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CHAPTER 1

INTRODUCTION

GUAN HUANAN
ZHANG ZHIHONG

Nanozymes are a class of nanomaterials with biocatalytic function, which can catalyse the substrate of natural enzymes and serve as enzyme substitutes based on specific nanostructures. Nanozymes have both the physical and chemical properties of nanomaterials and the catalytic function similar to enzymes, combining the advantages of natural enzymes and artificial enzymes. Nowadays, nanozymes have become a new research hotspot, and more than 1200 kinds of nanozymes have been developed. Therefore, the research on their catalytic mechanism should be more in-depth, and the application research should be gradually expanded from the initial detection to the nanozymes catalytic medicine, sensing and detection, green synthesis, new energy, environmental governance and other fields. This book introduces readers to the main progress of nanozymes since their discovery, and expects nanozymes to derive new technologies, products and commodities from new concepts and materials to provide highly sensitive, specific and reproducible tests for food and biological analysis and detection technologies, to serve human health and to drive the development of new disciplines.

Bioanalysis as an important topic of international concern, has gradually attracted widespread attention from people all over the world [1]. In the growing process of globalisation, the inadvertent adulteration of trace poisons has significantly increased the likelihood of food contamination. The harmful substances produced by food contamination can cause great harm to human health and lead to huge economic losses in the food industry around the world [2]. To further ensure food safety issues, organisations such as the World Health Organisation have developed regulations and legislation on food safety [3]. In addition, food analysis and testing techniques are constantly being innovated and reformed towards meeting the requirements of high sensitivity, specificity and reproducibility testing [4]. Among them, enzyme, as a natural biological catalyst, can catalyse a variety of biochemical reactions with high catalytic efficiency, substrate specificity and biocompatibility, and enzyme analysis is highly sensitive and selective, so it has been widely used in food analysis and testing to meet higher testing requirements [5,6].

Although natural enzymes have high catalytic efficiency and substrate specificity, they also have inherent defects such as poor stability, difficult preparation, high cost and low recovery efficiency [7]. In contrast, materials with enzyme-like activity have gradually gained popularity due to their low cost, good stability, and ease of design and regulation [8]. Enzyme-mimicking is a material with non-protein-like chemical composition, which uses synthetic polymers to mimic the structure, properties, principle of action and the chemical reaction process of enzymes in living organisms [9]. Nobel laureates CJPederson, DJCram, and JMLehn jointly proposed the "subject-object" chemical theory and supramolecular chemical theory, which laid an important theoretical foundation for enzyme simulation [10]. Mimetic enzymes are expected to be alternatives to natural enzymes because of their similar catalytic properties and their ability to address the limitations of natural enzymes [11]. At present, a lot of results have been achieved in the research based on mimetic enzymes, according to which mimetic enzymes can be broadly classified into two categories: conventional mimetic enzymes and nano-mimetic enzymes [12].

Conventional mimetic enzymes are not only superior to natural enzymes in terms of acid and alkali resistance, thermal stability, and cost, but also their ability to satisfy catalytic assay needs can be applied in a large number of real food assays [13,14]. Since its discovery in 1891, cyclodextrin (CD) has attracted many scientists to study it due to its special structure and properties [15]. CD is a cyclic oligosaccharide consisting of multiple glucose molecules linked by α-(1,4) glycosidic bonds, with an exohydrophilic and endohydrophobic ring structure [16]. Due to this special structure, it is able to better envelope the guest molecules and has a certain degree of selectivity for the guest molecules [17]. Although CD have unique enzyme-like recognition and catalysis, their hydrophobic surface area in contact with the surface of a given guest molecule is limited compared to most enzymes, resulting in low catalytic activity [18]. Therefore, researchers have started to improve the catalytic activity of the mimetic enzymes using functional group modification, formation of complexes with metals, and synthesis of cyclodextrin polymers [19,20]. Ma et al. prepared polydopamine/hemoglobin-β-cyclodextrin (PDA/Hemin-CD) supramolecular assemblies to mimic the natural peroxidases [21]. The results showed that PDA/Hemin-CD had good peroxidase activity and molecular recognition properties and was successfully used for cholesterol detection. Xing et al. prepared Ag-β-cyclodextrin-graphene oxide ternary nanocomposites (Ag-β-CD-GO) by homogeneous redox-active self-assembly method [22]. The prepared Ag-β-CD-GO exhibited excellent peroxide mimetic enzyme activity, high stability and non-toxicity, and was effectively used for the monitoring of toxic Hg^{2+} in natural water, beverage and juice samples.

Porphyrins are macromolecular heterocyclic compounds with four pyrrole unit molecules connected by four methyl bridges, they can be classified into three main groups: porphyrins, porphyrin derivatives and metalloporphyrins [23]. Compared to porphyrin monomers, porphyrin-based compounds have significant advantages and can significantly enhance porphyrin functions [24]. Among them, metalloporphyrins and their derivatives have become hotspots for enzyme-mimetic studies due to their conjugated large π-bond electronic system, variable metal valence and strong coordination ability of the central metal [25,26]. Lai et al. successfully synthesized a manganese-ion modified porphyrin metal-organic skeleton by a simple coordination method [27]. Using its oxidase-like properties, a simple and sensitive colorimetric method for the detection of glutathione (GSH) was established, which was successfully applied to the determination of human serum samples and food-grade GSH.Wang et al. synthesized an iron-based porphyrin porous organoskeleton via an alkylation reaction between 5,10,15,20-tetrakis (4-aminophenyl) porphyrin and trichloramine, and established a sensitive and simple colourimetric assay for H_2O_2 and glucose. It provides a simple strategy for the detection of hydrogen peroxide-related biomolecules [28].

