Inorganic nanoparticles with enzyme-mimetic activities for biomedical applications

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\textbf{A B S T R A C T}
Spurred by the recent advances in the chemical synthesis of nanomaterials, a group of inorganic nanoparticles with enzyme-mimetic activities has emerged as a new candidate to lead the future of nanomedicine. These so-called nanozymes have several advantages over their natural counterparts, such as more robust catalytic activities over wide ranges of pH and temperature, more economical production cost, and higher design flexibility through the integration and modification of various functional molecules and nanomaterials. To help readers understand this rapidly expanding field, we herein provide a short overview of the enzyme-mimetic activities of inorganic nanoparticles and their applications, with an emphasis on ceria and iron oxide nanoparticles, two of the most widely used nanozymes. Properties of other inorganic nanoparticle-based nanozymes are also briefly summarized. Finally, their current limitations and future outlook are discussed.

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1. Introduction

Thanks to the advancement in the inorganic nanoparticle synthesis that has shown unprecedented progress in the last three decades [1–3], diverse technological applications of the nanoparticles have been newly devised and renovated to provide with more advanced products and services. The early efforts mostly have been devoted to controlling the size and shape of nanoparticles because their physical and chemical properties are closely related to their dimensions, surface-to-volume ratios, and shapes. For instance, the band gap of a semiconducting nanomaterial increases with decreasing its dimension below its Bohr radius [4,5]. A ferro- or ferrimagnetic nanomaterial also experiences marked changes in its collective magnetic property as the size shrinks below its single-domain limit and superparamagnetic limit [6,7]. Among various synthetic approaches that have been developed to prepare inorganic nanoparticles, the so-called “hot-injection” method and “heat-up” method have proven their effectiveness in obtaining size and shape uniformity with high crystallinity [8,9]. Both methods share a common concept of separated nucleation and growth processes carried out in supersaturated high temperature organic solvents [10,11]. Although steadily advancing, understanding of in-depth mechanisms underlying each method is still incomplete [12–14]. Such a complication is partly due to the participation of weak forces and interactions during the nanoparticle synthesis, which would otherwise be non-significant outside the nanoscale range [15]. A lack of proper in situ techniques to accurately characterize nanoparticles and their interactions at their early formation stages also complicates the situation. Since elucidation on synthetic methods is beyond the scope of this review, readers are suggested to refer to other reviews dedicated to the subject [10,16].

Nonetheless, it is still worth taking a look at the roles of capping ligands used in the synthesis of nanoparticles. A capping ligand is a molecule that coordinates on the surface of nanoparticles to prevent their aggregation by providing repulsive forces between the nanoparticles and to change their growth rates and directions by modulating the diffusion of growing sources to the nanoparticle surface. Therefore, surface-capping ligands are regarded as essential components in the controlled synthesis of inorganic nanoparticles.

The presence of surface-binding ligands on inorganic nanoparticles has a significant implication in their catalytic use. Unlike optical, electric, or magnetic properties, the catalytic activities of nanoparticles are substantially affected by the surface structures and their accessibility to reactants. In some cases, the catalytic performance of ligand-capped nanoparticles can be lower than their bulk counterparts despite their higher specific surface area, as exemplified by the thiol-capped gold nanoparticles [17]. The strong chemical affinity between gold and thiols can block the catalytically active sites on the gold surface from interacting with other chemicals—the phenomenon known as catalyst poisoning [18,19]. Inevitably, the use of uniformly controlled inorganic nanoparticles as catalysts needs the following considerations: development of improved surface structures of nanoparticles, suitable modification methods such as ligand exchange or decomposition, techniques to deposit of nanoparticles onto substrates, and efficient surface functionalization methods. All of the above criteria have shown substantial progress up to now [20–22], and consequently, numerous practical applications along with research fields in environmental, electrocatalytic, photocatalytic, and energy-related areas have benefited from the availability of diverse inorganic nanoparticle-based catalysts [23–26].

