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OpenCourseWare (2023)

CHEMISTRY II

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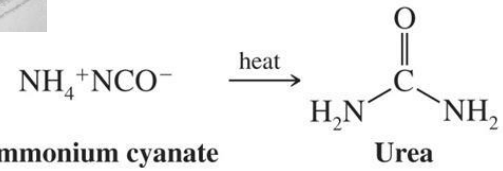
BIOPHYSICS AND CATALYSIS

Contents

Introduction

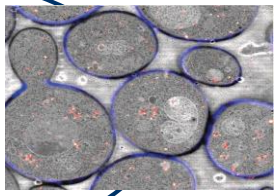
Biochemistry *is the study of the molecules and chemical reactions of life. It is the discipline that uses the principles and language of chemistry to explain biology at the molecular level.*

1828 Friedrich Wöhler synthesized the organic compound urea



Enzymes

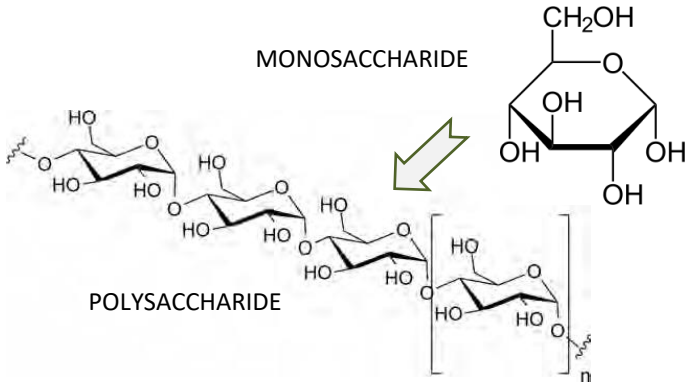
1897 Buchner showed that extracts of yeast cells could catalyze the fermentation of the sugar glucose



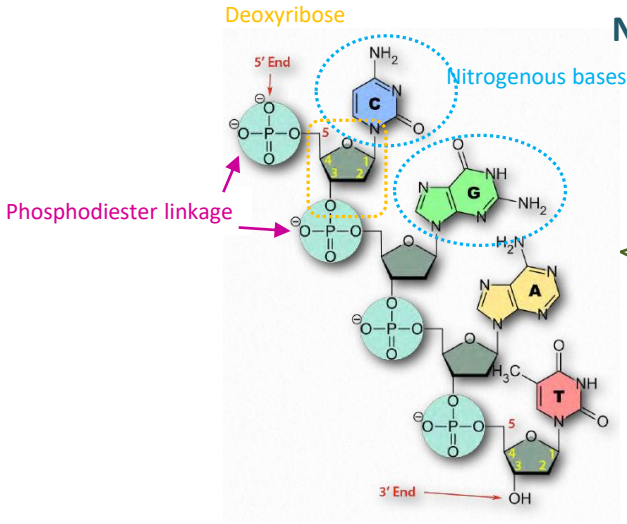
DNA

1944 Oswald Avery, Colin MacLeod, and Maclyn McCarty extracted deoxyribonucleic acid (DNA) from a toxic strain of the bacterium Streptococcus pneumoniae. DNA is the molecule that carries genetic information.

