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REVIEW

Oxidoreductase-Like Nanozymes: From Biosensing to Molecular Mechanisms in Disease Therapy

Mao Cao[1], Jiazi Ma[1], Yong Yang[1], Mengjie Cheng[1], Jianwei Liu[2], Zhifeng Pan[1], Zhongjun Du[1,3]

[1]Shandong Academy of Occupational Health and Occupational Medicine, Shandong First Medical University & Shandong Academy of Medical Sciences, Ji'nan, Shandong, 250062, People's Republic of China; [2]Public Health Monitoring and Evaluation Institute, Shandong Provincial Center for Disease Control and Prevention, Ji'nan, Shandong, 250000, People's Republic of China; [3]Occupational Disease Department, Harbin Second Hospital, Harbin, Heilongjiang Province, 150026, People's Republic of China

Correspondence: Zhongjun Du; Zhifeng Pan, Shandong Academy of Occupational Health and Occupational Medicine, Shandong First Medical University & Shandong Academy of Medical Sciences, No. 18877 Jingshi Road, Lixia District, Jinan, Shandong, 250062, People's Republic of China, Email duzhongjun@sdfmu.edu.cn; duzj1981@163.com; 18653199172@163.com

Abstract: Nanozymes, a class of nanomaterials capable of mimicking the functions of natural enzymes, have garnered significant attention in biomedical fields because of their stable catalytic activity, high efficiency, low cost, and tunable enzyme-like properties. In recent years, advances in nanotechnology have led to the development of numerous nanozymes with redoxase-like activities, which have been widely applied in biosensing and disease treatment, demonstrating considerable potential. In this review, we first summarize the redoxase-like activity of nanozymes. From the perspective of redox regulation, we discuss the catalytic mechanisms of nanozymes in biosensing applications, elaborate on the molecular mechanisms involved in tumor therapy, including the induction of apoptosis and ferroptosis, and examine their catalytic pathways in antibacterial and anti-inflammatory treatments. Finally, we also discuss the current limitations and future challenges of nanozymes in biomedical applications, aiming to provide insights for the rational design and clinical translation of next-generation nanozyme-based platforms.

Keywords: nanozymes, redoxase-like, biosensing, tumor treatment, antibacterial

Introduction

Enzymes, a class of biological catalysts with protein or RNA structures, play essential roles in organism metabolism and maintain the normal progression of various biochemical reactions.[1] Owing to their high catalytic efficiency, enzymes are widely employed in medicine. For example, oxidoreductases have been utilized to construct platforms for biosensing, food production, disease treatment, and other applications.[2] However, the inherent limitations of natural enzymes, such as unstable catalytic activity, high production costs, and complex preparation technologies, have significantly restricted their broader use in the biomedical field.[3] Therefore, it is necessary to find suitable substitutes that can mimic the catalytic activity of natural enzymes for biomedical applications.

Nanozymes are nanomaterials that can imitate the catalytic activity of natural enzymes. The concept of "nanozymes" was first introduced by Manea et al in 2004.[4] Since then, nanozymes have attracted considerable attention as promising substitutes for natural enzymes. With advances in nanotechnology, a wide variety of nanomaterials that mimic natural enzymatic activity have been developed, including noble metal nanozymes (such as Au and Pt nanozymes),[5] single-atom nanozymes (such as FeN_5 and CoN_x),[6,7] metal oxide nanozymes (like Fe_3O_4 for magnetite and CeO_2 for cerium oxide),[8–10] metal sulfide nanozymes,[11] carbon-based nanozymes such as graphene,[12] and metal–organic frameworks (MOFs).[13] They function as analogues of natural oxidoreductases, exhibiting peroxidase (POD), oxidase (OXD), catalase (CAT), and superoxide dismutase (SOD)-like activities, and have thus shown remarkable potential in biomedical research.

Compared with natural enzymes, nanozymes offer several advantages, including stable catalytic performance, low production cost, and ease of modification, making them excellent candidates as enzyme substitutes. Due to their ability to regulate reactive oxygen species (ROS), chemically reactive molecules containing oxygen, they are valuable, nanozymes

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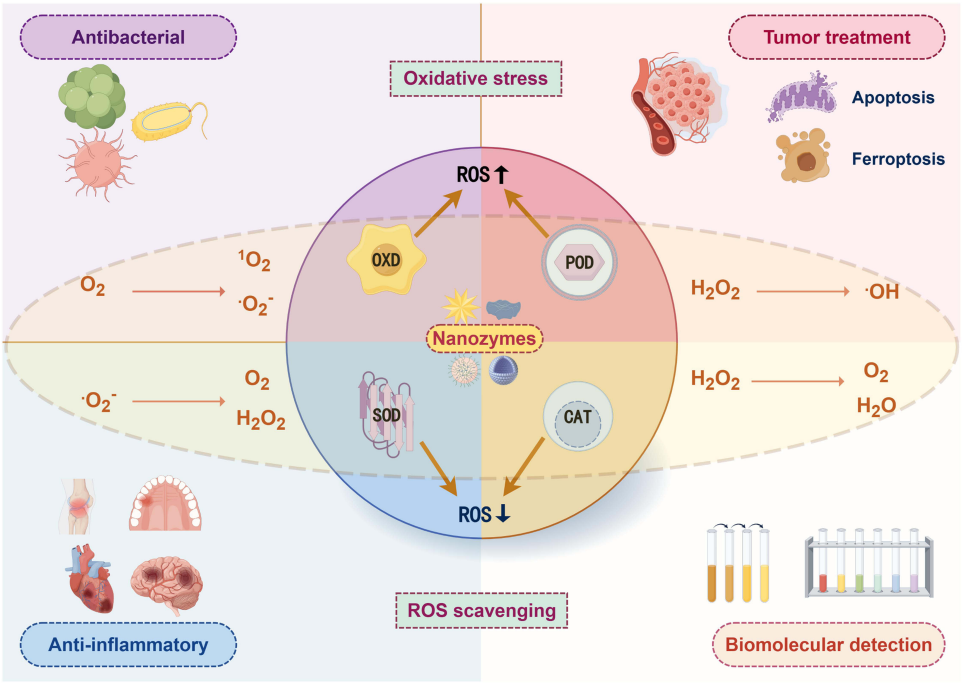
Graphical Abstract



have been applied across numerous fields, particularly in biomedicine, where they have demonstrated significant value in biosensing, disease treatment, antibacterial strategies, and anti-inflammatory applications.[14] Consequently, investigating the biomedical applications of nanozymes holds substantial medical importance.

To better understand the catalytic mechanism of nanozymes, in this review, we summarize the redoxase-like activity of nanozymes and highlight their potential applications in the biomedical field. Specifically, we focus on the perspective of oxidative stress and the regulation of redox metabolism by nanozymes. First, we outline the fundamental redoxase-like activities of nanozymes, including POD, OXD, CAT, and SOD-like activities. Next, we systematically discuss their biomedical applications in biosensing, tumor therapy, antibacterial treatment, and antioxidant therapy. Subsequently, we critically evaluate the current challenges in biocompatibility, targeting efficiency, and clinical translation. Finally, we propose future research directions to advance the development and application of nanozymes in precision medicine.

Redoxase-Like Activity of Nanozymes

Peroxidase-Like Activity

Peroxidase (POD) is an enzyme that utilizes hydrogen peroxide (H_2O_2) as an electron acceptor to catalyze the oxidation of substrates, generating hydroxyl radicals (·OH) in the process.[15] A classic example is horseradish peroxidase (HRP), which oxidizes the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB) to its oxidized form (oxTMB) in the presence of H_2O_2. In 2007, Yan et al reported that magnetite nanoparticles (Fe_3O_4) possess intrinsic POD-like activity and can similarly catalyze substrate oxidation in the presence of H_2O_2.[8] For example, they catalyze the oxidation of TMB (producing a blue color) and diaminobenzidine (DAB) (producing a brown precipitate). The catalytic mechanism resembles that of HRP, following a "ping-pong mechanism", wherein ferrous ions in the iron oxide structure play a central role by binding and releasing the first product before interacting with the second.

Since this discovery, an increasing number of researchers have sought to regulate the POD-like activity of nanozymes by modifying nanomaterial structures and engineering distinct active sites. For example, Li and colleagues[16] used a reverse thermal sintering process to atomize platinum nanoparticles (Pt NPs) into thermally stable platinum single-

atom nanozymes (PtTS-SAzymes), fully exposing the metal catalytic sites. Thus, compared with their nanoparticle counterparts, the resulting PtTS-SAzymes exhibited significantly enhanced POD-like activity. Moreover, Lou and colleagues[17] reported that gold nanoparticles (Au NPs) also display POD-like activity, which can be modulated by adjusting the environmental conditions such as pH, temperature, particle size, and surface modifications. This tunability improved the sensitivity of the Au NPs in the enzyme-linked immunosorbent assay (ELISA), opening new avenues for nanozyme-based immunoassays.

Oxidase-Like Activity

Oxidases (OXD) are a class of enzymes that catalyze the oxidation of substrates using oxygen (O_2) as the electron acceptor.[18] In terms of catalytic activity characterization, unlike peroxidases, oxidases do not require H_2O_2 to catalyze substrate oxidation. For example, in the presence of O_2, OXD can oxidize TMB to produce a blue color. This oxidation typically involves the generation of reactive oxygen species (ROS), such as superoxide anion ($\cdot O_2^-$) or singlet oxygen ($^1O_2$), as intermediates. For example, Lu and colleagues[7] synthesized a series of cobalt single-atom nanozymes with varying nitrogen coordination numbers and reported that the Co–N_3–C active site exhibited optimal oxidase-like activity. In the presence of O_2, it effectively oxidized TMB to produce a blue product. The proposed catalytic mechanism involves the adsorption and dissociation of O_2 molecules on the Co–N_3–C active site. This finding offers valuable insight into regulating the enzyme-mimicking activity of nanozymes at the atomic scale.

Additionally, oxidases can be categorized on the basis of their specific substrates, such as glucose oxidase (GOx) and glutathione oxidase (GSH-Ox). For example, Santamaria and colleagues[19] synthesized core–shell Au–Pt and Pt nanodendrites via a templating polymer-assisted method. Under neutral pH conditions, these nanostructures mimic the catalytic activity of GOx, which oxidizes glucose to gluconic acid and H_2O_2. Tian and colleagues[20] developed a Pd nanozyme-modified hydrogenated TiO_2 (H-TiO_2@Pd) nanozyme system. This system exhibits GSHOx-like activities, which significantly deplete glutathione (GSH) to downregulate the protein expression of glutathione peroxidase 4 (GPX4) for tumor treatment. Therefore, through a deep understanding of oxidase catalytic mechanisms and precise atomic-scale regulation, the design of high-performance biomimetic nanozymes has become feasible. This provides a solid theoretical foundation and innovative material platform for developing novel biosensors and advanced biomedical applications, such as highly effective tumor treatment strategies. Future research is expected to further optimize the structure of the active center and its microenvironment, thereby expanding their/the broader application prospects in fields such as precision diagnosis and catalytic therapy.

Catalase-Like Activity

CAT is an enzyme that catalyzes the decomposition of H_2O_2 into water (H_2O) and O_2.[21] As a type of ROS, H_2O_2 can induce the peroxidation of proteins, lipids, and mitochondrial DNA, leading to cellular and tissue damage.[22] Consequently, nanozymes with CAT-like activity can act as ROS scavengers, showing promise in the development of antioxidant therapies such as those that promote wound healing and alleviate inflammation. The first report of nanomaterials exhibiting CAT-like activity was by He et al[23] who demonstrated that gold nanoparticles (Au NPs) display POD-like activity under acidic conditions and transition to CAT-like and superoxide dismutase (SOD)-like behaviors as the pH increases. Moreover, by modifying the surface coatings of the Au NPs, their CAT-like activity could be effectively regulated. This work provides a foundation for the development of Au NP-based drug delivery platforms.