Although traditional mimetic enzymes are able to overcome the defects of natural enzyme instability, they still suffer from more complex synthesis, single catalytic active site, low catalytic efficiency and difficulties in separation and recovery [29]. In 2007, Gao et al. reported the peroxidase-like activity of ferromagnetic nanoparticles (Fe_3O_4NPs) and indicated that the catalytic activity of inorganic NPs is similar to that of nature's specifically acting as the presence of H_2O_2, these nanoparticles can directly trigger and accelerate the oxidation of peroxidase substrates [30]. Wei and Wang coined the term "nano-enzymes" to describe NPs with the ability to mimic enzyme action [31]. Compared with natural enzymes and traditional mimetic enzymes, nanoenzymes are more stable, less costly, and easier to modify than natural enzymes and traditional mimetic enzymes, in addition to their good recognition ability and biocatalytic activity. Therefore, the application of nano-mimetic enzymes has emerged [32].

Typically, nanomimetic enzymes can be classified into two categories: oxidoreductases and hydrolases [33]. Oxidoreductases are a class of enzymes that can catalyse biological oxidation/reduction reactions, such as oxidases and peroxidases. Their ability to interact with substrates and transfer electrons from one molecule to another facilitates redox reactions [34]. Zhan et al. constructed a novel colorimetric system for the detection of dimethoate by using the oxidase mimetic activity of cubic shaped Ag_2O [35]. In this system, Ag_2O has oxidase-mimicking activity and can catalyse the substrate 3,3',5,5'-tetramethylbenzidine (TMB) to produce a small amount of blue product (oxTMB). And after the addition of dimethoate, the dimethoate could promote the electron transfer from Ag_2O to dissolved oxygen, accelerate the release of $\cdot O_2^-$ radicals and $^1O_2$, and promote the catalytic oxidation of the colour-developing substrate. The results showed that the method has good sensitivity and the detection limit was as low as 14 μg/L, which can achieve accurate and reliable analysis of residual dimethoate in food. Yuan et al. prepared Fe/4Cu-MOF nano-enzymes by co-precipitation method using Fe^{3+} and Cu^{2+} as the central ions, and 1,3,5-benzenetricarboxylic acid (H_3BTC) as organic ligands to construct a catalytic colorimetric enzyme for detecting typhoid fever and Salmonella typhimurium [36]. Among them, Fe/4Cu-MOF has peroxidase mimetic activity, which can catalyse the oxidation of TMB to produce a visible blue colour. Under the optimised conditions, the catalytic activity of Fe/4Cu-MOF decreased proportionally to the concentration of Salmonella typhimurium with the linear range of 3.6×10^2~3.6×10^7 CFU/mL and the detection limit of 1.0×10^2 CFU/mL, which was successfully applied to the detection of Salmonella typhimurium in chicken and beef.

Hydrolysis enzymes are enzymes that catalyse hydrolysis reactions, such as nucleases and proteases. They can break chemical bonds in the substrate molecule to produce two or more small molecule products [37]. Guan et al. constructed a tetrahedral-based Pb^{2+}sensitive DNAzyme sensor (TPS) for Pb^{2+} detection by using Pb^{2+}dependent GR-5 DNAzyme and DNA tetrahedral structure [38]. In the absence of Pb^{2+}, the fluorescent moiety fluorescein amide (FAM) maintained a close relationship with the quencher black hole quencher (BHQ-1), and the FAM fluorescence was effectively burst. However, when Pb^{2+} is present, DNAzyme exhibits catalytic activity and cleaves the substrate chain, allowing FAM to separate from BHQ-1 and release fluorescence. Therefore, the sensor was able to achieve Pb^{2+}specific detection with a linear range of 0-500 nmol/L and a detection limit of 1 nmol/L. Hou et al. constructed a fluorescent sensor for the detection of uric acid using papain [39]. In this method, papain catalyses the oxidation of phenylenediamine (OPD) to 2,3-

diaminophenyldiazine (DAP) and emits a distinct yellow fluorescence. Under the optimal conditions, the linear range of the method was 10~1000 μmol/L, and the detection limit was 4.6 μmol/L. The method was successfully applied to the detection of real samples, paving the way for the application of papain as a catalyst for the fluorescence detection of different target biomolecules.

Mimetic enzymes are stable, easy to prepare and environmentally tolerant, which to some extent solves the defects of natural enzymes that are easy to inactivate and difficult to prepare. With the rapid development of nanotechnology, nano-enzymes with enzyme-mimicking properties and nanomaterial features have been widely used in biochemical engineering, sensor development, environment, agriculture and food. Compared with natural enzymes and traditional mimetic enzymes, nano-mimetic enzymes are simpler to prepare, more catalytically active, more stable, and less costly, which overcomes the limitations of natural enzymes and traditional enzymes in food analysis. Although a large number of studies have been conducted using nano-mimetic enzymes for detection, their catalytic principles and applications in analytical detection in food are still rarely reported. Therefore, this paper takes nano-mimetic enzymes as an entry point to systematically discuss the catalytic mechanism of nano-mimetic enzymes and the application of various types of nano-mimetic enzymes in food analysis and detection, put forward nano-mimetic enzymes and their challenges in food analysis and detection, and give an outlook on their future development trend.