Despite their promising competency and the widespread use of catalytic inorganic nanoparticles in the areas mentioned above, relatively less progress has been made in biomedical applications. To some extent, especially for the nanoparticles obtained in high temperature organic solvents, it can be ascribed to the hydrophobic nature of the surface capping ligands. The as-synthesized nanoparticles with hydrophobic ligands cannot provide colloidal stability in aqueous solutions where target biomolecules are dispersed in, preventing effective interactions between the nanoparticles and their target molecules. Accordingly, numerous surface modification methods for producing hydrophilic moiety onto the nanoparticle surface, in addition to the direct synthetic methods for producing various nanoparticles in aqueous solutions, have emerged in order to promote effective interactions between the nanoparticles and target molecules [20–25]. However, the utilization of the catalytic properties of nanoparticles, compared to the usage of their optical and magnetic properties, still has received little attention in biomedical applications. It is only recently that a few types of inorganic nanoparticles have come into the spotlight for their ability to catalyze chemical reactions as enzyme-mimics that can be utilized for biodetection and disease therapy [27–29]. These catalytic nanoparticles, namely nanozymes, can behave as artificial enzymes in biological systems and have several advantages such as more robust catalytic activities over wide ranges of pH and temperature, more economical production cost, and facile property tuning by size- and shape-controlled synthesis and surface modification [30–35]. It is highly probable that these nanozymes can provide another breakthrough in the field of nanomedicine. In that perspective, it would be timely to review the key aspects of the nanoparticle-based enzyme-mimics. Here, we provide a short overview of the enzyme-mimetic activities of inorganic nanoparticles and their applications, with an emphasis on ceria and iron oxide nanoparticles, two of the most widely used nanozymes. Properties of other inorganic nanoparticle-based nanozymes are also briefly summarized. Finally, their current limitations and future outlook are discussed.

2. Ceria nanoparticles

Ceria (CeO₂) has the fluorite structure, in which Ce⁴⁺ ions constitute the face centered cubic array and O²⁻ ions fill the tetrahedral interstitial sites. As often observed for other metal oxides, its stoichiometry is affected by the available oxidation states of the constituent cations (i.e., Ce³⁺ and Ce⁵⁺) and the partial oxygen pressure of the surrounding environment. The consequence is the formation of oxygen vacancies and the reduction of some Ce⁴⁺ ions to Ce³⁺ to match the charge balance. The redox reactions associated with these Ce³⁺ and Ce⁵⁺ ions are reversible so that they provide a basis for the catalytic activities observed in ceria [36,37]. However, because the effective ionic radius of Ce³⁺ is ~17.8% larger than that of Ce⁴⁺ for 8-coordination environments [38], which originates from the presence of an electron in 4f orbital for Ce⁴⁺, the conversion of Ce⁵⁺ to Ce³⁺ in ceria involves a huge increase in stress for the associated atomic lattice. Therefore, only a small portion of Ce³⁺ can be incorporated in ceria, limiting the overall catalytic activities. This limitation is somewhat relieved for nanoscale ceria,
as nanomaterials can afford lattice stains much better than bulk materials owing to their increased surface-to-volume ratios and to the fact that surface atomic lattices are softer than those in bulk [39]. As such, many types of ceria-based nanomaterials have been reported as highly catalytic, and their mechanisms in oxidation and/or reduction reactions have been extensively investigated together with their oxygen storage capability and ionic conductivity [40–42]. While most of the efforts have mainly focused on applications in automotive exhaust converters and solid oxide fuel cells [37], a new type of application recently has emerged to attract the researchers in the field of nanobiotechnology and nanomedicine. At the center of those biomedical applications are the superoxide dismutase-, catalase-, oxidase-, and peroxidase-mimetic activities of ceria nanoparticles [43–45].

Superoxide dismutase is an enzyme that dismutates superoxide anions (O$_2^-$) into oxygen (O$_2$) and hydrogen peroxide (H$_2$O$_2$) [46]. Catalase is an enzyme that dismutates H$_2$O$_2$ into O$_2$ and water (H$_2$O) [47]. Accordingly, the result of the sequential superoxide dismutase- and catalase-mimetic reactions by ceria nanoparticles is the elimination of O$_2$ and H$_2$O$_2$, the two major forms of reactive oxygen species (ROS). Although ROS are essential for key biological functions such as immune response and cell signal transduction [48,49], many diseases including cancers, sepsis, cardiovascular and neurodegenerative diseases are related to the oxidative stress by excessively produced ROS [48–52]. Therefore, regulating ROS at proper levels can be emphasized as a critical step for the prevention and treatment of the diseases. The exact mechanisms of substrate binding, coordination, and reaction dynamics of the ROS-scavenging activities on the ceria surface have not yet been thoroughly studied. However, it is widely accepted that the regenerative redox switching between Ce$^{4+}$ and Ce$^{3+}$ ions plays a pivotal role in the use of ceria nanoparticles as a very effective antioxidant to reduce the ROS-induced oxidative stress [53].