CARBOHYDRATES

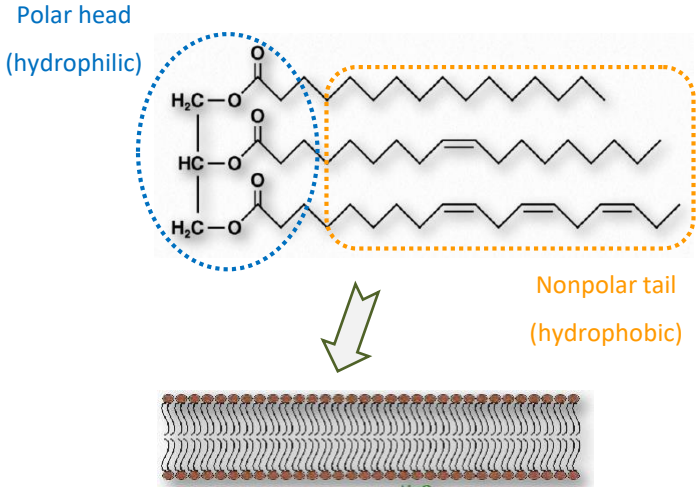


NUCLEIC ACIDS

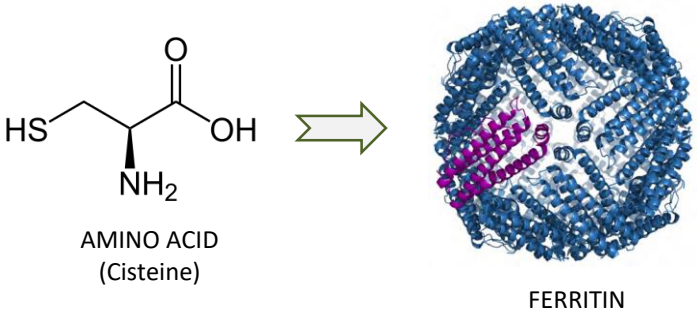


TYPES OF MACROMOLECULES

LIPIDS

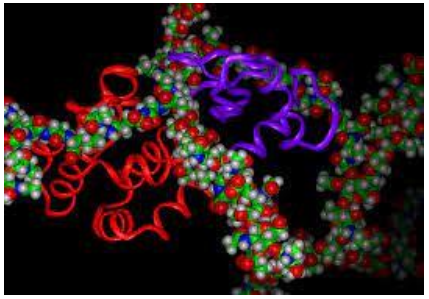


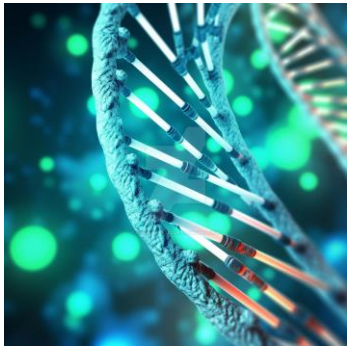
PROTEINS



Introduction

Computer Modelling



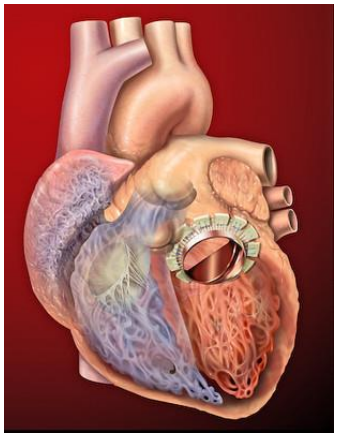


DNA Analysis and Structure

***Biophysics** is the field that applies the theories and methods of physics to understand how biological systems work.*

Bioengineering, Nanotechnologies, Biomaterials

Medical Applications



Bioenergetics and Thermodynamics

Bioenergetics *is the quantitative study of energy transductions—changes of one form of energy into another—that occur in living cells, and of the nature and function of the chemical processes underlying these transductions.*

Laws of Thermodynamics: *Biological Processes*

First Law: is the principle of the ***Conservation of Energy****. For any physical or chemical change, the total amount of energy in the universe remains constant; energy may change form or it may be transported from one region to another, but it cannot be created or destroyed.*

Second Law: the universe always tends toward increasing disorder, in all natural processes, the ***Entropy*** *of the universe increases.*

Standard Free-Energy Changes are Additive

$$\Delta G'^{0}_{total} = \Delta G'^{0}_{1} + \Delta G'^{0}_{2}$$

$$\begin{array}{ll} (1)\ A \rightarrow B & \Delta G'^{0}_{1} \\ (2)\ B \rightarrow C & \Delta G'^{0}_{2} \\ \hline Sum:\ A \rightarrow C & \Delta G'^{0}_{1} + \Delta G'^{0}_{2} \end{array}$$

Example:

$$Glucose + P_i \rightarrow glucose\ 6-phosphate + H_2O \quad \Delta G'^{0} = 13.8\ kJ/mol$$

$$ATP + H_2O \rightarrow ADP + P_i \quad \Delta G'^{0} = -30.5\ kJ/mol$$

$$\begin{array}{l} (1)\ Glucose + P_i \rightarrow glucose\ 6-phosphate + H_2O \\ (2)\ ATP + H_2O \rightarrow ADP + P_i \\ \hline Sum:\ ATP + glucose \rightarrow ADP + glucose\ 6-phosphate \end{array}$$

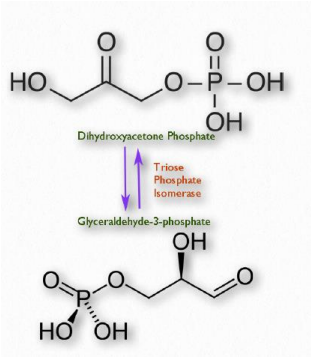
$$\Delta G'^{0} = 13.8 \frac{kJ}{mol} + (-30.5\ kJ/mol) = -16.7\ kJ/mol$$ EXERGONIC

Energy stored in ATP is used to drive the synthesis of glucose 6-phosphate

Introduction: Enzymes as Biocatalysts

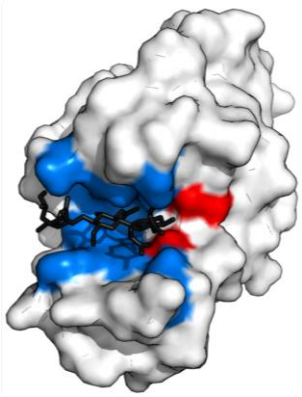
Enzymes *are extraordinarily efficient, selective biological catalysts.*

Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to wáter)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C–C, C–S, C–O, and C–N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor



Triose phosphate isomerase-catalyzed reaction

How does an Enzyme work?



