uc3m | Universidad Carlos III de Madrid

OpenCourseWare (2023)

# CHEMISTRY II

Verónica San Miguel Arnanz

Teresa Pérez Prior

Berna Serrano Prieto

Department of Materials Science and Engineering and Chemical Engineering

# BIOPHYSICS AND CATALYSIS

## 1. BIOCHEMISTRY

Biochemistry delves into the intricacies of life by exploring the molecules and chemical reactions that underlie biological processes. This field employs the principles and language of chemistry to decipher the molecular intricacies of biology. Notably, biochemists have proposed that identical chemical compounds and central metabolic processes are present in organisms as diverse as bacteria, plants, and humans. This revelation establishes that the fundamental tenets of biochemistry are shared across all living entities. While scientists often focus their research on specific organisms, the insights gained apply to various species.

The genesis of biochemistry as a dynamic scientific discipline occurred only within the last century. By the end of the 19$^{th}$ century, many chemicals produced in living organisms had been identified, but in the subsequent decades, biochemistry crystallized into an organized field. Biochemists have since elucidated numerous chemical processes integral to life. The growth of biochemistry and its impact on other disciplines is poised to continue well into the 21$^{st}$ century.

In 1828, Friedrich Wöhler's synthesis of urea from ammonium cyanate demonstrated for the first time that compounds exclusive to living organisms could be created from common inorganic substances.

Furthermore, two pivotal breakthroughs stand out in the history of biochemistry—the recognition of enzymes as catalysts and the acknowledgment of nucleic acids as carriers of genetic information. Identifying enzymes as biological reaction catalysts, a milestone partly attributed to Eduard Buchner's research, challenged the prevailing belief that only living cells could catalyze complex biological reactions. Buchner's 1897 demonstration that yeast cell extracts could catalyze the fermentation of glucose marked a turning point.

Half a century later, the second major breakthrough came with the revelation of nucleic acids as carriers of genetic information. In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty extracted deoxyribonucleic acid (DNA) from a toxic strain of Streptococcus pneumoniae. Mixing this DNA with a non-toxic strain of the same bacterium demonstrated the transformative role of nucleic acids in information transmission.

Biological macromolecules typically manifest as polymers, which result from the condensation of numerous smaller organic molecules, or monomers, entailing the removal of water elements. Each monomer integrated into the macromolecular chain is termed a *residue*. In certain instances, like specific carbohydrates, a singular residue is iteratively replicated, whereas in proteins and nucleic acids, an assortment of residues is sequentially connected. The repetition of the same enzyme-catalyzed reaction facilitates the addition of each residue in a polymer. Consequently, all residues in a macromolecule are oriented in the same direction, and the chemical composition at the ends of the macromolecule is distinct. Macromolecules exhibit properties markedly distinct from those of their constituent monomers.

There exist four major classes of biomolecules:

i. **Carbohydrates:** Primarily composed of carbon, oxygen, and hydrogen, carbohydrates encompass simple sugars (monosaccharides) and their polymers (polysaccharides). Monosaccharides and polysaccharide residues are characterized by multiple hydroxyl groups, rendering them polyalcohols. Common monosaccharides typically feature five or six carbon atoms, and carbohydrates serve as potent energy sources.

ii. **Nucleic acids**: Constructed from polymers of nucleotides, nucleic acids comprise a nitrogenous base, a pentose sugar, and a phosphate group. Polymerized nucleotides form DNA and RNA, serving as genetic material.

iii. **Lipids:** Comprising lengthy hydrocarbon chains, lipids generally exist as esters of fatty acids and constitute the fundamental components of biological membranes. Lipid molecules harbor substantial energy content, functioning as energy storage entities.

iv. **Proteins**: Heteropolymers cover strings of amino acids, and proteins are formed through peptide bonds between the carboxyl and amino groups of successive amino acids. Proteins exhibit diverse structures with four hierarchical levels with 20 different amino acids contributing to their composition.

## 2. BIOPHYSICS

Biophysicists pursue developing methodologies to combat diseases, address global hunger, create sustainable energy sources, design cutting-edge technologies, and unravel numerous scientific enigmas. In essence, biophysicists stand at the forefront of tackling age-old human challenges as well as future problems.

For instance, the elucidation of DNA's structure in 1953 through biophysics was pivotal, revealing DNA's role as a blueprint for life. Presently, we can decode DNA sequences from thousands of humans and various living organisms. Biophysical techniques play a crucial role in analyzing and interpreting the vast datasets generated. Regarding computer modeling, biophysicists actively devise and employ computer modeling methods to visualize and manipulate the shapes and structures of complex molecules like proteins and viruses. This information is vital for developing new drug targets and understanding how proteins mutate, leading to tumor growth.

