CHAPTER-1

Introduction

1. Introduction

Enzymes are indispensable biocatalysts that govern virtually every biochemical reaction essential to life.[1] Enzymes function with remarkable specificity and efficiency in all living organisms, facilitating metabolic transformations, regulatory pathways, and signalling cascades under mild physiological conditions. The study of enzymes, or enzymology, not only deepens our understanding of biological systems but also provides foundational tools for numerous applications across biotechnology, medicine, industrial chemistry, and environmental science.[2] As catalysts, enzymes lower the activation energy of chemical reactions, thereby accelerating reaction rates by factors ranging from 10^6 to 10^{12} compared to their uncatalyzed counterparts.[3] The catalytic power, selectivity, and mild operational conditions of enzymes underscore their superiority over traditional chemical catalysts, aligning well with the principles of green chemistry. Accordingly, enzymes are increasingly exploited in synthetic biology, pharmaceutical production, food processing, biofuel generation, and waste management, among other sectors.

1.1 Definition of Enzymes: An enzyme is a biological macromolecule—typically a protein, though some RNA molecules also exhibit catalytic activity (ribozymes)—that accelerates the rate of a specific biochemical reaction without being consumed in the process. Enzymes act by stabilizing the transition state of a reaction, thereby reducing its activation energy and increasing the rate at which equilibrium is attained.[4]

The classical definition proposed by J.B. Sumner in 1926, following the crystallization of urease, established that enzymes are proteins with specific catalytic functions. This understanding was later expanded with the discovery of catalytic RNAs, highlighting that catalytic activity is not limited to proteins.[5] Regardless of their molecular nature, enzymes remain highly specific to their substrates and operate under tightly regulated temperature, pH, and ionic strength conditions.[6,7]

1.2 Naming Conventions: Enzymes are systematically named by the International Union of Biochemistry and Molecular Biology (IUBMB) based on the type of reaction they catalyze and the nature of their substrate. Each enzyme is assigned a unique Enzyme Commission (EC) number that provides a hierarchical classification across four levels:

- **First number**: General class of reaction (e.g., oxidoreductase, transferase)
- **Second number**: Type of substrate or functional group involved

- **Third number**: Nature of the acceptor or cofactor
- **Fourth number**: Serial identifier for the specific enzyme

For example, lactate dehydrogenase is designated as EC 1.1.1.27, where EC 1 denotes an oxidoreductase, and the subsequent numbers describe the specific reaction parameters.[8] In practice, enzymes are also named using a combination of substrate and reaction type, often ending with the suffix "-ase," such as lipase, polymerase, or protease. However, historical and trivial names, such as trypsin, pepsin, or catalase, are still widely used and accepted.[9,10]

1.3 Role of Enzymes in Nature: Enzymes are fundamental to sustaining life, serving as biological catalysts that accelerate chemical reactions in living organisms. They are essential for a variety of complex biochemical processes, enabling them to occur swiftly and efficiently at relatively low temperatures. Each enzyme is highly specific, typically acting on a particular substrate to yield a specific product. In nature, enzymes are integral to crucial functions such as digestion, energy production, DNA replication, and the detoxification of harmful substances. Without enzymes, the essential chemical reactions required for life would proceed at a pace too slow to sustain biological processes, rendering them indispensable to all life forms. Table 1 presents an overview of the six major classes of enzymes, emphasizing their unique catalytic functions and biological importance. These classes include oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases, each playing a vital role in facilitating and regulating the biochemical transformations necessary for life. Among these, oxidoreductases are the most widely studied and applied due to their remarkable ability to catalyze oxidation-reduction (redox) reactions. These enzymes mediate electron transfer between molecules, a fundamental process that underpins various metabolic pathways. Their redox capabilities make oxidoreductases particularly valuable in numerous biological and chemical contexts, including cellular respiration, detoxification, biosensing, and synthetic chemistry. Consequently,

Table 1: Types of Enzymes and Their Biological Role.

S. No.	Enzyme	Biological Role	Example
1	Oxidoreductase Enzymes	Defense against Oxidative Stress	Peroxidase
		Involved in plant defense mechanisms	Laccase
		Involved in plant defense Mechanisms and Enzymatic Browning of plants	Catechol Oxidase
		An antioxidant enzyme	Superoxide dismutase
2	Transferases	Atom/Group transfer	Acetate Kinase, Alanine deaminase, Transaminase
3	Hydrolases	Hydrolysis	Lipase
4	Lyases	Group removal	Oxalate decarboxylase, Isocitrate lyase
5	Isomerases	Isomerization	Glucose phosphate isomerase, alanine racemase
6	Ligase	Joining of molecules linked to the breakage of a pyrophosphate bond	Acetyl-CoA synthetase, DNA ligase

oxidoreductases have emerged as powerful biocatalysts, finding applications in both natural ecosystems and engineered systems for purposes ranging from environmental remediation to the development of advanced therapeutic and diagnostic tools.

Oxidoreductase Enzymes: Chemistry and Applications

Oxidoreductase enzymes are a versatile group of biocatalysts that facilitate redox reactions, playing vital roles in both biological systems and industrial applications. These enzymes are integral to various metabolic pathways, including glycolysis, the Krebs cycle, and oxidative phosphorylation. They are crucial in processes such as drug detoxification and photosynthesis. Their capacity to catalyze reactions under mild conditions makes oxidoreductases especially attractive for organic synthesis, offering more direct and environmentally friendly routes to target products compared to traditional chemical methods. Oxidoreductases encompass a variety of enzymes, including oxidases, dehydrogenases, peroxidases, and oxygenases. Each type exhibits specific substrate affinities and distinct catalytic mechanisms.[11] These enzymes facilitate a range of reactions, such as the insertion of oxygen, transfer of hydride ions, and extraction of protons, often utilizing cofactors such as NAD, FAD, or NADP.[12]

The utility of oxidoreductases extends to bioconversion, bioremediation, and the development of biosensors, largely due to their efficiency and specificity.[11] In organic synthesis, these enzymes enable the production of optically pure compounds, such as epoxides and alcohols, through stereoselective transformations. Many oxidoreductases contain transition metal ions that function as active sites and centers for electron transfer. For instance, copper present in nitrite reductase and molybdenum found in aldehyde oxidoreductase both facilitate substrate conversion and electron exchange.[13] The electron transfer pathways within these enzymes are complex and can be studied through molecular biology and spectroscopic techniques. Among the diverse group of oxidoreductase enzymes, peroxidases (POD) and oxidases, such as laccase and catalase, are well-studied due to their established crystal structures and catalytic mechanisms. The structure and chemistry of these enzymes are briefly discussed in the following sections.

1.4 Peroxidase (POD)

Peroxidases (EC 1.11.1.7) constitute a diverse and widespread group of heme-containing oxidoreductase enzymes that catalyze the reduction of hydrogen peroxide (H_2O_2) or organic peroxides by utilizing a wide range of electron-donating substrates. These enzymes play an essential role in various physiological and biochemical processes across different kingdoms of life, including detoxification, lignin degradation, wound healing, defense responses, and hormone metabolism.[14] The remarkable substrate versatility and oxidative capabilities of peroxidases have spurred their application in industrial, environmental, and biotechnological

processes, particularly those aligned with green chemistry. Among the best-studied members of this group are horseradish peroxidase (HRP) from *Armoracia rusticana*, lignin peroxidase from *Phanerochaete chrysosporium*, and myeloperoxidase from mammalian neutrophils. Each peroxidase possesses distinctive properties and physiological functions but operates through a common mechanistic paradigm based on the activation of peroxides and transfer of oxygen atoms to substrates. In this context, the investigation of peroxidases not only enhances our understanding of redox biology but also provides foundational tools for the design of bioinspired catalysts, biosensors, and bioremediation technologies.

1.4.1 Structure and Nature of POD:

Peroxidases are primarily characterized by the presence of a prosthetic heme group (iron protoporphyrin IX), which is responsible for their redox activity. The heme iron is coordinated typically via a histidine residue on the proximal side and often modulated by distal amino acids such as arginine and histidine that facilitate peroxide binding and activation.[15]

Structurally, plant peroxidases such as HRP consist of a single polypeptide chain (~300 amino acids), a heme cofactor, and several disulfide bridges that contribute to thermal and pH stability. Fungal peroxidases, particularly lignin and manganese peroxidases, contain additional domains and glycosylation sites that enhance their extracellular activity and environmental resilience.[16] Mammalian peroxidases, such as myeloperoxidase (MPO) and eosinophil peroxidase (EPO), are larger glycoproteins containing covalently linked heme and are specialized for immune defense.

Based on structural and functional similarities, peroxidases are broadly categorized into three classes:

- **Class I**: Intracellular peroxidases (e.g., cytochrome c peroxidase)
- **Class II**: Secretory fungal peroxidases (e.g., lignin peroxidase, manganese peroxidase)
- **Class III**: Secretory plant peroxidases (e.g., HRP)

These structural classes reflect both the evolutionary divergence and functional adaptations of peroxidases in different biological contexts.