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The article highlights the field of research on specific species of metalloporphyrins containing non-pyrrole heterocycles and provides an overview of the current state of research on their synthesis, structure and properties. A basis for further studies of these interesting and structurally diverse metalloporphyrin analogues is provided.

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CHAPTER 2

NANOZYME CATALYSIS MECHANISM

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CHEN YANYU

Nanozymes are nanomaterials that both function as enzymes and surface catalysts [1]. It is at the intersection of several fields such as bioenzymes, nanomaterials and surface catalysts. From an enzymatic point of view, enzymes are mainly proteins that are created by living cells and have strong catalytic activity. They are usually composed of amino acids, sugars, metal atoms and other cofactors with specific active sites, e.g., heme molecules coordinated in protein scaffolds are the active sites of many peroxide-related enzymes [2]. The heme center is occupied by metal atoms such as Fe, Cu, Mn or Zn, which are essential for catalysis. Furthermore, heme has substrate-binding sites generated by particular amino acid structures, which provide stereospace for cofactor coordination with metal atoms and substrate binding. This complex and precise structure gives the enzyme excellent catalytic activity and selectivity [3]. Nanozymes are a type of nanomaterial having intrinsic enzyme-like characteristics that, due to their nanoscale size, display markedly different physicochemical properties than bulk inert materials [4]. In particular, single-atom nanozymes with enzyme-like structures have excellent catalytic capabilities [5]. In addition, nanozymes can catalyze bioorthogonal reactions that cannot be handled by natural enzymes, possibly due to their multivalent components, multiple active sites, or abundant ligand structures [4]. Table 2.1 demonstrates the comparison of the structure and properties of natural enzymes and nanozymes.

Table 2.1. A comparison of the structure and characteristics of natural enzymes with nanozymes [4].

	Natural enzymes	**Nanozymes**
Essence	Majorly proteins	Nanomaterials
Composition	Amino acids, saccharides, metal atoms/ion, and cofactors.	Metal or nonmetal atoms/ion.
Structure characteristics	(a) With active site. e.g., metal atoms in heme's center. (b) Substrate binding site with specific structure.	(a) With active site. e.g., coordination unsaturated surface atoms. (b) Without specific substrate binding structure, except for chiral nanozymes.
Number of active sites	Limited	Multiple
Utilization of active sites	High	Low, except for single-atom nanozymes.
Catalytic characteristics	High catalytic activities and substrate specificity.	High catalytic activity, low substrate specificity.

Nanozyme activity is generally determined by their size, shape, and exposure to external stimuli (e.g. light, sound and heat) [6]. In terms of particle size, smaller particles have more activity. This is due to the fact that as the size of the nanoparticles decreases, the specific surface area increases significantly. The increased specific surface area results in a significant shift in surface atomic number and surface energy, influencing the catalytic activity of nanoparticles [7]. And as the particle size decreases, the proportion of surface atoms increases significantly and the proportion of surface atoms to total atoms changes [8]. The fast increase in surface atom ratio alters the character of nanoparticles. In addition, as the size decreases, many dangling and unsaturated bonds are created on the surface atoms. These surface atoms with high surface energy are very unstable, and react easily with other atoms, groups or substances, and demonstrate significant chemical and catalytic activity in nanozymes [9]. Morphologically, because the atomic coordination environment is intimately linked to the dangling bonds and crystal planes, the more saturated the coordination, the lower the catalytic activity, and vice versa [4]. In addition, certain external factors may stimulate the activity of the nanozymes due to the activation of the electrons upon gaining energy [10]. When a given energy is given, on the one hand, the ligand-unsaturated atoms on the surface of the nanoparticle are excited into more active atoms. On the other hand, as atomic motion accelerates, saturated atoms near the surface break away from their original bonds and become active. All of these factors may influence the active site of nanozymes, resulting in variations in catalytic activity. The active site is a surface active atom or site with high energy due to unsaturated coordination, which can form a strong binding ability with other atoms, groups or substances. Common active sites include ligand-unsaturated surface atoms and surface defects (e.g., steps, kinks, attached atoms, and vacancies) [11].

To date, a wide range of nanomaterials exhibiting notable enzyme-like activities, commonly referred to as nanozymes. These nanozymes have received great attention due to their robust stability, tunable activity, and cost-effectiveness compared to natural enzymes. The primary categories of nanozymes include hydrolytic enzymes, oxidases, peroxidases, catalases, and superoxide dismutases, each mimicking the catalytic function of their corresponding natural enzymes. Hydrolytic nanozymes catalyze the breakdown of complex biomolecules into simpler forms, akin to natural hydrolases, and have shown potential applications in bioremediation and food processing [12]. Oxidase nanozymes facilitate the oxidation of substrates in the presence of molecular oxygen, which makes them valuable for applications in environmental monitoring and biosensing [13]. Peroxidase nanozymes, one of the most extensively studied types, catalyze the breakdown of hydrogen peroxide (H_2O_2) to generate reactive oxygen species, and are widely used in biosensors and cancer therapy [14]. Catalase nanozymes decompose H_2O_2 into water (H_2O) and oxygen, offering applications in antioxidant therapies and environmental detoxification [15]. Superoxide dismutase nanozymes convert superoxide anions into less reactive species, playing a crucial role in oxidative stress management and disease treatment [16]. Typical nanozyme application materials and their characteristics are shown in Table 2.2. Despite these advances, the catalytic mechanisms underlying the activity of most nanozymes remain poorly understood. The absence of a comprehensive understanding of these mechanisms has hindered the development of more efficient and targeted nanozyme applications. Therefore, this section aims to analyze and summarize the catalytic mechanisms of commonly studied nanozymes, categorizing them based on their enzyme-like activities. A better understanding of these mechanisms will not only facilitate the design of advanced nanozymes with enhanced specificity and efficiency but also expand their application scope across diverse fields, including medicine, environmental science, and industrial biotechnology.