On the other hand, biophysics is critical in comprehending biomechanics, contributing to the design of improved prosthetic limbs and advanced nanomaterials for drug delivery. Moreover, it has been instrumental in developing life-saving treatments and devices, including kidney dialysis, radiation therapy, cardiac defibrillators, pacemakers, and artificial heart valves.

The nineteenth century witnessed the formulation of two fundamental laws of thermodynamics based on observations of energy interconversion. The first law, the conservation of energy, asserts that the total energy in the universe remains constant during any physical or chemical change. The second law, which describes the tendency toward increasing disorder (entropy) in the universe, takes various forms but consistently emphasizes this trend. Living cells and organisms, operating as open systems, exchange both material and energy with their surroundings, avoiding equilibrium. This continuous interaction explains how organisms create internal order while adhering to the second law of thermodynamics.

Cells operate at constant temperature and pressure, relying on free energy described by the Gibbs free-energy function G. This enables predictions about the direction, equilibrium, and work potential of chemical reactions at constant temperature and pressure. Heterotrophic cells derive free energy from nutrient molecules, while photosynthetic cells harness energy from absorbed solar radiation. Both types of cells convert this free energy into ATP and other energy-rich compounds for biological work.

Bioenergetics principles elucidate how thermodynamically unfavorable (endergonic) reactions can proceed by coupling them to highly exergonic reactions through a common intermediate. For instance, the synthesis of glucose 6-phosphate, an endergonic reaction, is driven forward by connecting it to the exergonic breakdown of ATP.

## 3. CATALYSIS

Enzymes are predominantly proteins except for a small group of catalytic RNA molecules. They serve as highly efficient and selective biological catalysts. Each living cell boasts a multitude of enzymes, numbering in the hundreds, that catalyze crucial life-sustaining reactions. Even the most basic organisms house numerous copies of various enzymes. Their catalytic ability relies on the integrity of their native protein conformation. Denaturation or dissociation into subunits typically leads to the loss of catalytic activity. Moreover, if an enzyme is disassembled into its constituent amino acids, its catalytic function is invariably destroyed. Hence, protein enzymes' primary, secondary, tertiary, and quaternary structures are indispensable for their catalytic activity.

Occasionally, the same enzyme may be known by different names, or conversely, distinct enzymes may share a common name. To address such ambiguities and accommodate the growing list of newly discovered enzymes, biochemists, through international consensus, have adopted a systematic naming and classification system. This system categorizes enzymes into six classes, each with subclasses based on the type of catalyzed reaction. Each enzyme is assigned a four-part classification number and a systematic name delineating the reaction it catalyzes.

### *3.1. Enzymes as Biocatalysts*

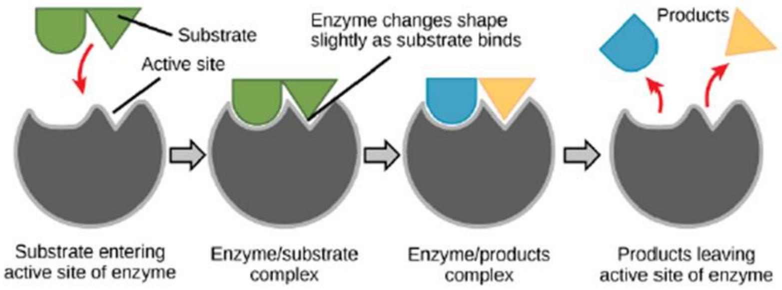
Enzymes play an important role in accelerating biochemical reactions, as most of these reactions struggle to proceed at significant rates under normal physiological conditions without the aid of enzymes. The primary function of enzymes lies in amplifying the rates of these reactions, typically achieving speeds twice as fast as their uncatalyzed counterparts. This catalytic ability is crucial for the functioning of living systems.

In biologically relevant environments, uncatalyzed reactions tend to be sluggish due to the inherent stability of biological molecules in the neutral-pH, mild temperature, and aqueous conditions within cells. Processes essential for food digestion, nerve signal transmission, or muscle contraction, lack practical rates without enzymatic catalysis. Enzymes exhibit high specificity towards their substrates, with some targeting related groups of substrates and others exclusively acting on a single compound.

Many enzymes display stereospecificity, acting exclusively on a particular stereoisomer of the substrate. However, the paramount aspect of enzyme specificity is the specificity of the reaction itself, ensuring the formation of products with exceptional purity (essentially 100%). This level of specificity surpasses the typical purity achieved in catalyzed reactions in organic chemistry. It serves to save cellular energy while preventing the accumulation of potentially harmful metabolic by-products.