Under other conditions, the oxidase- and peroxidase-mimetic activities of ceria nanoparticles are used for the oxidation of substrate molecules with the help of O$_2$ and H$_2$O$_2$, respectively [43]. Besides the oxidized forms of the substrates, H$_2$O/H$_2$O$_2$/O$_2$ and H$_2$O are generated, respectively, by the catalytic reactions. The production of H$_2$O$_2$ and O$_2$ by the oxidase-mimetic activity may not be desired because they are the deleterious species to be scavenged by the superoxide dismutase- and catalase-mimetic activities as mentioned previously. For this reason, applications based on oxidase-mimetic activity often have different goals from those based on ROS-scavenging activities. For example, the oxidase-mimetic activity of ceria nanoparticles can be used for biosensing and detection rather than cell protection. The peroxidase-mimetic activity can also have similar applications because both activities can induce oxidation-related substrate color changes that are useful for the colorimetric detection of biomolecules.

In this section, we will summarize some of the recent achievements regarding the nanzyme properties of ceria nanoparticles. A specific focus is given to their therapeutic and biosensing applications.

### 2.1. ROS-scavenging activities and applications

The ROS-scavenging activities of ceria nanoparticles have been applied to treat various ROS-related diseases and dysfunctions. Examples include ischemic stroke, Alzheimer's disease (AD), Parkinson's disease (PD), sepsis, and ionizing radiation-induced damages [54–64].

For the ischemic stroke treatment, the antioxidant capability of 3 nm-sized ceria nanoparticles encapsulated in phospholipid-polyethylene glycol (PEG) was evaluated using an in vivo rat model [56]. The PEG coating provided colloidal stability as well as protection from non-specific binding of blood compartments. Therefore, a significant amount of intravenously injected ceria nanoparticles could permeate the ischemic brain tissues by passing through the disrupted blood-brain barrier (BBB) after the induction of ischemic stroke. The ROS-scavenging activities of the ceria nanoparticles following their uptake into the intracellular space, which was also confirmed by in vitro experiments, could protect the cells from oxidative damages. As a result, ROS-induced ischemic injury and apoptotic cell death could be suppressed as reflected by the considerably reduced brain infarct volumes (Fig. 1A).

Although these results show the possibility of therapeutic ceria nanoparticles for treating various brain diseases/injuries, more practical non-invasive administration is required for their delivery through the intact BBB. In this regard, the work by Heckman et al. is worth noting in that they revealed that surface-customized ceria nanoparticles could cross the BBB [57]. To minimize the interactions with serum proteins, ceria nanoparticles were stabilized with both citric acid and ethylenediaminetetraacetic acid (EDTA) that binds strongly to the ceria surface. The obtained ceria nanoparticles exhibited a prolonged blood circulation time with reduced uptake by the reticuloendothelial system. Importantly, the ceria nanoparticles were found to accumulate in the brain in a dose-dependent manner, implying their transport through the BBB. Accordingly, they could be used as an excellent antioxidant agent against experimental autoimmune encephalomyelitis.

Recently, Bao et al. developed the ceria nanoparticles capable of crossing the BBB via receptor-mediated transcytosis [63]. Their design comprised the ceria nanoparticle as core and three different shell components: angiopep-2, PEG, and edaravone, each of which was added for enhanced BBB crossing, improved biocompatibility with longer blood circulation, and synergistic ROS scavenging, respectively. More timely and effective treatment of brain injury induced by the overproduced ROS in stroke was possible with this strategy, as demonstrated in vivo by a focal cerebral ischemic injury model of rats (Fig. 1B). Moreover, the long-term biosafety of the ceria nanoparticles was supported by the hematic and histological analyses in which no sign of adverse effects at 30 days post-treatment was shown.