Superoxide Dismutase-Like Activity

Superoxide dismutase (SOD) catalyzes the disproportionation of $\cdot O_2^-$ into O_2 and H_2O_2, serving as a key ROS scavenger in vivo that protects organisms from oxidative stress caused by ROS overaccumulation.[22] With advances in nanotechnology, numerous nanomaterials, including metal-based and metal oxide nanozymes, have been developed to mimic SOD-like catalytic activity.[24] These SOD-like nanozymes hold significant potential for the antioxidant treatment of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.[25]

Synergistic Effects Among Redoxase-Like Activities

Although the diverse catalytic characteristics of their redoxase-like activities (as summarized in Table 1), these nanozymes often exhibit synergistic effects in biological systems. This synergistic effect is primarily manifested in the cascade reactions among the multiple redoxase-like activities simulated by nanozymes, where the reaction product of one enzyme-like activity can serve as the substrate for another. These redoxase-like activities are interconnected through shared intermediate metabolites, forming a dynamic and interdependent reaction network. The specific mechanisms can be summarized as follows: (1) Synergy between OXD-like and POD-like activities: OXD-like activity generates H_2O_2 during catalysis, and the in situ-produced H_2O_2 can directly serve as the substrate for its own POD-like activity, thereby significantly enhancing the overall oxidative capacity of the system and constituting a self-supplying cascade catalytic process. (2) Synergy between SOD-like and POD-like activities: SOD-like activity converts $\cdot O_2^-$ into H_2O_2, which subsequently provides the substrate for POD-like activity. This synergistic mechanism is particularly important in antioxidant or inflammation-related environments. (3) Synergy between CAT-like and OXD-like activities: CAT-like activity decomposes H_2O_2 to produce O_2, alleviating local hypoxia and providing additional substrate O_2 for OXD-like activity. In turn, OXD-like activity catalyzes the generation of H_2O_2. Together, they form a positively reinforcing substrate cycle, enhancing the catalytic efficiency of the nanozyme. Additionally, peroxidase and catalase both utilize H_2O_2 as a substrate, enabling cascade reactions that increase the overall catalytic efficiency. Figure 1 illustrates the principal catalytic reactions and interrelationships among these redoxase-like activities of nanozymes.

As nanotechnology continues to evolve, nanomaterials capable of mimicking multiple redoxase activities have been developed. By leveraging cascade reactions among nanozymes, their biomedical applicability has been substantially expanded, fostering the integration of nanotechnology and medicine.[26] Furthermore, tailoring the size and morphology of nanozymes allows precise regulation of their catalytic properties, enhancing their adaptability to diverse and specific biological microenvironments.[27] These engineered nanozymes have considerable potential for a wide range of biomedical applications.

Biomedical Applications of Nanozymes

Biomolecular Detection and Assay

The commonly used strategies in biomolecular detection include electrochemical, colorimetric, and fluorescent methods, among others.[28] Many effective biosensing platforms have been established on the basis of the reactions catalyzed by natural enzymes. However, the shortcomings of natural enzymes, such as difficulty in extraction, purification, digestion and denaturation, limit their application.[29] Therefore, developing nanozymes with simpler preparation processes for biomolecular sensing can be an effective strategy. The construction of sensing platforms for biomolecules using nanozymes, relies primarily on colorimetric methods on the basis of their POD-like and OXD-like activities. These colorimetric methods determine the concentration of target molecules by monitoring changes in the color and absorbance of the substrate. This approach is simple, rapid, and yields stable results.

Table 1 Comparative Catalytic Mechanisms of Nanozymes Mimicking Oxidoreductases

Enzyme-Like Activity	POD	OXD	CAT	SOD
Main substrates	H_2O_2 + reducing substrate	O_2 + reducing substrate	H_2O_2	$\cdot O_2^-$
Main products	OH	$\cdot O_2^-$ + H_2O	H_2O+O_2	H_2O_2+O_2
Requires O_2	No	Yes	No	No
Requires H_2O_2	Yes	No	Yes	No
Reaction type	Redox	Oxidation	Dismutation	Dismutation
pH dependence	Acidic (pH 3.0–5.0)	Acidic or neutral	Neutral/alkaline (pH 7.0–10.0)	Neutral/alkaline (pH 7.0–10.0)
Typical nano-materials	Fe_3O_4 nanoparticles	Pt-based nanodendrites	Au nanoparticles	Mn Single-atom nanozymes
Reference	[8]	[19]	[23]	[24]

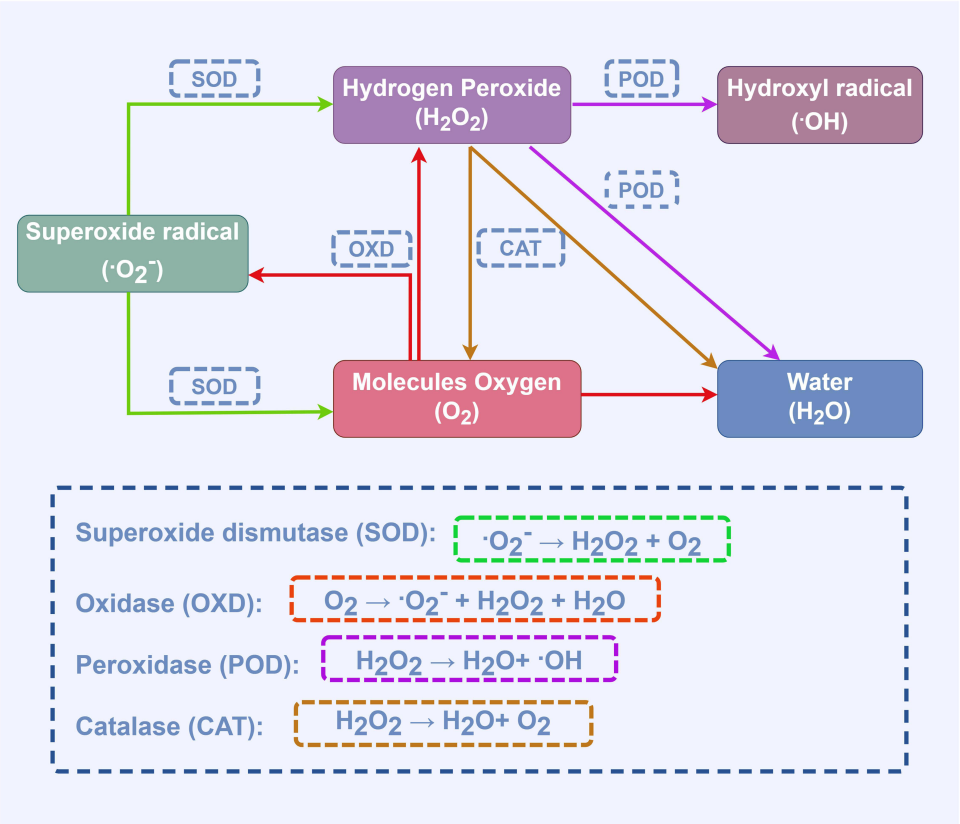


Figure 1 The catalytic reaction equations and interrelationships of various redoxases (POD, SOD, OXD, CAT) through representative products such as H_2O_2, ·OH, $·O_2^-$, O_2, H_2O. The green arrow indicates the SOD-like catalytic reaction, which uses $·O_2^-$ as a substrate to produce H_2O_2 and O_2. The red arrow represents the OXD-like catalytic reaction, which uses O_2 as an electron donor to generate $·O_2^-$ and H_2O_2. The purple and brown arrows denote POD-like and CAT-like activities, respectively, both of which use H_2O_2 as a substrate. The POD-like reaction produces ·OH and water, whereas the CAT-like reaction yields H_2O and O_2. In the dashed boxes represent the catalytic equations of different types of oxidoreductase-like activities of nanozymes: green for SOD-like, Orange for OXD-like, purple for POD-like, and brown for CAT-like. (by Figdraw).

Assays Based on POD-Like Activity

The use of nanozymes with POD-like activity to detect biomolecules (such as H_2O_2 and glucose) is a common colorimetric method. In the presence of H_2O_2, POD-like nanozymes catalyze the generation of ·OH radicals, which then oxidize a chromogenic substrate, causing a color change in the solution. When the target substance (which reacts with H_2O_2) is added, the resulting consumption or production of H_2O_2 alters the original color and absorbance of the solution. The concentration of the target substance was then calculated on the basis of this change. For example, Rostami, S. and colleagues[30] synthesized graphene nanoribbons (GNRs) that exhibit POD-like activity. In the presence of H_2O_2, GNRs can catalyze the oxidation of TMB to generate blue oxTMB. Therefore, a rapid and simple colorimetric method for dopamine (DA) detection was developed on the basis of the inhibition of TMB oxidation. The mechanism is that the reducing property of DA consumes H_2O_2 via a redox reaction, thereby inhibiting the GNR-catalyzed oxidation of TMB. As the concentration of DA increases, the generated oxTMB decreases. The DA concentration is determined on the basis of the fading of the solution color and the corresponding decrease in absorbance. The limit of detection (LOD) was 0.035 μM. This method demonstrates the potential of nanozymes in biosensing and analytical applications. Through the POD-like activity of nanozymes, many detection and sensing platforms for small molecules have been established. Table 2 shows the detection mechanism and LOD of the biomolecules on the basis of the POD-like activity of nanozymes.

In addition, many biomolecules oxidized by oxidases can usually generate corresponding oxidative substrates and H_2O_2. Owing to this characteristic, the cascade of POD-like nanozymes and oxidases can establish a sensing platform for a variety of biomolecules. The detection mechanism is based on the specificity of oxidase in the catalysis of its substrate to produce H_2O_2, which can serve as the substrate for nanozymes that exhibit POD-like activity. For example, Lee and

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Table 2 The Detection Mechanism and LOD of Biomolecules Based on the POD-Like Activity of Nanozymes

Nanozymes	Enzyme-Like Activity	Detection Substrate	Detection Mechanism	LOD	Reference
FeCDs/Mo SACs	POD	Uric Acid	Colorimetric-fluorescent dual-mode detection	0.03 μM	[31]
FeNCP/NW	POD	Acetylcholine	Colorimetric detection	0.96 μM	[32]
FeNi DSAs/N-CSs	POD	Cholesterol H_2O_2	Colorimetric-fluorescent dual-mode detection	0.19 mM 0.45 mM	[33]
Fe-Nx SANLISA	POD	Amyloid beta 1–40 (Aβ 1–40)	Immunosorbent assay	0.88 pg/mL	[34]
Fe-SAzyme	POD	Galactose	Colorimetric detection	10 μM	[35]
RuSe-N/C	POD	Alkaline phosphatase	Colorimetric detection	0.0102 mU/mL	[36]
MOF-808/Pt NPs	POD Acetylcholinesterase (AChE)	Acetylcholine	Colorimetric detection	5 μM	[37]
m-MEA nanozyme	POD	Prostate-specific antigens	Immunosorbent assay	1.20 pg/mL	[38]
Ir(III)/GO	POD	Pirimicarb (PIB)	Colorimetric determination	2.81 nM	[39]
Cu_2O@MOF	POD	Deoxynivalenol (DON)	Colorimetric-fluorescent dual-mode detection	0.0018 ng/mL	[40]
X-MoO_3-x NDs	POD	Xanthine oxidase	Colorimetric detection	0.019 U/mL	[41]
Bimetal MOFs	POD	Thiamphenicol	Colorimetric-fluorescent dual-mode detection	0.030 nM	[42]
Fe_3O_4/MWCNTs@Mo-CDs	POD	*Escherichia coli*	Colorimetric detection	0.978 CFU/mL	[43]
CoRh graphitic nanozyme	POD	Dopamine Ascorbicacid	Colorimetric detection	0.83 μM 1.0 μM	[44]

Notes: Colorimetric detection: Qualitative/semiquantitative analysis via solution color change. Immunosorbent assay: This method utilizes antigen- and antibody-specific reactions to capture target molecules. Colorimetric-fluorescent dual-mode detection: Dual-signal (color and fluorescence) for screening and precise quantification.

colleagues[45] synthesized chitosan-coated multibranch Au-Ag-Pt nanozymes (CCNPs) with POD-like activity, using TMB as a chromogenic substrate, which can be used to detect the concentrations of H_2O_2 and glucose. When CCNPs coexist with glucose oxidase, CCNPs can react with the H_2O_2 produced by the oxidation of glucose, forming ·OH to oxidize colorless TMB to blue oxTMB, thereby allowing determination of the glucose concentration in serum with an LOD of 0.289 mM. Figure 2 shows the synthesis process of the CCNPs and a schematic diagram of glucose detection. In addition, in order to achieve rapid detection of glucose, Huang and colleagues[46] prepared an agarose hydrogel containing N-CD/Fe_3O_4 nanozymes, GOx and TMB. Among them, the N-CD/Fe_3O_4 nanozymes can simulate POD activity. By a cascade reaction of GOx with POD-like activity, the agarose hydrogel can achieve visual detection of glucose in serum. This design provides a promising strategy for the visual detection of biomolecules.