Table 2.2. Typical nanozyme application materials and their characteristics.

Enzyme	Nanomaterial	Representative	Characteristic
Hydrolysis enzyme	Metal nanomaterials	Gold nanoparticles, silver nanoparticles	The properties of these metallic nanomaterials were utilized to catalyze the hydrolysis reaction.
	Carbon nanomaterials	Carbon nanotubes、graphene	Carbon nanomaterials are utilized to better contact with the substrate and promote the hydrolysis process by taking advantage of properties such as their large specific surface area.
	Oxide nanomaterials	Titanium dioxide, zinc oxide	The corresponding hydrolases perform the hydrolysis reaction based on the crystal structure, surface charge and other factors of the oxide nanomaterials.
Oxidase	Metal nanomaterials	Gold nanoparticles	Exhibits glucose oxidase-like activity and catalyzes the adsorption of glucose anions on its surface and affinity addition reactions with oxygen molecules.

	Cerium oxide nanoparticles	Catalytic oxidation of a wide range of amine substrates with a catalytic mechanism related to valence transitions of surface cerium ions and generation of superoxide anion radicals.
	Iron-based nanomaterials	Possesses ferric oxidase activity, which can alter intracellular ferrous ion concentration or cytochrome C redox state and regulate iron homeostasis and metabolism.
Carbon nanomaterials	Carbon nanotubes、graphene	With properties such as large specific surface area and unique electronic structure, it can function as an oxidase.
Metal-organic framework materials	Metal-organic framework	By choosing different metal nodes and organic ligands, metal-organic framework nanozymes with specific oxidase activities can be designed. Its pore structure can also restrict the domain substrate or catalytic activity center to enhance the catalytic performance.
Oxidized nanomaterials oxidase	Molybdenum oxide nanoparticles	Sulfite oxidase-like activity, catalytic oxidation of sulfite by valence conversion of molybdenum ions.
	Manganese dioxide nanomaterials	Oxidase-like activity that catalyzes the conversion of oxygen molecules into reactive oxygen radicals.

	Single-atom nanomaterial oxidase	M-N-C type monoatomic nanozymes such as Fe-N-C, Co-N-C, etc.	Metal atoms act as catalytically active centers and form stable structures with surrounding N and C atoms, exhibiting excellent oxidase activity.
Peroxidase	Metal nanomaterials	Iron-based nanomaterials such as iron tetraoxide nanoparticles	Iron can participate in redox reactions and catalyze the oxidation of substrates in the presence of H_2O_2.
		Gold nanoparticles	It can react with H_2O_2 to produce reactive oxygen species with strong oxidizing properties.
	Carbon nanomaterials	Carbon nanotube	Possesses unique electrical and chemical properties. Capable of mimicking the behavior of peroxidase when modified with appropriate functionalization.
		Graphene and its derivatives	Due to its large specific surface area and good electrical conductivity, it can effectively adsorb the substrate and transfer electrons during the catalytic reaction to promote the oxidation reaction of the substrate by H_2O_2.
	Oxide nanomaterials	Manganese dioxide nanomaterials	It is able to utilize its own chemical structure characteristics for catalysis, and can be used to optimize peroxidase activity by controlling its morphology, crystalline form, and other factors.

Catalase enzyme	Metal oxide nanomaterials	Cerium oxide nanomaterials	Its surface allows redox reactions and its catalytic activity is related to the change in valence of cerium.
		Manganese dioxide nanomaterials	Catalytic decomposition of H_2O_2 by valence changes of metal ions in metal oxides.
	Carbon nanomaterials	Carbon nanotube	The large specific surface area and unique electron-conducting properties can interact with H_2O_2 for efficient electron transfer.
Superoxide dismutase	Metal nanomaterials	Gold nanoparticles	The activity of superoxide dismutase can be mimicked by loading the active center-like structure of superoxide dismutase onto the gold nanoparticles surface.
	Metal oxide nanomaterials	Cerium dioxide nanoparticles	The unique redox property allows it to catalyze the disproportionation reaction of superoxide anion by Ce^{3+}/Ce^{4+} conversion on the surface to achieve the removal of superoxide anion.
	Carbon nanomaterials	Carbon nanotubes, graphene, etc.	It can be utilized as an electron transfer medium to promote the disproportionation reaction of superoxide anion and effectively scavenge superoxide anion.