The distinctive characteristic of an enzyme-catalyzed reaction is its confinement within a specific pocket on the enzyme, known as the active site. The substrate, the molecule upon which the enzyme acts, is bound within this active site. The active site's surface is adorned with amino acid residues containing substituent groups that facilitate substrate binding and catalyze its chemical transformation. Often, the active site completely encloses the substrate, isolating it from the surrounding solution.

The *Lock and Key Theory* describes the interaction between enzymes and substrates as a precise mechanism (Figure 1). According to this theory, the substrate is akin to a key that perfectly fits into the preexisting lock of the enzyme's active site, which site is already shaped to accommodate the substrate, and the two fit together with precision. This fitting process leads to the formation of an enzyme-substrate complex, a temporary intermediate state in the catalytic process. The enzyme undergoes subtle conformational changes during this complex formation to further facilitate the catalytic reaction.



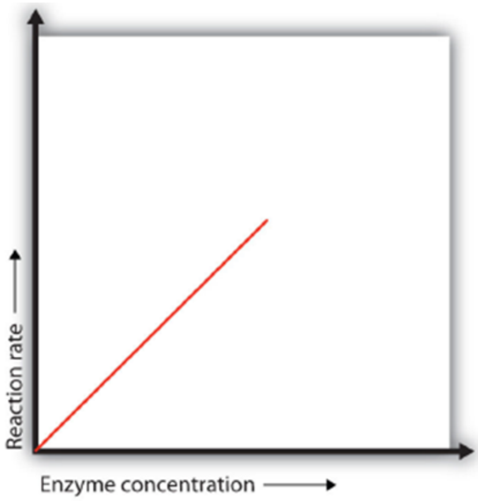

Source: Biochemistry: Free For All, 2018. Kevin Ahern, Indira Rajagopal, and Taralyn Tan, Oregon State University. https://open.umn.edu/opentextbooks/textbooks/866.

*Figure 1.* Lock and Key Theory.

The Lock and Key Theory emphasizes the specificity of the interaction, highlighting that each enzyme is tailored to recognize and bind a particular substrate with high precision. This specificity is essential for the enzyme's function, ensuring that only the correct substrate can initiate the catalytic process within the active site.

### *3.2. Enzyme Kinetics*

One of the initial breakthroughs in biochemistry arose from exploring enzyme kinetics, revealing the exclusive binding nature of specific substrates to particular enzymes. Let's delve into a straightforward enzymatic reaction unfolding in two distinct stages: first, the formation of an enzyme–substrate complex (ES) as the enzyme binds with a substrate, and second, the transformation of the substrate into the reaction product through the catalytic action of the protein. A specific rate characterizes each step, and the overall pace of an enzymatic reaction hinges on the concentrations of both the substrate and the enzyme catalyst. When the enzyme amount is significantly less than the substrate amount, the reaction's progress is contingent upon the enzyme's quantity. Figure 2 shows that an increase in enzyme concentration correlates with a swifter reaction. These conditions prove valuable in determining enzyme concentrations, with enzyme activity easily assessed by comparing it to a reference curve. Under these experimental circumstances, there are ample substrate molecules, ensuring each enzyme molecule binds to a substrate, creating a saturated condition known as saturation of E with S.




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

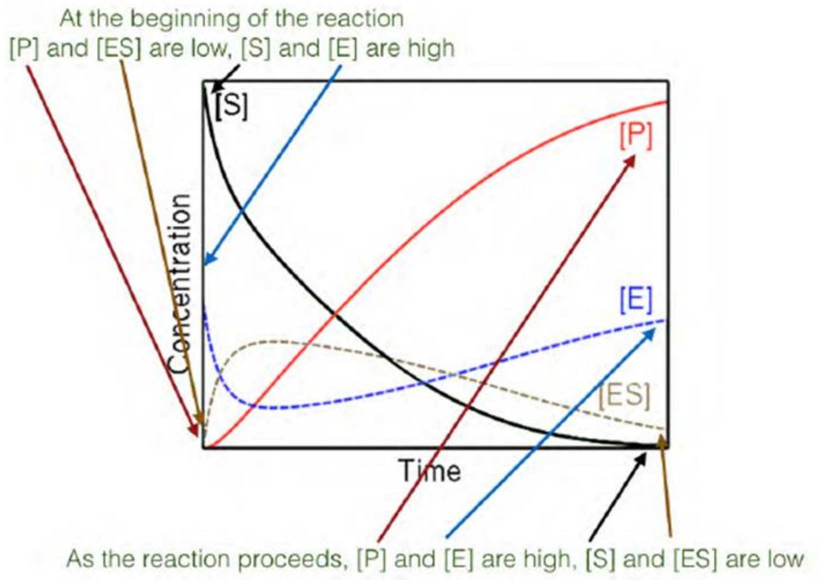
*Figure 2.* Effect of enzyme concentration ([E]), on the initial velocity (v) of an enzyme-catalyzed reaction at a fixed, saturating [S].

