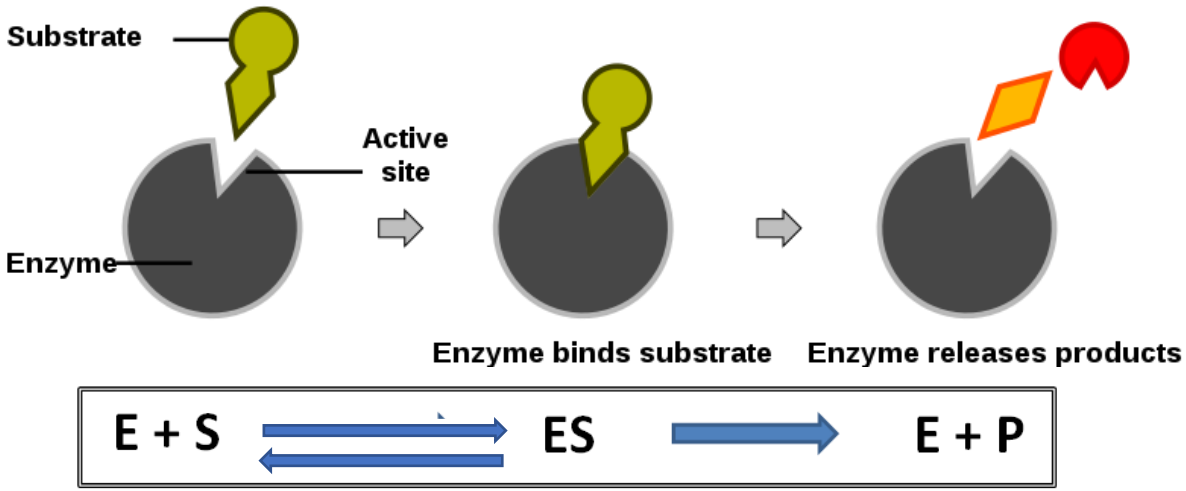


How is enzyme activity measured?

$$V=-d[S]/dt = d[P]/dt$$

Diagram source: courseslumenlearning.com

1. Enzyme kinetics and reaction rate.



How is enzyme activity measured?

$$V=-d[S]/dt = d[P]/dt$$

1-By measuring the **disappearance of substrate or the emergence of product**

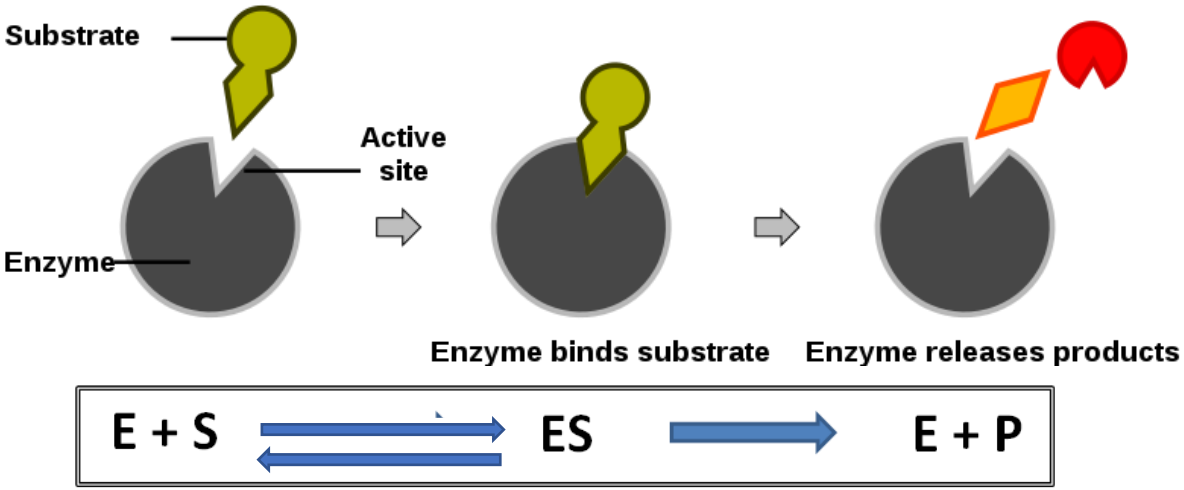
2-In the presence of very small quantities of enzyme, 10^{-8} and 10^{-12} M, quantities that are known to be catalytic.

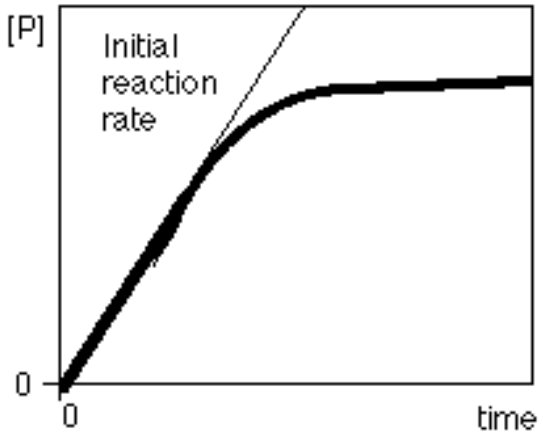
3 - Under optimal conditions of pH, temperature, presence of cofactors, etc.

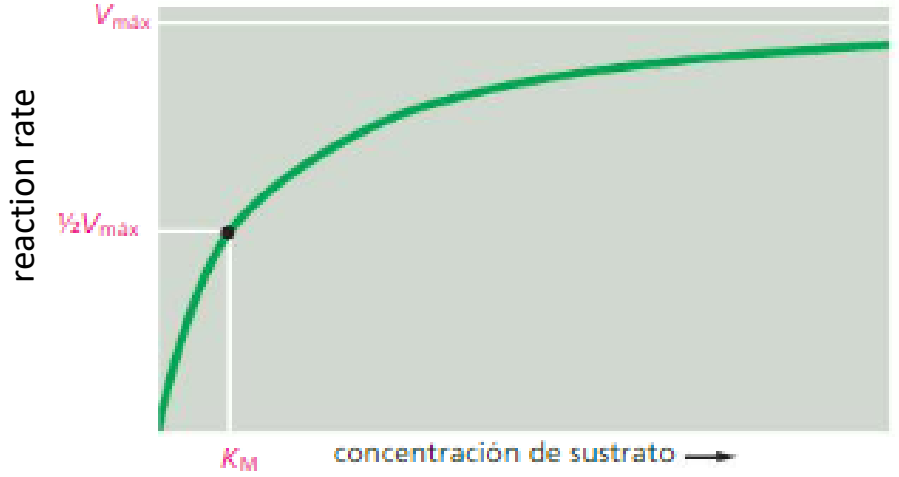
4 – By saturating conditions of substrates are used

5 – By determining the **initial** reaction **rate**, V_0

2. Measurement of the rate of an enzymatic reaction.



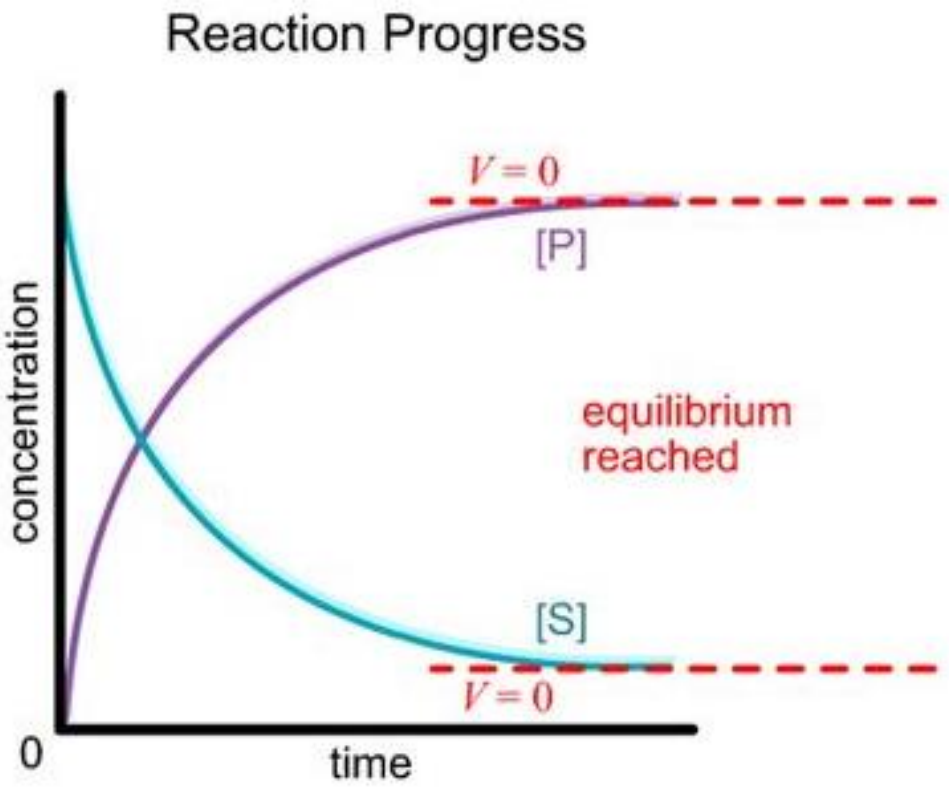




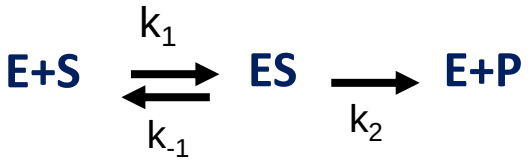
Introducción a la Biología Celular, Bruce Alberts et al

Diagram source: wikibooks.org

2. Measurement of the rate of an enzymatic reaction.



The rate of product formation is directly proportional to the disappearance of the substrate.