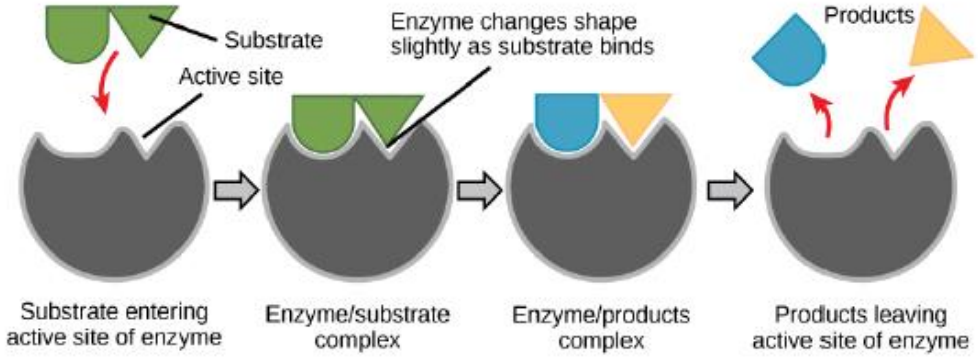
Binding of a substrate to an enzyme at the active site.
The enzyme chymotrypsin, with bound substrate in red (PDB ID 7GCH).

Enzymes enhance the **rates** of biological reactions.

Substrate is bound in the **active site** of the enzyme.

High **specificity** in the interaction between enzyme and substrate:

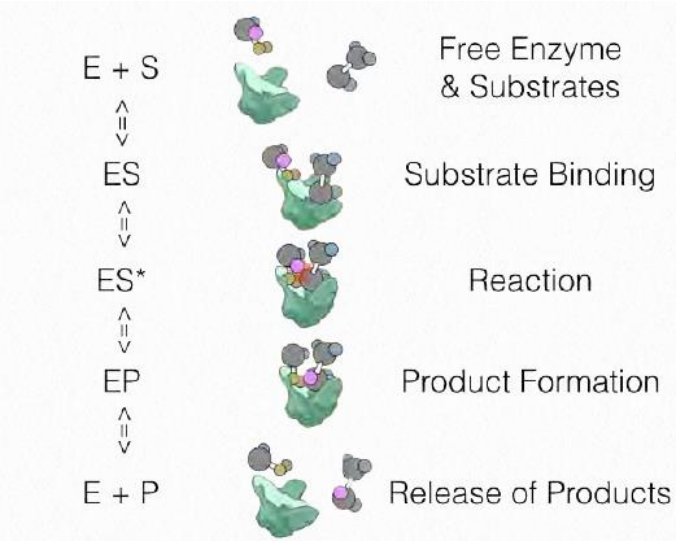
Lock and Key Theory



Lock and Key Theory *The substrate fits into a preformed active site on the enzyme. An* ***enzyme-substrate complex*** *is formed as an intermediate*

Enzyme Kinetics

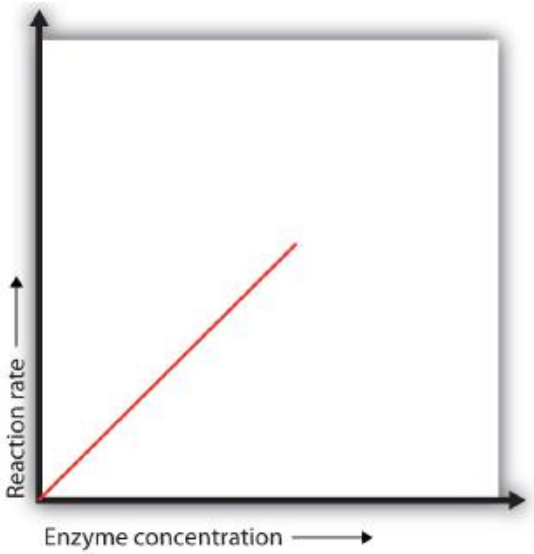
Enzymatic reaction



1. *The conversion of a substrate (S) to a product (P), catalyzed by an enzyme (E). Enzyme binds a substrate to form an **enzyme–substrate complex (ES)**.*

2. *The **reaction rate** is affected by the concentration of **enzyme** but not by the concentration of the other reactant, S, if [S] is very high.*