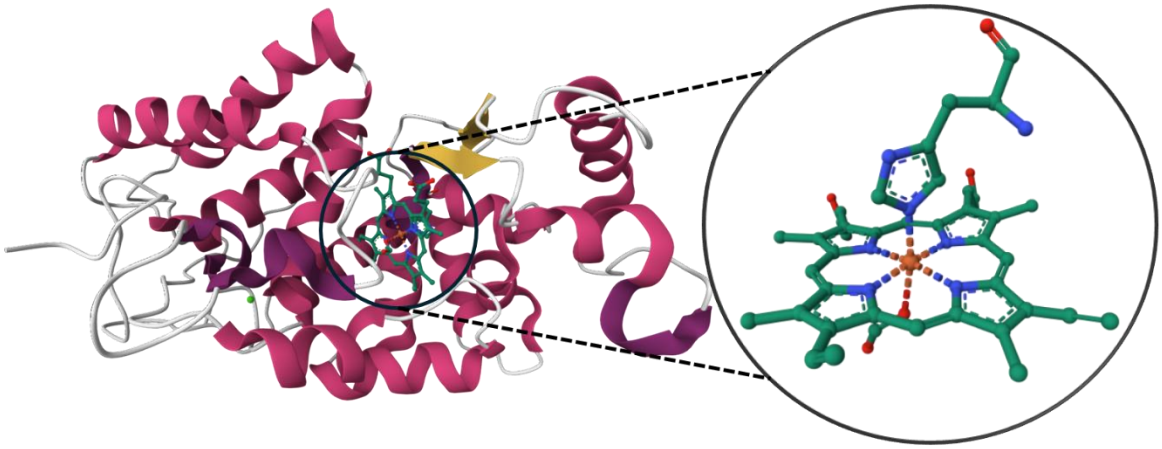

Figure 1. Crystal structure of horseradish peroxidase (PDB: 1W4W) and its catalytic active site.[17]

1.4.2 POD Catalytic Reaction and Mechanism of Action

The general catalytic reaction catalyzed by peroxidases involves the oxidation of an organic substrate (AH) using hydrogen peroxide as the oxidant:

$$\mathbf{H_2O_2 + 2\ AH \rightarrow 2\ H_2O + 2\ A}$$

In this reaction:

- H_2O_2 serves as the oxidizing agent.

- AH represents the reducing substrate, which may include compounds such as phenols and aromatic amines etc.

- A denotes the oxidized product, which is often characterized by its color or fluorescence.

The reaction proceeds through a well-characterized, three-stage catalytic cycle involving the formation of transient redox intermediates:

1. Resting state enzyme (Fe^{3+}) reacts with H_2O_2 to form Compound I, an oxo-ferryl species (Fe^{4+}=O) with a porphyrin π-cation radical.
2. Compound I oxidizes one molecule of the reducing substrate (AH_2) to generate Compound II (Fe^{4+}=O).
3. Compound II undergoes a second one-electron reduction by another AH_2 molecule to regenerate the resting enzyme (Fe^{3+}).

This mechanism allows peroxidases to facilitate the oxidation of a wide range of phenolic, aromatic, and inorganic substrates under mild conditions, making them highly attractive for green catalytic applications.[14,18]

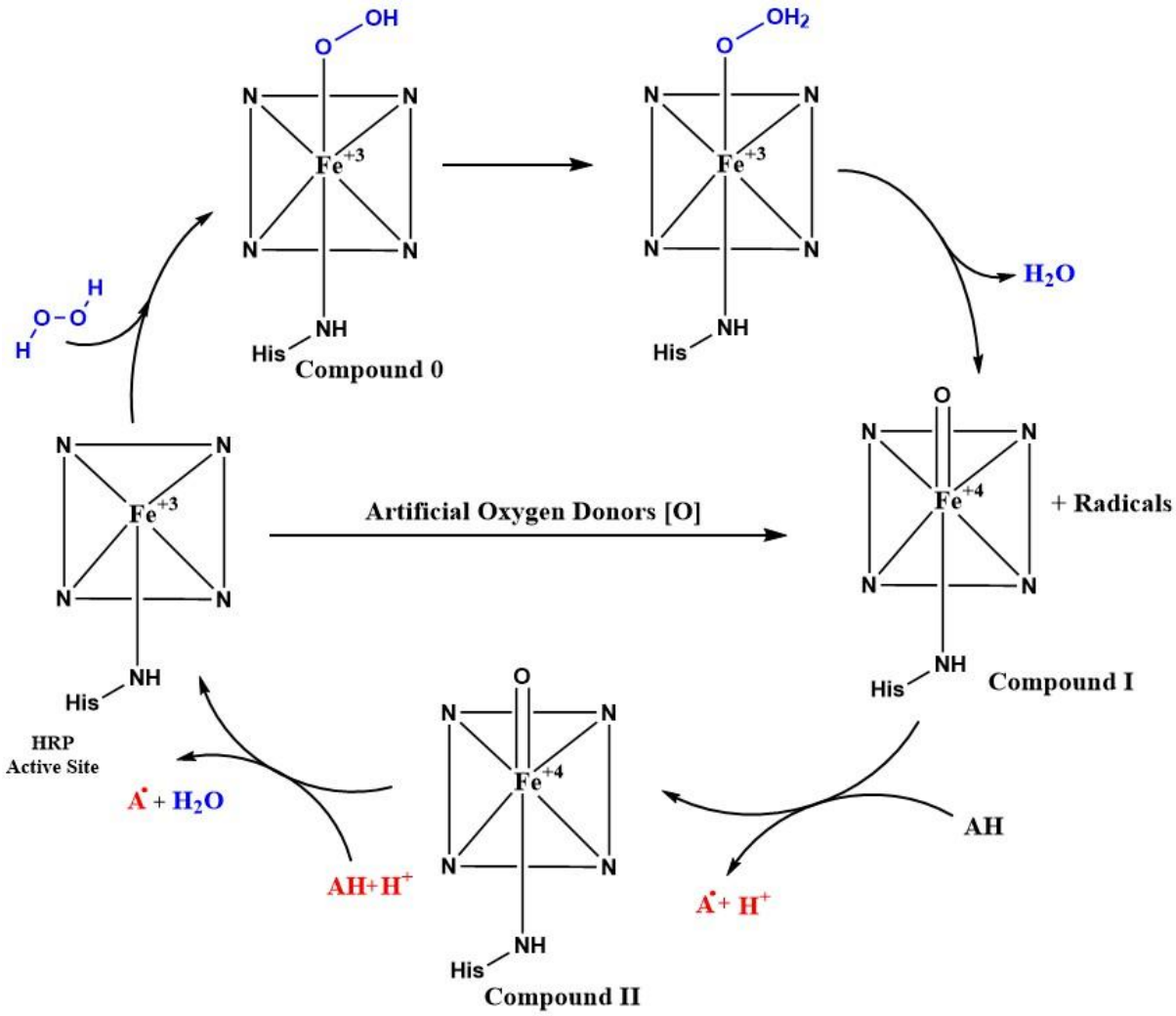

Figure 2. Well reported Proposed catalytic cycle of catechol oxidase.[18]

1.4.3 Biocatalytic Kinetics and Colorimetric Assay Methods of POD

The analysis of peroxidase kinetics is critical for deciphering the mechanism of action, optimizing reaction conditions, and enabling rational design of bioassays and biosensors. Classical Michaelis–Menten kinetic models have long been applied to characterize peroxidase-catalyzed transformations, yet their catalytic behavior often exhibits deviations due to substrate inhibition, enzyme inactivation, or multiphasic kinetics under high peroxide concentrations. This necessitates the application of more refined kinetic models and multi-substrate systems.[19] Complementing kinetic studies, colorimetric assay methods using chromogenic substrates like

ABTS, TMB, or o-phenylenediamine have become standard in enzyme kinetics, immunoassays, and high-throughput screening.[20]

Classical Michaelis–Menten Kinetics: The catalytic mechanism of peroxidases, particularly horseradish peroxidase (HRP), involves a two-substrate (ping-pong) reaction scheme. In its simplest form, the steady-state kinetics of peroxidases are modeled using the Michaelis–Menten equation:

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

where ν is the reaction rate, V_{max} is the maximal rate, K_m is the Michaelis constant, and [S] is the substrate concentration. However, due to the bi-substrate nature of the peroxidase reaction—typically involving H_2O_2 as an oxidant and a chromogenic electron donor (AH) as a reducing substrate—a more accurate model is the bi-substrate ping-pong mechanism.[19]

$E + H_2O_2 \rightarrow$ Compound I + H_2O

Compound I + AH → Compound II + $A^{\cdot}$

Compound II + AH → E + $A^{\cdot}$ + H_2O

Here, Compound I and Compound II are high-valent redox intermediates, characteristic of peroxidase catalysis. The formation and turnover of these intermediates are often rate-limiting and can be influenced by both substrate identity and reaction conditions.