Upon initiating an enzyme-catalyzed reaction by mixing substrate and enzyme, the absence of product during the initial stages allows us to disregard the reverse reaction. The reaction can be briefly described by reaction kinetics governed by rate constants $k_1$ and $k_{-1}$, dictating the association and dissociation rates, respectively.

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

The velocity measured during this brief interval constitutes the initial velocity, a concept explored earlier. The formation and dissociation of ES complexes happen swiftly due to non-covalent bond dynamics. At the same time, the substrate-to-product conversion usually represents the rate-limiting step involving chemical alterations to the substrate.

In the context of Michaelis-Menten kinetics, velocity is measured as initial velocity ($v_0$), focusing on the early stages of the reaction before equilibrium sets in, mitigating the impact of the reverse reaction. Two interconnected assumptions underlie this approach: an abundance of substrate compared to enzyme concentration and conditions favoring a faster substrate conversion to product over substrate binding. The progress curves in the figure (Figure 3) showcases the initial velocities obtained from progress curves, emphasizing the dependency on enzyme concentration rather than substrate concentration under conditions of high initial substrate concentration.

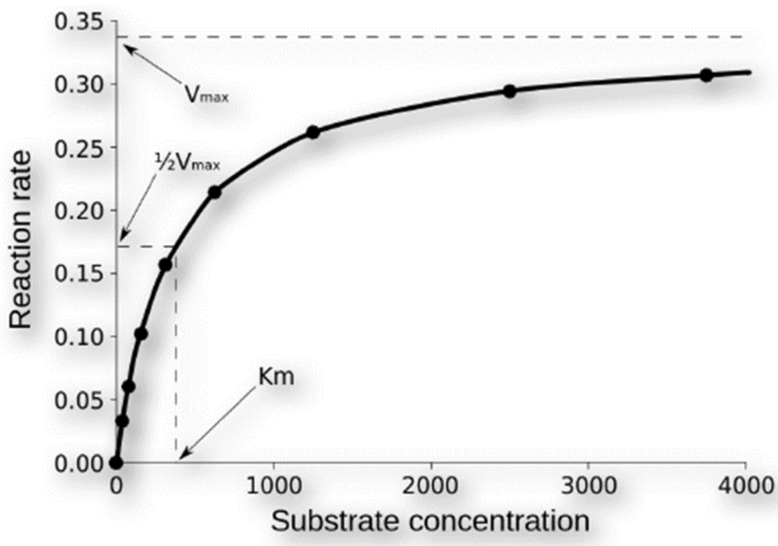



Source: Biochemistry: Free For All, 2018. Kevin Ahern, Indira Rajagopal, and Taralyn Tan, Oregon State University. https://open.umn.edu/opentextbooks/textbooks/866.

*Figure 3.* Change in concentration of reaction materials over time.

### *3.3. The Michaelis-Menten Equation*

In Figure 4, the typical outcome illustrates the relationship between the initial velocity of the reaction and substrate concentration ([S]). The initial step involves a bimolecular interaction where the enzyme and substrate combine to form an ES complex. At high substrate concentrations (the right side of the curve in the figure), the initial velocity remains relatively constant despite adding more substrate, indicating enzyme saturation. The reaction's initial rate shows little change when substrate concentration is further increased, suggesting that the enzyme, present in a lower concentration than the substrate, has become the limiting factor. Conversely, at low substrate concentrations (the left side of the curve in the figure), the curve approximates a steeply rising straight line. In this region, the initial velocity is highly dependent on substrate concentration, as most enzyme molecules have yet to bind substrate and the formation of the ES complex hinges on substrate concentration.




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

*Figure 4.* Change in initial reaction rate materials over substrate concentration.

The comprehensive rate equation, known as the *Michaelis–Menten equation*, defines the connection between the initial velocity of a reaction and substrate concentration. As shown in the figure, the Michaelis constant (Km) represents the substrate concentration at half-maximum velocity. The enzyme is half-saturated when [S] equals Km.