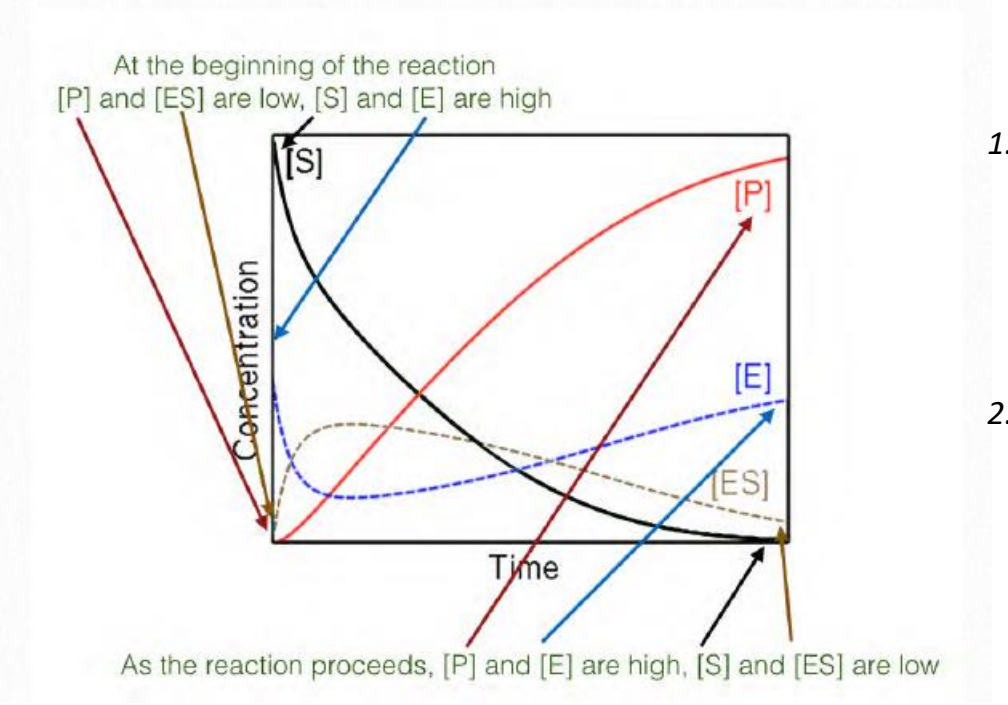
Effect of enzyme concentration ([E]), on the initial velocity (v) of an enzyme-catalyzed reaction at a fixed, saturating [S].



Mechanism: A kinetic Approach

Kinetic parameters

$$E + S \underset{K_{-1}}{\overset{k_1}{\rightleftarrows}} ES \overset{k_2}{\rightarrow} E + P$$



Change in concentration of reaction materials over time.

1. *The concentration of product [P], increases as the reaction proceeds. The **initial velocity of the reaction v_0** is the slope of the initial linear portion of the curve.*

2. *The **rate of the reaction doubles when twice as much enzyme** is added to an otherwise identical reaction mixture.*

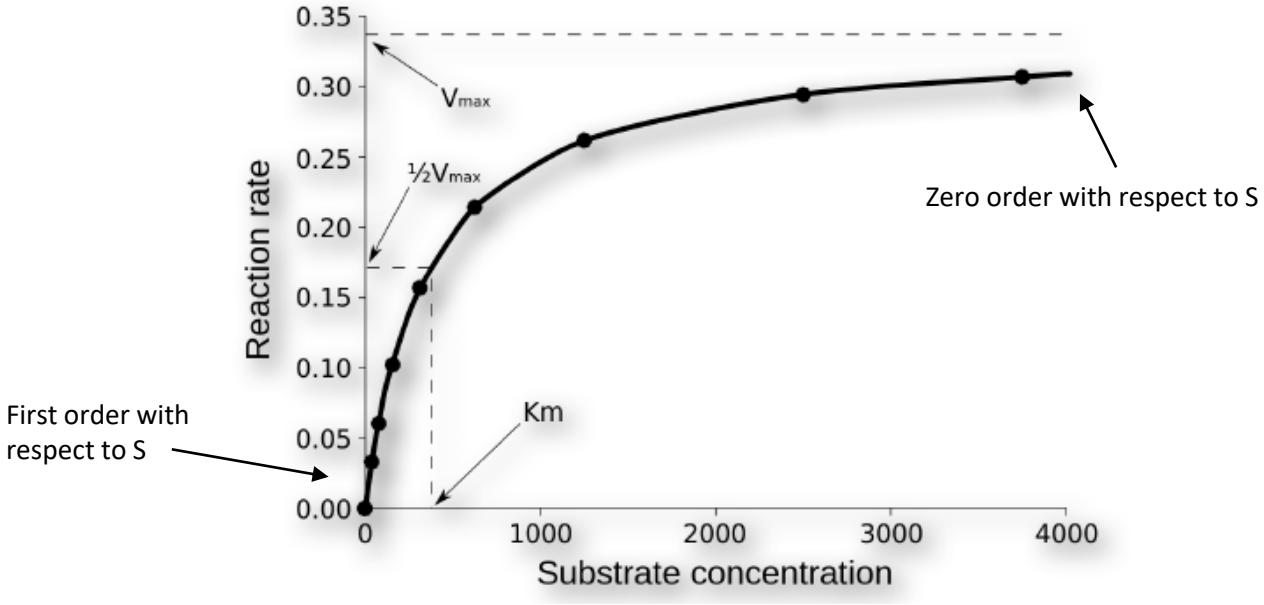
The Michaelis-Menten Equation

$$E + S \underset{K_{-1}}{\overset{k_1}{\rightleftarrows}} ES \overset{k_2}{\rightarrow} E + P$$