Deviations and Advanced Models: Under certain experimental conditions—such as high concentrations of hydrogen peroxide—HRP exhibits substrate inhibition or enzyme inactivation, which deviates from the classical kinetics. Excess H_2O_2 can oxidize the enzyme irreversibly to inactive forms (Compound III or ferryl radicals), necessitating the use of modified kinetic models. To account for such complexities, non-Michaelis–Menten models (e.g., Hill equation, cooperative models, or inhibitory kinetics) are applied. Some peroxidase systems also exhibit sigmoidal kinetics, suggesting allosteric behavior or multi-enzyme interactions under in vitro or immobilized conditions.[20]

In addition to kinetic constants (K_m, V_{max}), mechanistic parameters like turnover number (kcat) and catalytic efficiency (k_{cat}/K_m) are key descriptors for comparing catalytic performance across natural, engineered, and nanozyme systems.

Colorimetric Assay Methods: Colorimetric assays are among the most widely used techniques for evaluating peroxidase activity due to their simplicity, sensitivity, and adaptability to high-throughput formats. These assays rely on the oxidation of chromogenic substrates to produce colored or fluorescent products, which are easily quantifiable using spectrophotometry.

Common Chromogenic Substrates: Several chromogenic substrates are routinely employed for peroxidase assays:

1. **3,3′,5,5′-Tetramethylbenzidine (TMB)**: Colorless substrate oxidized to a blue chromophore ($\lambda_{max} \approx 650$ nm), then yellow upon acidification ($\lambda_{max} \approx 450$ nm). Common in ELISA.
2. **2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)**: Forms a green radical cation ($\lambda_{max} \approx 414$ nm). Soluble in aqueous buffers and suitable for continuous assays.
3. **o-Phenylenediamine (OPD)**: Yields a yellow-orange product ($\lambda_{max} \approx 492$ nm). Frequently used in immunoenzymatic assays.
4. **Guaiacol or Pyrogallol**: Simple phenolic substrates forming brown-colored oxidized products. Often used in classical enzymology and plant peroxidase activity assays.

The choice of substrate depends on the application, sensitivity requirement, pH stability, and interference susceptibility. ABTS and TMB are favored in biosensor development and microplate assays due to their rapid and stable color development.

Assay Conditions and Optimization: The performance of colorimetric assays depends on several factors:

- **pH and temperature**: Optimal activity of HRP is generally observed around pH 6.0–7.0 and 25–37°C. However, thermal or pH stability may vary based on enzyme source and modification.
- **Buffer composition**: Phosphate or citrate buffers are typically used to maintain consistent pH and ionic strength.
- **H_2O_2 concentration**: Excess peroxide may lead to enzyme inactivation; hence, substrate concentrations must be optimized empirically.

- **Reaction time**: Initial rates are measured within the linear range of product formation (typically 5–10 min) to avoid substrate depletion or side reactions.

Quantitative assays are often conducted by monitoring absorbance changes (ΔA/min) at the relevant wavelength using UV–Vis spectrophotometry or microplate readers. Enzyme activity is expressed in Units (U), defined as the amount of enzyme catalyzing the transformation of 1 μmol of substrate per minute under specified conditions. Understanding the biocatalytic kinetics and assay methodologies of peroxidase enzymes is foundational to their rational utilization in both scientific and industrial applications. While the classical Michaelis–Menten model offers valuable insights into enzyme–substrate interactions, the dynamic nature of peroxidase catalysis under various oxidative regimes necessitates more nuanced modeling approaches. Simultaneously, the development and refinement of colorimetric assays have empowered researchers to explore peroxidase function with high sensitivity, throughput, and adaptability. The synergy of kinetic modeling and robust assay design continues to drive innovation in enzyme engineering, diagnostics, and sustainable catalysis, making peroxidase

1.4.4 Biological Significance of POD

Peroxidases are integral to diverse physiological functions. In plants, Class III peroxidases are involved in lignin biosynthesis, auxin catabolism, defense against pathogens, and oxidative stress regulation. The generation of reactive oxygen species (ROS) and oxidative cross-linking of cell wall proteins by peroxidases strengthens plant tissues and imparts resistance to environmental and microbial stresses.[21] In fungi, Class II peroxidases such as lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP) participate in the oxidative depolymerization of lignin, enabling the breakdown of complex plant biomass and facilitating carbon cycling in ecosystems.[22] In mammals, peroxidases such as myeloperoxidase (MPO), lactoperoxidase (LPO), and eosinophil peroxidase (EPO) play critical roles in innate immunity. MPO, for instance, catalyzes the production of hypochlorous acid (HOCl) from chloride ions and H_2O_2, contributing to microbial killing by neutrophils.[23]

The broad biological distribution and functional diversity of peroxidases underscore their evolutionary importance and multifaceted biochemical utility.

1.4.5 Industrial and Biotechnological Relevance

The high specificity and oxidative capacity of peroxidases have led to their deployment in numerous industrial and environmental processes. Among the most notable applications are:

- **Bioremediation and Environmental Detoxification**: Peroxidases are effective in the degradation of phenolic pollutants, endocrine disruptors, dyes, and aromatic hydrocarbons. Lignin peroxidase and HRP have been used to remove phenols from industrial effluents, demonstrating high efficiency and eco-friendliness.[24]
- **Analytical and Diagnostic Biosensors**: HRP is widely employed in enzyme-linked immunosorbent assays (ELISA), biosensors, and chemiluminescence-based diagnostics due to its ability to generate colored or luminescent products in the presence of substrates like tetramethylbenzidine (TMB) or luminol.[20]
- **Wastewater Treatment**: Immobilized peroxidases are utilized in the treatment of industrial wastewater to remove color and toxicity by catalyzing oxidative polymerization and precipitation of dyes and phenolic compounds.[25]
- **Synthetic Chemistry and Biocatalysis**: Peroxidases are used in stereoselective oxidation reactions, oxidative coupling, and epoxidation, serving as green alternatives to harsh chemical oxidants. Their ability to catalyze the formation of complex molecules from simple precursors makes them valuable in pharmaceutical synthesis and fine chemical manufacturing.[26]
- **Nanobiotechnology**: Recent studies have demonstrated the integration of peroxidases with nanomaterials to create hybrid biocatalytic systems with enhanced stability, activity, and reusability. Additionally, nanozymes mimicking peroxidase activity are being developed for biomedical diagnostics and therapeutics.[27]

Hence, peroxidases represent a functionally versatile and structurally diverse class of enzymes that play pivotal roles in biological redox processes and offer promising applications in modern biotechnology. Their unique ability to catalyze oxidative reactions under ambient conditions, coupled with their substrate adaptability and environmental compatibility, positions them as indispensable tools in industrial catalysis, environmental remediation, and biosensing. With continued advances in enzyme engineering, immobilization strategies, and nanotechnology, peroxidase-based systems are poised to contribute significantly to sustainable technologies and bioinspired innovations in the coming decades.

1.5 Laccase Enzyme

Laccases (EC 1.10.3.2) are multicopper oxidases capable of oxidizing a broad range of phenolic and non-phenolic substrates while concomitantly reducing molecular oxygen to water. First discovered in the Japanese lacquer tree *Rhus vernicifera* over a century ago,

laccases have since been identified in fungi, plants, insects, and bacteria. Their ecological ubiquity and broad substrate specificity have rendered them a subject of sustained research interest in biochemistry, enzymology, and green biotechnology.

1.5.1 Structure and Nature of Laccase

Laccases are glycoproteins typically ranging from 50 to 130 kDa, composed of three conserved cupredoxin-like domains that house four copper atoms categorized into three types: Type 1 (T1), Type 2 (T2), and a pair forming a Type 3 (T3) site.[28] The T1 site accepts electrons from the substrate, while the T2/T3 trinuclear copper cluster facilitates the four-electron reduction of dioxygen to water. The blue color of many laccases arises from the intense absorbance at ~610 nm attributed to the T1 copper center. Fungal laccases, particularly from Trametes versicolor and Pleurotus ostreatus, are the most extensively studied due to their high redox potential and industrial relevance. Bacterial laccases, although often less redox-efficient, exhibit superior thermostability and tolerance to alkaline pH and inhibitors.[29]

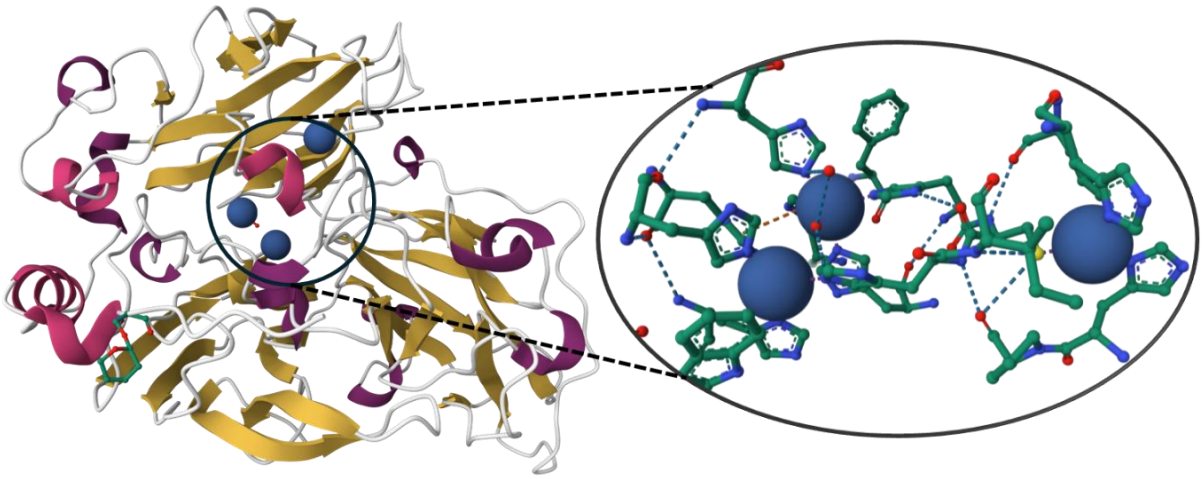

Figure 3. Crystal structure of laccase from coprinus cinereus (PDB: 1A65) and its catalytic active site.[30]