Kwon et al. paid attention to the treatment of mitochondrial dysfunction that originates from the oxidative stress in neurodegenerative diseases [60]. To deliver 3 nm-sized ceria nanoparticles to mitochondria, triphenylphosphonium (TPP) was conjugated to the phospholipid-PEG-coated ceria nanoparticles. TPP is a small molecular lipophilic cation with a strong positive charge, and compounds attached to TPP have been known to show improved mitochondrial uptake [65]. The driving force for such behavior is the direction of the electric potential of the mitochondrial inner membrane that is more suited for the inward transport of positively charged compounds. In experiments using a 5XFAD transgenic AD mouse model, the TPP-conjugated ceria nanoparticles were locally administered to the subicula of the AD mouse by stereotaxic injection. Their mitochondrial localization was verified in vitro and in vivo by taking confocal microscope images of the cells treated with the ceria nanoparticles co-conjugated with fluorescein isothiocyanate (FITC) and by transmission electron microscope (TEM) analysis of the mouse brain tissues, respectively. When the mouse brains were investigated two months after the injection, much reduced neuronal cell death as well as alleviated neuroinflammation were observed, indicating the neuroprotective effects by the mitochondrial ROS-scavenging TPP-conjugated ceria nanoparticles and their potential as a regenerative antioxidant for AD treatment (Fig. 1C).

Besides the ROS-induced oxidative stress, aggregation of amyloid-β (Aβ) peptides has been considered as another important cause of AD-related neurotoxicity. Multifunctional therapeutic agents that can simultaneously inhibit the Aβ aggregation and...
eliminate ROS have thus been sought after. The polyoxometalate-coated ceria nanoparticles reported by Guan et al. is such an example [66]. A polyoxometalate is a transition metal-based oxide complex that can inhibit the fibrillation of Aβ peptides by preferentially binding to Aβ monomers [67]. As such, they can degrade Aβ aggregates as well. Owing to the bifunctional ability to disintegrate Aβ and scavenge ROS, the prepared hybrid nanoparticles showed a better utility in diminishing the Aβ-induced toxicity than regular ceria nanoparticles, as evidenced by the in vitro cell and in vivo mouse studies (Fig. 1D).

Li et al. proposed a novel strategy to employ a dual delivery platform in which ceria nanoparticles and metal chelators are used to reduce the oxidative stress and the metal ion-induced aggregation of Aβ, respectively, to treat AD [68]. Mesoporous silica nanoparticles (MSNs) loaded with clioquinol, a metal chelator, were conjugated with glucose-coated ceria nanoparticles using the covalent bonding between glucose and phenylboronic acid on the surface of the MSNs. The covalent bonds broke upon contact with H2O2, and subsequent release of the ceria nanoparticles and metal chelators reduced the Aβ-induced cytotoxicity in vitro. The combination of ceria nanoparticles and metal chelators showed enhanced therapeutic effects compared to each individual component alone.

Despite the frequent use of ROS-scavenging ceria nanoparticles for neuroprotection, the detailed mechanism by which the neuroprotective effects can be achieved requires further elucidation. One such effort was attempted by Zeng et al. [69]. In their study, the change in the phenotype of microglial cells from pro-inflammatory to anti-inflammatory after treatment with ceria nanoparticles was attributed to blocked activation of neurotoxic

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**Fig. 1.** (A) Schematic illustration (a) and transmission electron microscope (TEM) image (b) of ceria nanoparticles (scale bar: 5 nm). Stained images of reduced infarct volumes after treatment with ceria nanoparticles in vivo (c). Reproduced with permission from Ref. [56]. Copyright 2012 John Wiley and Sons. (B) Schematic diagram of the design of ceria nanoparticles (a). Time-dependent change in the concentration of ceria nanoparticles in brain tissues (b). Photo images of triphenyltetrazolium chloride (TTC)-stained brains treated with different types of ceria nanoparticles (c). Reproduced with permission from Ref. [63]. Copyright 2018 American Chemical Society. (C) Schematic illustration of TPP-conjugated ceria nanoparticles for AD treatment (a). Fluorescence images of coronal brain sections stained with anti-NeuN antibody (red) and showing FITC-conjugated TPP-ceria nanoparticles (green) (scale bars: 30 μm) (b). Fluorescence images of gliosis in brain tissues stained with glial fibrillary acidic protein (GFAP) (red) and ionized calcium-binding adapter molecule 1 (Iba-1) (blue), and showing FITC-conjugated TPP-ceria nanoparticles (scale bars: 30 μm) (c). Reproduced with permission from Ref. [60]. Copyright 2016 American Chemical Society. (D) Schematic diagram of the preparation of hybrid ceria nanoparticles for Aβ degradation (a). ROS generation in PC12 cells of each treatment group. (*p < 0.05, **p < 0.01, and ***p < 0.001) (b). Scanning electron microscope (SEM) images of PC12 cells treated with Aβ40 peptides only (control) (top) and Aβ40 peptides and hybrid ceria nanoparticles (bottom) (c). Reproduced with permission from Ref. [66]. Copyright 2016 Elsevier.
inflammatory responses by which the co-cultured neuron cells were protected (Fig. 2A). The high efficiency in cellular uptake as well as the superior ROS-scavenging activities of ceria nanoparticles also contributed to enhancing the neuroprotective effects.