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

Michaelis-Menten Equation

K_m: Michaelis constant



Derivation of the Michaelis-Menten Equation

$$E + S \underset{K_{-1}}{\overset{k_1}{\rightleftarrows}} ES \overset{k_2}{\rightarrow} E + P$$

Steady-State Derivation

$$ES \overset{k_2}{\rightarrow} E + P \qquad V_0 = k_2[ES] \quad \textit{(Eq 1)}$$

Rate-limiting step

$v_f = k_1 [E][S]$

$[E] = [E]_{total} - [ES]$

$v_d = k_{-1} [ES] + k_2 [ES]$

Formation and Decomposition of ES: Kinetic Considerations

Rate of ES formation = Rate of ES decomposition

Steady-State Approximation

$$k_1([E]_{total} - [ES])[S] = (k_{-1} + k_2)[ES] \quad \textit{(Eq 2)}$$

Rearrangement of Eq 2

$$\frac{k_{-1} + k_2}{k_1} = K_m = \frac{([E]_{total} - [ES])[S]}{[ES]} \quad \textit{(Eq 3)}$$

K_m Michaelis Constant

$$\frac{k_{-1} + k_2}{k_1} = K_m = \frac{([E]_{total} - [ES])[S]}{[ES]} \quad \textit{(Eq 3)}$$

$$[ES]K_m = ([E]_{total} - [ES])[S] \quad \textit{(Eq 4)}$$

$$[ES](K_m + [S]) = [ES]_{total}[S] \quad \textit{(Eq 5)}$$

$$[ES] = \frac{[E]_{total}[S]}{K_m + [S]} \quad \textit{(Eq 6)}$$

Steady-State Concentration

$$v_0 = k_2[ES] = \frac{k_2[E]_{total}[S]}{K_m + [S]} \quad \textit{(Eq 7)}$$

$$V_{max} = k_2[E]_{total} \quad \textit{(Eq 8)}$$

***Maximum rate**: the concentration of S is very high, the molecules of E are present as ES*

$$\boxed{V_0 = \frac{V_{max}[S]}{K_m + [S]}} \quad \textit{(Eq 9)}$$

Michaelis-Menten Equation

Catalytic Constant (k_{cat})

At high substrate concentration the overall velocity of the reaction is V_{max}

$$V_{max} = k_{cat}[E]_{total}$$

$$\boxed{k_{cat} = \frac{V_{max}}{[E]_{total}}}$$

k_{cat} Catalytic constant

*Number of moles of substrate converted to product per second per mole of enzyme or **turnover number**.*

Enzyme	Turnover Number (per second)
Carbonic anhydrase	600,000
3-Ketoesteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymostrypsin	100
DNA Polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

Representative values of k_{cat} (The catalytic constants are given only as orders of magnitud)

Michaelis Constant (K_m)

$$\frac{k_{-1} + k_2}{k_1} = K_m = \frac{([E]_{total} - [ES])[S]}{[ES]} \qquad \textit{(Eq 3)}$$

$$E + S \underset{K_{-1}}{\overset{k_1}{\rightleftarrows}} ES \overset{k_2}{\rightarrow} E + P$$

1. *If $k_2 << k_{-1}$, k_2 can be neglected and* ***$K_m = k_{-1} / k_1$***

   K_m ***Equilibrium constant*** *for the dissociation of the ES complex to E + S*

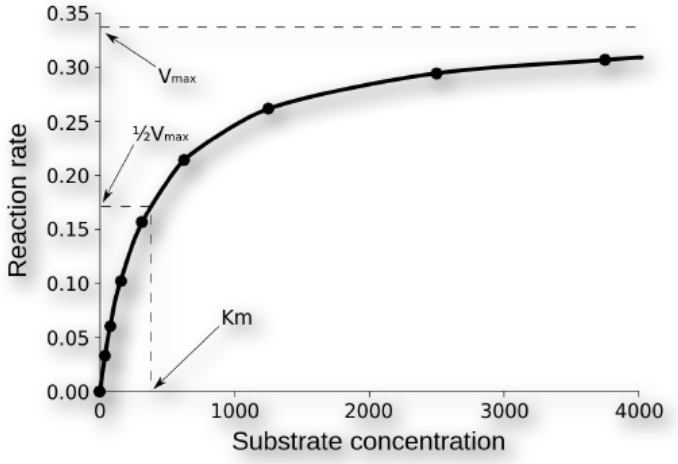
2. *K_m is a measure of the* ***affinity of E for S***.