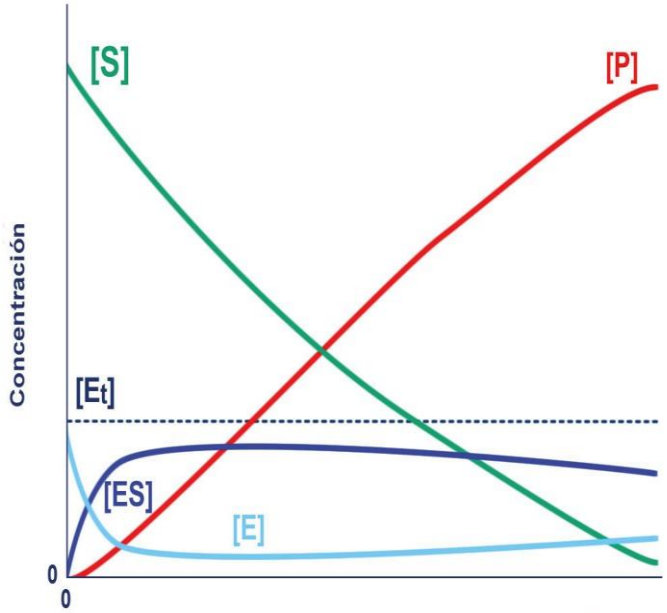
To define the rate of the reaction we need to define a steady state, where [ES] is constant.

2. Measurement of the rate of an enzymatic reaction.

STEADY STATE in the enzyme reaction: WHEN THE CONCENTRATION OF THE COMPLEX **[ES]** REMAINS CONSTANT.

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

Vformation [ES]= Vdisappearance [ES].



Briggs and Haldane, 1925

The rate of change of [E] and [ES] with respect to time can be considered 0, compared to the rate of change of [S] or [P]. That is, d[ES]/dt=0.

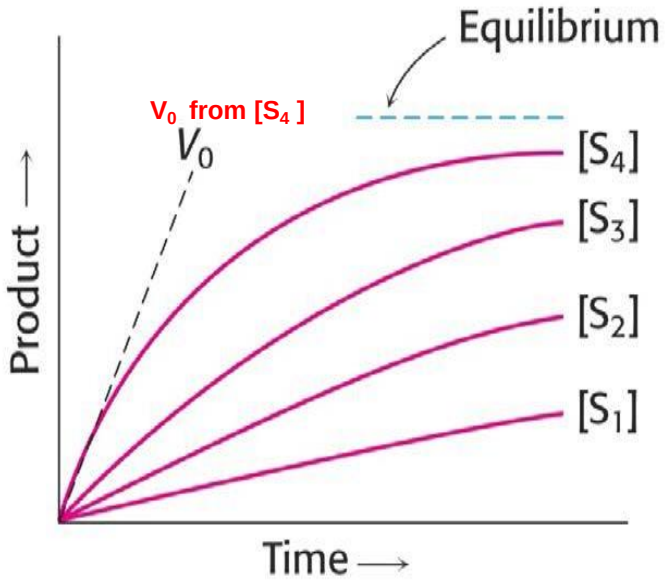
$$ES \xrightarrow[k_2]{} E + P$$

$$V = [ES]k_2$$

2. Measurement of the rate of an enzymatic reaction.

The steady state in enzyme kinetics: assumption of application of the Michaelis-Menten equation.

The ES complex is in a steady state when the entire enzyme is in ES form. In this case, the rate of P formation is maximal for a given [S].



For substrate concentration the initial speed is different.

The enzymes that behave in this way follow the ***MICHAELIS-MENTEN*** model.

Biochemistry Stryer 7th edition

Michaelis Menten?

Michaelis Menten?

Leonor Michaelis

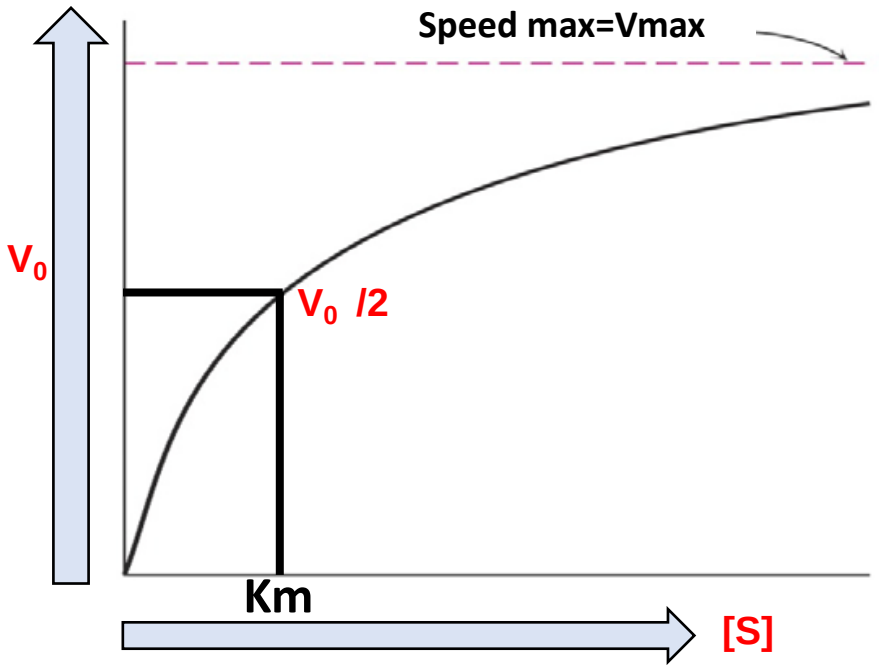
Maud Menten

https://www.um.es/eubacteria/Los_cuatro_mosqueteros_de_la_cinetica_enzimatica_Eubacteria34.pdf

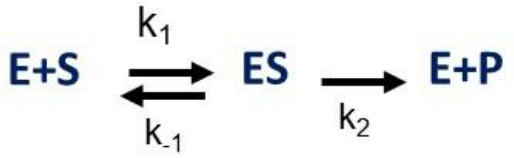
2. Measurement of the rate of an enzymatic reaction.

2.1. The Michaelis-Menten Model

The Michaelis-Menten equation governs enzymatic reactions.
Most enzymes exhibit Michaelis-Menten type kinetics, in which the graph of initial velocity (V_0) versus substrate concentration ([S]), is of the hyperbolic type.