On the other hand, by expanding the concept of subcellular localization-specific scavenging of ROS, Kwon et al. investigated the roles of mitochondrial, intracellular, and extracellular ROS in the pathogenesis and progression of PD [64]. Three types of ceria nanoparticles were prepared. The first type, phospholipid-PEG-coated ceria nanoparticles, which were also used for treating ischemic stroke in the previous study, could internalize into the cells, but not into the mitochondria due to their negatively charged surface. Therefore, they were able to scavenge intracellular ROS only. On the contrary, the second type, TPP-conjugated ceria nanoparticles, could selectively scavenge mitochondrial ROS as demonstrated previously for the AD treatment. The extracellular ROS were scavenged by the third type, ~300 nm-sized ceria nanoparticle clusters, which did not show...
any cellular uptake. When these three types of ceria nanoparticles were applied to the striata of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mice, a significant increase in the retention of tyrosine hydroxylase (TH) was observed for the mice treated with the ceria nanoparticles or the TPP-conjugated ceria nanoparticles, evidencing the neuroprotective effects of the nanoparticles of both types. The ceria nanoparticle clusters were able to reduce neuroinflammation as with the other two types of the ceria nanoparticles. However, their ineffectiveness for protecting TH from ROS-induced oxidative damages suggested that intracellular and mitochondrial ROS were more important treatment targets than extracellular ROS for PD therapy (Fig. 2B). Although the extracellular ROS scavenging by large cluster particles does not look much useful compared with the other two, the ability to decrease inflammation outside of the cells can still find a good use for some applications as recently reported for the blood Aβ peptide cleansing for AD treatment [70].

The ROS-scavenging activities of ceria nanoparticles could be further enhanced by incorporating dopant cations into the ceria lattice. The criterion for the dopant choice is the smaller ionic size than Ce3+ so that the lattice strain accompanying the reduction of Ce4+ to Ce3+ is relieved and a higher amount of Ce4+ ions is allowed in the ceria nanoparticles. In fact, this kind of doping strategy has long been tried for ion conductors because the increased Ce4+ concentration in ceria also increases the number of oxygen vacancies that contribute to the ionic conductivity of the material [71]. Since the superoxide dismutase-mimetic activity of ceria nanoparticles is facilitated at high Ce4+/Ce3+ ratio, while their catalase-mimetic activity is favored at low Ce4+/Ce3+ ratio [53], it is believed that there is an optimal fraction of Ce3+ for maximized ROS-scavenging. In a systematic study on zirconium (Zr)-doped ceria nanoparticles by Soh et al., it was found that Ce0.7Zr0.3O2 (7CZ) nanoparticles possessed the best in vitro ROS-scavenging activities compared with other compositions [61]. Such a large improvement in ROS-scavenging performance could render the Zr-doped ceria nanoparticles as a promising therapeutic agent for sepsis treatment. The intravenously administered 7CZ nanoparticles treat two different animal models of sepsis, which are lipopolysaccharide (LPS)-induced endotoxemia rat model and cecal ligation and puncture (CLP)-induced bacteremia mouse model, significantly increased the survival rates of the rats for 14 days, confirming their anti-inflammatory effects by scavenging ROS (Fig. 2C).