1.5.2 Catalytic Reaction and Mechanism

Laccases catalyze one-electron oxidation reactions, coupling the four-electron reduction of molecular oxygen to water. The general catalytic mechanism involves the abstraction of one electron from the reducing substrate at the T1 site, followed by intramolecular electron transfer to the T2/T3 trinuclear cluster, where oxygen is ultimately reduced.[31]

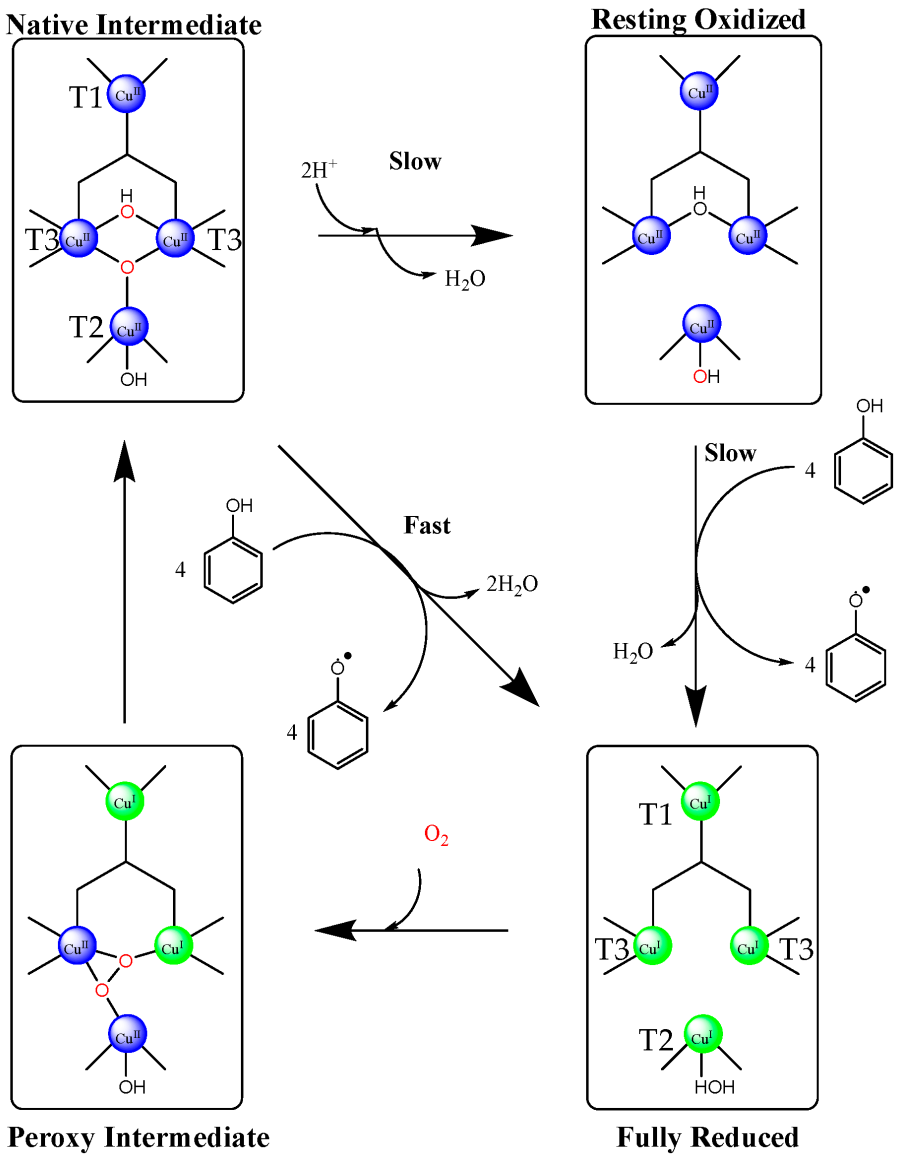

Figure 4. Laccase catalytic cycle depicting the native intermediate (fully reduced oxygen), the oxidized resting state, the fully reduced form (after substrate oxidation), and the peroxy intermediate (partial oxygen reduction). Phenol is used as a model substrate. Cu^{II}—blue, Cu^{I}—green.[33]

The reaction scheme is typically:

Substrate (phenol, amine, etc.) + O_2 → Oxidized product + H_2O

Laccases require no co-substrate other than oxygen and can act on a wide variety of phenolic compounds, polyphenols, aromatic amines, and even inorganic ions. In the presence of redox mediators (such as ABTS, HBT, TEMPO), laccases can also oxidize non-phenolic substrates, significantly expanding their catalytic range—a phenomenon referred to as the "laccase-mediator system" (LMS).[32]

1.5.3 Biocatalytic Kinetics and Colorimetric Assay Methods of LAC

Understanding the kinetic behaviour of laccases is essential to fully exploit their catalytic efficiency and substrate specificity in various biotechnological applications. Likewise, robust and sensitive assay methods are crucial for the qualitative and quantitative determination of laccase activity, particularly in enzyme engineering, immobilization studies, and high-throughput screening of variants or mimetic catalysts.

The kinetic behaviour of laccases generally follows Michaelis–Menten kinetics, described by

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

where ν is the reaction rate, V_{max} is the maximal rate, K_m is the Michaelis constant, and [S] is the substrate concentration. The turnover number (k_{cat}) and catalytic efficiency (k_{cat}/K_m) vary depending on substrate type, pH, temperature, and enzyme source.[34,35]

Common Substrates

- **2,4-DP+ 4-AP:** 2,4-Dichlorophenol (2,4-DP), in conjunction with 4-aminoantipyrine (4-AP), serves as the reference substrate for this analysis. In this instance, 2,4-DP is identified as the primary substrate. Through the process of laccase-catalyzed oxidation, 2,4-DP is transformed into a radical product that subsequently reacts with 4-AP. This interaction yields a red-colored dye, termed antipyrilquinoneimine, which exhibits a distinctive broad absorbance peak centered at 510 nm.nm
- **ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid))**: Produces a green radical cation ($ABTS^{\bullet+}$) with strong absorbance at 420 nm. One of the most widely used substrates due to high solubility and stability.
- **Syringaldazine**: Produces a purple product with maximum absorbance at 530 nm. Preferred for detection in neutral to alkaline pH ranges. Sensitive to trace enzyme concentrations and useful for plant or fungal laccase detection.

- **Guaiacol**: Forms a reddish-brown polymeric product upon oxidation ($\lambda_{max} \approx 470$ nm). A classical substrate used in lignin degradation studies.
- **Catechol and o-Dianisidine**: Produce colored quinones or chromophoric intermediates, widely used in analytical and environmental chemistry.
- **DMP (2,6-dimethoxyphenol)**: Another popular substrate for basic kinetic studies ($\lambda_{max} \approx 469$ nm), ideal for studies in lignin model degradation.

Assay Procedure and Optimization: Standard laccase assays are performed in buffered solutions (typically acetate buffer, pH 3.0–6.0 for fungal laccases), and involve continuous or endpoint absorbance measurements. Activity is typically expressed in Units (U), defined as the amount of enzyme that oxidizes 1 μmol of substrate per minute under defined conditions. Microplate-based formats are often used for high-throughput analysis of enzyme variants or inhibitor screening.