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

Now, let's derive the Michaelis–Menten equation, utilizing the steady-state derivation proposed by Briggs and Haldane. This approach posits a steady state during which the rate of ES complex formation equals its decomposition rate, maintaining a constant ES concentration. The initial velocity is used in this derivation, assuming a negligible product concentration ([P]). The steady-state derivation involves solving Equation 1 for [ES] using measurable terms such as the rate constant ($k_2$), total enzyme concentration ([E]total), and substrate concentration ([S]). Assuming [S] is greater than [E]total but not necessarily saturating, the concentration of [ES] becomes constant soon after mixing a small amount of enzyme with the substrate until [S] approaches [E]total. Equation 2 expresses these dynamics mathematically. By rearranging Equation 2 to collect rate constants, we obtain Equation 3, where the ratio of rate constants on the left-hand side is the Michaelis constant.

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

$$ES \overset{k_2}{\rightarrow} E + P \qquad V_0 = k_2[ES]$$ *(Eq 1)* *Rate-limiting step*

$v_f = k_1 [E][S]$

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

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

↓

Rate of ES formation = Rate of ES decomposition

*Steady-State Approximation*

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

↓ *Rearrangement of Eq 2*

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

The derivation proceeds through several steps (Expanding Eq. 3, collecting [ES] terms, and rearranging), resulting in Equation 6, which describes the steady-state [ES] concentration in terms that can be experimentally measured. Substituting this [ES] value into the velocity equation (Equation 1) yields Equation 7.

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

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

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

$$[ES] = \frac{[E]_{total}[S]}{K_m + [S]}$$ *(Eq 6)* *Steady-State Concentration*

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

When substrate concentration is high, leading to enzyme saturation, adding more substrate minimally affects the reaction velocity. The only way to increase velocity is by adding more enzyme. Equation 8, defined as the maximum velocity under these conditions, is then substituted into Equation 7, resulting in the familiar form of the Michaelis–Menten equation (Eq. 9). This form of the Michaelis–Menten equation adequately describes data from kinetic experiments.

$$V_{max} = k_2[E]_{total}$$ *(Eq 8)* ***Maximum rate****: the concentration of S is very high, the molecules of E are present as ES*

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$ *(Eq 9)* ***Michaelis-Menten Equation***

At elevated substrate concentrations, the overall velocity of the reaction reaches its maximum, denoted as $V_{max}$, and the rate becomes dictated by the enzyme concentration. The rate constant observed under these saturated conditions is termed the *catalytic constant*, $k_{cat}$, defined as the number of moles of substrate converted to product per second per mole of enzyme (or per mole of active site for a multisubunit enzyme).

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

In simpler terms, $k_{cat}$ signifies the maximum number of substrate molecules transformed into product by each active site per second, often referred to as the *turnover number*. This constant is a quantitative measure of how rapidly a specific enzyme can catalyze a given reaction, representing the number of reactions per second that a single enzyme active site can catalyze. The unit for $k_{cat}$ is $s^{-1}$, and its reciprocal represents the time required for one catalytic event. It's crucial to note that knowledge of enzyme concentration is imperative to determine $k_{cat}$.

In the case of a straightforward reaction, the limiting step is the conversion of ES to yield P + E, and $k_2$ equals $k_{cat}$. However, many enzyme reactions exhibit more complexity, and if one step stands out as the rate-limiting one, its rate constant becomes the $k_{cat}$ for that reaction. In scenarios where the mechanism is intricate, $k_{cat}$ may involve a combination of various rate constants. This underscores the need for a distinct rate constant, $k_{cat}$, to describe the overall rate of enzyme-catalyzed reactions, although, in most cases, $k_{cat}$ approximates $k_2$ effectively. Enzymes, often potent catalysts, typically possess $k_{cat}$ values ranging from $10^2$ to $10^3$ $s^{-1}$. Some enzymes exhibit extraordinary catalytic prowess with $k_{cat}$ values reaching $10^6$ $s^{-1}$ or even higher.

*Meanings of Michaelis constant*

The Michaelis constant, $K_m$, carries multiple meanings:

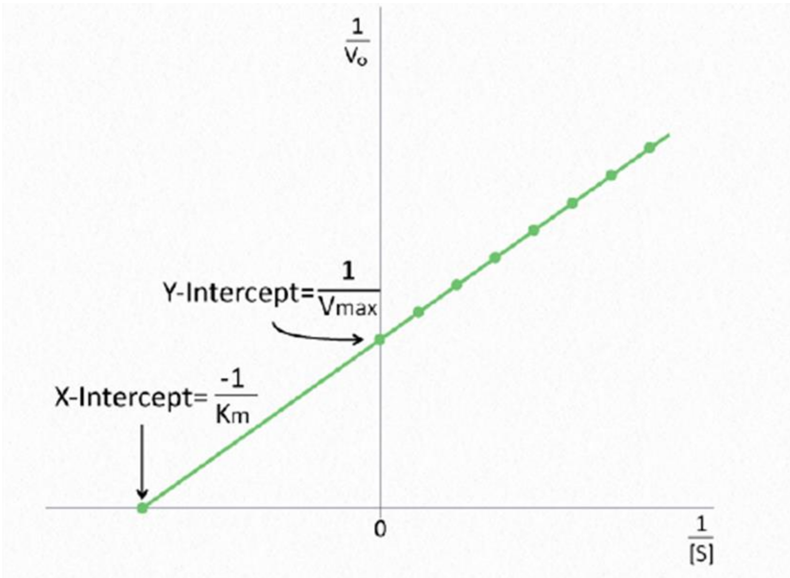
- When the rate constant for product formation ($k_2$) is considerably smaller than $k_1$ or $k_{-1}$, $K_m$ approximates $k_{-1}/k_1$. In this scenario, $K_m$ represents the equilibrium constant for the dissociation of the ES complex to E + S.
- $K_m$ serves as a measure of the enzyme's affinity for the substrate. A lower $K_m$ value indicates a tighter binding of the substrate.
- Additionally, $K_m$ is one of the parameters influencing the shape of the $v_0$ vs. [S] curve, representing the substrate concentration at which the initial velocity is half the $V_{max}$ value.

*Lineweaver-Burk Equation. Measurement of Kinetic Parameters*

$K_m$ values can be employed to differentiate between different enzymes catalyzing the same reaction. To obtain reliable kinetic constants, the substrate concentration ([S]) points must be spread both below and above $K_m$ to generate a hyperbolic curve. Determining $K_m$ or $V_{max}$ directly from a graph of initial velocity versus concentration is challenging due to the asymptotic approach of the curve.

The Michaelis–Menten equation can be reformulated to derive values for $V_{max}$ and $K_m$ from straight lines on graphs (Figure 5). The commonly used transformation is the double-reciprocal, or Lineweaver–Burk plot, where the values of $1/v_0$ are plotted against 1/[S]. The absolute value of $1/K_m$ is obtained from the intercept of the line at the x-axis, while the value of $1/V_{max}$ is obtained from the y-intercept. Although double-reciprocal plots are not the most accurate methods for determining kinetic constants, they offer a comprehensible approach and present recognizable patterns for studying enzyme inhibition—an essential aspect of enzymology.

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




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

*Figure 5.* Double-reciprocal (Lineweaver-Burk) plot.

## 4. INHIBITION MECHANISMS

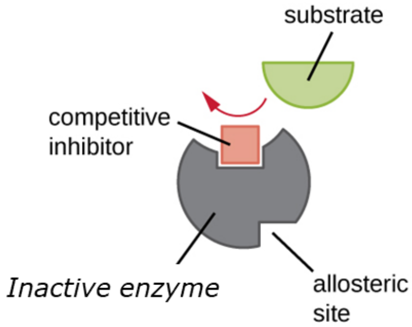
An enzyme inhibitor (I) is a compound that binds to an enzyme, disrupting its activity. Inhibition can occur by impeding the formation of the ES complex or obstructing the chemical reaction that forms the product. Inhibitors are typically small molecules that bind reversibly to the enzyme they affect. Natural enzyme inhibitors in cells play crucial roles in metabolic regulation. Artificial inhibitors are employed in experiments to explore enzyme mechanisms and unravel metabolic pathways. Enzyme inhibitors are also found in certain drugs and numerous poisons.

While some inhibitors form covalent bonds with enzymes, causing irreversible inhibition, most biologically relevant inhibition is reversible. Reversible inhibitors attach to enzymes through the same non-covalent forces as substrates and products. Their distinction from irreversible inhibitors lies in their easy removal from enzyme solutions, achieved through dialysis or gel filtration. The equilibrium between free enzyme (E) and inhibitor (I) and the EI complex is characterized by a dissociation constant ($k_i$), referred to as the inhibition constant.

Reversible inhibition comes in basic types: competitive, uncompetitive, and noncompetitive. The experimental distinction of these types is based on their observable effects on the kinetic behavior of enzymes.

### *4.1. Competitive Inhibition*

In competitive inhibition, the inhibitor selectively binds to free enzyme molecules not engaged with any substrate (Figure 6). In this scenario, only the ES complex can proceed to form the product, and the formation of an EI complex redirects the enzyme from its normal pathway.