Relieving the oxidative stress induced by ROS has also been proven to be beneficial not only for disease treatment but also for wound healing. Ceria nanoparticles, as a regenerative antioxidant with excellent ROS-scavenging properties, have thus been actively applied for that purpose. An example is the work by Chigurupati et al. [72], in which ceria nanoparticles promoted the infiltration of leukocytes, migration and proliferation of keratinocytes, fibroblasts, and vascular endothelial cells, and thereby angiogenesis. Accelerated wound healing after daily topical application of ceria nanoparticles was demonstrated using an in vivo mouse model. Furthermore, the treatment did not disturb other intrinsic wound healing processes such as pathogen killing and clearance, emphasizing their versatility. Such wound healing property can be integrated with a tissue adhesive property to obtain a synergistic effect. In a study by Wu et al., ceria nanoparticles were immobilized onto the surface of mesoporous silica nanoparticles with a known characteristic of tissue adhesion [73]. The generated nanoassembly particles maintained the tissue adhesive properties of the silica and ROS-scavenging activities of the ceria nanoparticles, exhibiting a high performance in tissue barrier structure restoration and oxidative stress mitigation by bridging tissue matrices and by managing ROS levels in the microenvironment, respectively. All these effects were reflected in the in vivo rat model results, in which high-quality cutaneous wound healing, such as reduced scar formation and augmented morphogenesis of skin appendages, was achieved (Fig. 2D).

2.2. Oxidative activities and applications

While numerous applications of ceria nanoparticles as nanozymes rely on their use as ROS-scavenging antioxidants, some studies have focused on their roles in catalyzing oxidation reactions. For instance, their oxidase- and peroxidase-mimetic activities have received increasing attention [74–79].

With respect to the oxidase-mimetic activity, a study suggested that the oxidation of substrate molecules, such as 3,3’,5,5’-tetramethylthiophenylbenzidine (TMB) and 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABT), by ceria nanoparticles under acidic conditions resulted in the dissolution of the ceria nanoparticles, implying that the reactions were not actually catalytic [80]. It was later revealed that there were two contributing pathways [81]. One was the oxidation by the hydroxyl radicals (·OH) that were generated through the Fenton-like catalytic reaction of ceria nanoparticles. The other was the direct oxidation in acidic solutions by the ceria nanoparticles containing Ce4+ ions, accompanied by the concomitant Ce3+ dissolution. Therefore, it is speculated that the oxidase-mimetic activity of ceria nanoparticles is pH-dependent. In other words, substrate oxidation at low pH involves the non-recyclable dissolution of ceria nanoparticles and that at neutral or basic pH is based on the catalytic activity of the ceria nanoparticles [43]. However, a more thorough investigation on the molecular mechanism of the oxidase-mimetic activity of ceria nanoparticles is needed for clarification.

Nevertheless, many studies have already been conducted to make use of the oxidation effect by ceria nanoparticles. For example, Asati et al. reported an immunooassembly capable of detecting folate receptor- or epithelial cell adhesion molecule (EpCAM)-expressing tumor cells (Fig. 3A) [74,75]. In a different format, Cheng et al. developed a bioassay scheme that could detect proton-producing enzymes [82]. In their approach, the oxidase-mimetic activity of the ceria nanoparticles could be controlled by the acidity of the reaction solution, which was on par with the previous reports. When acetylcholinesterase, an enzyme that produces acetic acid as a proton source by hydrolyzing acetylcholine substrate, exists with its substrate in a neutral pH solution, the oxidase-mimetic activity of the ceria nanoparticles works to convert TMB into blue-colored products. In contrast, the ceria nanoparticles do not induce any TMB color change in the absence of the enzyme. This regulatory effect on the oxidase-mimetic activity can be utilized for the colorimetric detection of proton-producing/consuming enzymes and their inhibitors (Fig. 3B).

Other than the bioassays, Xu et al. proposed an interesting method of drug delivery for cancer therapy [83]. Ferrocene-functionalized mesoporous silica nanoparticles were loaded with 5-fluorouridine, an antitumor drug, and their pores were capped with β-cyclodextrin-modified ceria nanoparticles via the complexation between ferrocene and β-cyclodextrin [84]. The oxidase-mimetic activity of the ceria nanoparticles was activated after their cellular uptake and exposure to the acidic microenvironment of tumor cells, which eventually led to the formation of ferrocenium ions and the detachment of the pore-capping ceria nanoparticles from the mesoporous silica surface. As a result, the loaded drugs could be released for chemotherapy. In addition, the ferrocenium ions have anticancer activity, while the ceria nanoparticles themselves can cause oxidative stress to the cancer cells. Overall, greatly enhanced therapeutic efficacy could be obtained (Fig. 3C).