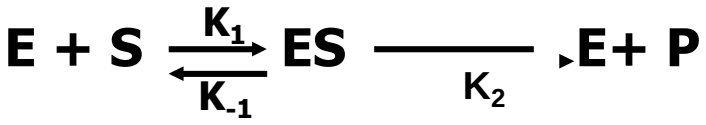
The representation of V_0 with respect to [S] is a hyperbola



$$V = k_2 [ES]$$

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

Origin of the Michaelis-Menten equation



In the steady state:

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

Michaelis constant

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

$$[ES]=[E]_t\frac{[S]}{(K_M+[S])}$$

Initial Speed: $V_0=k_2[ES]$

V_0 is maximum when $[ES]=[E_t]$.

$$[E]=[E]_t-[ES]$$

$$V_0=k_2[ES]=k_2[E]_t\frac{[S]}{(K_M+[S])} \leftarrow k_2[E]_t=V_{maxima}$$

Michaelis-Menten equation (hyperbolic curve):

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

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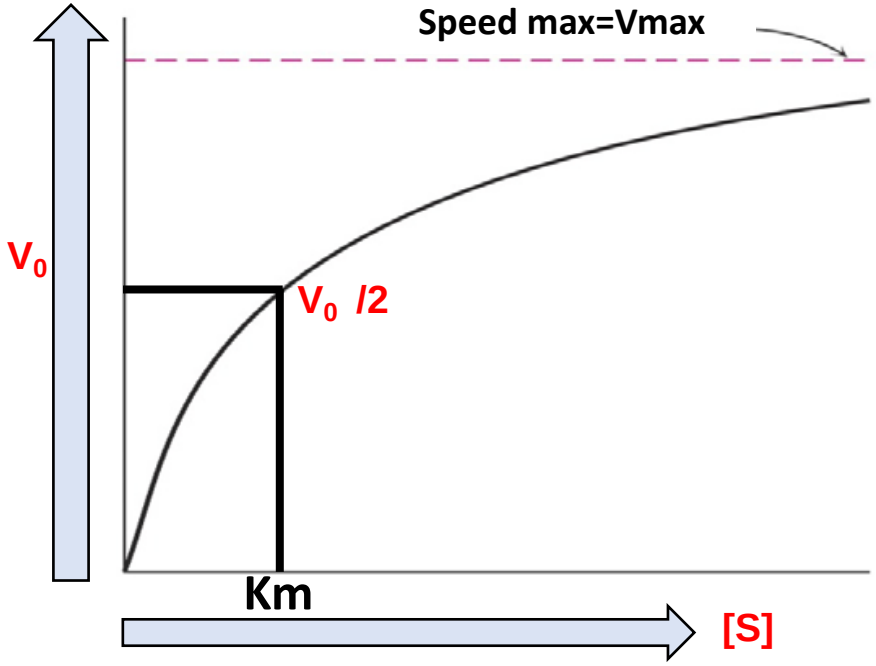


The representation of V_0 with respect to [S] is a hyperbola

MICHAELIS-MENTEN EQUATION

$$V_0 = \frac{Vmax \times [S]}{Km + [S]}$$

2. Measurement of the rate of an enzymatic reaction.

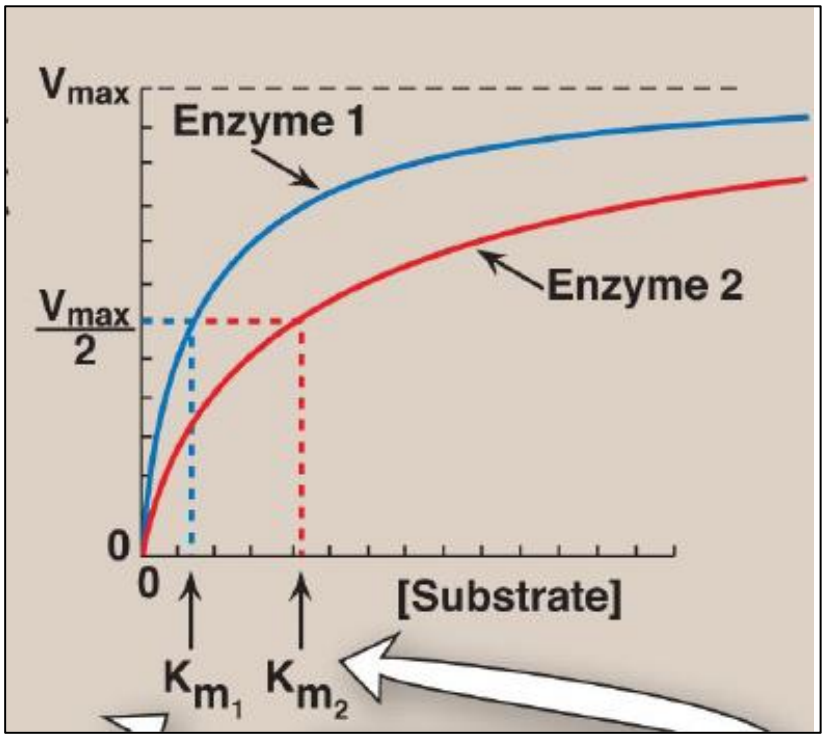
2.2. Concepts and meaning of the constants Vmax, Km and Kcat

MEANING OF V_{max}

It is the theoretical maximum speed that is never reached and is constant.

It requires very high substrate concentrations, and for all enzyme molecules to be bound to substrate.

In the Michaelis-Menten equation representation, the curve of V_0 versus [S] is asymptotically close.



From: Harvey, R., Ferrier, D. Bioquímica (Lippincot Ilustrated Reviews) 7ª Edition, 2017

2. Measurement of the rate of an enzymatic reaction.

2.2. Concepts and meaning of the constants Vmax, Km and Kcat

MEANING OF K_m

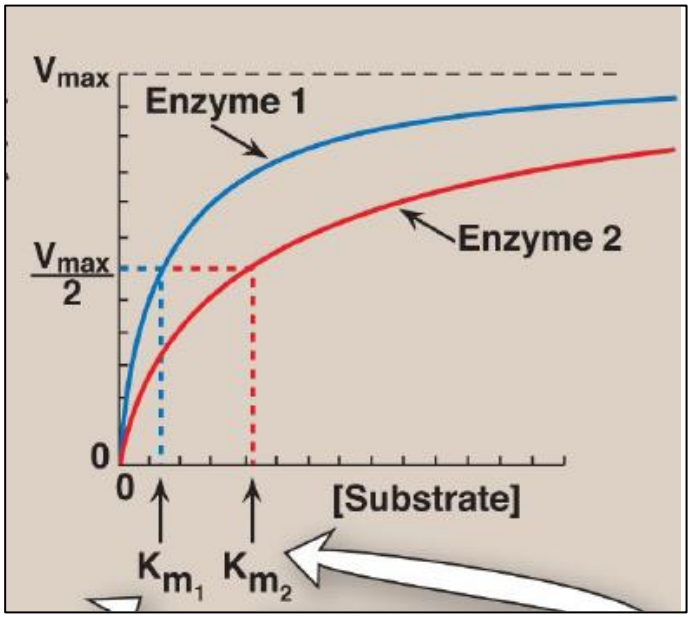
Substrate concentration at which the rate of **Vmax/2** is reached**.**

The K_m may range in values from 10^{-1} to 10^{-6} M, depending on the enzyme.

The value of K_m for an enzyme depends on the particular substrate, and on other variables such as temperature or pH.

K_m establishes an approximate value for the intracellular level of the substrate. The reaction rate is very sensitive to changes in [S] in the K_m range

K_m is usually related to an enzyme's affinity for the substrate.



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2. Measurement of the rate of an enzymatic reaction.

2.2. Concepts and meaning of the constants Vmax, Km and Kcat

MEANING OF K_m

Example: hexokinase and glucokinase have different affinities for glucose.

Km of **HEXOQUINASE is very low at 0.1 mM,** this works for glycolytic purposes when glucose concentration is low.
Km of **GLUCOQUINASE, 5 mM,** will function at high concentrations and for GLUCOGENIC (storage) purposes.