1.5.4 Biological Significance

Laccases play vital roles in multiple physiological and ecological processes. In fungi, they are instrumental in lignin degradation, morphogenesis, pigment formation, and pathogenesis. Plant laccases contribute to lignification, wound healing, and defense responses against pathogens. Bacterial laccases are often involved in copper homeostasis and detoxification of phenolic compounds in extreme environments.[36] Their involvement in lignin metabolism is of particular importance, as lignin is one of the most recalcitrant components of plant biomass. Laccase-producing white-rot fungi are among the few organisms capable of degrading lignin efficiently, positioning laccases as key agents in natural carbon recycling.[37]

1.5.5 Industrial and Biotechnological Relevance of Laccase

The robust catalytic activity and environmentally benign nature of laccases have led to their widespread application in several industrial and environmental domains. In the textile industry, laccases are used for dye decolorization and fabric bleaching under mild, eco-friendly conditions.[38] In pulp and paper industries, laccase-based processes have been employed for biobleaching and delignification, replacing harsh chemical treatments and reducing environmental impact.[39] In bioremediation, laccases effectively degrade a variety of environmental pollutants, including endocrine disruptors, phenols, bisphenols, and synthetic dyes, making them valuable for wastewater treatment and soil detoxification (https://doi.org/10.1007/s10532-008-9185-3). Their utility in biosensor technology has also grown, particularly in detecting phenolic pollutants and developing laccase-based biofuel cells

due to their oxygen-reducing capabilities.[40] Moreover, synthetic organic chemistry increasingly employs laccases for oxidative coupling reactions and green synthesis of bioactive compounds, especially in the formation of biaryl linkages and polymeric materials.[41]

Therefore, Laccases are multifunctional biocatalysts with remarkable substrate flexibility, eco-compatibility, and industrial utility. Their ability to catalyze green oxidation reactions under mild conditions, along with the continuous discovery and engineering of novel isoforms, underscores their value in the transition towards more sustainable biotechnological processes. Given their profound biological roles and broad application potential, the study of laccases continues to be a pivotal and promising area within enzymology and green chemistry.

1.6 Catechol Oxidase (CO)

Catechol oxidase (EC 1.10.3.1), also referred to as polyphenol oxidase (PPO), is a type of copper-containing oxidoreductase enzyme responsible for the oxidation of o-diphenols (such as catechol) to the corresponding o-quinones using molecular oxygen as the electron acceptor. This reaction is central to enzymatic browning in fruits and vegetables and is also involved in plant defense mechanisms. As a member of the type-III copper protein family, catechol oxidase shares structural and mechanistic features with other copper enzymes like tyrosinase and laccase but exhibits distinct substrate specificity and catalytic behavior.[28,42]

Catechol oxidase has attracted significant attention in recent years not only for its role in plant physiology but also for its emerging applications in biotechnology, environmental remediation, and bioanalytical sensing. Its catalytic promiscuity, oxygen-dependent oxidation mechanism, and ability to generate reactive quinones make it a valuable biocatalyst in multiple domains.

1.6.1 Structure and Nature

Catechol oxidases are typically found in the plastids of plant cells, particularly within the thylakoid lumen, and exist as soluble or membrane-associated glycoproteins. Structurally, catechol oxidase is a homodimer or homotetrameric protein with each subunit containing two conserved copper ions coordinated by histidine residues, which form the active site.[43] The enzyme possesses a binuclear type-3 copper center, composed of two Cu atoms (CuA and CuB) each ligated by three histidine residues. The active site is buried within the protein matrix, facilitating the binding of diphenolic substrates and O_2. X-ray crystallographic studies of catechol oxidases from *Ipomoea batatas* (sweet potato) and *Vitis vinifera* (grapevine) have revealed a highly conserved architecture among plant PPOs.[44] Unlike tyrosinases, catechol

oxidases lack monophenolase activity and are unable to hydroxylate monophenols to o-diphenols, which distinguishes their catalytic role.

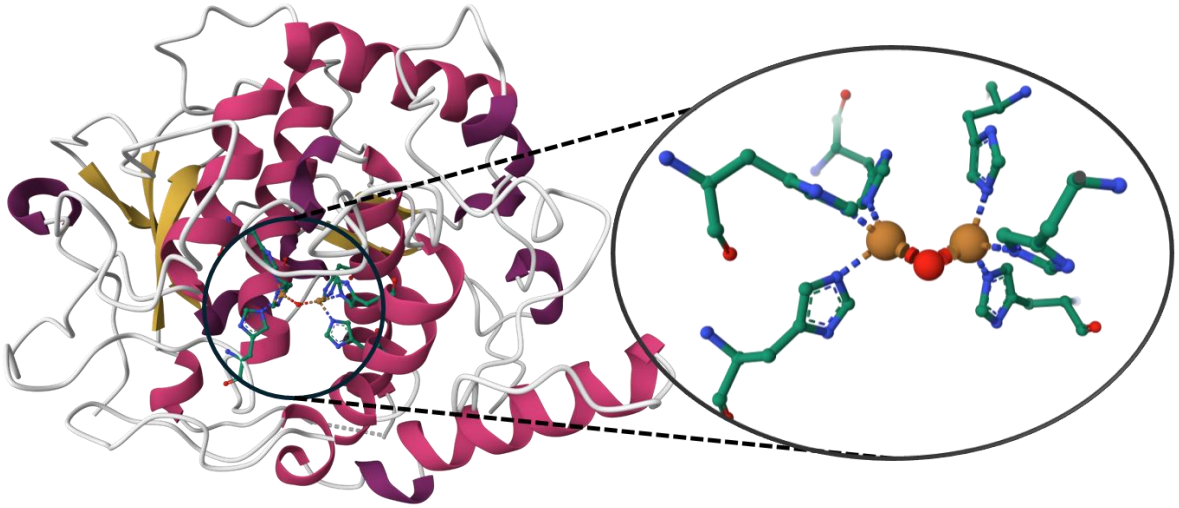

Figure 5. Crystal structure of Catechol oxidase from ipomoea batatas (sweet potatoes) (PDB: 1BT1) and its catalytic active site.[44]

1.6.2 Catalytic Reaction and mechanism of action

Catechol oxidase catalyzes the oxidation of o-diphenols (such as catechol, dopamine, or L-DOPA) to o-quinones, with molecular oxygen acting as the terminal electron acceptor and reduced to water. The overall reaction can be represented as:

2 Catechol + O_2 → 2 o-Quinone + 2 H_2O

The catalytic cycle involves two-electron oxidation steps where electrons are transferred from the diphenolic substrate to the Cu(II) ions at the active site, which are subsequently reduced. These electrons are then transferred to molecular oxygen, which is reduced to water, restoring the oxidized state of the enzyme and allowing for continuous catalysis.[28,42]

The catalysis proceeds through three redox states of the copper active site:

1. **Met state** (inactive, resting state: Cu(II)-Cu(II))
2. **Oxy state** (active: Cu(II)-O_2^{2-}-Cu(II))
3. **Deoxy state** (reduced: Cu(I)-Cu(I))

Upon substrate binding, catechol is oxidized to quinone through two sequential single-electron transfers. The quinone products can undergo non-enzymatic polymerization to form brown melanins or other pigments, contributing to browning reactions in plant tissues.

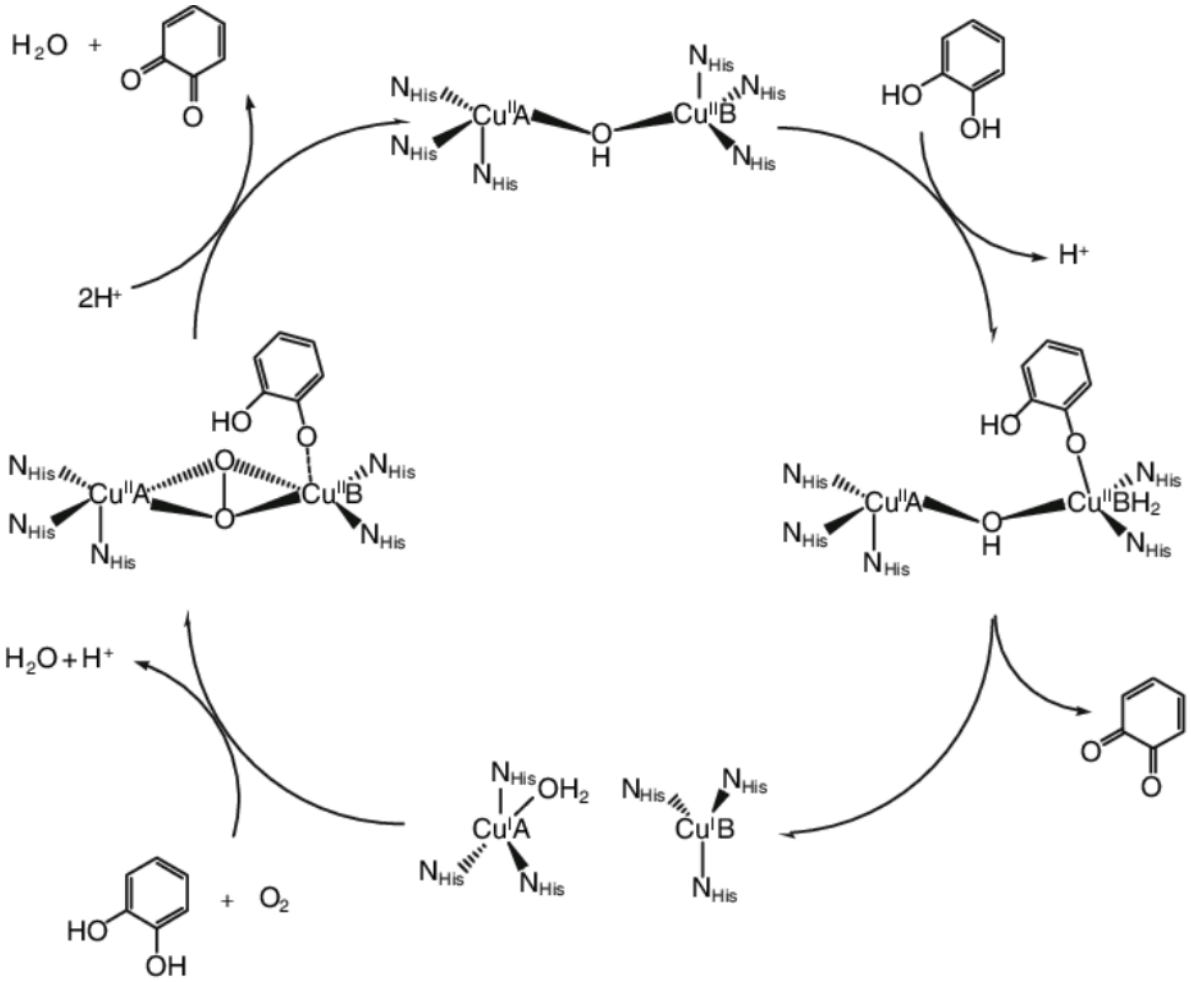

Figure 6. Well reported Proposed catalytic cycle of catechol oxidase.[85]

1.6.3 Biocatalytic Kinetics and Colorimetric Assay Methods of CO

Catechol oxidase generally follows Michaelis–Menten kinetics under initial-rate conditions.