The peroxidase-mimetic activity of ceria nanoparticles has also brought various applications. Many of the applications deal with the molecular sensing in similar ways to the oxidase-mimetic activity-based bioassays due to the oxidizing nature of the
peroxidase-mimetic activity. One of the typical molecules that have been frequently detected is glucose. The oxidation of glucose by glucose oxidase produces H$_2$O$_2$ with which the peroxidase-mimetic activity of ceria nanoparticles induces a substrate color change for a colorimetric assay. Hence, this setup can be understood as a two-step process that necessitates the coexistence of glucose oxidase in the solution, and the ceria nanoparticles that function as a replacement to horseradish peroxidase (HRP) used in conventional assays (Fig. 3D). Of course, the peroxidase-mimetic activity was demonstrated to work in sandwich-type immunoassays as well for detecting antigens [78]. Further improvement on the peroxidase-mimetic activity was achieved by doping or facet-selective synthesis of ceria nanoparticles [85,86].
3. Iron oxide nanoparticles

Iron oxide nanoparticles have been investigated extensively for various biomedical applications including medical diagnosis, drug delivery, therapy, magnetic separation, and catalysis [87]. In particular, they have gained much attention because of their FDA approval for clinical use as magnetic resonance imaging (MRI) contrast agents and nutritional iron supplements [88]. Various synthetic approaches including precursor precipitation in aqueous solution, hydrothermal, thermal decomposition, and electrochemical methods have been reported to produce high-quality iron oxide nanoparticles [89–92]. Notably, the thermolysis of iron precursors using a heat-up process can yield uniformly sized iron oxide nanoparticles in a multigram scale in a single reaction [9]. Additionally, recent advances in surface engineering based on the understanding of biochemistry and medicinal chemistry endow iron oxide nanoparticles with new opportunities for biomedical applications [93].

3.1. Enzyme-mimetic activities

In 2007, the pioneering work by Gao et al. demonstrated that the intrinsic peroxidase-mimetic catalytic activity of Fe₃O₄ nanoparticles could oxidize organic substrates such as TMB, 3,3'-diaminobenzidine (DAB), and o-phenylenediamine dihydrochloride (OPD) with the assistance of H₂O₂ for immunoassay (Fig. 4A) [28]. Their catalytic activity, similar to that of HRP, depended on the concentration of H₂O₂, pH, and temperature, adhering to the typical Michaelis-Menten kinetics while exhibiting the characteristics of the ping-pong mechanism. They reported that the peroxidase-mimetic activity of the Fe₃O₄ nanoparticles was maintained over wide ranges of pH and temperature compared with HRP. In addition, they demonstrated that antibody-conjugated Fe₃O₄ nanoparticles could be utilized as a new type of immunoassay agent for capture, separation, and detection due to their magnetic properties. This work prompted developments in various peroxidase-mimetic nanomaterials and their applications.

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**Fig. 4.** (A) TEM images of Fe₃O₄ nanoparticles with peroxidase-mimetic activity (a). Photo images of organic substrates (TMB, DAB, OPD) oxidized by the peroxidase-mimetic activity of the Fe₃O₄ nanoparticles with H₂O₂ (b). Reproduced with permission from Ref. [28]. Copyright 2007 Springer Nature. (B) The proposed reaction mechanism of the peroxidase-mimetic activity of Fe₃O₄ nanoparticles with the assistance of H₂O₂. Reproduced with permission from Ref. [95]. Copyright 2010 Elsevier. (C) Optical images and relative peroxidase-mimetic activity for TMB oxidation depending on Fe₃O₄ nanoparticle structure: cluster spheres (a), triangular plates (b), octahedra (c), and without any Fe₃O₄ nanoparticle (d). Reproduced with permission from Ref. [97]. Copyright 2011 John Wiley and Sons. (D) Schematic diagram of peroxidase-mimetic activity of dumbbell-like Pt₄₈Pd₅₂-Fe₃O₄ nanoparticles (left), and photo images of the color change of the PBS solutions (pH = 5.2) containing H₂O₂ and TMB oxidized by Pt₄₈Pd₅₂-Fe₃O₄ (a), Fe₃O₄ (b), Pt₄₈Pd₅₂ (c) nanoparticles, and without nanoparticles (d). Reproduced with permission from Ref. [101]. Copyright 2013 John Wiley and Sons. (E) Selective oxidization of substrates by Fe₃O₄ (a), Fe₃O₄ with TMB-imprinted negatively charged MIP (T-MIPneg) (b), and Fe₃O₄ with ABTS-imprinted positively charged MIP (A-MIPpos) (c) in the presence of H₂O₂. Reproduced with permission from Ref. [104]. Copyright 2017 American Chemical Society. (F) Photo images of the color change after TMB oxidation in pH 4.0 NaH₂PO₄ buffer at 40 °C for 15 min catalyzed by H₂O₂ only (a), H₂O₂ with GO-Fe₃O₄ nanocomposites (b), and GO-Fe₃O₄ nanocomposites only (c). Reproduced with permission from Ref. [105]. Copyright 2012 RSC Publishing.
Furthermore, iron oxide nanoparticles were later found to exhibit catalase-mimetic properties as well, as reported by Chen et al. [94]. They synthesized dimercapto succinic acid (DMSA)-coated γ-Fe2O3 and Fe3O4 nanoparticles which had pH-dependent dual catalytic properties. Both nanoparticles exhibited peroxidase-mimetic activities to produce OH from H2O2 under acidic lysosome-like conditions while Fe3O4 nanoparticles showed higher activity than that of γ-Fe2O3 nanoparticles. The catalase-mimetic behaviors of both nanoparticles were observed under neutral pH conditions as they dismutated H2O2 into H2O and O2.