2.1 Hydrolysis enzyme

Hydrolytic enzymes are a class of biological catalysts that specialize in cleaving specific chemical bonds such as ester, amide/peptide, and glycosidic bonds through hydrolysis reactions. They are extensively utilized in industrial production for processes such as polymer degradation, food processing, and biofuel production [17]. One distinguishing feature of hydrolytic enzymes is the unique structural configuration of their active sites, which are designed to interact with specific substrates. Unlike other enzymes, hydrolytic enzymes often possess active site regions that facilitate highly specialized binding interactions with substrate molecules [18]. This structural specialization allows for the precise alignment of the substrate within the active site, ensuring optimal conditions for catalysis. The hydrolysis process catalyzed by these enzymes is facilitated by their ability to lower the activation energy barrier of the reaction. When the substrate reaches the enzyme's active site, it becomes concentrated, and the interaction between the enzyme and substrate induces conformational changes in both molecules. This interaction often alters the electron density in specific groups of the substrate molecule, creating electron tension. This electron tension makes the targeted chemical bond, such as an ester or glycosidic bond, more labile and susceptible to cleavage. As the reaction progresses, the enzyme forms a transient enzyme-substrate covalent intermediate. The intermediate is structurally similar to the transition state of the reaction and is intrinsically unstable, making it easier for the substrate to overcome the activation energy barrier, accelerating the hydrolysis process and ultimately generating the target product [19]. Through this mechanism, hydrolytic enzymes exemplify the efficiency of enzymatic catalysis, transforming substrates with remarkable speed and precision. Their unique structural and mechanistic properties underline their pivotal role across diverse fields, and further study into their catalytic processes could enhance their application potential in cutting-edge technologies such as bioengineering, environmental remediation, and precision medicine.

2.1.1 Nanozymes with phosphoric ester hydrolysis enzyme activity

As a typical class of readily hydrolyzable substrates, phosphate esters are important in the field of biochemistry and nanozymatic research. Most of the research efforts targeting phosphate hydrolysis have focused on modeling the properties and behaviors of phosphoric ester hydrolysis. As a new type of catalyst, phosphoric ester hydrolysis nanozymes have diverse catalytic mechanisms. For example, metal-oxide nanozymes are able to effectively promote the breaking of phosphate bond through acid-base catalysis and redox catalysis by oxygen vacancies or metal ions on the surface. In contrast, metal-organic framework (MOF) nanozymes cleverly utilize the Lewis acidic sites of metal nodes to activate the phosphate bond of the substrate, thus realizing the catalytic effect [20-22]. These diverse catalytic mechanisms provide abundant ideas and methods for the study of phosphate hydrolysis nanozymes. Through a simple cluster metallization strategy, Xu et al. mixed MOF-808 with aluminum nitrate at 85°C to obtain MOF-808-Al nanozymes with strong Lewis acid Al^{3+} sites, as shown in Figure 2.1 [23]. The relative concentration of acid sites on the surface of the nanozymes was established by characterisation. It was demonstrated that the addition of an Al^{3+} site might raise the Lewis acidity of the nanozymes by more than twofold. MOF-808-Al has excellent activation and nucleophilic attack ability of ethyl paraoxon substrate, and its hydrolysis rate is 1923.08 mol/(L·min), which is an order of magnitude higher than that of MOF-808 (188.14 mol/(L·min)), showing very high catalytic activity. Furthermore, MOF-808-Al has great biocompatibility and works well to reduce the toxicity of organophosphorus nerve agents while also healing cell tissue damage.

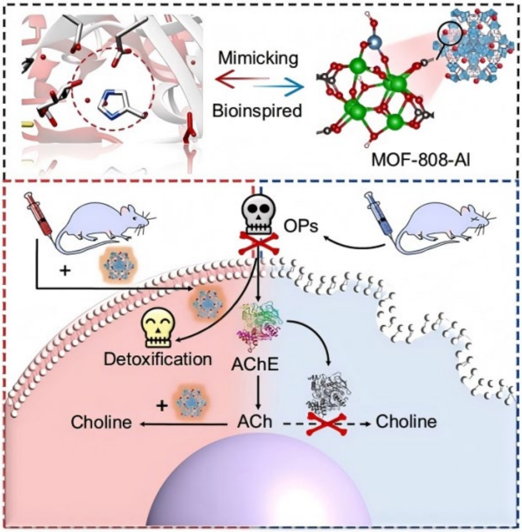

Figure 2.1 Schematic diagram of the bionic active center of MOF-808-Al and its neuroprotective effect against organophosphates [23].