In conclusion, many biosensing detection platforms for small molecules have been developed on the basis of the cascade effect of POD-like activity and OXD activity of nanozymes. For example, Zhao and colleagues[47] coupled cholesterol oxidase with a histidine-modified magnetic Fe_3O_4 nanozyme to detect the concentration of human serum cholesterol. Similarly, Zhu and colleagues[48] used perovskite oxide nanozymes prepared via the sol–gel method to simulate POD activity, combined with three oxidases (creatine kinase, creatinine kinase and creatinine oxidase) to achieve visual detection of human serum creatinine, with an LOD of 0.09 μM. However, biosensor platforms utilizing POD-like nanozymes offer a highly sensitive new strategy for small molecule detection with great application potential, and the repeatability and stability of the nanozymes themselves in real-world environments remain the major obstacles hindering their transition from proof of concept to market.

Assays Based on OXD-Like Activity

Many antioxidant molecules in the body, such as biothiols and ascorbic acid (AA), are closely associated with overall health. As crucial antioxidants, they can eliminate ROS and maintain the oxidative balance in cells. Abnormal levels of these molecules may indicate specific diseases, including cancer, Parkinson's disease, and liver disease.[49,50] Therefore, sensitive detection of biological antioxidant molecules is vital for biomedical applications. Leveraging the reducing properties of antioxidants, colorimetric methods based on the OXD-like activity of nanozymes offer rapid and stable detection.

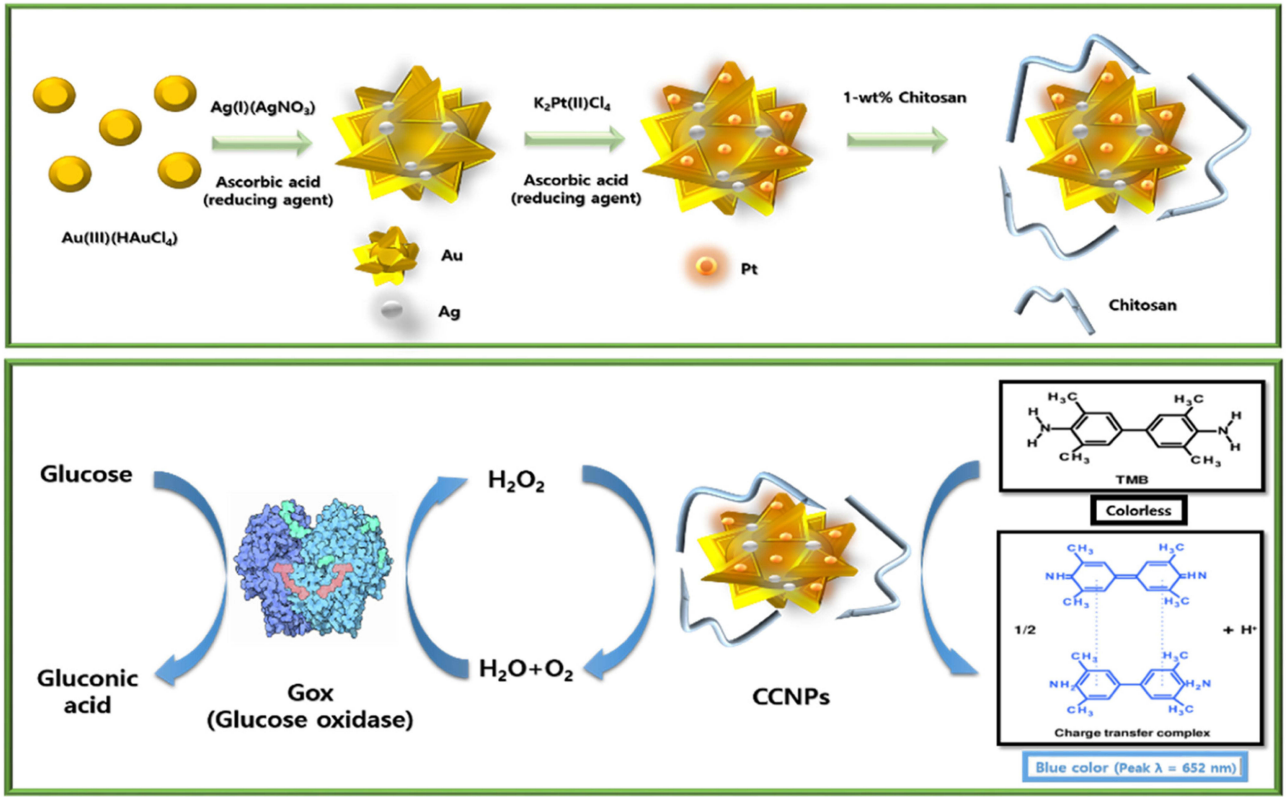

Figure 2 Illustration of the reaction cascade for glucose detection using CCNPs. The synthesis process of CCNPs and the detection mechanism of H_2O_2 and glucose. Reprinted with permission from,[45] copyright 2022, ACS Omega.

For example, Gu and colleagues[51] synthesized an iron–nitrogen–carbon single-atom nanozyme (Fe–N–C SAzyme) via a high-temperature pyrolysis strategy. This SAzyme exhibited excellent OXD-like activity, enabling the oxidation of colorless TMB to blue oxTMB. Therefore, on the basis of the antioxidant capacity of biomolecules and with TMB as a chromogenic substrate, a colorimetric platform for the detection of AA and GSH was established. The detection mechanism for AA exploits its reducing activity to convert blue oxTMB into colorless TMB. The AA concentration was determined by measuring the change in solution absorbance, and an LOD of 0.1 μM was achieved. For GSH, a colorimetric sensor was developed on the basis of its inhibitory effect on the OXD-like activity of Fe-N-C SAzymes, achieving an LOD of 1.3 μM. This design offers a novel approach for applying nanozymes in biosensing.

Notably, the level of GSH in the tumor microenvironment (TME) is often greater than that in normal cells. Chen and colleagues[52] synthesized a porous single-atom iron enzyme (pFeSAN) using hemoglobin as an iron source and template via a biomimetic strategy. pFeSAN exhibits OXD-like activity, enabling rapid colorimetric detection of GSH in tumors with an LOD of 2.4 nM. The detection mechanism relies on the color change of the chromogenic substrate TMB. pFeSAN oxidizes colorless TMB to blue oxTMB, whereas GSH, which has antioxidant capacity, reduces blue oxTMB back to colorless TMB. On the basis of this pFeSAN-GSH assay, this approach achieved accurate detection of intracellular GSH at the millimolar level and visualization of tumor regions, demonstrating the great potential of pFeSAN for clinical tumor diagnosis. Figure 3 shows the synthesis process of pFeSAN and the process of detecting GSH.

Biosensing technology utilizing nanozymes with OXD-like activity provides a highly sensitive platform for rapid detection of antioxidant molecules. However, this approach fundamentally relies on the reducing capacity of antioxidants, which may result in insufficient specificity for certain biomolecules and susceptibility to interference from other reducing agents in complex biological samples. Furthermore, nanozymes still face notable limitations in terms of catalytic stability, substrate selectivity, and biocompatibility under real biological conditions. These factors collectively hinder the transition of such analytical methods from laboratory research to practical clinical applications.

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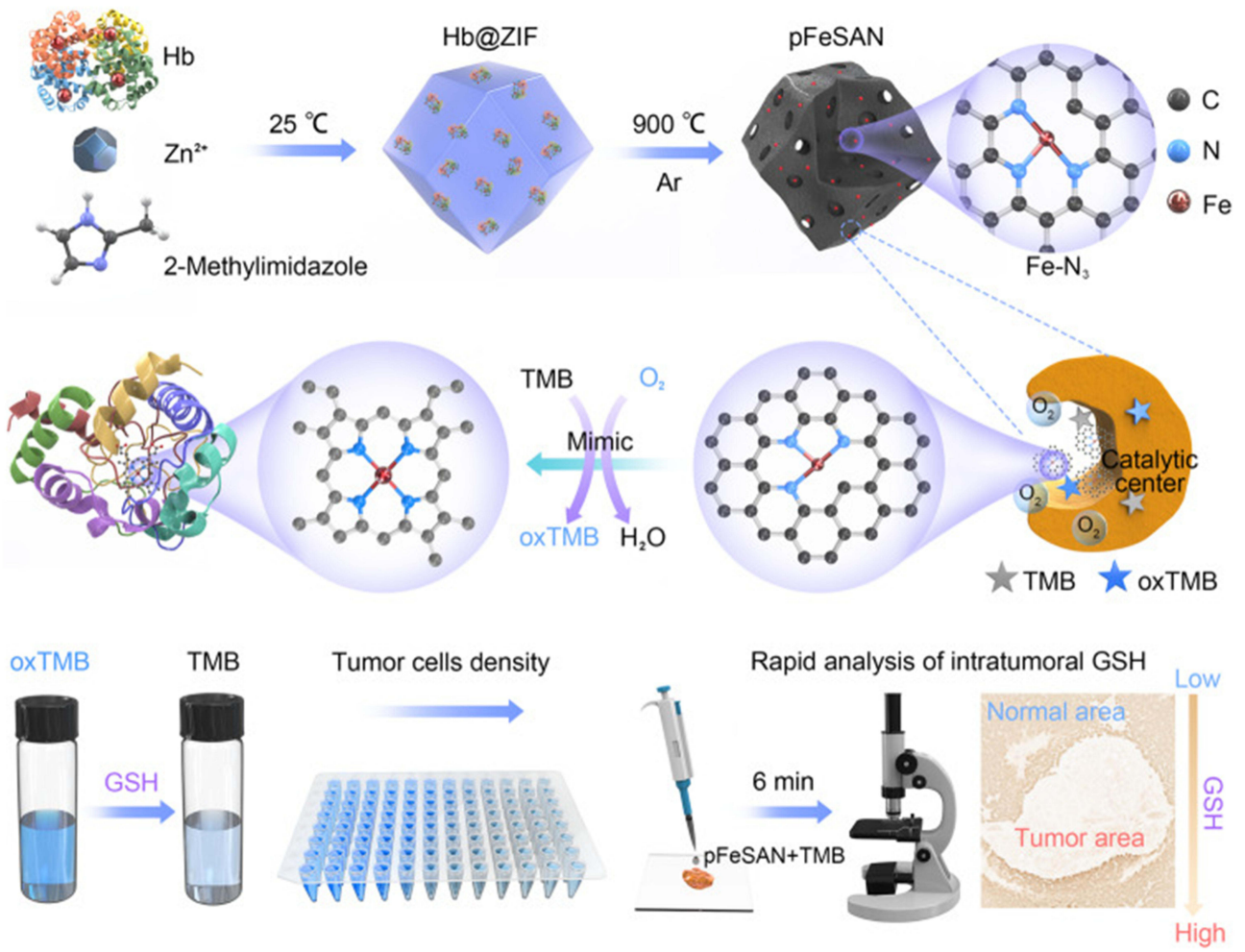

Figure 3 Schematic illustration of the synthesis process and detection effects of pFeSAN. The pFeSAN was synthesized via a two-step method using Hb@ZIF-8 as a precursor, in which mesoporous structures with Fe–N_3 sites were formed. The pFeSAN-based biosensing system enables rapid detection of GSH in tumors. Reprinted with permission from,[52] copyright 2023, Nature Communications.