Colorimetric Assay Methods: Colorimetric assays are the most widely adopted approach to determine catechol oxidase activity, based on the detection of coloured o-quinone products formed from catechol derivatives. These reactions are monitored spectrophotometrically and allow for real-time and end-point quantification of enzymatic activity. Substrates Commonly Used

- **Catechol**: Oxidation yields brown o-quinone products with absorbance at ~420 nm. Simple, inexpensive, and highly reactive substrate.
- **L-DOPA (L-3,4-dihydroxyphenylalanine)**: Produces dopachrome (λmax ≈ 475 nm), a highly chromogenic product. Preferred for high sensitivity assays, especially in plant or fungal systems.
- **Pyrogallol (1,2,3-trihydroxybenzene)**: Oxidation leads to yellowish-brown products ($\lambda_{max} \approx 420$ nm).
- **Dopamine or Tyrosine Derivatives**: Can be used to study catechol oxidase-like monophenolase activity in complex matrices.

Assay Conditions and Optimization: Standard assay conditions involve measuring the increase in absorbance over time at specific wavelengths (e.g., 420 nm for catechol or 475 nm for L-DOPA). Reaction conditions are typically optimized as follows:

- Buffer: Phosphate or citrate-phosphate buffers, pH 5.5–7.0
- Temperature: 25–40°C, depending on enzyme stability
- Substrate concentration: Typically, 1–10 mM to cover linear response range
- Reaction time: 2–5 minutes for initial-rate determination

The enzyme activity is expressed in units (U), where 1 U is defined as the amount of enzyme that catalyzes the formation of 1 μmol of o-quinone per minute under assay conditions. The molar extinction coefficient (ε) of the product is used to calculate enzyme activity from the observed change in absorbance (ΔA/min).

1.6.4 Industrial and Biological Relevance

Catechol oxidase is increasingly recognized for its versatility in biotechnological and industrial applications. One of the most prominent areas is its application in biosensor development. Catechol oxidase-based electrochemical and optical biosensors are employed for the detection of phenolic pollutants in industrial wastewater due to their high selectivity and eco-compatibility.[45] In the food industry, catechol oxidase activity is both a challenge and a tool. While enzymatic browning is undesirable in fresh produce, controlled oxidation processes are used in the fermentation and flavors development of products such as tea, coffee, and cocoa.[46] Moreover, the enzyme has been proposed for use in bio-based adhesives, crosslinking agents, and enzyme-mediated polymerization due to its ability to produce quinones that can undergo

further polymerization reactions. The environmental sector also benefits from catechol oxidase's oxidative power. The enzyme has demonstrated the ability to degrade phenolic and aromatic contaminants, contributing to greener strategies for wastewater detoxification and soil remediation.[47] Immobilized catechol oxidase systems, particularly when combined with nanomaterials or biopolymers, have improved operational stability and reusability in bioreactors.

Hence, Catechol oxidase is a structurally conserved and functionally significant enzyme with an expanding role in plant biology and biotechnology. Its distinctive ability to catalyze the oxidation of diphenols into quinones under ambient conditions, using molecular oxygen, reflects a green and efficient catalytic process. From plant defense and pigment biosynthesis to biosensing and pollutant degradation, catechol oxidase offers vast potential for research and application. Continued exploration into its catalytic mechanisms, structural diversity, and biotechnological adaptability is expected to further its relevance in green chemistry, agriculture, and environmental science.

2. Limitations of Natural Oxidoreductases and the Need for Artificial Enzyme Mimics:

Natural oxidoreductases, such as peroxidase, laccase, and catechol oxidase, represent a fundamental class of redox enzymes that catalyze electron transfer reactions involving molecular oxygen, hydrogen peroxide, and a wide range of phenolic and aromatic substrates. These enzymes play critical roles in biological systems and have been extensively studied for their applications in industrial catalysis, biosensing, environmental remediation, and biomedical diagnostics.[20,48] Despite their high specificity and catalytic efficiency, the direct translation of these enzymes into *in vitro* and applied systems faces significant limitations related to stability, cost, scalability, and operational robustness. These drawbacks have motivated a paradigm shift towards the development of artificial enzymes, or enzyme mimics, that can emulate the catalytic behaviour of their natural counterparts while overcoming the associated limitations.[49]

2.1 Limitations of Natural Oxidoreductases in In Vitro Applications

2.1.1 Structural Fragility and Environmental Instability: Natural oxidoreductases are proteinaceous macromolecules that exhibit a narrow operational window of pH, temperature, and ionic strength. Many of these enzymes undergo denaturation or inactivation under non-physiological conditions that are common in industrial or environmental settings. For instance, horseradish peroxidase (HRP), although widely used in biosensing and immunoassays, is

unstable at temperatures above 50 °C and is highly susceptible to oxidative inactivation in the presence of excess hydrogen peroxide, a paradoxical limitation for a peroxidase.[50] Similarly, fungal laccases—despite their broad substrate specificity—are active primarily in acidic to mildly neutral pH ranges, making them less effective in alkaline wastewater treatment or polymerization processes.[51] Catechol oxidases, typically derived from plant sources, also suffer from instability under process conditions and are prone to product inhibition due to quinone accumulation, further limiting their catalytic longevity.[42]

2.1.2. Expensive Production and Purification Processes: Large-scale production of oxidoreductase enzymes often involves recombinant expression systems, complex downstream processing, and cold-chain logistics to preserve enzymatic activity. The use of native extraction methods is not scalable, and recombinant production can introduce issues of misfolding or post-translational modification requirements. These production challenges lead to high costs, thereby limiting the feasibility of deploying these enzymes in cost-sensitive sectors such as environmental monitoring and large-volume wastewater treatment.[52]

2.1.3. Short Shelf-Life and Reusability Constraints: The lack of long-term storage stability and poor reusability hinder the integration of oxidoreductases into continuous-flow reactors and sensor platforms. Many enzymes lose activity within days or weeks, particularly when stored under non-ideal conditions. Attempts at enzyme immobilization to improve reusability often lead to reduced catalytic efficiency due to conformational constraints or diffusion limitations.[53]

2.1.4. Substrate Specificity and Operational Rigidity: While substrate specificity is a hallmark of natural enzymes, it can also be a disadvantage in synthetic or analytical chemistry contexts where broad-spectrum catalysis is preferred. Natural oxidoreductases typically show selectivity toward phenolic or aromatic compounds with specific redox potentials, limiting their utility in oxidizing a broader range of industrial substrates. In addition, natural enzymes rarely tolerate organic solvents or extreme redox environments, further reducing their versatility.[31]

3. Emergence of Artificial Enzyme Mimics: A Strategic Necessity

The pressing limitations of natural oxidoreductases in *in vitro* applications have prompted a significant research shift toward designing robust artificial enzymes that not only mimic the catalytic functions of their biological counterparts but also exhibit enhanced physicochemical resilience, tunable reactivity, and structural adaptability.[34,54,55] Two major categories of artificial oxidoreductase mimics have emerged: nanozymes inorganic nanomaterials with enzyme-like activities) and biomolecular supramolecular catalysts (self-assembled peptide-, DNA-, RNA-, or hybrid-based materials with catalytic properties). These systems are

engineered to emulate the active site geometry, redox properties, and catalytic mechanisms of natural enzymes, while offering superior stability and functionality under non-physiological conditions.