2.1.2 Nanozymes with proteolytic enzyme-like activity

The catalytic mechanism of nanozymes exhibiting proteolytic enzyme-like activity primarily relies on the hydrolysis of peptide bonds within protein molecules. These nanozymes mimic the catalytic function of natural proteolytic enzymes by breaking down complex proteins into smaller peptides or amino acids through specific active centers and well-defined catalytic processes [24]. The ability of proteolytic nanozymes to decompose proteins into peptides or amino acids makes them valuable in a wide range of applications. In the biomedical field, they can be used for protein digestion in proteomics research, drug delivery systems, and therapeutic treatments targeting specific protein aggregates. In environmental science, these nanozymes have potential for the degradation of protein-based waste, contributing to sustainable waste management practices. In summary, nanozymes with proteolytic enzyme-like activity emulate the sophisticated catalytic mechanisms of natural enzymes, enabling the hydrolysis of peptide bonds with high efficiency and specificity. Their unique properties and versatility open up promising avenues for applications in biotechnology, medicine, and environmental remediation. Wang et al. used zirconium sulfate tetrahydrate and 3,3',5,5'-tetracarboxylic diphenylmethane as precursors to manufacture MIP-201 zirconium-based MOF material with square octahedral topological network structure via hydrothermal method [25]. The strong Lewis acid zirconium site increased the hydrolysis rate of the dipeptide substrate by more than three orders of magnitude. The study discovered that MIP-201 can selectively cut 5 peptide bonds out of 153 amino acids in myoglobin, indicating high selectivity, and is expected to be an efficient and practical alternative for selectively hydrolyzed proteins in proteomics and biotechnology. Inspired by the synergistic effect of various cofactors in the natural enzyme activity pocket, Yuan et al. introduced serine with hydroxyl side chain into the zinc metal site of zinc azolate framework (ZAF) material through pre-synthesis method, and obtained ZAF (Ser) nanozymes with high catalytic activity (Figure 2.2A) [26]. Among them, the serine side chain hydroxyl group can form hydrogen bond with the azo ligand to form the second amino acid coordination microenvironment. Mixtures such as Zn^{2+} and ligands alone do not show significant activity, and ZAF (Ser) is more active than ZAF and Zn Ser (Figure 2.2B). This study indicated that the active expression of ZAF (Ser) is not only regulated by the metal site of Lewis acid in the primary coordination environment, but also the formation of hydrogen bonds in the second amino acid coordination environment can further enhance its catalytic activity. The experimental results of removing hydrogen bonds by increasing temperature also prove that the presence of hydrogen bonds is a key reason for the higher activity of ZAF (Ser) than ZAF (Figure 2.2C). ZAF (Ser) nanozymes offer advantages such as high stability, recyclability and a wide range of substrates, making them a potential substitute for future artificial enzyme and protein engineering designs.

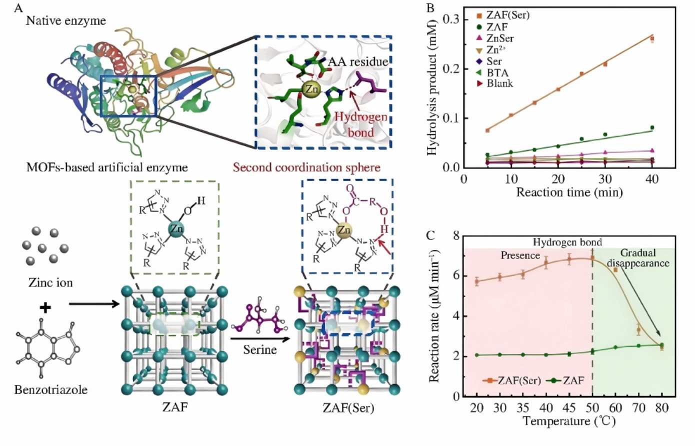

Figure 2.2 (A) Design of the artificial hydrolytic enzyme ZAF (Ser); (B) Control experiments showing the catalytic activities of ZAF (Ser), ZAF, Zn Ser, and the corresponding building blocks under identical concentrations; (C) Disappearance of hydrogen bonds induced by temperature [26].

2.1.3 Nanozymes with glucoside hydrolysis enzyme activity

Glucoside hydrolysis enzymes play a critical role in sugar metabolism within organisms, facilitating the breakdown of polysaccharides into simpler sugars and their derivatives with low degrees of polymerization [27]. These enzymes are essential for various biological processes, including energy production, cell signaling, and carbohydrate metabolism. Similarly, nanozymes with glucoside hydrolysis enzyme-like activity mimic these natural enzymes, offering a robust alternative for catalyzing the hydrolysis of glycosidic bonds. Nanozymes with glucoside hydrolysis activity are designed with specific active sites and structural features that enable them to recognize and bind glycoside substrates selectively. Once the substrate is bound, the nanozymes facilitate a series of chemical reactions that weaken and ultimately cleave the glycosidic bond. This process releases sugar molecules or other hydrolysis products, replicating the function of natural enzymes [28]. Glucoside hydrolysis nanozymes emulate the natural enzymatic process of breaking glycosidic bonds with high specificity and efficiency. Their structural adaptability and catalytic robustness position them as promising tools in biotechnology, energy production, and medical research, bridging the gap between natural enzyme functionality and artificial catalytic systems. Yu et al. annealed copper(I) phosphide (Cu_3P) nanoparticles obtained by mixing copper oxide nanoparticles with sodium hypophosphite and annealing at 300°C, which were able to catalyze glycosidic bond breaking as a glycoside hydrolysis enzyme mimic, as shown in Figure 2.3 [29]. Cu_3P nanoparticles had high catalytic activity against glycoside substrates in acidic circumstances, and experimental results and theoretical calculations confirmed that their catalytic pathways were similar to those of natural enzymes.