In conclusion, while nanozyme-based biosensors offer distinct advantages over traditional enzymatic assays, they also present inherent limitations concerning sensitivity, selectivity, and real-sample applicability. Regarding sensitivity, the high surface area and robustness of nanomaterials enable nanozymes to maintain catalytic activity and amplify signals under extreme conditions, often resulting in lower detection limits. However, their catalytic efficiency (K_{cat}/K_m) generally remains inferior to that of natural enzymes, potentially leading to slower response times or requiring higher material loadings to compensate for lower substrate affinity.[53] In terms of selectivity, natural enzymes benefit from precise protein-binding pockets, whereas unmodified nanozymes typically lack such specificity due to their radical-mediated catalytic mechanisms. Nonetheless, surface functionalization, such as introducing molecularly imprinted polymers onto nanozyme surfaces, can have an impact on the nanozymes' catalytic activity and recognition capabilities.[54] Concerning real-sample applicability, nanozymes exhibit enhanced stability and resistance to proteolysis, making them more robust in complex matrices like environmental water samples. However, the propensity of nanoparticles to form protein coronas in biological fluids can block active sites and alter catalytic activity.[55] Thus, the translation of nanozyme-based sensors to real-world applications requires thorough validation to ensure reproducibility and accuracy.

Molecular Mechanism of Nanozymes in the Treatment of Tumors

The incidence and mortality of malignant tumors are high. Traditional treatments such as radiotherapy and chemotherapy have been widely used in clinical practice, but their therapeutic efficacy is often suboptimal, and side effects remain a significant concern.[56] With advances in modern medicine, cancer therapies based on ROS-induced oxidative stress have

demonstrated considerable potential. Several emerging approaches, including photodynamic therapy,[57] photothermal therapy,[58] chemodynamic therapy,[59] and sonodynamic therapy,[60] can generate ROS to induce oxidative stress within tumors, thereby achieving a certain degree of tumor-specific cell death and reducing treatment-related side effects. Nevertheless, the efficacy of these strategies is severely limited by the TME, which is characterized by conditions such as hypoxia and a high concentration of glutathione.[61] Therefore, ROS-based tumor therapy requires further exploration.

ROS are a series of oxygen-containing substances with oxidative properties, such as H_2O_2, $\cdot O_2^-$, $^1O_2$, and ·OH.[62] As key regulators of the cellular redox balance, ROS can trigger oxidative stress when the imbalance between their production and clearance occurs.[63] ROS play dual roles in cancer development. At moderate levels, they act as crucial signaling molecules by regulating pathways related to tumor cell proliferation and survival, thereby promoting tumor occurrence and progression. However, when excessive amounts of ROS accumulate, they cause oxidative stress. This leads to irreversible damage, such as DNA breaks, lipid peroxidation, and protein denaturation, disrupting cellular homeostasis and inducing apoptosis or necrosis.[64,65] This dose-dependent dual role makes ROS potentially ideal therapeutic targets in tumor therapy.

Studies have shown that nanozymes (especially redox nanozymes) have potential applications in the treatment of tumors.[66] Many nanozymes are responsive to hydrogen peroxide and acidic conditions, among other properties, which allows for the precise regulation of the TME. However, a key point that remains to be clarified is the molecular mechanism by which the enzyme-like activity of nanomaterials regulates the levels of metabolites and signaling pathways associated with tumor redox metabolism. Currently reported mechanisms of nanozyme-mediated tumor therapy primarily involve the induction of apoptosis, ferroptosis, and pyroptosis. This chapter reviews the molecular mechanisms by which nanozymes modulate intracellular ROS levels to induce tumor cell death, including apoptosis and ferroptosis. These findings highlight nanozyme-induced oxidative stress as a unique therapeutic mechanism against malignant tumors.

Nanozyme-Induced Apoptosis via Oxidative Stress

Apoptosis is one of the most important antitumor mechanisms of nanozymes. The core mechanism of nanozyme-induced apoptosis in tumor cells involves the OXD-like and POD-like activities of nanozymes, which catalyze a series of chemical reactions, generating a large amount of ROS and thereby disrupting the redox balance of tumor cells. This intense oxidative stress can then induce apoptosis by damaging mitochondria, lysosomes, and other organelles, as well as by regulating the expression of apoptosis-related proteins.

Nanozyme-Induced Mitochondrial Dysfunction

Mitochondria, the "powerhouses" of cells, generate ATP through oxidative phosphorylation and play pivotal roles in processes such as apoptosis and calcium signaling.[67] Mitochondrial dysfunction is a key mechanism in nanozyme-induced tumor cell apoptosis. Studies have shown that when nanozymes enter tumor cells, they utilize their enzyme-like activity to catalyze the production of ROS. The oxidative stress caused by ROS increases mitochondrial membrane permeability and decreases the mitochondrial membrane potential (MMP), leading to mitochondrial dysfunction and tumor cell apoptosis. For example, Gao and colleagues[68] developed ultrasmall gold and iron oxide nanoparticles coloaded into dendritic mesoporous silica nanoparticles (DMSN-Au-Fe_3O_4 NPs). In this system, the Au NPs with GOx-like activity catalyze the oxidation of β-D-glucose into gluconic acid and H_2O_2, while the resulting H_2O_2 is subsequently catalyzed by POD-like Fe_3O_4 NPs to generate highly toxic ·OH via a Fenton-like reaction. These radicals damage the DNA and mitochondria of 4T1 breast cancer cells, thereby inducing tumor cell apoptosis. Zhu and colleagues[69] developed a polyethylene glycolated Mn-based single-atom enzyme (Mn/PSAE) that mimics the activity of multiple enzymes for tumor treatment. First, through the cascade of CAT-like and OXD-like activities, Mn/PSAE generates $\cdot O_2^-$. Second, its POD-like activity catalyzes the conversion of intracellular H_2O_2 into ·OH. The resulting $\cdot O_2^-$ and ·OH radicals damage the mitochondrial membrane, and ultimately trigger tumor cell apoptosis. Moreover, Mn/PSAE has a photothermal effect. The synergy between this photothermal therapy (PTT) and catalytic therapy enables complete tumor ablation in vivo.

TME-Responsive Nanozyme Design for Apoptosis

Notably, the unique microenvironment of tumors, characterized by hypoxia, high levels of H_2O_2, the overexpression of GSH, elevated glucose, and an acidic pH, provides favorable conditions for tumor growth, metastasis, and invasion but

also limits the efficacy of nanozymes in treating tumors.[70] Therefore, designing tumor-catalytic therapeutic strategies on the basis of the characteristics of the TME and the oxidoreductase-like activity of nanozymes represents a new breakthrough for tumor therapy. For example, Cai and colleagues[71] synthesized a Co single-atom nanozyme (Co-SAs@NC) on nitrogen-doped porous carbon. This nanozyme exhibits not only CAT-like activity, which catalyzes the decomposition of H_2O_2 in tumor cells to produce O_2 but also OXD-like activity, which converts O_2 into cytotoxic $\cdot O_2^-$ radicals. Under acidic conditions (pH = 6), Co-SAs@NC generate a large amount of ROS through a dual-enzyme cascade. The generation of $\cdot O_2^-$ radicals significantly induced tumor cell apoptosis in vitro. In vivo studies demonstrated a tumor inhibition rate of 66% in the catalytic treatment of breast tumors. This design leverages the ability of CAT-like nanozymes to catalyze H_2O_2 decomposition to alleviate hypoxia in the TME, thus highlighting the promising potential of nanozyme-based cascade reactions for cancer therapy. Lei and colleagues[72] synthesized CeO_2@Au-PEG nanocomposites by modifying CeO_2@Au nanorods with polyethylene glycol (PEG). The CeO_2@Au-PEG nanocomposite exhibited GOx-like activity, enabling it to convert glucose in tumor cells into gluconic acid and H_2O_2. This process not only cuts off the energy supply of tumor cells but also provides endogenous H_2O_2. Furthermore, CeO_2@Au-PEG possesses POD-like activity, which converts the generated H_2O_2 into highly cytotoxic ·OH radicals to kill tumor cells. Cong and colleagues[73] synthesized a ruthenium (Ru) nanozyme loaded with atorvastatin (ATO). The Ru nanozyme possesses POD-like activity, enabling it to catalyze the decomposition of H_2O_2 to generate ·OH radicals and O_2. Additionally, it exhibits GOx-like activity, allowing it to catalyze the breakdown of glucose in the TME to produce gluconic acid and H_2O_2. This process achieves an endogenous self-supply of H_2O_2 in tumors. Moreover, the generated gluconic acid helps maintain the acidic tumor microenvironment, which in turn promotes the enzyme-like activity of the nanozyme. Overall, this design achieves self-supply of H_2O_2 and O_2 in the TME through a cascade of the multiple enzyme-like activities of the nanozyme. Furthermore, it consumes glucose to implement starvation therapy for tumors, thereby improving the efficiency of nanozyme-based tumor treatment. In conclusion, increasing the self-supply of O_2 and H_2O_2 in the tumor microenvironment further enhances the ROS generation capacity of nanozymes and improves their tumor killing efficacy. These findings demonstrate that the cascade reactions of nanozymes in response to the tumor microenvironment hold great potential for tumor therapy.

Calcium Overload as an Apoptotic Trigger

In addition to inducing oxidative stress and mitochondrial dysfunction via ROS, nanozymes can also trigger apoptosis by causing calcium overload in mitochondria. Calcium ions (Ca^{2+}) play a critical role in apoptosis, as their accumulation in mitochondria can lead to dysfunction, rupture, and apoptosis.[74] Nanozymes can disrupt calcium homeostasis in tumor cells by generating ROS, which impair the function of calcium channels and pumps, thereby accelerating apoptosis. For example, Wang and colleagues[75] engineered two-dimensional $Ca^{2+}Mn_8O_{16}$ nanosheets (CMO NSs) as high-performance nanozymes, which can mimic the catalytic activities of glutathione peroxidase, catalase, oxidase, peroxidase, and glucose oxidase. CMO nanosheets release exogenous Ca^{2+} and induce endogenous Ca^{2+} accumulation through POD-like and OXD-like activities, collectively causing Ca^{2+} overload, which triggers apoptosis. Dong C and colleagues[76] developed a calcium fluoride nanozyme with POD-like activity to enhance tumor therapy via calcium overload. The mechanism involves the introduction of exogenous Ca^{2+} to regulate intracellular calcium channels in tumor cells. The release of exogenous calcium and the production of ROS through POD-like activity promote intracellular calcium accumulation, ultimately leading to mitochondrial dysfunction and apoptosis caused by calcium overload.