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

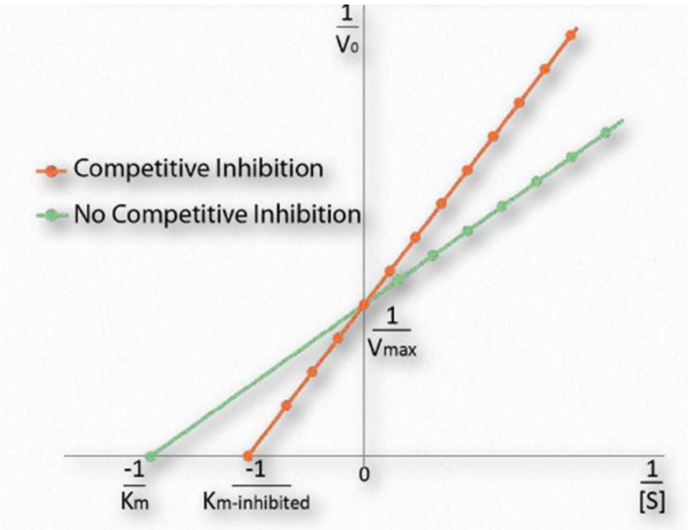
$$or\ E + I \overset{k_i}{\rightleftarrows} EI$$

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

*Figure 6.* Enzyme and inhibitor compete for the same substrate.

When a competitive inhibitor is bound to an enzyme, the corresponding substrate molecule cannot bind to that enzyme. Conversely, the binding of a substrate to an enzyme prevents the binding of an inhibitor. Essentially, substrate (S) and inhibitor (I) compete for binding to the enzyme molecule, often occupying the same site on the enzyme, known as the active site. Increasing the concentration of substrate (S) diminishes the amount of EI complex. At sufficiently high substrate concentrations, the enzyme can still become saturated, resulting in the same maximum velocity in the presence or absence of an inhibitor.

It has been demonstrated that the concentration of substrate at half-saturation is $K_m$. Consequently, in the presence of escalating concentrations of a competitive inhibitor, $K_m$ increases. The greater the presence of the competitive inhibitor, the more substrate is required for half-saturation, denoted as the apparent $K_{mapp}$. On a double-reciprocal plot (Figure 7), the addition of a competitive inhibitor manifests as a decrease in the absolute value of the x-axis intercept ($1/K_m$), while the y-intercept ($/V_{max}$) remains constant.

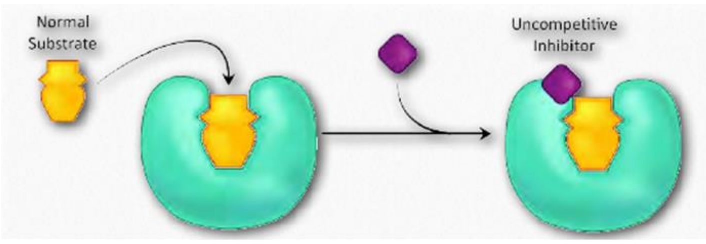



Source: Biochemistry: Free For All, 2018. Kevin Ahern, Indira Rajagopal, and Taralyn Tan, Oregon State University. https://open.umn.edu/opentextbooks/textbooks/866.

*Figure 7.* Double reciprocal plot for competitive inhibition.

### *4.2. Uncompetitive Inhibition*

Uncompetitive inhibitors exclusively bind to the ES complex and do not associate with free enzyme molecules. The inhibitor can bind to either the enzyme (E) or the enzyme-substrate complex (ES), rendering the enzyme inactive upon binding (Figure 8). Even though the EI complex can still interact with the substrate (S), no product is generated in this state.




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

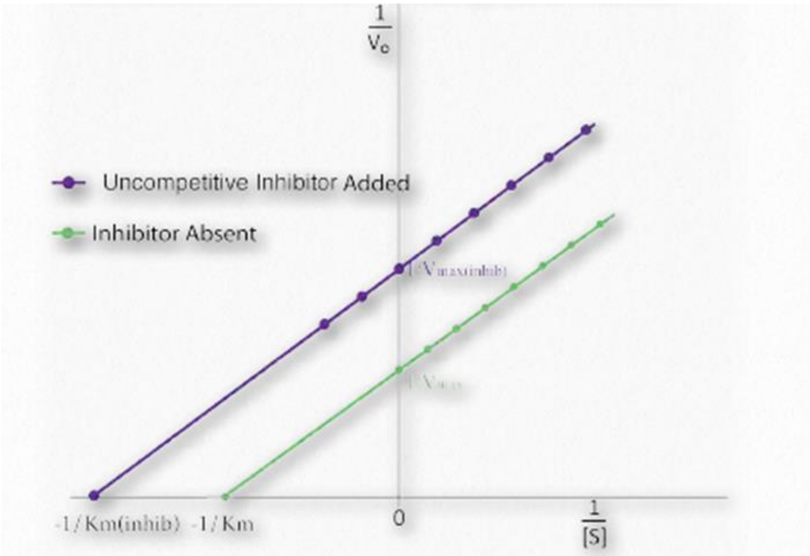
$$or\ ES + I \overset{k_i}{\rightleftarrows} ESI$$

*Inactive enzyme*

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

*Figure 8.* Uncompetitive inhibitor binding only to ES, not to free enzyme.