   The lower the value of K_m, the more tightly the substrate is bound.

3. *From the curve:*

   K_m = [S] *when $v_0 = V_{max}/2$*

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



Lineweaver-Burk Equation: Measurement of Kinetic Parameters

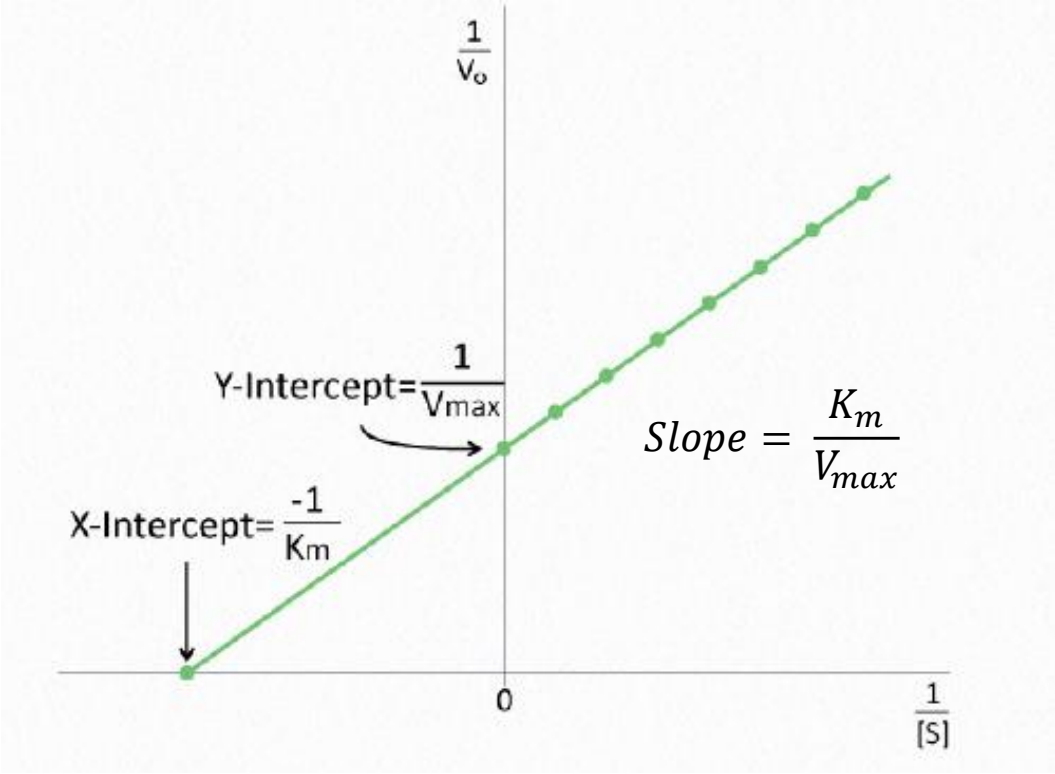
$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \quad \textit{(Eq 9)}$$

Michaelis-Menten Equation

Linear transformation

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]}$$

Lineweaver-Burk Equation



Double-reciprocal (Lineweaver-Burk) plot.

Inhibition Mechanisms: A Kinetic Approach

*An **enzyme inhibitor** (I) is a compound that binds to an enzyme and interferes with its activity*

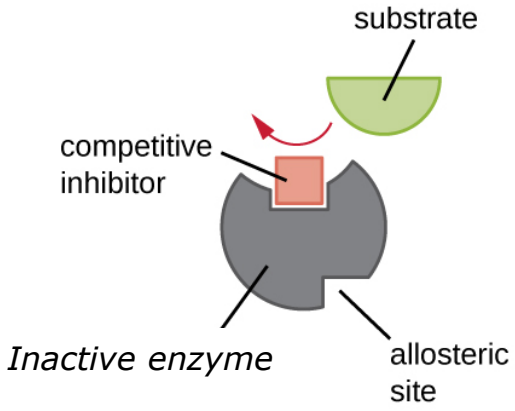
- Prevent the formation of ES complex.
- Block the chemical reaction that leads to the formation of product.

Types of Reversible Inhibition

Type of inhibitor	Effect
Competitive (I binds to E only)	Raises K_m Vmax remains unchanged
Uncompetitive (I binds to ES only)	Lowers V_{max} and K_m Ratio of V_{max}/K_m remains unchanged
Noncompetitive (I binds to E or ES)	Lowers V_{max} K_m remains unchanged