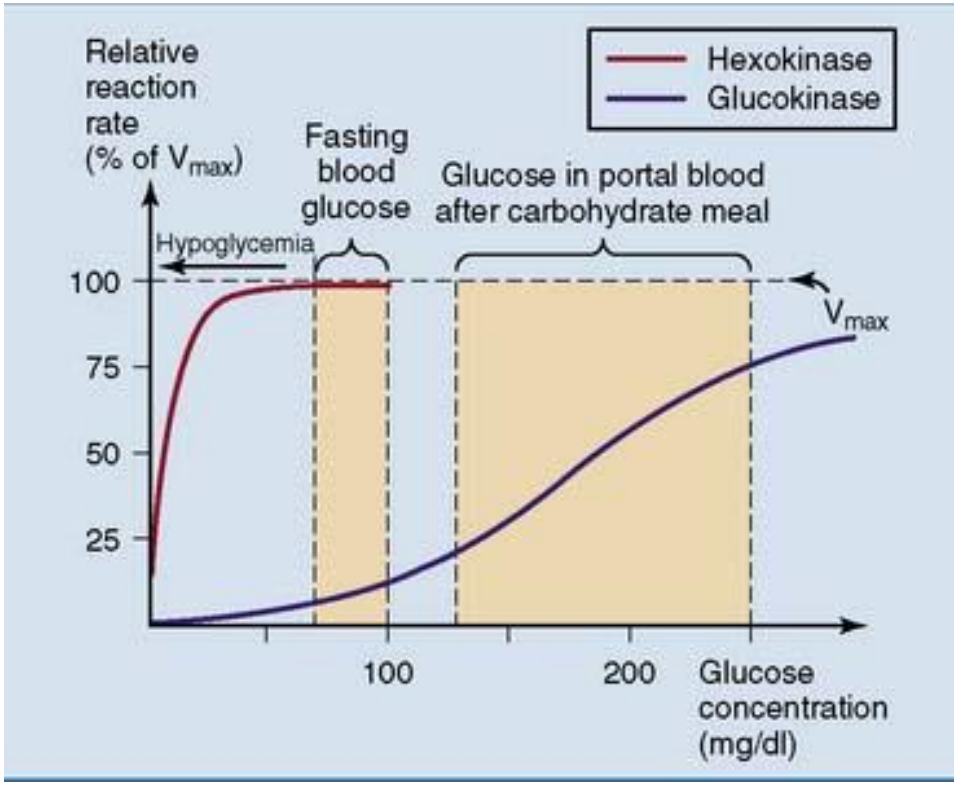

Diagram source: basicmedicalkey.com

2. Measurement of the rate of an enzymatic reaction.

2.1. The Michaelis-Menten Model

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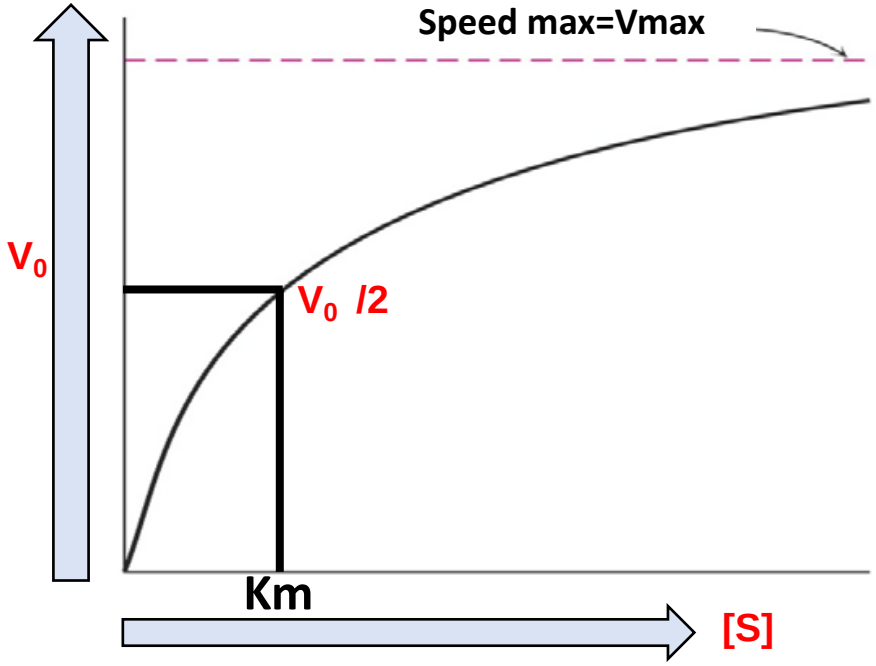

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MICHAELIS-MENTEN EQUATION

$$V_0 = \frac{Vmax \times [S]}{Km + [S]}$$

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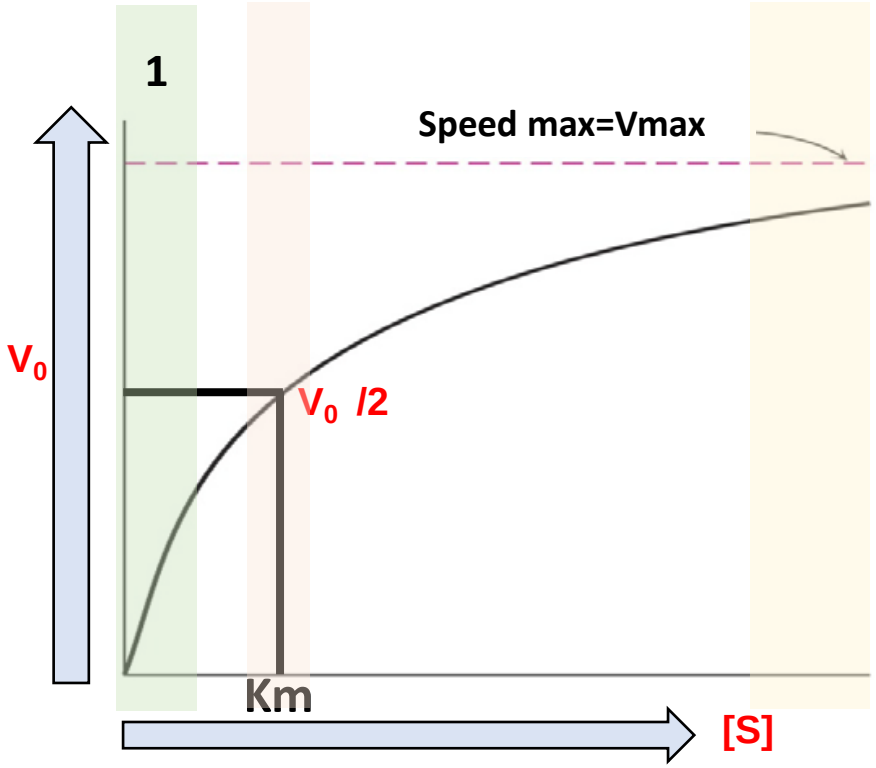


The representation of V_0 with respect to [S] is a hyperbola

Explanation of experimental data with the M-M equation:

1.- [S] very low below the Km

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

[S] is negligible versus Km and then V_0 increases linearly with [S]:

$$V_0 = cte(Vmax/Km)x[S]$$

2.- [S] very HIGH and much HIGHER than Km

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

$$V_0 = Vmax$$

3.- If $V_0 = Vmax/2$ then

$$V_0 = \frac{V_{max}}{} = \frac{V_{max} \times [S]}{Km + [S]} \rightarrow \textbf{Km=[S]}$$

2. Measurement of the rate of an enzymatic reaction.

2.2. Concepts and meaning of Vmax, Km and Kcat constants

K_{CAT} or TURNOVER NUMBER

Number of substrate molecules converted to product per enzyme molecule per unit time, under substrate saturation conditions.

$Kcat=Vmax/[E]_T$. The units are $seconds^{-1}$.

2. Measurement of the rate of an enzymatic reaction.

2.3. Linearization of the Michaelis-Menten equation

Lineweaver-Burk plot

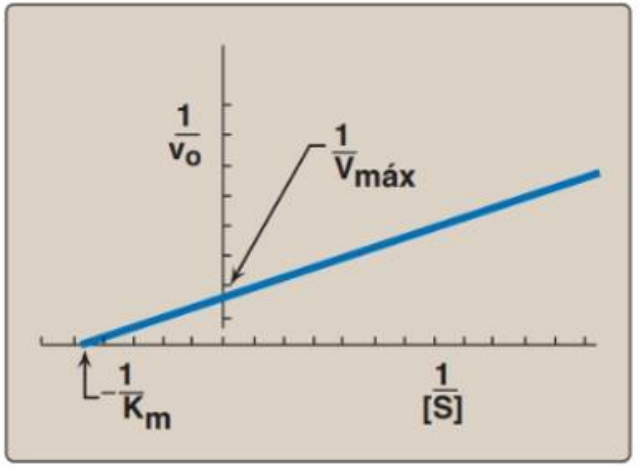
$$\frac{1}{v_o} = \frac{K_m}{V_{máx}\,[S]} + \frac{1}{V_{máx}}$$

This is an **inverse** representation **of V_0 versus [S]:**

$1/V_0$ is plotted against 1/[S] and a straight line is obtained. This is also called a double-reciprocal plot.

This is **useful for calculating Km and Vmax more accurately** and the mechanism of action of enzyme inhibitors.

The intercept on the **x-axis is equal to -1/Km,**
The **y-axis** intercept **is equal to 1/Vmax**.
The slope **m = Km/Vmax**



From: Harvey, R., Ferrier, D. Bioquímica (Lippincot Ilustrated Reviews) 7th Edition, 2017

What is the purpose of inverse representation?

When V_0 is plotted against [S], it is not always possible to determine when Vmax has been achieved due to the gradual upward slope of the curve at high substrate concentrations.