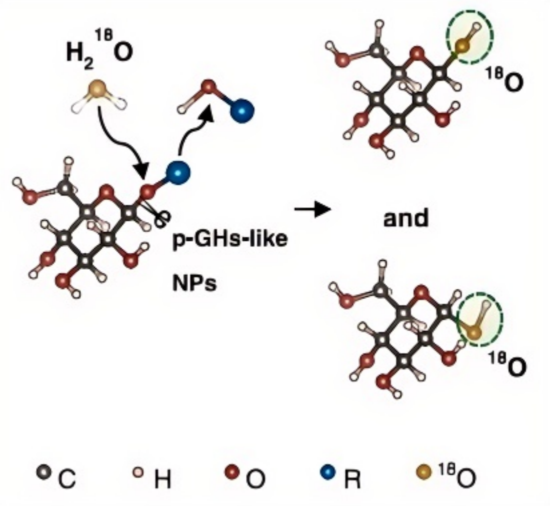

Figure 2.3 Schematic diagram of hydrolysis glycosidic bond [29].

2.2 Oxidase

Oxidase, a unique and highly specialized biocatalyst, plays a critical role in facilitating oxidation reactions. By utilizing molecular oxygen or other oxidizing agents, oxidase catalyzes the transformation of substrates into oxidized products while generating byproducts such as H_2O, H_2O_2, or superoxide anions ($O_2^{-\cdot}$) as part of the reaction process [30]. This remarkable enzymatic functionality enables oxidase to drive essential biochemical transformations with high efficiency and precision. One of the most noteworthy characteristics of oxidase is its ability to act directly on substrates without requiring the addition of external reactive agents like H_2O_2. This feature is particularly significant, as H_2O_2, while a common reagent in many oxidative processes, can pose risks to both the reaction system and the surrounding environment due to its oxidative and potentially harmful properties [31]. By eliminating the need for such hazardous intermediates, oxidase ensures that the catalytic process is not only safer but also more environmentally friendly. This distinct advantage enhances the suitability of oxidase-based reactions for applications in biochemistry, biotechnology, and related fields. Moreover, the catalytic mechanism of oxidase offers a high degree of operational convenience. The direct use of molecular oxygen as an electron acceptor simplifies reaction setups and reduces the complexity of the process. This streamlined approach not only minimizes the need for additional reagents but also lowers operational costs, making oxidase an attractive option for large-scale and industrial applications [32]. Oxidase stands out as a versatile and efficient biocatalyst that combines safety, convenience, and specificity. Its ability to catalyze oxidation reactions without harmful intermediates underscores its significance in advancing biochemical and industrial processes. This makes oxidase an indispensable tool in the pursuit of innovative and sustainable solutions across a wide range of scientific and technological fields.

2.2.1 Glucose oxidase

Glucose oxidase (GOD) is a flavin-containing oxidoreductase known for its ability to catalyze the oxidation of *β-D*-glucose into *D*-glucono-δ-lactone, utilizing molecular oxygen (O_2) as an electron acceptor and producing H_2O_2 as a byproduct (Figure 2.4) [33]. This reaction proceeds through a two-step mechanism: reduction and oxidation, each with distinct rate-limiting steps that define the overall catalytic efficiency of the enzyme. In the reduction step, *β-D*-glucose binds to the active center of GOD, initiating a sequence of reactions facilitated by key amino acid residues. The histidine residue in the active center acts as a base, attracting protons from the first hydroxyl group of glucose. This proton transfer destabilizes the glucose molecule, enabling the transfer of a hydrogen ion from the C_1 atom of glucose to the N_5 position of the flavin ring within the active center of GOD.

This proton transfer is the rate-limiting step of the reduction reaction, determining the speed at which the flavin ring is reduced. The subsequent oxidation step involves the transfer of electrons from the reduced flavin ring to molecular oxygen. This process generates a flavin semiquinone radical and a superoxide anion intermediate. The electron transfer step, which involves interactions between the flavin ring and O_2, is the rate-limiting step of the oxidation reaction. In this catalytic process, *β-D*-glucose is oxidized to *D*-glucono-δ-lactone, which is then hydrolyzed into *D*-gluconic acid in the presence of lactonase. The cofactor flavin adenine dinucleotide (FAD) plays a central role in this enzymatic cycle. During the reduction step, FAD is reduced to fatty acid desaturase 2 ($FADH_2$) as it accepts electrons from glucose. In the oxidation step, molecular oxygen interacts with $FADH_2$ in the active site, oxidizing it back to FAD while simultaneously reducing O_2 to H_2O_2. This regeneration of FAD ensures the enzyme's continuous catalytic activity and highlights its role as a critical electron shuttle within the reaction [34-36]. This dual-step mechanism not only underscores the intricate nature of GOD's enzymatic function but also its efficiency in facilitating glucose oxidation. The production of H_2O_2 as a byproduct is particularly significant in biosensing applications, where it serves as a measurable signal for glucose concentration. The specificity and well-characterized catalytic mechanism of GOD have made it a cornerstone in the development of glucose biosensors, diagnostic tools, and other biotechnological applications.

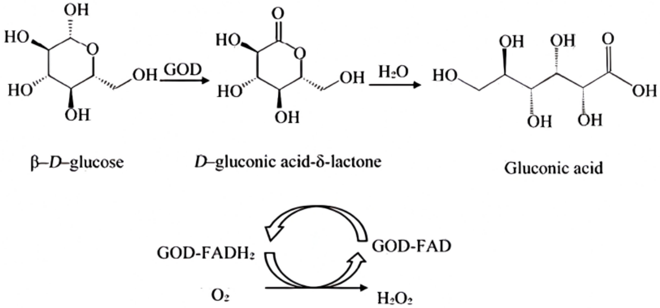

Figure 2.4 Catalytic mechanism of GOD [33].