Regulation of Apoptotic Protein Expression

The Bcl-2 family of proteins are regulators of apoptosis and include both pro- and antiapoptotic members. Under normal physiological conditions, the Bcl-2 protein located in the outer membrane of mitochondria can inhibit the release of cytochrome c, while the apoptotic protein Bax exists in the cytoplasm in the form of a monomer.[77] Upon receiving apoptotic signals such as mitochondrial damage, the proapoptotic members of the Bcl-2 protein family (Bax and Bak) are activated, translocate to the mitochondria, and undergo oligomerization to form channels that release cytochrome c. Cytochrome c, an apoptotic protease activator (Apaf-1), and caspase-9 then assemble to form the "apoptosome", a complex that leads to the

activation of caspase-9. Activated caspase-9 subsequently triggers downstream caspase effectors (caspase-3 and caspase-7) to execute the apoptosis program.[78]

Studies have demonstrated that nanozymes can regulate the expression of apoptosis-related proteins to induce tumor cell apoptosis. For example, Luo and colleagues[79] successfully developed an Au@Pd nanozyme with POD-like activity. This nanozyme effectively catalyzes the production of ROS from excess H_2O_2 in the tumor microenvironment. It induces tumor cell apoptosis by modulating mitochondrial apoptosis-related proteins, including downregulating the antiapoptotic protein Bcl-2, upregulating the proapoptotic protein Bax, and increasing the expression of the apoptosis-related gene p53. These results demonstrate the potent antitumor effect of the Au@Pd nanozyme. Liu and colleagues[80] developed an iron-based nanozyme (Fe_3O_4-OA-DHCA-PEI-MAN@DSF) to induce apoptosis in hepatocellular carcinoma (HCC) cells. This mechanism allows the accumulation of the small-molecule drug disulfiram (DSF) within hepatocellular carcinoma cells. DSF-loaded magnetic nanocubes induce apoptosis in liver cancer cells by downregulating the expression of Bcl-2, matrix metalloproteinase 9 (MMP9), matrix metalloproteinase 2 (MMP2), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) proteins while increasing the expression of Caspase-3 and Bax and promoting the formation of ROS. Thus, the synthesized composite iron-based nanozyme Fe_3O_4-OA-DHCA-PEI-MAN@DSF achieves targeted therapy for liver cancer cells.

In conclusion, nanozymes primarily induce tumor cell apoptosis by triggering oxidative stress. The core mechanism involves their OXD-like and POD-like activities, which catalyze the generation of abundant ROS and disrupt the intracellular redox balance. This intense oxidative stress instigates apoptosis through two pathways: first, by impairing mitochondrial function through membrane potential dissipation and calcium ion overload, and second, by modulating the expression of apoptosis-related proteins, such as downregulating the antiapoptotic protein Bcl-2 and upregulating the proapoptotic protein Bax, thereby activating the caspase cascade. Furthermore, nanozyme design strategically exploits the tumor microenvironment, employing cascade catalytic reactions to achieve self-supply of O_2 and H_2O_2 while consuming glucose, which collectively amplifies ROS generation and ultimately enhances the tumor-killing efficacy. Figure 4 summarizes the mechanism of nanoenzyme-induced apoptosis in tumor cells.

Molecular Mechanism of Ferroptosis Induced by Nanozymes

Ferroptosis is an iron-dependent form of cell death triggered by lipid peroxide (LPO) accumulation. This process relies on excessive intracellular iron ion accumulation and oxidative stress.[81] Tumor cells, characterized by elevated ROS levels and abnormal iron metabolism, exhibit heightened sensitivity to ferroptosis.[82] Therefore, inducing ferroptosis in tumor cells has emerged as a promising therapeutic strategy. Nanozymes induce ferroptosis by acting as both iron and ROS suppliers, thereby disrupting the redox balance of cells.

ROS Generation and Lipid Peroxidation Initiation

In nanozyme-based tumor therapy research, ferroptosis is primarily induced through the accumulation of ROS, which directly triggers LPO in tumor cells. Nanozymes exhibit POD-like and OXD-like activities, catalyzing the production of ROS such as ·OH and $\cdot O_2^-$. These ROS can further attack polyunsaturated fatty acids (PUFAs), which are abundant in cell membranes, triggering lipid peroxidation chain reactions that induce the formation of LPO. This process activates ferroptosis, thereby achieving tumor therapy.[83]

For example, Yuan and colleagues[84] developed an MOF-based magnetic nanozyme (PZFH) platform. PZFH exhibits multienzyme cascade activity, in which a light-triggered OXD-like activity catalyzes the generation of $\cdot O_2^-$. This species is subsequently converted into H_2O_2 via SOD-like activity, ultimately producing ·OH through field-enhanced POD-like-catalyzed reactions. The generated ·OH radicals lead to LPO accumulation. This approach provides novel insights for ferroptosis-related nanomedicine research. Carvalho, S. M. et al[85] synthesized cobalt-doped iron oxide nanozymes (Co-MIONs) characterized by a carboxymethyl cellulose (CMC) biopolymer. Co-MIONs possesses a supramolecular eco-colloidal nanostructure that can simulate peroxidase activity for the biocatalytic killing of glioma cells. The cytotoxicity of Co-MION nanozymes was first demonstrated in an in vitro 2D culture of U87 brain cancer cells. Owing to its POD-like activity, Co-MION generates highly toxic ·OH, which induces LPO and ferroptosis in tumor cells, thereby effectively killing cancer cells.

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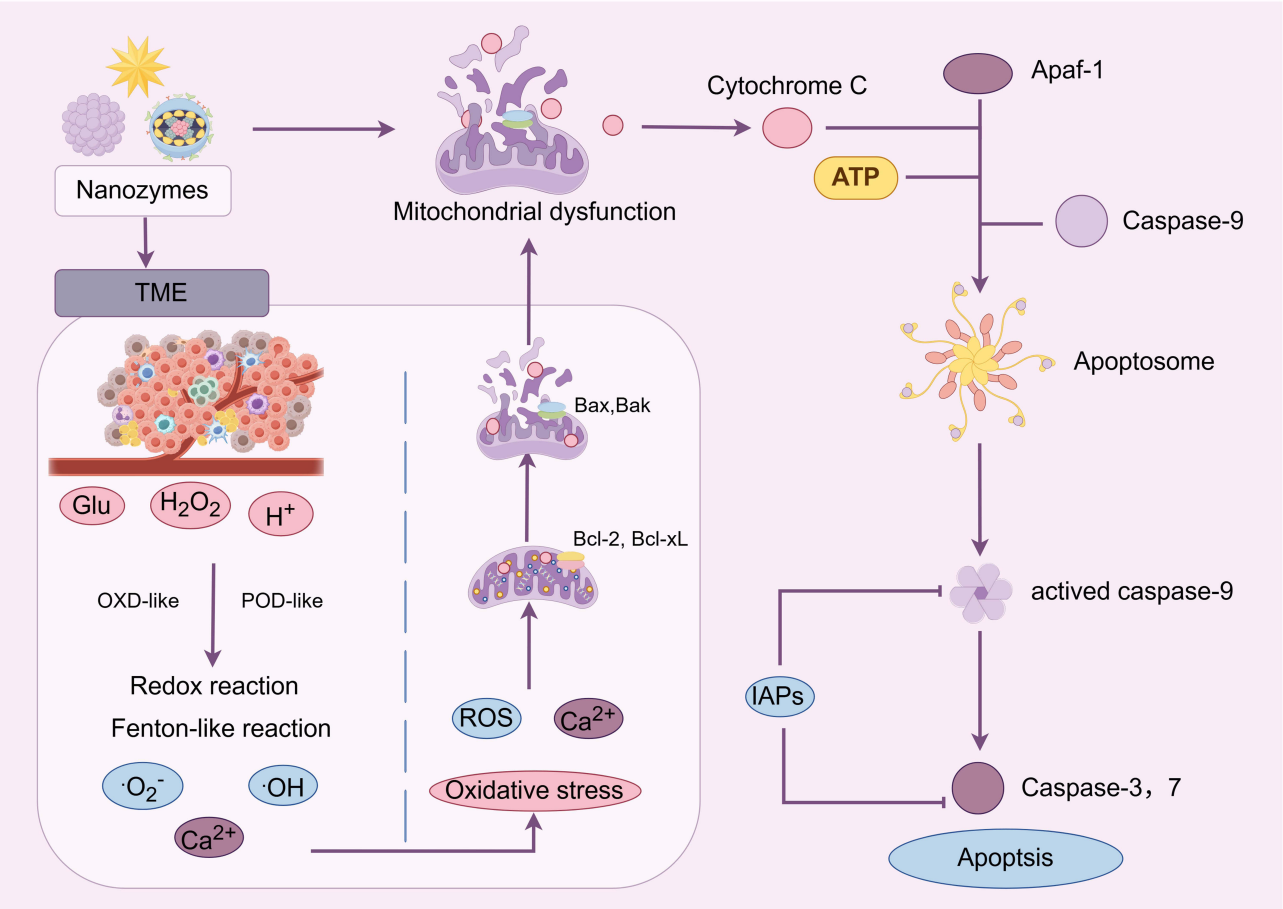

Figure 4 Mechanism of nanozyme-induced tumor cell apoptosis. Nanozymes catalyze the generation of abundant ROS through redoxase-like activity. The resulting ROS overload induces mitochondrial dysfunction and regulates the expression of apoptotic proteins, thereby initiating apoptosis. (by Figdraw).

Weakening the Antioxidant Capacity of the Tumor

ROS-induced ferroptosis holds great promise in tumor therapy. However, under normal physiological conditions, the antioxidant defense system in the TME can counteract ROS-induced oxidative stress. The regulation of ferroptosis by tumor cells primarily involves the system Xc− and the glutathione redox system. System Xc− is a cystine/glutamate transporter widely distributed in the phospholipid bilayer and is composed of two subunits: solute carrier family 7 member 11 (SLC7A11) and solute carrier family 3 member 2 (SLC3A2).[86] This system imports cystine into the cell in exchange for exporting glutamate out of the cell at a 1:1 ratio, thus promoting the synthesis of GSH.[87] The synthesized GSH serves as a cofactor for GPX4 to eliminate ROS. GPX4 utilizes GSH to catalyze the reduction of lipid peroxides into harmless lipid alcohols.[88] This antioxidant activity protects the structural integrity and function of cell membranes from peroxide-induced damage. Therefore, nanozymes can induce ferroptosis by destroying the antioxidant system of tumor cells for tumor treatment.

Compared with normal cells, tumor cells maintain redox balance through high concentrations of GSH, which promotes cancer cell proliferation and poses challenges for cancer treatment. Therefore, many researchers have developed nanomaterials that can simulate the activity of GSH-Ox, which can weaken the ability of tumors to respond to antioxidant stress by catalyzing the oxidation of GSH in the TME.[89] For example, Meng and colleagues[90] developed a pyrite nanozyme with POD-like activity, which has an extremely high affinity for H_2O_2 and effectively catalyzes the conversion of H_2O_2 into ·OH radicals in the TME, leading to oxidative damage in tumor cells. In addition, pyrite nanozymes mimic GSH-Oxd activity and promote the depletion of GSH in tumor cells. This weakens the cellular antioxidant defense capacity, induces LPO, and thereby activates both the ferroptosis and the apoptosis pathways. With dual enzyme activities, pyrite nanozymes form a cascade catalytic reaction platform in the TME, enabling tumor treatment through massive ROS generation and GSH depletion, which collectively induce tumor cell ferroptosis.