3. Phenomenon of cooperativity

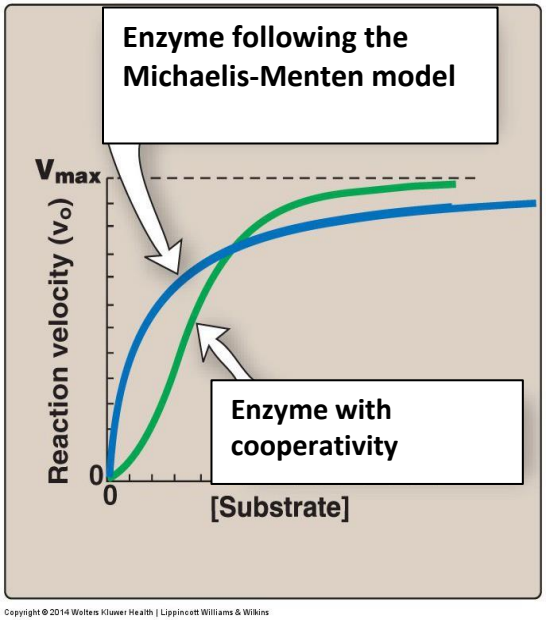
3.1. Kinetics that do not behave according to the Michaelis-Menten model: enzymes with cooperativity.

Some enzymes exhibit **a sigmoid curve of V_0 versus [S]** or **sigmoid kinetics**. This phenomenon indicates cooperativity in the binding of the substrate to the active site.

Cooperativity: when the binding of one substrate molecule to the enzyme affects the binding of subsequent substrate molecules.
This behaviour is more common in **multimeric enzymes** with several active sites.

Positive cooperativity: The binding of one substrate molecule **increases the affinity** of the other active sites for the substrate.

Negative cooperativity: The binding of one substrate molecule **decreases the affinity of** the other active sites for the substrate.



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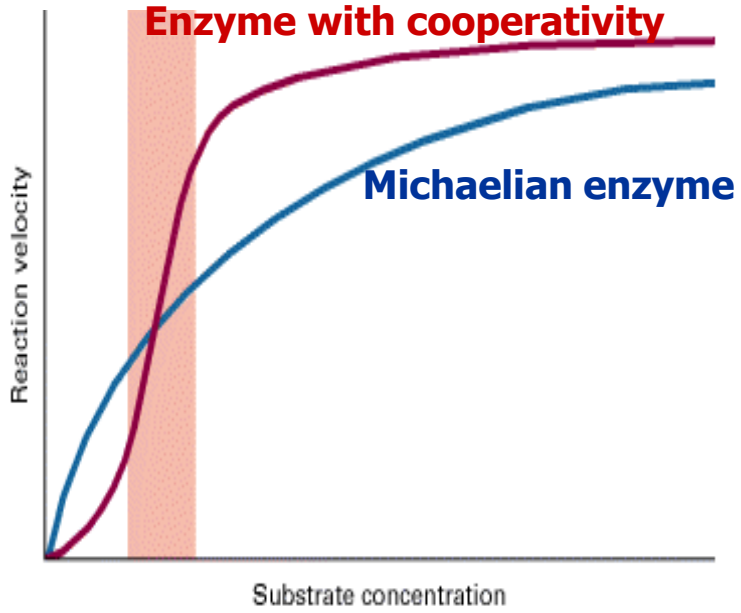
3. Phenomenon of cooperativity

3.1. Kinetics that do not behave according to the Michaelis-Menten model: enzymes with cooperativity.

The phenomenon of **cooperativity and sigmoidal behaviour** in enzyme kinetics appears in **multimeric enzymes that are also allosteric (a concept we will see later).**
They have several active sites for substrate binding but these sites are not independent.



Affinity increases as sites are occupied: Sigmoid curve

The representation of V_0 vs [S] has sigmoidal behaviour.

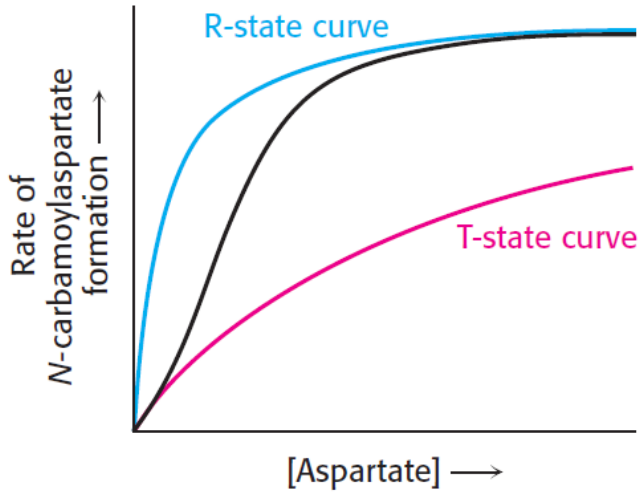
Vmax/2 is referred to as $K_{0.5}$

3. Phenomenon of cooperativity

3.2. Co-operative models

Cooperativity and allosteric effects can be explained by two models:
Monod-Wyman-Changeux **concerted** model. **MWC**
Koshland-Nemethy-Filmer **sequential** model. **KNF**

<u>Both postulate that</u>:
The enzyme subunits exist in one of two conformations:
Tense (T) conformation: lower affinity
Relaxed conformation (R): higher affinity to substrate



Explanation of kinetic behaviour

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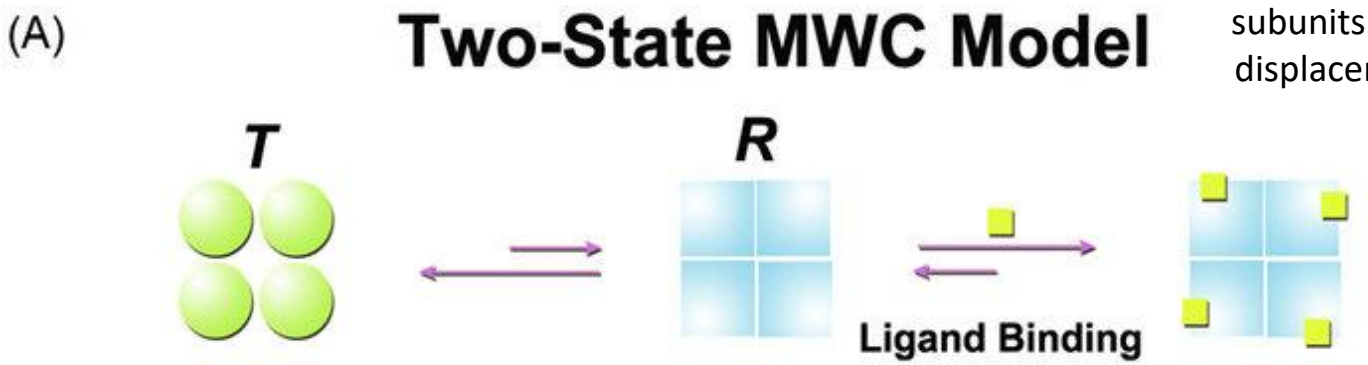
3. Phenomenon of cooperativity

3.2. Co-operative models

Tense (T) conformation: low-affinity
Relaxed conformation (R): high affinity

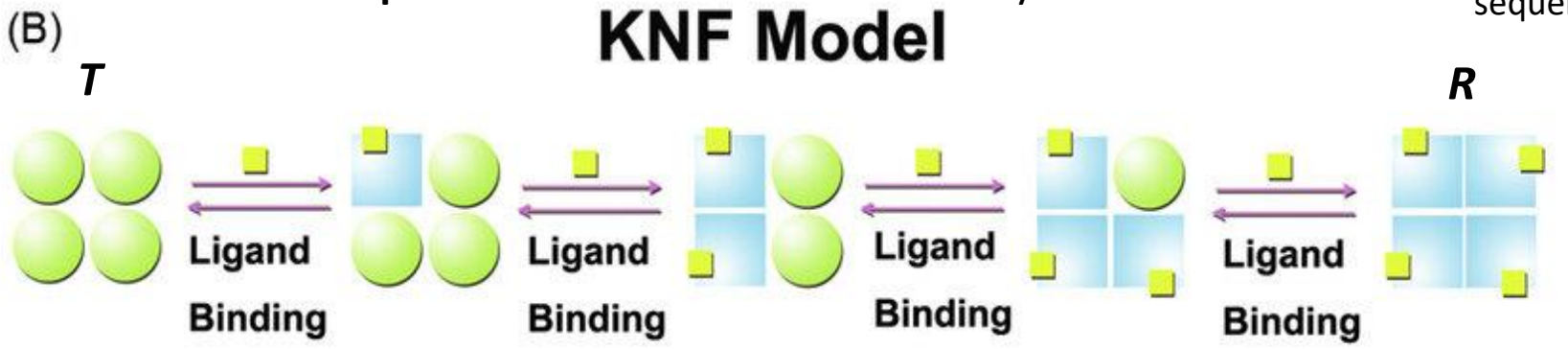
Concerted model: Monod-Wyman-Changeux

Upon binding of the first ligand, the subunits change to the R state by displacement of the equilibrium.