Gold nanoparticles (AuNPs) are structurally distinct from the active site of GOD, but have GOD-like activity. It is highly efficient in the oxidation of glucose to create H_2O_2 and has sparked significant interest in biological detection and treatment. In 2004, Rossi and colleagues proposed that "naked" AuNPs may catalyze the production of glucose into gluconic acid and H_2O_2 in the presence of O_2 [37]. The controlled experiments showed that other metal nanomaterials had no obvious catalytic ability for glucose oxidation. Subsequently, through a series of explorations, they gave a reasonable explanation for this phenomenon in 2006 based on the promotion of bases and the generation of H_2O_2, and proposed the molecular activation mechanism of gold catalysis, as shown in Figure 2.5 [38]. The hydrated glucose anion reacts with gold surface atoms to generate electron-rich gold, which then activates molecular oxygen via nucleophilic attack. The Au^+-O_2^- or Au^{2+}-O_2^{2-} pairs of gold dioxide intermediates can be used as a bridge to convert electrons from glucose to dioxygen. In this way, the reaction product is finally formed.

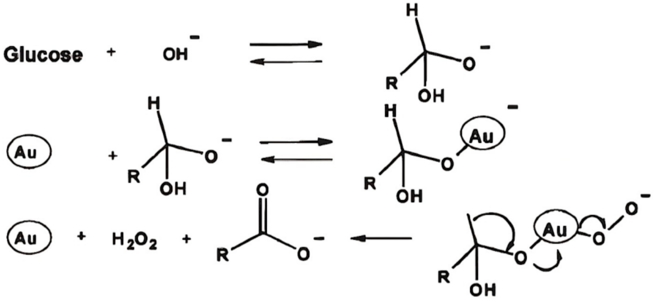

Figure 2.5 The catalytic mechanism of AuNPs as GOD mimics [38].

Huang et al. suggested a new colorimetric sensor array based on AuNPs with GOD-like activity for the detection of monosaccharides, as shown in Figure 2.6 [39]. AuNPs can employ O_2, $ABTS^{+}\cdot$ or $[Ag(NH_3)_2]^+$ as electron acceptors to catalyze the oxidation of various monosaccharides at varying rates, causing different color development processes, resulting in a cross-response signal. Combining linear discriminant analysis and hierarchical cluster analysis, the sensor array can distinguish different monosaccharides or their mixtures, and the confusion matrix shows that the sensor array has good discrimination performance. At the same time, the sensor array's neural network regression model may be utilized to forecast the concentration of monosaccharide components in the mixture. The approach has promise in industrial, medicinal, and biological monosaccharide detection applications.

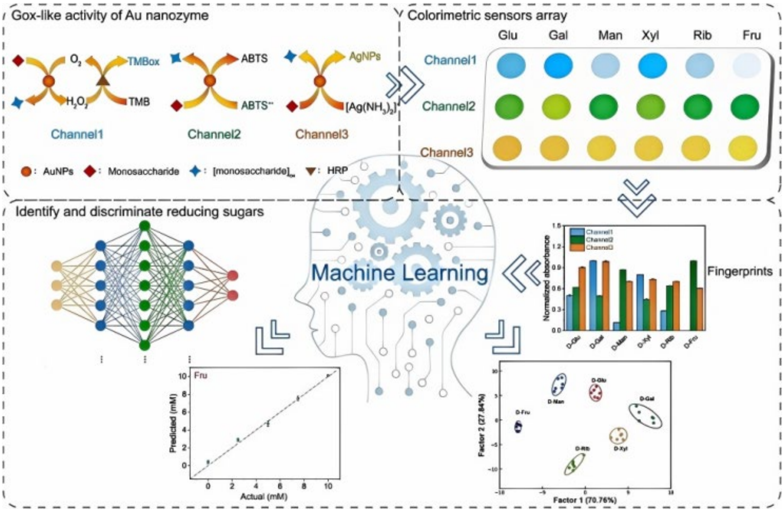

Figure 2.6 Principles of identification of monosaccharides by sensor array based on GOx-like activity of AuNPs and machine learning [39].

2.2.2 Sulfite oxidase

Sulfite oxidase (SuOx) is a molybdenum-containing enzyme found in mitochondria that catalyzes the oxidation of hazardous sulfites to sulfate utilizing cytochrome C as an electron acceptor. The catalytic process involves molybdenum cofactors in the enzyme's active core. In the catalytic process, the sulfite binds to the molybdenum atom, the active center of the enzyme, and the oxidation state of the molybdenum atom changes to promote electron transfer. Sulfites are oxidized while electrons are passed to isoelectron acceptors for cytochrome C through an electron transport chain within the enzyme, and eventually oxygen acts as a terminal electron acceptor to produce H_2O, while sulfites are converted to sulfate. This electron transport is essential for sustaining physiological processes like redox equilibrium within cells [40]. SuOx plays a key role in intracellular detoxification, and a shortage of it may cause certain diseases. Tremel and his team revealed in 2014 that molybdenum trioxide nanoparticles have similar activity as SuOx under normal physiological settings, turning harmful sulfites into sulfates [41]. The catalytic mechanism for this transition is depicted in Figure 2.7. From this we can see that the sulfite ion first binds to the molybdenum oxide nanoparticle. Subsequently, during the reduction of Mo^{VI} to Mo^{IV}, sulfite ions are oxidized to sulfate