3.1 Nanozymes (Metal and Metal Oxide-Based Nano Catalysts):

The term "nanozyme" refers to nanostructured materials that possess intrinsic enzyme-like catalytic activity. Among these, metal and metal oxide nanoparticles have gained prominence for their peroxidase-, laccase-, or catechol oxidase-mimicking capabilities. These nanozymes leverage their high surface area-to-volume ratios, quantum-size effects, and surface redox-active sites to facilitate catalytic transformations with high turnover and operational robustness.[49,56,57] For instance, Fe_3O_4 nanoparticles exhibit peroxidase-like activity, catalyzing H_2O_2-dependent oxidation of chromogenic substrates such as TMB and ABTS under acidic conditions. Similarly, CeO_2 (ceria) nanoparticles can catalyze both oxidase and catalase reactions due to the dynamic redox cycling between Ce^{3+} and Ce^{4+} ions at oxygen vacancy-rich surfaces.[58] Copper-based nanostructures (e.g., CuO, Cu_2O, and Cu-doped MOFs) have been reported to mimic laccase and catechol oxidase activity through redox cycling of Cu(I)/Cu(II) centers, analogous to the type-3 copper active sites in natural enzymes.[59–62]

These nanozymes offer multiple advantages:

- Operational stability across broad pH and temperature ranges
- Resistance to proteolytic degradation
- Scalability via wet chemical or solvothermal synthesis
- Multifunctionality, such as magnetism, fluorescence, or surface functionalizability for biosensing

Moreover, rational engineering of nanozyme structure and surface chemistry—via doping, heterostructure formation, or surface ligand modification—allows precise control over their redox potential, substrate affinity, and catalytic selectivity.[56,63]

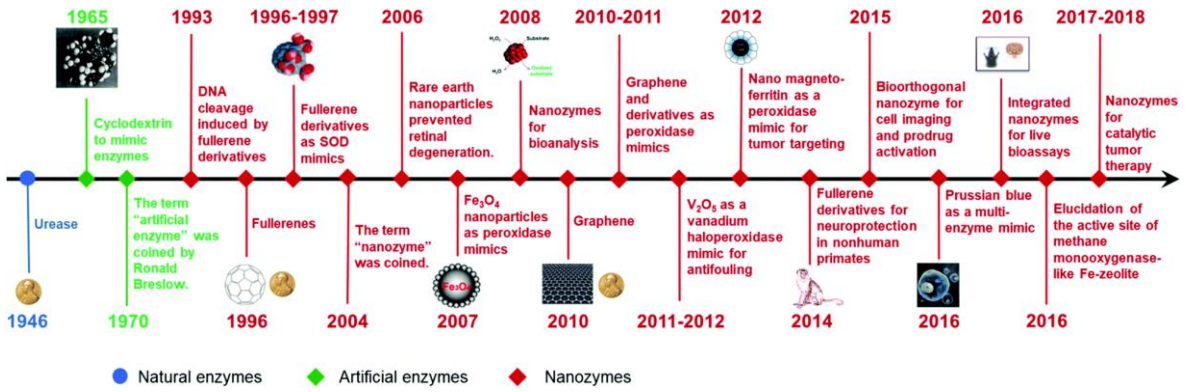

Figure 7. A brief timeline for the development of nanozymes (natural enzymes and artificial enzymes are listed for comparison). Adapted with permission from Ref.[61]

4. Biomolecular Supramolecular Catalysts (Peptides, Nucleic Acids, and Hybrids): Alongside inorganic nanozymes, a new frontier in artificial enzyme development involves biomolecular self-assembly, wherein short peptides, nucleic acids (DNA/RNA), or their hybrid constructs are programmed to self-organize into nanoarchitectures with catalytic function. These supramolecular systems often capitalize on non-covalent interactions (e.g., hydrogen bonding, π–π stacking, electrostatics) to assemble into ordered nanostructures that can mimic enzyme active sites or create confined microenvironments conducive to catalysis. Peptide-based artificial enzymes have been designed using sequences rich in histidine, cysteine, or tyrosine residues—mimicking the coordination environment of metal centers in natural oxidoreductases.[64–68] These metallopeptide assemblies, upon complexation with transition metal ions (e.g., Cu^{2+}, Fe^{3+}), can reproduce laccase- or peroxidase-like activity.[69] Importantly, peptide nanostructures (e.g., nanotubes, nanofibers, hydrogels) offer biocompatibility, chirality, and responsiveness to external stimuli, making them ideal candidates for biomedical and biosensing applications.[70–72] DNA-based enzyme mimics—also known as DNAzymes or deoxyribozymes—are catalytic oligonucleotides that utilize the folded secondary structure of DNA, often stabilized by metal ion cofactors, to perform oxidation or cleavage reactions. While traditionally known for nucleic acid catalysis, certain guanine-rich DNA sequences complexed with hemin have been shown to mimic peroxidase activity, forming G-quadruplex/hemin DNAzymes with broad utility in biosensors.[73–75] These systems offer high programmability and target recognition through Watson-Crick base pairing, enabling sequence-specific molecular detection. More recently, hybrid biomolecular mimics that integrate peptide motifs with DNA scaffolds or attach functional metal complexes to nucleic acid frameworks have been developed. These peptide-DNA conjugates provide spatial control over active site geometry and allow multivalent catalysis, bridging the structural rigidity of DNA with the chemical versatility of peptides.[76,77]

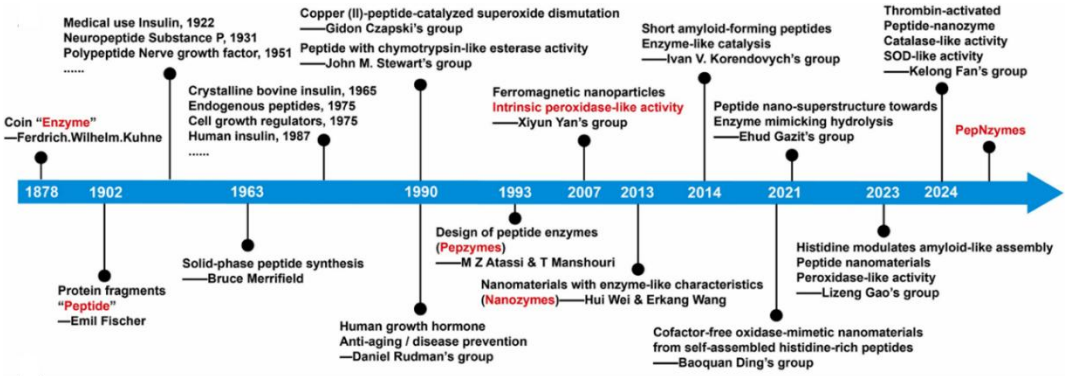

Figure 8. Timeline of critical events of peptides, enzyme-like peptides, nanozymes, PepNzymes, *etc*. adapted from the ref.[68]

Despite the progress made in the development of artificial enzyme mimics, current nanozymes and biomolecular supramolecular catalysts are constrained by fundamental and practical shortcomings. The mechanistic ambiguity, fabrication complexity, biocompatibility concerns, and low substrate selectivity of nanozymes, along with the high cost, instability, and poor reusability of biomolecular catalysts, present significant barriers to real-world application. These limitations emphasize the critical need for a new class of enzyme mimics that integrate the simplicity, robustness, and modularity of materials science with the functional finesse of enzymology. The work presented in this thesis aims to address this gap through the design, synthesis, and functional evaluation of simple biomolecular architectures, offering a platform for efficient, affordable, and sustainable biocatalysis.

5.Rational Design of Bionanozymes: Bioinspired Supramolecular Catalysts with Enzyme-Mimetic Functionality:

The remarkable catalytic efficiency and specificity of enzymes arise primarily from the unique chemical and spatial arrangements of functional groups within their active sites. These active sites are located in well-defined three-dimensional clefts or pockets, typically composed of amino acid residues that, although distant in the primary sequence, are brought into close proximity through intricate tertiary folding. Within these catalytic cavities, functional moieties work in concert, often in the presence of cofactors—either organic or inorganic—to achieve precise substrate binding and catalytic transformation. The interplay of structural hierarchy, conformational dynamics, and electronic tuning allows enzymes to facilitate complex biochemical reactions under mild conditions with unparalleled efficiency and selectivity.

Inspired by this exquisite structure–function paradigm and aiming to address the inherent limitations of natural enzymes—such as poor stability under non-physiological conditions, limited reusability, and high production costs—this thesis presents a novel class of artificial enzyme mimics termed bionanozymes. These systems are based on the bottom-up assembly of simple biomolecular building blocks, often in combination with ionic cofactors, to create supramolecular nanoassemblies with enzyme-mimetic biocatalytic activity. The work focuses on the design, synthesis, and functional exploration of these minimalistic, robust, and modular biocatalysts, with applications spanning biosensing, environmental remediation, and biomedical diagnostics.

6. Bionanozymes: Definition and Construction Strategy:

Bionanozymes are structurally ordered nanoassemblies constructed through bottom-up self-assembly of biomolecular components that possess inherent or cofactor-assisted catalytic potential. These materials emulate enzyme-like functions without the need for large, complex protein scaffolds. Instead, they rely on non-covalent interactions—including hydrogen bonding, π–π stacking, hydrophobic interactions, and metal coordination—to organize the functional groups into spatially defined architectures that resemble natural enzymatic active sites. Unlike natural enzymes, where the active site is a direct result of folding a long polypeptide chain into a compact globular structure, bionanozymes achieve a similar catalytic architecture by modularly assembling short, well-defined molecular units. This approach allows for precise tailoring of the catalytic environment, offering control over substrate access, reaction selectivity, and microenvironment polarity.