Sequential model: Koshland-Nemethy-Filmer

The subunits are changed sequentially



S.L.J.Zhang *Comprehensive Medicinal Chemistry III*
2017, Pages 276-296

3. Phenomenon of cooperativity

3.3. Allosteric enzymes. Concept.

Allosteric enzymes are proteins with a quaternary structure, they have more than one active and catalytic site, and their activity is regulated by allosteric modules.

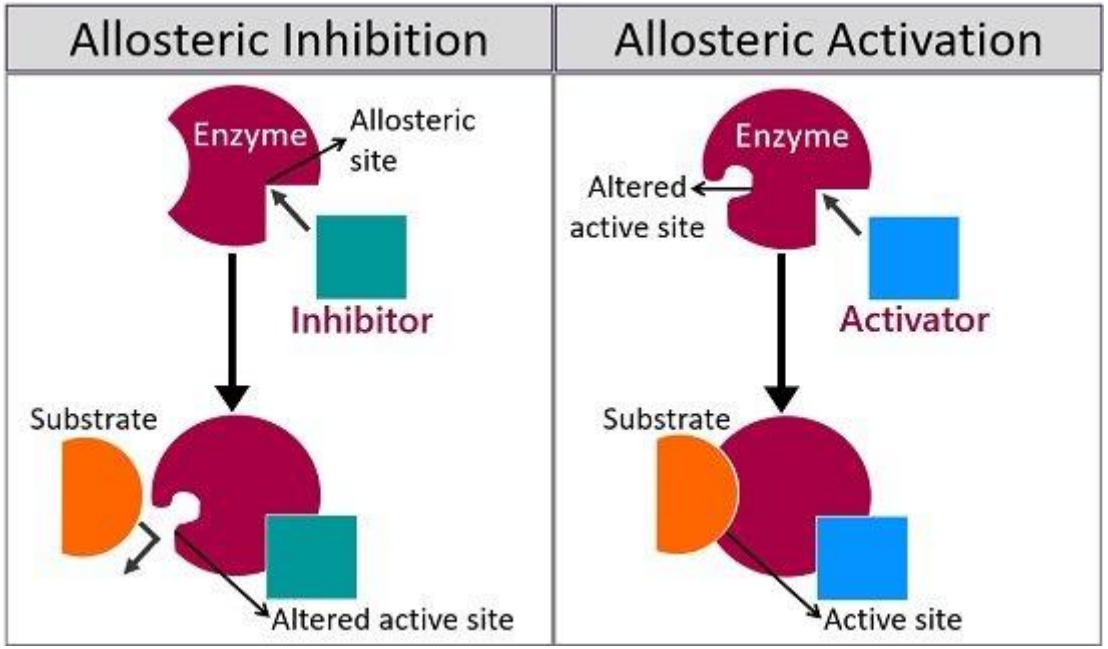


Diagram source: biologyreader.com

3. Phenomenon of cooperativity

3.3. Allosteric enzymes. Concept.

Allosteric enzymes can bind molecules other than substrates. They are usually referred to as ligands or allosteric effects, which can modulate enzyme activity.

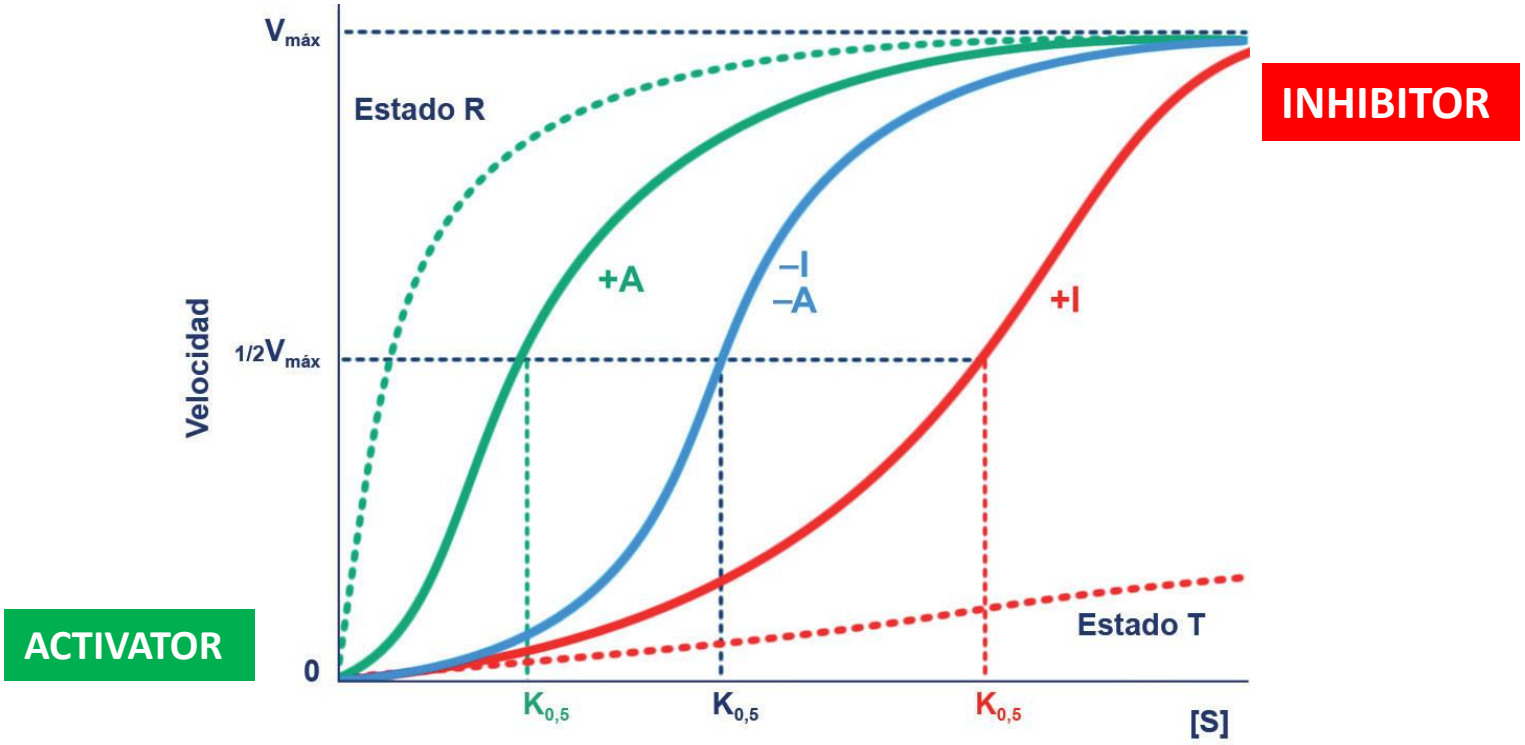
ALLOSTERISM: a property of enzymes and other proteins that undergoes a conformational change upon binding of a ligand such that the affinity for that ligand or another ligand is affected.

ALLOSTERIC EFFECTOR: this is the ligand that induces the conformational change – a binding modulator.

The activity of allosteric enzymes can be modulated by the binding of other ligands.

3. Phenomenon of cooperativity

3.3. Allosteric enzymes. Regulation of enzyme activity.



3. Phenomenon of cooperativity

3.3. Allosteric enzymes. Regulation of enzyme activity.

Homotropic effectors

- The active site is also the regulatory site

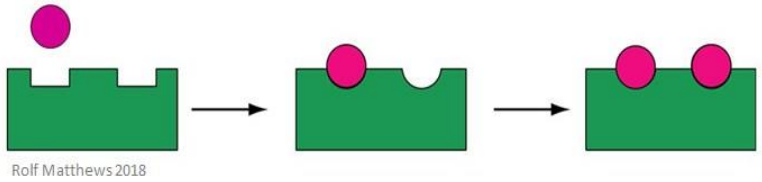
- Binding at one site affects affinity at others (dependent)

If the homotropic effector facilitates binding, this is **positive cooperativity**

If it hinders binding, this is **negative cooperativity**

They are usually multimeric proteins with a binding site on each protein subunit.