Competitive Inhibition

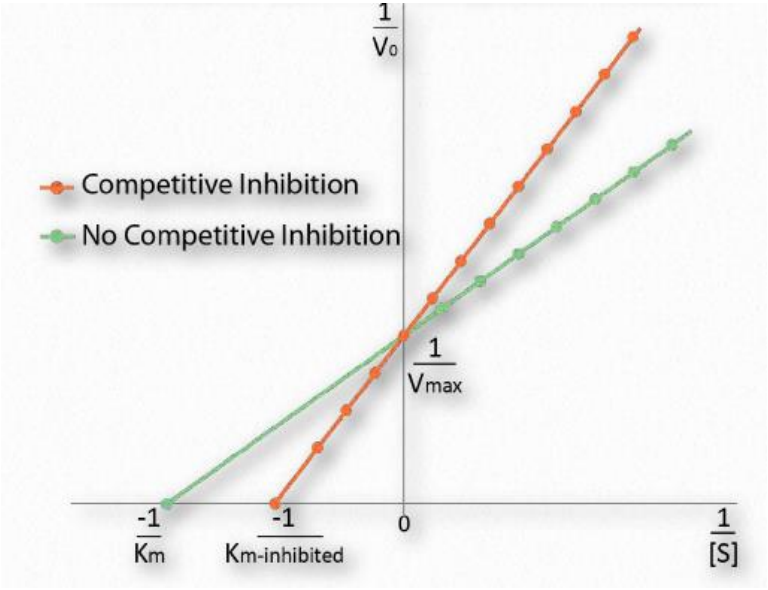
(I binds to E)



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

$$or\ E + I \overset{k_i}{\rightleftarrows} EI$$

S and I compete for binding to the enzyme molecule



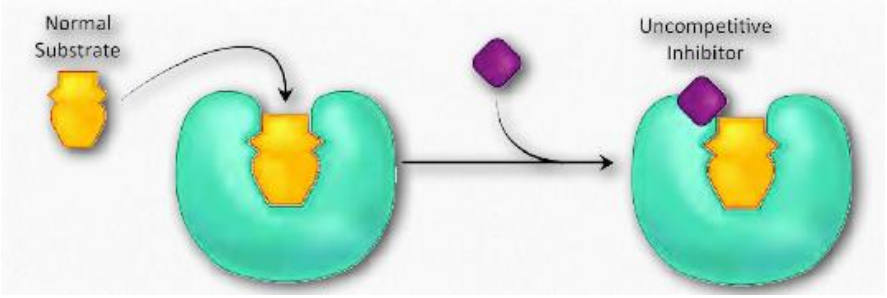
Double reciprocal plot

***V_{max}* remains unchanged and *K_m* increases.** The green line labeled "Control" is the result in the absence of inhibitor. The red line is the result in the presence of inhibitor.

$$\frac{1}{V_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

Uncompetitive Inhibition

(I binds to ES)

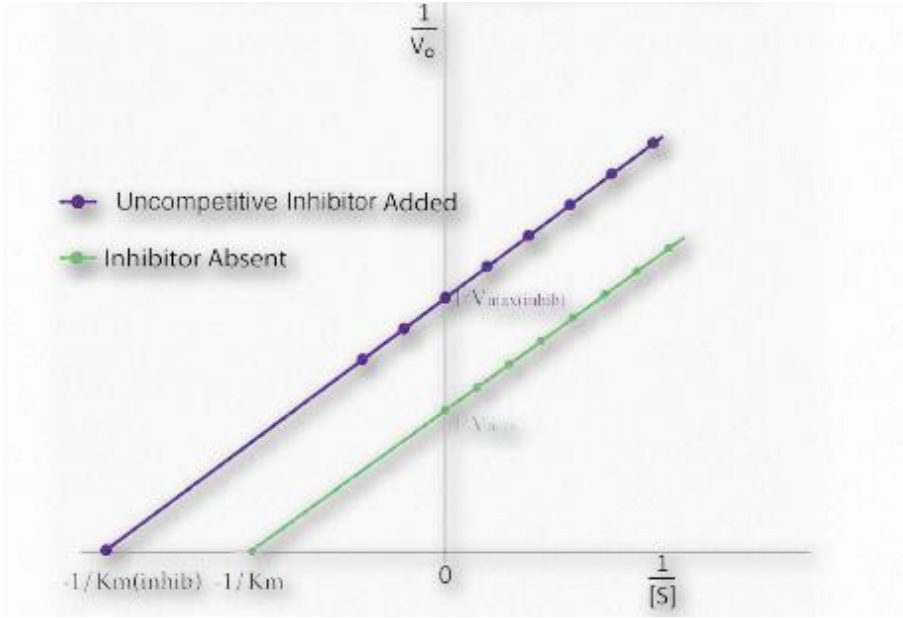


Inactive enzyme

$$E + S \underset{K_{-1}}{\overset{k_1}{\rightleftarrows}} ES \overset{k_2}{\rightarrow} E + P$$

$$or\ ES + I \overset{k_i}{\rightleftarrows} ESI$$

Inhibitors bind only to ES, not to free enzyme



Double reciprocal plot

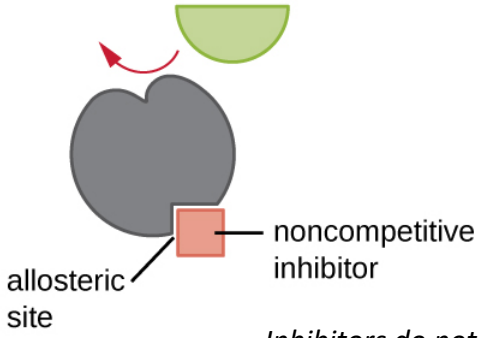
Both ***V_{max}*** **and** ***K_m*** **decrease** (i.e., the absolute values of both $1/V_{max}$ and $1/K_m$ obtained from the y and x intercepts, respectively, increase).