In addition to depleting GSH, suppressing the Xc− system and cysteine uptake impaired GSH synthesis, in turn decreasing GPX4 activity and the cellular antioxidant capacity. For example, Li and colleagues[91] developed a Cu_2O@Au nanozyme that exhibited excellent GOx-like and POD-like activity. First, the Cu_2O@Au nanozyme was used for starvation therapy and as a peroxidase mimic for chemodynamic therapy (CDT), resulting in the production of ·OH. The nanozyme consumes glucose at the tumor site to block the tumor's energy supply, continuously produces H_2O_2, and lowers the pH to increase the efficiency of CDT, thereby initiating a cascade reaction that leads to a storm of ROS. Additionally, the Cu_2O@Au nanozyme consumes GSH and reduces the expression of the SLC7A11 protein to decrease cancer cell uptake of cysteine, further enhancing the burst of ROS, which induces LPO in tumor cells and ultimately leads to ferroptosis. Zhang and colleagues[92] used FeOOH nanoshuttles coloaded with Au nanodots and Fe-apigenin (Ap) complexes (FeOOH@Fe-Ap@Au NSs) to regulate the SLC7A11/GSH/GPX4 axis and achieve ferroptosis-mediated tumor therapy. In this system, the Au nanodots exhibit GOx-like activity, consuming large amounts of glucose. This further limits NADPH production and suppresses cystine/cysteine uptake via the SLC7A11/GSH/GPX4 axis. In addition, the efficient delivery of exogenous iron ions by FeOOH@Fe-Ap@Au NSs amplifies ferroptosis through a Fenton-like reaction, producing ·OH.

In conclusion, nanozymes with redoxase-like activity show great potential in tumor ferroptosis therapy. On the one hand, they utilize highly expressed substances in the TME, such as H_2O_2, GSH, and glucose, as raw materials for catalytic reactions. Through a series of enzyme-like cascade reactions, highly toxic ROS are generated, leading to LPO. On the other hand, nanozymes consume glucose and GSH in the TME, which cuts off the tumor's energy supply and weakens its antioxidant capacity. This enhances the efficacy of nanozyme-induced ferroptosis, thereby achieving therapeutic effects on tumors and offering a promising strategy for cancer treatment. Figure 5 shows the molecular mechanism of nanozyme-induced ferroptosis.

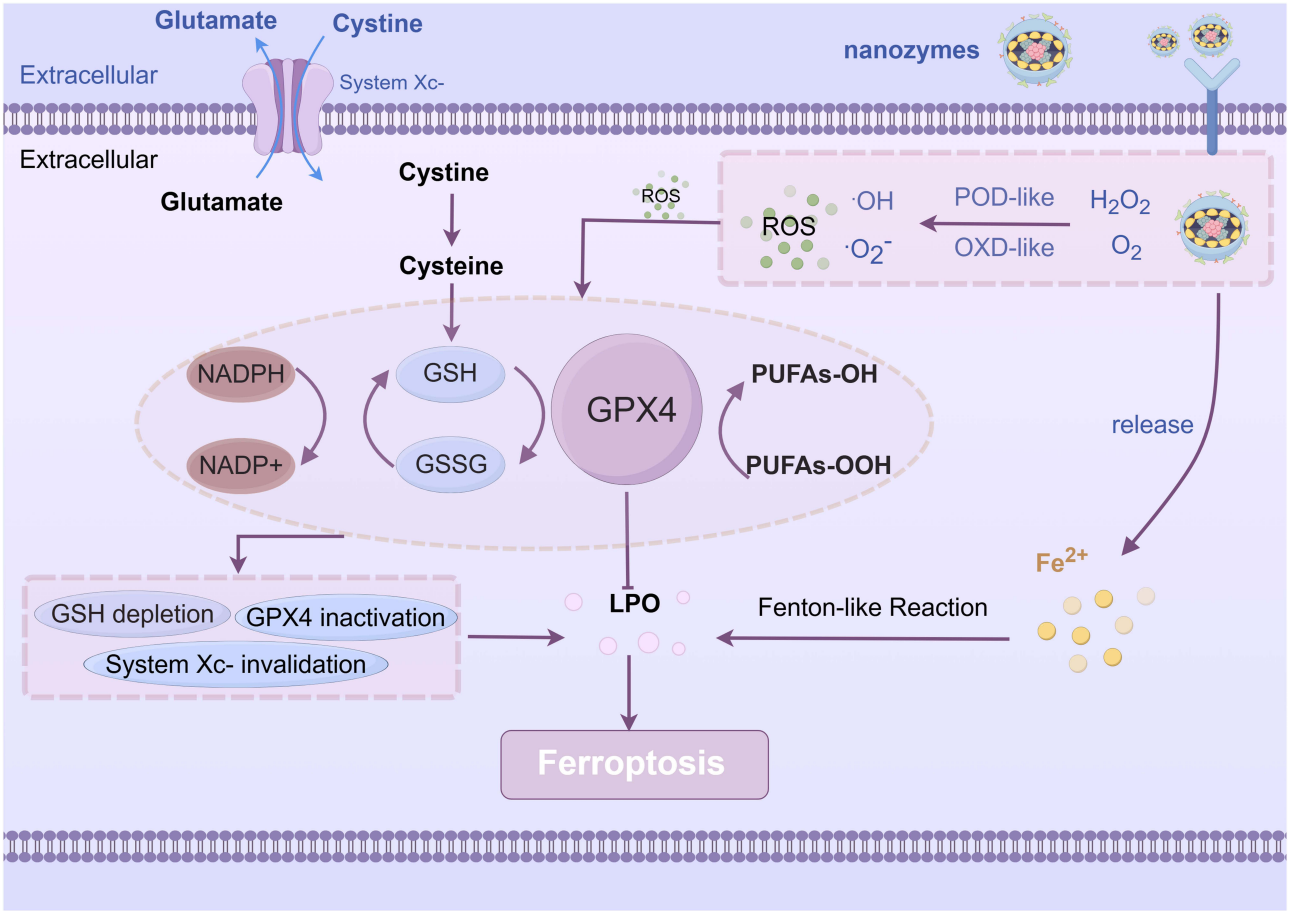

Figure 5 Schematic diagram of the molecular mechanism of nanozyme-induced ferroptosis. Involving the catalysis of ROS generation, GSH depletion, redox and iron metabolism homeostasis, GPX4 inactivation, the induction of LPO and ferroptosis. (by Figdraw).

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Interaction Between Nanozyme-Induced Apoptosis and Ferroptosis

The therapeutic efficacy of nanozymes in tumor treatment does not rely on a single cell death pathway but is achieved through the complex interplay between ROS-mediated apoptosis and ferroptosis.[93,94] Specifically, nanozymes utilize their redoxase-like activities to catalyze the overproduction of ROS. These ROS serve as key signaling and effector molecules, simultaneously triggering two cell death pathways: On the one hand, ROS attack polyunsaturated fatty acids in the cell membrane, initiating an LPO chain reaction, leading to GSH depletion and inactivation of GPX4, thereby inducing ferroptosis. On the other hand, ROS cause mitochondrial and DNA damage, resulting in decreased MMP, release of cytochrome c, and subsequent activation of the caspase cascade, mediating apoptosis. The mutual promotion and crosstalk between these two modes of death ultimately synergize to achieve efficient tumor cell killing, providing a theoretical basis for the application of nanozymes in cancer therapy. Figure 6 summarizes the interplay between ROS-mediated apoptosis and ferroptosis in nanozyme-based cancer therapy.

Nanozymes Generate ROS for Antibacterial Treatment

Bacterial infections have persistently threatened human health. However, the widespread use of antibiotics in clinical practice has resulted in the emergence of drug-resistant strains, which present a significant clinical problem.[95] Therefore, the development of new broad-spectrum antimicrobial agents to address the problem of bacterial resistance is urgently needed. With the development of nanotechnology and nanomedicine, many bionic antibacterial nanozymes are expected to become broad-spectrum antimicrobial agents. After reaching the site of bacterial infection, the nanozymes catalyze H_2O_2 to generate ROS, which destroy bacterial nucleic acids, proteins, polysaccharides, and other biological macromolecules, thereby affecting the structural integrity of bacteria and leading to bacterial death.[96] For example, Lian et al[97] prepared a Mo-doped ZIF-8 nanozyme that generates ·OH in the presence of a low dose of H_2O_2. This nanozyme exhibited significant inhibitory effects against both gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria. Song and colleagues[98] developed a $NiCo_2O_4$ nanozyme with an adaptive hierarchical nanostructure, which has excellent POD-like catalytic activity. The $NiCo_2O_4$ nanozyme can capture bacteria of various shapes via physical–mechanical interactions with its nanostructure. This ability allows it to exert a broad-spectrum and potent antibacterial effect.

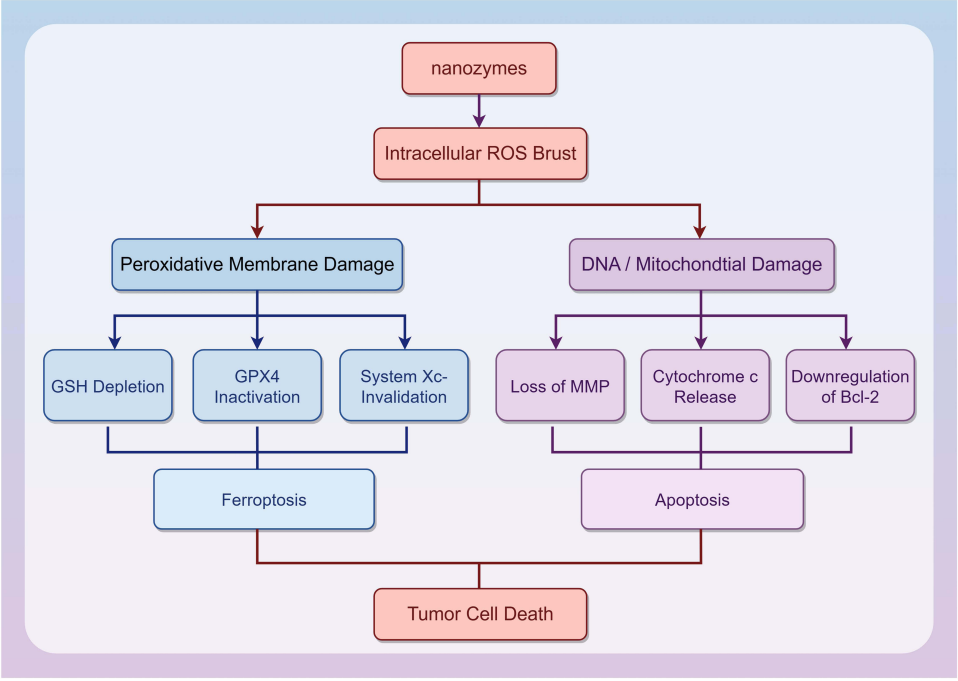

Figure 6 Flowchart of the synergistic mechanism by which nanozymes-inducesd apoptosis and ferroptosis. Nanozyme disrupts redox homeostasis by generating ROS, which simultaneously inactivates the GPX4-mediated ferroptosis defense and impairs mitochondrial function to trigger the intrinsic apoptotic pathway. The synergistic interaction between ferroptosis and apoptosis results in tumor suppression. (by Figdraw).

The potential of nanozymes in antibacterial therapy is further evidenced by their tunable enzymatic activity and ability to target bacteria, which can be precisely regulated via rational design of their dimensions, morphology, composition, and surface properties. For example, Hu and colleagues[99] prepared the UsAuNPs/MOFs nanozyme via the in-situ reduction of ultrasmall gold nanoparticles on a two-dimensional metal–organic framework. The UsAuNPs/MOFs have an ultrasmall size and exhibit excellent POD-like activity, which allows them to efficiently generate ·OH for antibacterial treatment at lower H_2O_2 concentrations. As a result, it exhibits excellent antibacterial activity against both gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria. In vivo experiments demonstrated that it significantly accelerated the healing of bacterium-infected wounds. This study suggests a promising strategy for promoting the clinical translation of nanocatalytic antibacterial therapy. Figure 7 shows the preparation process and an antibacterial schematic diagram of the UsAuNPs/MOFs nanozyme.