HOMOTROPIC EFFECT ON EQUIVALENT SITES



conformational change that facilitates the binding of the second molecule

The binding of O_2 favours binding at the other sites.

3. Phenomenon of cooperativity

3.3. Allosteric enzymes. Regulation of enzyme activity.

Heterotropic effectors

- This is another molecule that alters the affinity of enzyme-substrate binding

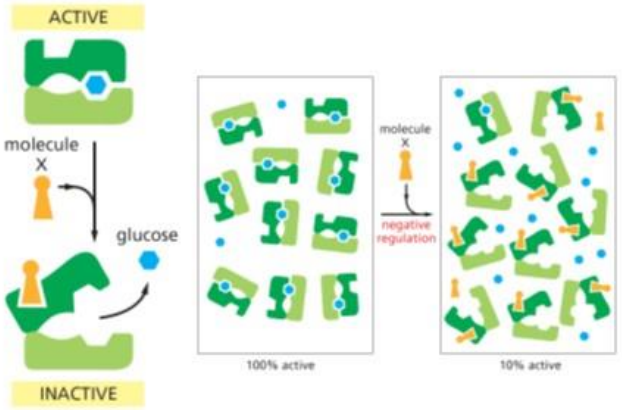
- Can inhibit or activate binding

- They bind to a regulatory site, which is different from the active site of the enzyme.

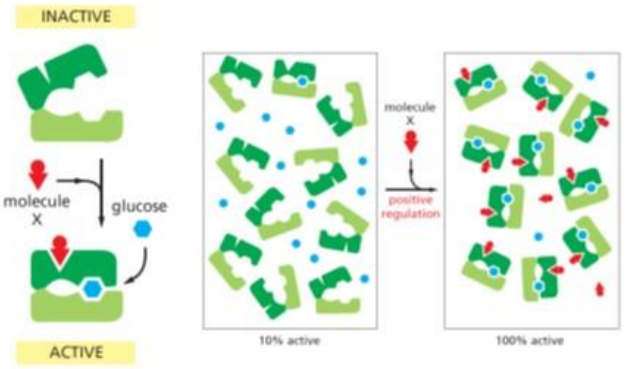
If the heterotropic effector facilitates binding, this is **positive cooperativity.**

If it hinders binding, this is **negative cooperativity.**

NEGATIVE HETEROTROPIC EFFECT



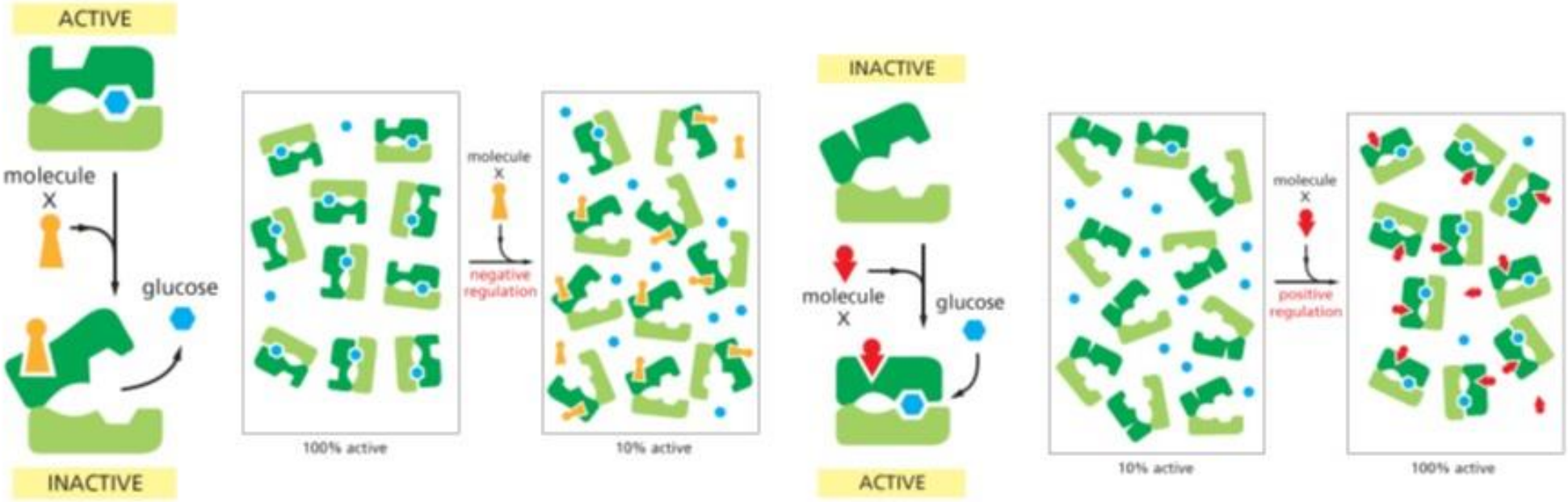
POSITIVE HETEROTROPIC EFFECT



Molecular Biology of the Cell, 6th edition, Bruce Alberts

NEGATIVE HETEROTROPIC EFFECT

POSITIVE HETEROTROPIC EFFECT



Molecular Biology of the Cell, 6th edition, Bruce Alberts

3. Phenomenon of cooperativity

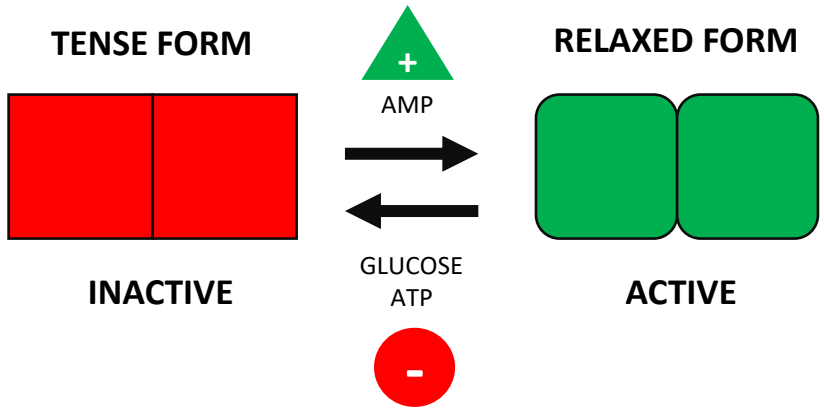
3.3. Allosteric enzymes. Examples.

Glycogen phosphorylase: key enzyme in glycogenolysis

It has an **allosteric regulation** system that responds to a low energy load.

Glucose and ATP: favourable energy conditions shift enzyme equilibrium towards the tense form.
Negative allosteric effectors/inhibitors.

AMP: poor energy conditions, shifts the balance to the active, relaxed form.
Positive allosteric effect activator.