In uncompetitive inhibition, the maximum reaction velocity ($V_{max}$) is reduced (1/$V_{max}$ is increased) due to the conversion of some enzyme molecules (E) to the inactive form ESI. Since the inhibitor (I) binds to the ES complex, adding more substrate does not alleviate the decrease in $V_{max}$. Uncompetitive inhibitors also diminish the $K_m$ value because the equilibrium for both ES and ESI formation shifts towards the complexes upon inhibitor binding. Experimentally, the lines on a double-reciprocal plot depicting varying concentrations of an uncompetitive inhibitor exhibit the same slope, indicating proportionally decreased values for $K_m$ and $V_{max}$ (Figure 9). This type of inhibition typically arises in multisubstrate reactions.

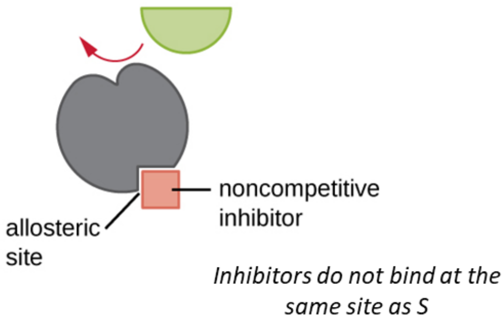



Source: Biochemistry: Free For All, 2018. Kevin Ahern, Indira Rajagopal, and Taralyn Tan, Oregon State University. https://open.umn.edu/opentextbooks/textbooks/866.

*Figure 9.* Double reciprocal plot for uncompetitive inhibition.

### *4.3. Noncompetitive Inhibition*

Noncompetitive inhibitors can bind to either free enzyme (E) or the enzyme-substrate complex (ES), forming inactive EI or ESI complexes, respectively. Unlike substrate analogs, these inhibitors do not bind at the same site as the substrate (S) (Figure 10).




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

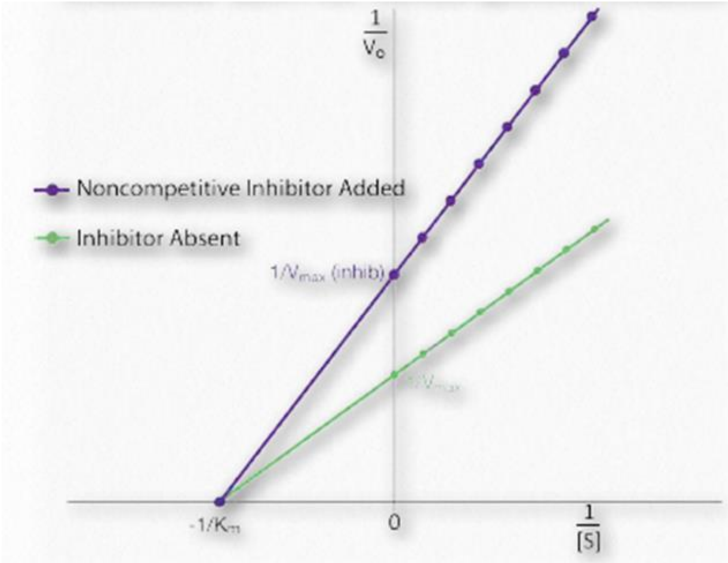
$$or\ E + I \overset{k_i}{\rightleftarrows} EI + S \rightleftarrows ESI$$

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

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

*Figure 10.* Inhibitor binding to E or ES, forming inactive EI or ESI complexes.

In the classic scenario of noncompetitive inhibition, there is an apparent reduction in $V_{max}$ ($1/V_{max}$ seems to increase) without any alteration in $K_m$. On a double-reciprocal plot (Figure 11), the lines representing classic noncompetitive inhibition intersect at the x-axis point corresponding to $-1/K_m$. This common x-axis intercept indicates an unaffected $K_m$. The impact of noncompetitive inhibition is the reversible titration of active enzyme molecules (E and ES) with the inhibitor (I), essentially removing them from the solution. Notably, this inhibition cannot be overcome by adding substrate (S). Although classic noncompetitive inhibition is rare, instances are known among allosteric enzymes. In these cases, the noncompetitive inhibitor likely induces a conformational change in the enzyme, allowing it to bind S but preventing it from catalyzing any reaction.




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

*Figure 11.* Double reciprocal plot for noncompetitive inhibition.