Moreover, Feng and colleagues[100] developed spherical mesoporous Fe-N-C single-atom nanozymes for antibacterial applications via a soft template strategy. The mesoporous structure significantly enhances the performance of nanozymes. These Fe-N-C nanozymes exhibit not only excellent POD-like activity, enabling the conversion of H_2O_2 into highly toxic ·OH but also a carbon framework with high infrared photothermal conversion efficiency. This photothermal effect increases the local reaction temperature, thereby further improving the catalytic activity of the nanozymes. Consequently, the Fe-N-C single-atom nanozymes achieve enhanced antibacterial efficacy, significantly reducing the viability of *Escherichia coli* and *Staphylococcus aureus* when combined with photothermal treatment.

In conclusion, nanozymes inhibit bacterial activity by producing ROS in the bacterial microenvironment and are potential candidates for treating drug-resistant bacteria. However, ROS-based antimicrobial therapy faces several key challenges. First, the issue of catalytic efficiency and limited functional scope: Many nanozymes exhibit suboptimal activity under physiological conditions, and those relying on a single enzyme-like function often struggle to maintain a sustained and effective ROS storm due to limited substrate availability or cofactor dependency. Second, the biofilm barrier: The dense extracellular polymeric substance matrix of biofilms not only physically impedes ROS penetration but also scavenges ROS, thereby greatly weakening the bactericidal efficacy against deeply embedded persister cells. Third, the potential for bacterial resistance: Although ROS exert broad-spectrum bactericidal effects through multitarget

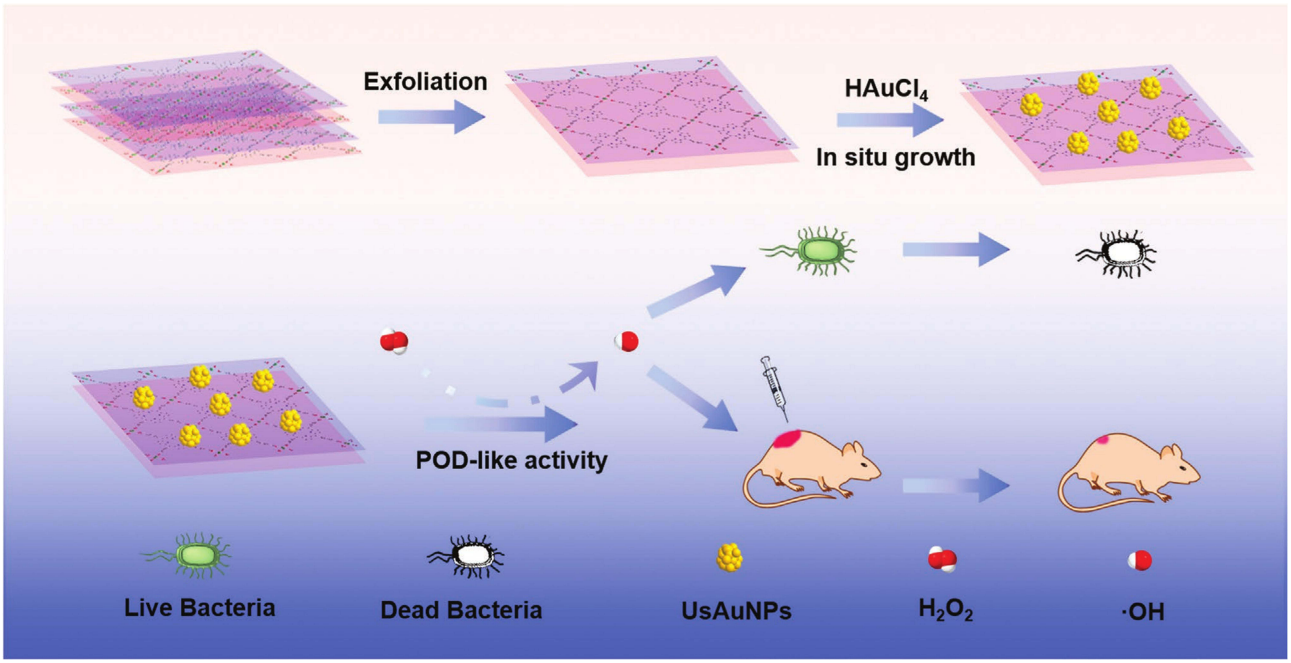

Figure 7 Schematic illustration of the preparation and POD-like activity of the UsAuNPs/MOFs hybrid for antibacterial therapy. The formed UsAuNPs/MOFs hybrid features both the advantages of UsAuNPs and ultrathin 2D MOFs, displaying remarkable POD-like activity toward H_2O_2 decomposition into ·OH for antibacterial therapy. Reprinted with permission from,[99] copyright 2020, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

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damage, prolonged exposure to sublethal ROS levels can activate bacterial stress responses, potentially leading to adaptive resistance and reduced therapeutic vulnerability.

To overcome these bottlenecks, future nanozyme design should move beyond single-function catalysis toward multifunctional synergistic platforms. For instance, constructing nanozymes with cascaded enzyme-like activities can continuously supply H_2O_2 in situ while generating highly toxic ·OH, thereby enhancing catalytic efficiency and addressing the issue of limited substrates. Furthermore, integrating photothermal properties into nanozyme design offers a dual advantage: the localized hyperthermia not only disrupts biofilm integrity to facilitate deeper ROS penetration but also creates a synergistic bactericidal effect that lowers the required ROS dose, thereby mitigating the risk of inducing bacterial resistance.[101] Ultimately, optimizing such multi-mechanistic synergistic strategies will be key to advancing nanozyme-based therapies for clinical application against biofilm-associated and drug-resistant infections.

Antioxidant and Other Disease Treatments

ROS play key physiological and pathological roles in organisms. They mediate damage primarily through two mechanisms. First, ROS can directly oxidize biological macromolecules such as proteins, lipids, nucleic acids, and carbohydrates, leading to cellular dysfunction and even death. Second, ROS function as important signaling molecules, and dysregulation of their levels can perturb cellular signaling pathways.[102] High levels of ROS in the local microenvironment of inflammatory diseases, such as periodontitis and pancreatitis, exacerbate inflammatory progression.[103] Studies have shown that the CAT-like and SOD-like activities of nanozymes can eliminate ROS for anti-inflammatory therapy, making them highly promising for clinical application.

For example, He and colleagues[104] prepared a copper-based nanozyme hydrogel (Cu_2Se/F127 hydrogel) with SOD-like activity and investigated its therapeutic efficacy in the treatment of skin wounds. In vitro tests revealed that the Cu_2Se/F127 hydrogel effectively eliminated ROS and nitrogen species (RNS) and promoted the migration of fibroblasts as well as tube formation in vascular endothelial cells. In vivo experiments demonstrated that the hydrogel accelerated acute wound healing in mice, facilitated hemostasis, upregulated CD31 expression, and downregulated the levels of TNF-α and IL-6. Jin and colleagues[105] proposed a CuZnHis nanozyme for enhancing artificial SOD activity. These assemblies, prepared via an entropy-driven self-assembly method with an optimized Cu catalytic site, exhibited a catalytic activity at least 5.4 times greater than of natural Cu-Zn-SOD. In male animal models, CuZnHis assemblies promoted macrophage polarization from the M1 phenotype to the M2 phenotype and the expression of anti-inflammatory factors, which inhibited periodontitis progression. Wang and colleagues[106] noted that oxidative stress-induced inflammation may be involved in the pathogenesis of osteoarthritis (OA). They designed a Mn_3O_4 nanozyme (Mn_3O_4@CS hydrogel) with dual SOD-like and CAT-like activities for OA treatment. These results demonstrated that this nanozyme significantly alleviated arthritis in mouse models by reducing oxidative stress. Figure 8 illustrates the synthesis process of the Mn_3O_4 nanozyme and its therapeutic mechanism against OA.

With the rapid development of nanomedicine, nanozymes have shown great potential for clinical application in the field of antioxidant therapy because of their unique catalytic activity and good biocompatibility. Compared with traditional antioxidants, the nanozymes have greater stability, adjustable catalytic efficiency, and multifunctional synergistic therapeutic capacity, giving them significant advantages in the treatment of ROS-related diseases.[107] Nanozymes have been studied in various disease models. For example, in the treatment of ulcerative colitis, nanozymes can relieve intestinal oxidative stress and inflammation by effectively removing excessive ROS.[108] In models of myocardial ischemia and reperfusion injury, nanozymes can reduce the damage caused by free radicals to cardiomyocytes and significantly improve heart function.[109] In addition, nanozymes also show good therapeutic results in the treatment of neurodegenerative diseases and acute liver injury.[110] These studies not only validate the antioxidant mechanism of nanozymes but also provide an important experimental basis for future clinical translation.

Beyond these established disease models, antioxidant nanozymes exhibit substantial therapeutic potential in several emerging areas. In the realm of neuroprotection, nanozymes capable of crossing the blood-brain barrier can mitigate neuronal oxidative damage by scavenging excess ROS, offering novel interventional strategies for neurodegenerative diseases such as Alzheimer's and Parkinson's.[111] In cardiovascular diseases, beyond myocardial ischemia-reperfusion injury, nanozymes are being explored for conditions like atherosclerosis, where they can alleviate oxidative stress and inflammation in vascular endothelial cells, thereby stabilizing plaques and slowing disease progression.[112] Furthermore,