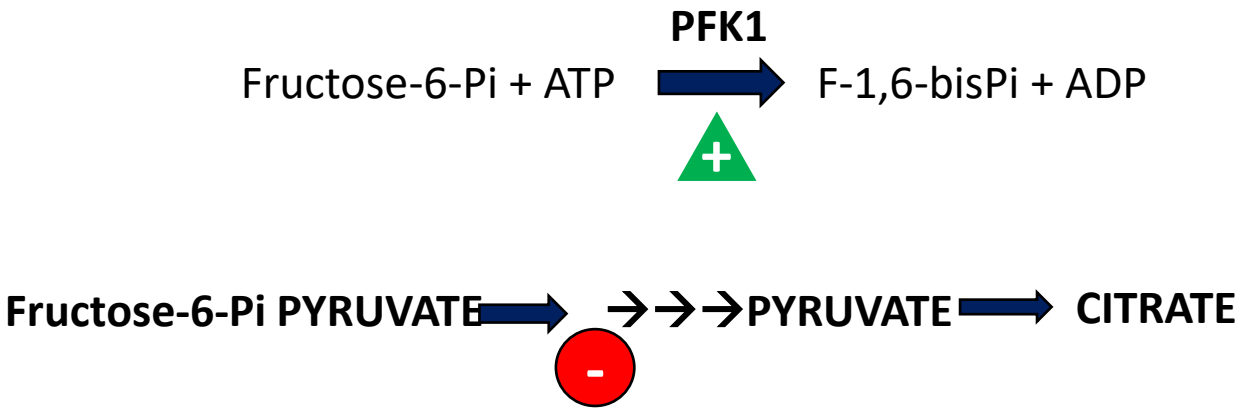


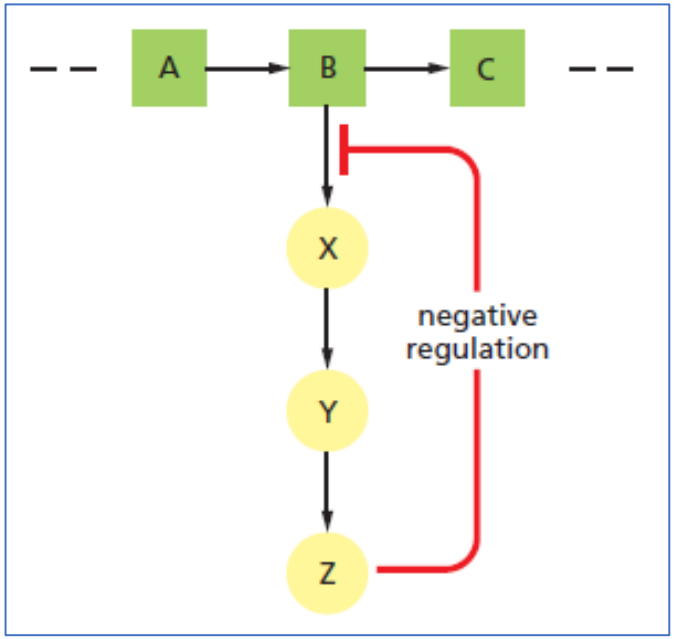
3. Phenomenon of cooperativity

3.3. Allosteric enzymes. Examples.

Phosphofructokinase 1 (PFK1) – involved in glycolysis

This glycolytic enzyme is allosterically inhibited by citrate, which is not a substrate for the enzyme. This is an example of feedback inhibition, in this case by citrate.





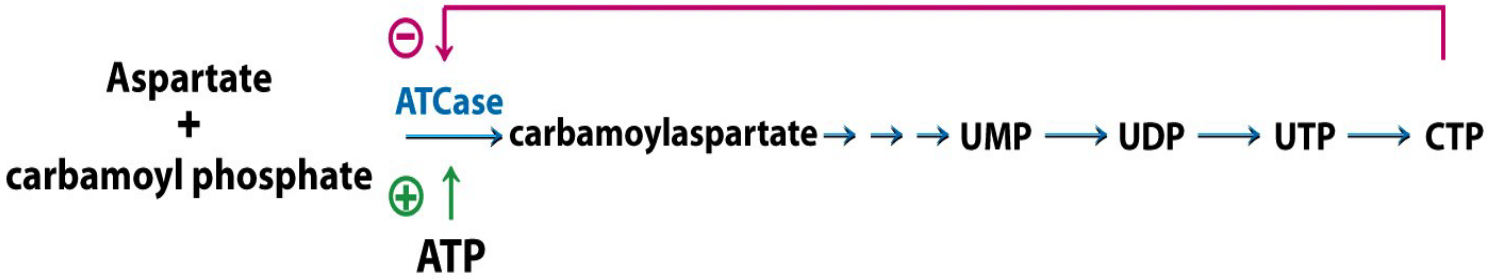
Introducción a la Biología Celular, Bruce Alberts et al

3. Phenomenon of cooperativity

3.3. Allosteric enzymes. Examples.

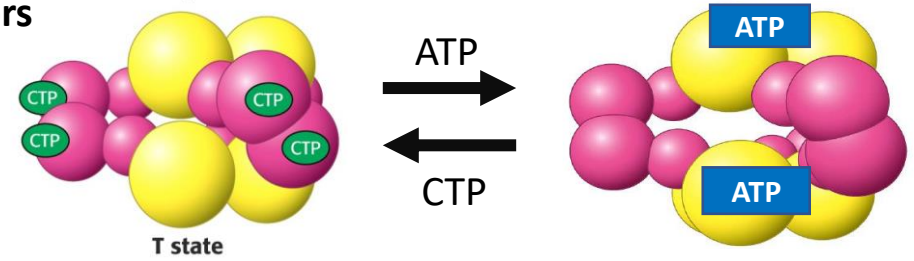
Aspartate transcarbamoylase (ATCase): an important enzyme in the synthesis of pyrimidine nucleotides

ATCase catalyses the regulated step of the pathway, is **inhibited by the end product CTP**, and is activated by **ATP** (purines).



ALLOSTERIC SUBUNIT: YELLOW, linking the effectors

CATALYTIC SUBUNIT: PINK



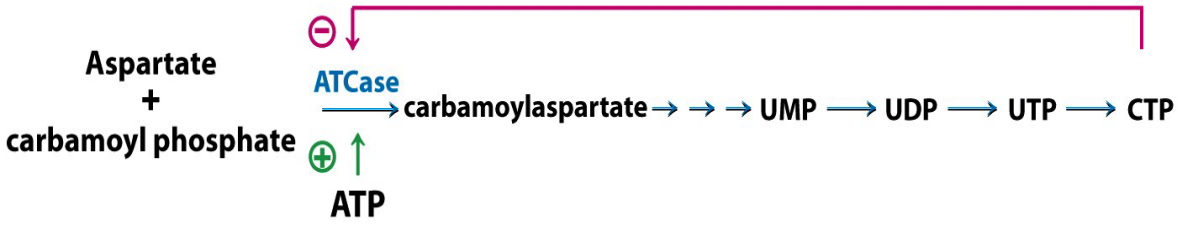
Mechanism of CTP inhibition: Stabilises **T-shapes**

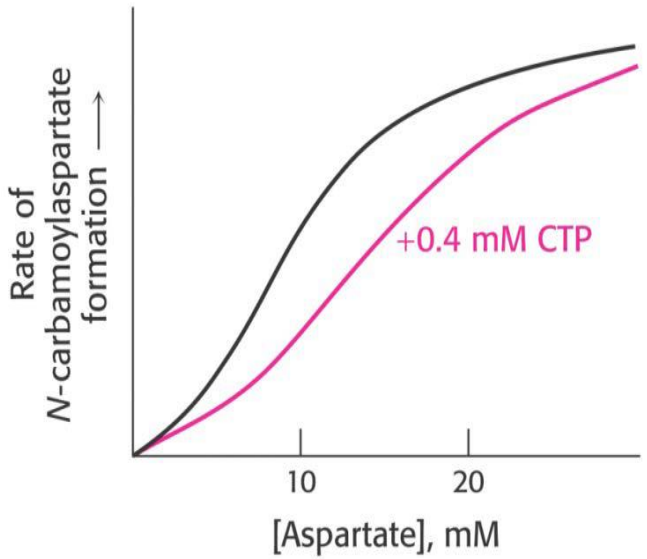
Mechanism of ATP activation: Stabilises **R-shapes**

3. Phenomenon of cooperativity

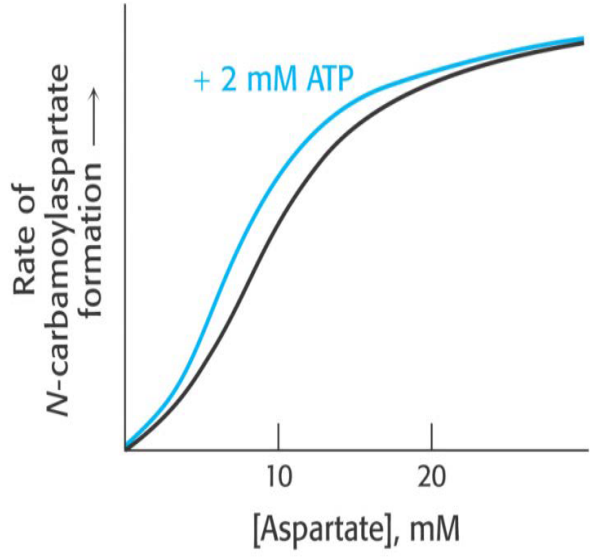
3.3. Allosteric enzymes. Examples.

Aspartate transcarbamoylase (ATCase) – an enzyme important in the synthesis of pyrimidine nucleotides





Lower activity and less sensitive curve to substrate concentration



Higher activity and more sensitive curve to substrate concentration

https://www.youtube.com/watch?v=PmDX52dA4hw

4. Types of protein-ligand interactions

P-L interactions:
- are specific
- occur with weak bonds: Van der Waals, hydrogen bridges, hydrophobic and ionic bonds

The functionality of proteins depends on dynamic properties:

1. Flexibility: ability to modulate a large number of weak links.

2. Conformational changes: small variations of the tertiary structure that have an effect on the functionality/activity of proteins

3. Interactions: which may be reversible interactions with ligands (inhibitors, activators, substrates, etc.) or protein-protein interactions.

The functions of ligand-binding proteins are affected by ligand binding.