The ratio K_m/V_{max}, **the slope of the lines, remains unchanged.**

$$\frac{1}{V_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

Noncompetitive Inhibition

(I binds to E or ES)



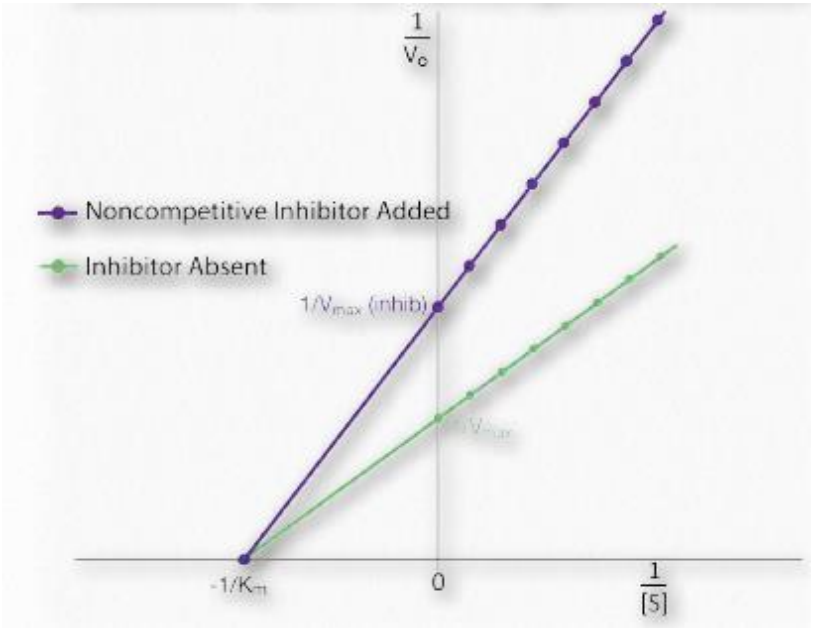
Inhibitors do not bind at the same site as S

$$E + S \underset{K_{-1}}{\overset{k_1}{\rightleftarrows}} ES \overset{k_2}{\rightarrow} E + P$$

$$or\ E + I \overset{k_i}{\rightleftarrows} EI + S \rightleftarrows ESI$$

$$or\ ES + I \overset{k_i}{\rightleftarrows} ESI$$

Inhibitors can bind to E or ES, forming inactive EI or ESI complexes



Double reciprocal plot

V_{max} decreases**, but **K_m remains the same.

$$\frac{1}{V_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

Image Credits

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Slides 4, 8 (isomerase reaction), 9 (enzyme), 10 (enzyme reaction), 11 (graph), 12 (graph), 15 (table), 16-17 (graph), 19-21(graph),:

- Biochemistry: Free For All, 2018. Kevin Ahern, Indira Rajagopal, and Taralyn Tan, Oregon State University. https://open.umn.edu/opentextbooks/textbooks/866. .

Slide 5:

- Computer modeling: EMSL, CC BY-NC-SA 2.0 DEED, https://www.flickr.com/photos/emsl/4281867363.
- DNA analysis and structure: Coolarts223, CC BY 3.0 DEED, https://www.deviantart.com/coolarts223/art/Dna-helix-Macro-photography-965829819.
- Bioengineering, Nanotechnologies, Biomaterials: UCSD Jacobs School of Engineering , CC BY-NC 3.0 US DEED, https://biomat.net/site/human-brain-organoids-implanted-into-mouse-cortex-respond-to-visual-stimuli-for-first-time/.
- Medical applications: Patrick J. Lynch, CC BY 2.0 DEED, https://www.flickr.com/photos/patrlynch/450141959.

Slides 7, 11-17, 19-21:

- Equations made by the authors.

Slide 9:

- Lock and key theory: Principles of Biology, Lisa Bartee; Walter Shriner; and Catherine Creech, Open Oregon Educational Resources, https://openoregon.pressbooks.pub/mhccmajorsbio/.

Slides 10, 19 (image), 21 (image):

- Graph: CHE 301: Biochemistry, Hernan D. Biava, Brevard College, https://chem.libretexts.org/Courses/Brevard_College/CHE_301_Biochemistry.