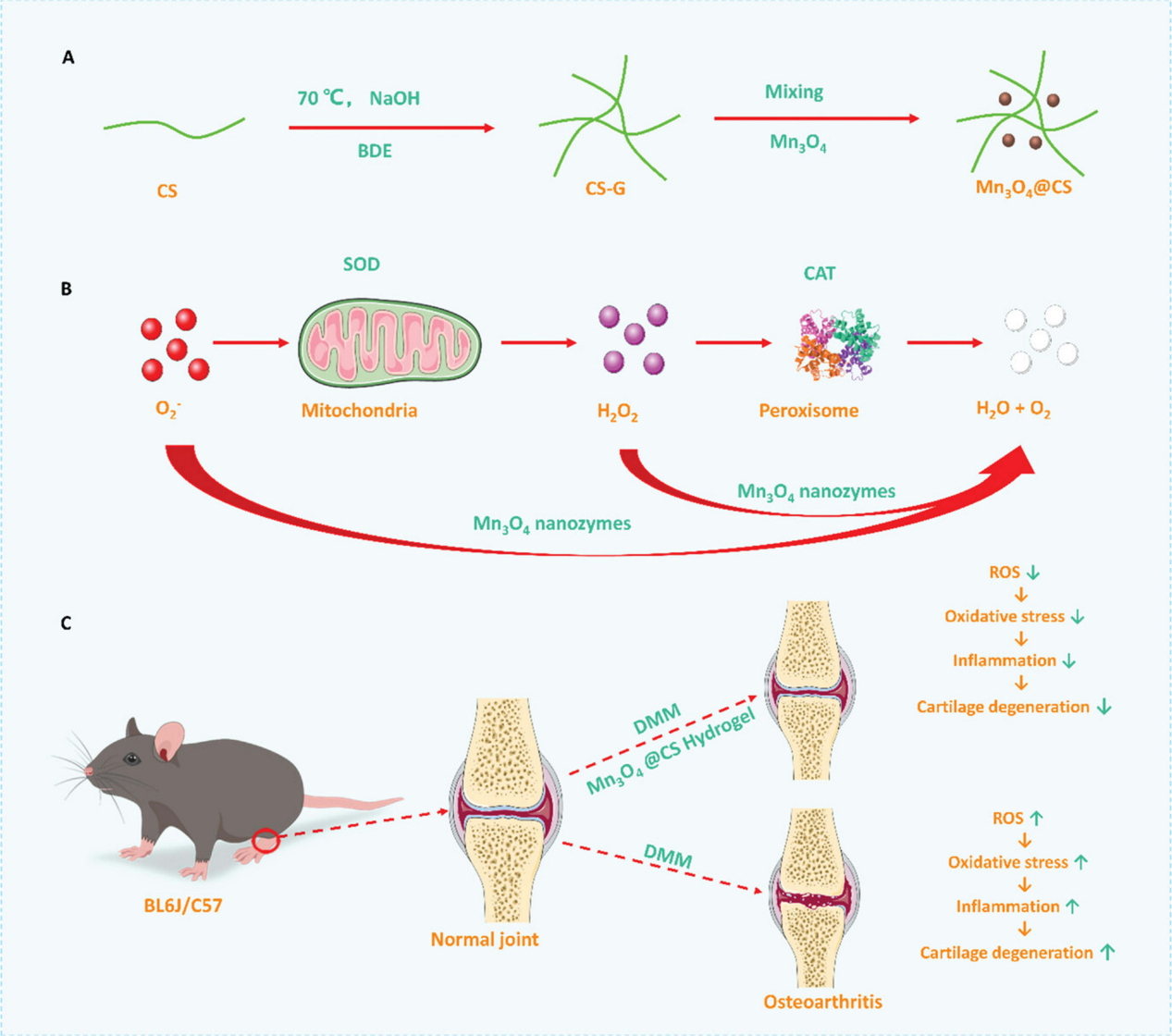

Figure 8 Graphical abstract of the Mn_3O_4 nanozyme-based therapeutic strategy for osteoarthritis. (**A**) The synthetic process of the Mn_3O_4@CS hydrogel. (**B**) The catalytic pathway of the enzyme-like activity of the Mn_3O_4@CS hydrogel. (**C**) The mechanism by which the Mn_3O_4@CS hydrogel eliminates ROS for OA treatment. The red circle represents the normal joint microenvironment of BL6J/C57 mice. By establishing a medial meniscus (DMM) osteoarthritis model, the DMM group exhibited elevated levels of ROS, increased oxidative stress and inflammation, leading to observed cartilage degeneration (Green upward arrow). In contrast, the Mn_3O_4@CS hydrogel group effectively alleviated cartilage degeneration by reducing ROS levels, decreasing oxidative stress and inflammatory responses (Green downward arrow). Reprinted with permission from,[106] copyright 2023. Advanced Science published by Wiley-VCH GmbH 2023.

In metabolic disorders such as impaired wound healing in diabetes, nanozymes can improve the microenvironment of wounds and promote tissue repair by regulating redox balance, highlighting their potential as a multifunctional therapeutic platform.[113] These emerging applications underscore the broad potential of nanozymes as a versatile platform for redox modulation. With the cross-integration of materials science and biomedicine, the application prospects of nanozymes in precision medicine and personalized therapy will be even broader.

Limitations and Challenges in Nanozyme Research

Nanozymes, a class of nanomaterials that can simulate the activity of natural enzymes, have shown great potential in biomedical field, such as biosensing, disease treatment and antibacterial activity. However, many limitations and challenges remain in the process of transforming from basic research to clinical application.

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Biosafety and Biocompatibility of Nanozymes

First, the biosafety and biocompatibility of nanozymes need to be evaluated. Nanozymes are usually composed of metals, metal oxides or carbon-based materials, which have not been fully evaluated in terms of long-term retention, metabolic pathways, degradation products and potential accumulation toxicity in organisms. Therefore, long-term biocompatibility and toxicity studies are needed to evaluate the potential side effects of nanozymes in organisms. Furthermore, within the complex biological microenvironment, nanozymes are likely to contact biological fluids and adsorb various proteins on their surfaces, forming a "protein corona". The protein corona may change the surface properties of nanozymes; mediate their processes of distribution, transport, metabolism, and clearance in vivo; and affect their biosafety. However, how protein coronas influence the catalytic activity of nanozymes is not yet systematically or comprehensively understood. The design and application of nanozymes should consider the microenvironment of the organism and the interactions between nanozymes and biomolecules.

Targeting Efficiency and Side Effectsof Nanozymes

In terms of nanozyme targeting, the traditional "EPR effect" (enhanced permeability and retention effect) refers to the phenomenon where macromolecules or nanoparticles (such as nanozymes) passively extravasate through the porous or leaky vasculature of tumors and are subsequently retained due to impaired lymphatic drainage. This effect has long been considered the fundamental mechanism behind the tumor-targeting ability of nanozymes. However, passive targeting strategies that rely on the enhanced EPR effect are generally inefficient. Although surface modification with targeting molecules (such as antibodies or peptides) can increase nanozyme accumulation in target tissues, the overall delivery efficiency remains suboptimal. Therefore, achieving highly efficient and targeted delivery of nanozymes remains a significant challenge.

Biological Applicability of Nanozymes

Nanozyme, as an inorganic nanomaterial, requires comprehensive consideration for its applicability in biomedical fields. Currently, nanozyme research lacks well-defined acceptable ranges for particle size and dosage, which significantly hinders their clinical translation. From the perspective of biological distribution, particle size plays a decisive role in the in vivo behavior of nanozymes. Particle sizes less than 5–10 nm are readily cleared by the kidneys; however, this excessively rapid clearance may hinder the achievement of effective concentrations at the target site. Particle sizes exceeding 200 nm are highly susceptible to uptake by macrophages in the liver and spleen, thereby potentially inducing hepatosplenotoxicity. Therefore, identifying an "acceptable particle size" for biomedical application is crucial in current nanozyme research. Furthermore, traditional enzymology defines dosage in units of enzyme activity (U), whereas nanozyme research often reports only mass concentration (eg, mg/kg) due to the lack of a unified standard for activity units, and the reported dosages of nanozymes also vary significantly. Therefore, determining an "acceptable dose" that ensures therapeutic efficacy while avoiding toxic side effects is crucial. The uncertainty of particle size and dosage will ultimately affect the biosafety of nanozymes. An ideal nanozyme should exert its effects at the target site and then be safely metabolized and excreted from the body without causing systemic toxicity. Future research should focus on establishing a unified standard for nanozyme activity units to address these translational challenges and facilitate clinical application.

Challenges in Clinical Translation of Nanozymes

In clinical translation, nanozymes still face challenges such as regulatory hurdles, poor scalability, and low stability. First, regulatory approvals from agencies such as the FDA and EMA require rigorous testing of safety, efficacy, and quality, which can be time-consuming and costly. In addition, the commercial production of nanozymes must ensure consistent quality and activity. However, their catalytic activity is highly dependent on size, morphology, and surface modification. It is extremely challenging to precisely control these parameters during synthesis while maintaining high consistency and reproducibility across batches. This challenge significantly hinders the clinical translation and commercialization of nanozymes. The development of cost-effective and repeatable synthesis methods is critical for commercial viability. Most

importantly, extensive preclinical studies and multiphase clinical trials are essential to establish the efficacy and safety of nanozymes in biomedical applications. However, most current research remains at the in vitro and animal model levels. To achieve true clinical application, fundamental breakthroughs are still needed in material design, targeting strategies, and safety evaluation systems.

In conclusion, as novel nanomaterials that mimic natural enzyme activities, nanozymes have significant potential for biomedical applications. However, their clinical translation still faces critical challenges. Current research needs to systematically address core issues, including biosafety, targeted delivery efficiency, and the stability of nanozymes during large-scale preparation. In the future, through interdisciplinary integration and innovation, optimized material design, biomimetic strategies, and the establishment of standardized evaluation systems, breakthroughs in translating nanozymes from basic research to clinical applications are expected. This will ultimately provide a new generation of tools for precision medicine.

Conclusion

In conclusion, nanozymes, which exhibit distinctive redoxase-like properties, have shown significant promise in various biomedical applications. This review systematically elucidates the catalytic mechanisms of nanozymes, including POD-like, OXD-like, CAT-like and SOD-like activities, and discusses their wide application in biomedical fields, from high-sensitivity biosensing and precision tumor therapy to high-efficiency antibacterial strategies and anti-inflammatory therapy.

First, the POD-like and OXD-like activities of nanozymes can catalyze the production of ROS, such as hydroxyl radicals and superoxide anions. These radicals possess strong oxidizing properties, enabling colorimetric detection of biomolecules. Second, nanozymes can increase intracellular oxidative stress levels through ROS generation, thereby facilitating tumor therapy and antibacterial treatment. In addition, their CAT-like and SOD-like activities enable antioxidant treatments, such as relieving inflammation through ROS scavenging. In short, by mimicking the catalytic functions of natural enzymes, nanozymes exhibit remarkable advantages such as high stability, tunable catalytic performance, and cost-effectiveness, making them ideal candidates for next-generation diagnostic and therapeutic platforms.

Despite these advances, the clinical translation and broader application of nanozymes still face several critical challenges. These include the need for more systematic elucidation of their catalytic mechanisms at the atomic level, improvement of their substrate specificity and long-term stability within complex biological environments, enhancement of targeted delivery efficiency, and the development of standardized, large-scale preparation processes.

Looking forward, driven by the cross-integration of nanotechnology and biomedicine, nanozymes are expected to become an important tool for disease diagnosis and treatment. To bridge the gap between fundamental research and clinical reality, future research should prioritize the following directions: First, the rational design of smart nanozymes with microenvironment-responsive activities should be explored. Such next-generation nanozymes could be engineered to selectively switch their catalytic activity "on" or "off" in response to specific pathological triggers (eg, pH, H_2O_2 concentration, or hypoxia), thereby enhancing therapeutic efficacy while minimizing off-target toxicity. Second, establishing standardized protocols for nanozyme characterization is urgently needed. A unified framework for evaluating and reporting catalytic activity (including turnover numbers and Michaelis-Menten constants) and biosafety profiles would not only facilitate cross-study comparisons but also accelerate regulatory approval and industrial translation. By addressing these priorities, nanozyme research can move toward the rational design of more biomimetic and clinically translatable nanocatalysts.

Abbreviations

MOFs, Metal–organic frameworks; POD, Peroxidase; CAT, Catalase; OXD, Oxidase; SOD, superoxide dismutase; H_2O_2, Hydrogen peroxide; OH, Hydroxyl radical; HRP, Horseradish peroxidase; TMB, 3,3',5,5'-tetramethylbenzidine; oxTMB, The oxidized form of TMB; DAB, Diaminobenzidine; NPs, Nanoparticles; SAzymes, Single-atom nanozymes; Pt NPs, Platinum nanoparticles; Au NPs, Gold nanoparticles; ELISA, Enzyme-linked immunosorbent assay; O_2, Oxygen; ROS, Reactive oxygen species; O_2^-, Superoxide anion; $^1O_2$, Singlet oxygen; GOx, Glucose oxidase; GSH-Ox, Glutathione oxidase; H_2O, Water; GSH, Glutathione; GPX-4, Glutathione peroxidase 4; DA, Dopamine; LOD, Limit of detection; AA, Ascorbic acid; TME, Tumor microenvironment; MMP, Mitochondrial membrane potential; PEG, Polyethylene glycol; Ca^{2+}, Calcium ion;

ApaF-1, Apoptotic protease-activating factor 1; HCC, Hepatocellular carcinoma; DSF, Disulfiram; LPO, Lipid peroxide; SLC7A11, Solute carrier family 7 member 11; SLC3A2, Solute carrier family 3 member 2; Glu, Glucose; RNS, Reactive nitrogen species; OA, Osteoarthritis.

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Author Contributions

Mao Cao: Writing-original draft, Writing - review & editing, Methodology, Visualization, Conceptualization, Formal analysis, Data curation. Jiazi Ma: Conceptualization, Formal analysis, Data curation. Yong Yang: Conceptualization, Formal analysis, Data curation. Mengjie Cheng: Conceptualization, Formal analysis, Data curation. Jianwei Liu: Funding acquisition, Formal analysis, Data curation. Zhifeng Pan: Funding acquisition, Formal analysis, Data curation. Zhongjun Du: Writing - review & editing, Resources, Funding acquisition, Conceptualization.

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Disclosure

The authors declare that they have no competing interests.

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