The resulting supramolecular nanoassemblies exhibit a high degree of structural hierarchy and functional adaptability. Multiple reactive sites can be integrated into the same scaffold, either randomly distributed or periodically arranged, depending on the nature and stoichiometry of the constituent building blocks. The architecture can be engineered to resemble a heterogeneous catalytic reactor, in which catalysis occurs in a tailored nanoscale environment—often at a phase boundary between hydrophilic and hydrophobic domains. This tunability represents a powerful strategy to control catalytic behavior at the molecular level.

7.Design Principles and Key Advantages

Several defining characteristics distinguish bionanozymes from traditional artificial enzymes and natural protein-based enzymes:

- **Structural Simplicity and Tunability**

  Bionanozymes are composed of simple, readily available biomolecular units that are easy to chemically modify. By altering the building blocks, cofactors, or assembly conditions, one can rationally adjust the catalytic properties, selectivity, and stability of the resulting nanozyme. This modularity greatly simplifies the design process and enhances functional versatility.

- **Proximity-Induced Catalysis**

  Through self-assembly, multiple reactive centers can be positioned in close proximity to the substrate binding site. This spatial arrangement not only accelerates reaction kinetics but also enables stereoselective or regioselective transformations, depending on the molecular orientation within the nanoassembly.

- **Operational Simplicity and Cost-Efficiency**

  Compared to protein enzymes, which require complex expression systems and purification protocols, bionanozymes can often be synthesized and assembled under mild, aqueous conditions using inexpensive starting materials. This renders the process scalable and economically viable for practical applications.

- **Tailorable Microenvironment**

  The amphiphilic nature of many biomolecular building blocks allows for the creation of distinct microdomains—hydrophilic, hydrophobic, or zwitterionic—within the catalytic scaffold. These localized environments can be designed to mimic biological compartments, enhancing reaction rates, stability, and substrate compatibility.

- **Versatility in Cofactor Integration**

  The incorporation of metal ions (e.g., Fe^{3+}, Cu^{2+}, Zn^{2+}) as cofactors provides redox functionality reminiscent of natural metalloenzymes. The coordination of metal ions to the nucleophilic or electron-rich functional groups of the biomolecules can significantly alter their electronic transitions, enabling activation of oxygen species or redox-mediated transformations that are characteristic of oxidoreductase enzymes.

8.Structural Mimicry of Natural Enzymes

One of the key challenges in artificial enzyme design lies in mimicking the active site architecture of natural enzymes, which have been evolutionarily optimized over billions of years. In protein enzymes, catalytic residues are typically non-contiguous in the primary sequence but are brought into proximity through specific folding patterns. Reproducing such a spatial arrangement via a bottom-up approach requires precise control over intermolecular interactions during self-assembly. Bionanozymes address this challenge by using **pre-**organized building blocks capable of forming stable, predictable supramolecular architectures. The intermolecular assembly of amino acids or nucleobases leads to the convergence of functional groups in three-dimensional space, effectively generating artificial active sites. In these systems, cooperative interactions among multiple catalytic residues—sometimes aided by a metal ion or small-molecule cofactor—enable complex transformations such as substrate oxidation, electron transfer, or hydrolysis, depending on the mimic being constructed.

Importantly, the random or periodic distribution of catalytic motifs within the nanoassembly allows for a more diverse range of substrate interactions than in conventional enzymes, where the catalytic site is fixed within a single location. This flexibility and plasticity can be harnessed

to perform tandem reactions, or even multi-substrate catalysis, within a single supramolecular platform.

8.1 Choice of Building Blocks: Biomolecular Simplicity Meets Functional Sophistication:

The fundamental design philosophy behind bionanozymes lies in the use of biologically relevant, structurally diverse, and functionally rich molecular precursors. Among these, amino acids and nucleobases are particularly attractive due to their abundance, water solubility, biocompatibility, and programmability.

- **Amino acids and peptides** provide functional diversity through their side chains (e.g., imidazole in histidine, phenol in tyrosine, thiol in cysteine), many of which are directly involved in natural enzymatic catalysis. Their chiral nature and secondary structure motifs (e.g., β-sheets, α-helices) also enable enantioselective catalysis when assembled appropriately.
- **Nucleobases (e.g., adenine, guanine)** offer multiple hydrogen bonding sites, metal-binding pockets, and π-conjugated systems that facilitate electron delocalization and redox activation. They are also ideal scaffolds for stacked, layered, or G-quadruplex-type structures, which can provide multivalent catalytic surfaces.

These molecules inherently carry the information necessary for self-assembly, and their modular nature allows for fine-tuning of geometry, charge distribution, and hydrophobicity, all of which are critical for catalytic performance.

8.2 Functional Integration and Applications:

The development of bionanozymes represents a convergence of bioinspired design, materials chemistry, and enzyme engineering. Once assembled, these supramolecular catalysts exhibit a wide range of functionalities depending on their composition and environmental conditions. For instance, the incorporation of Cu^{2+} ions can impart peroxidase-, laccase-, or catechol oxidase-like activity, enabling applications such as:

- Colorimetric biosensing (e.g., detection of glucose, H_2O_2, dopamine)
- Reactive oxygen species (ROS) scavenging in therapeutic settings
- Degradation of environmental pollutants, such as phenols or dyes
- Synthetic biocatalysis under aqueous, ambient conditions

The simplicity of their construction, combined with their catalytic robustness, positions bionanozymes as promising candidates for scalable, sustainable, and clinically translatable enzyme mimics.

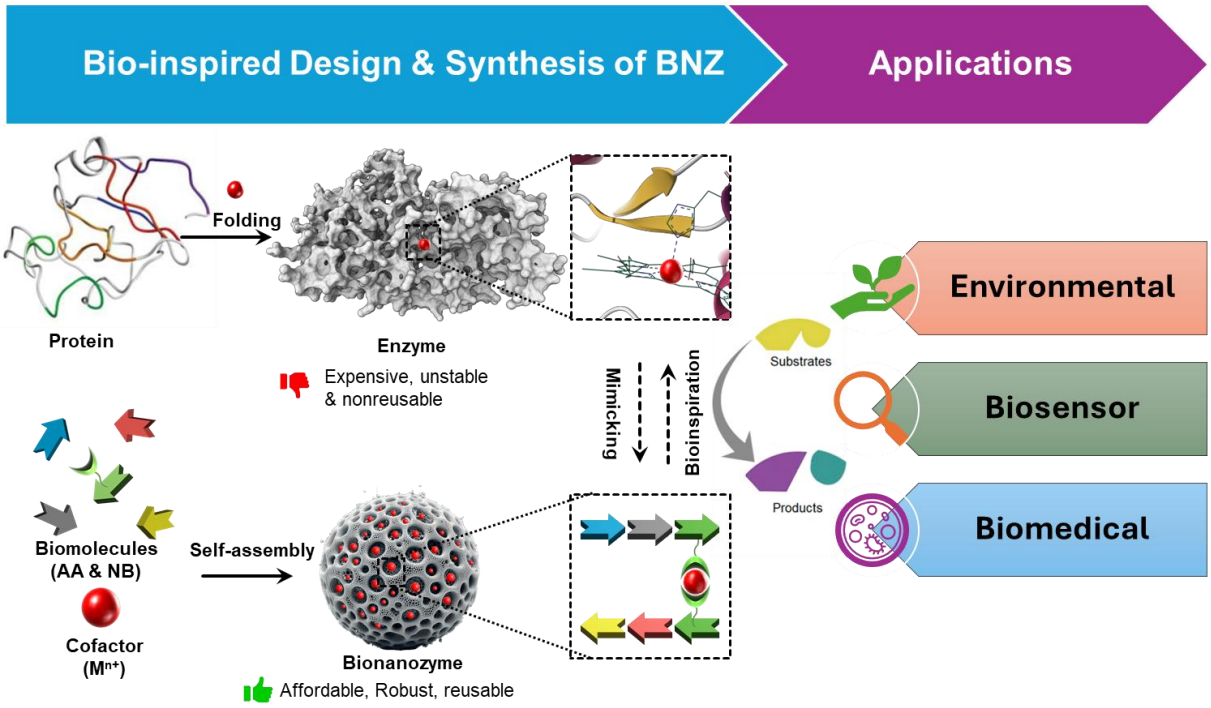

Figure 9. Overview of bioinspired design, synthesis and applications of bionanozymes presented in this thesis. Where AA = amino acids, NB = Nucleobases.

9. Thesis Outlook

This thesis contributes to the rapidly growing field of enzyme mimics by presenting a systematic framework for the design, synthesis, and functional application of bionanozymes. Through a combination of rational molecular engineering, self-assembly techniques, and cofactor integration, a new class of minimalistic yet potent artificial enzymes have been developed. These systems offer a paradigm shift in how enzyme mimics are conceptualized—not as complex folded macromolecules but as modular, self-assembled catalytic platforms derived from simple biomolecular components.

The results presented herein lay the groundwork for future development of bionanozymes as next-generation biocatalysts with applications in environmental, industrial, and biomedical domains.

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