Wang et al. proposed a plausible mechanism built upon the Fenton and Haber-Weiss reactions for the peroxidase-mimetic activity of iron oxide nanoparticles [95]. Using electron spin resonance (ESR) analysis, they revealed that Fe3+ and Fe3+ can activate the adsorbed H2O2 on the surface of an iron oxide nanoparticle to generate OH and superoxide/hydroperoxy radical. The O2-/HO2 radicals contributed more to the degradation and mineralization of organic pollutants (Fig. 4B). They also found that the catalytic activity was not derived from leached free Fe ions as the Fe concentration in the supernatant was too low for Fenton reaction to proceed. Other groups also reported that the peroxidase-mimetic property of iron oxide nanoparticles resulted from the nanoparticle surface as a heterogeneous Fenton system rather than from the free Fe ions [28,29].

To maximize the catalytic efficiency of iron oxide nanoparticles, several groups focused on modulating their size, morphology, composition, and surface. In general, the smaller the particle size, the better the catalytic efficiency because smaller nanoparticles possess a larger surface-to-volume ratio. Peng et al. compared three different-sized Fe3O4 nanoparticles with average diameters of 11, 20, and 150 nm, and found that their catalytic activity increased with decreasing the nanoparticle size [98]. The morphological difference can also affect the catalytic activities of iron oxide nanoparticles. Liu et al. evaluated the shape effect on the peroxidase-mimetic activity of iron oxide nanoparticles by comparing cluster spheres, octahedra, and triangular plates [97]. In their study, the peroxidase-mimetic activities followed the order of cluster spheres > triangular plates > octahedra. This trend is closely related to the shape-dependent exposure of active Fe ions and/or crystallographic planes (Fig. 4C).

One way of changing compositions of nanoparticles for the enhanced catalytic activity of iron oxide nanoparticles includes doping with other elements. Battacharya et al. demonstrated that Mn2+-doped ferrite nanoparticles have enhanced peroxidase-mimetic activity and magnetic moment in proportion to the amount of Mn2+ added [98]. Su et al. also showed that magnetic ZnFe2O4 nanoparticles have higher peroxidase-mimetic activity and better stability, while maintaining an excellent dispersibility and a quicker separation capability, in urine glucose detection compared with HRP and other peroxidase-mimetic nanozymes [99]. In a study by Mumtaz et al., it was reported that the peroxidase-mimetic activities of dopamine (DOPA)-functionalized mixed ferrite nanoparticles for followed the order of CoFe2O4-DOPA > MnFe2O4-DOPA > CuFe2O4-DOPA > NiFe2O4-DOPA > Fe3O4-DOPA [100].

Other than the compositional change of nanoparticles, the formation of heterointerface can be effective. Sun et al. reported that dumbbell-like Pt8Pd32-Fe3O4 nanoparticles exhibit higher peroxidase-mimetic activity than that of a simple physical mixture of Pt8Pd52 nanoparticles and Fe3O4 nanoparticles (Fig. 4D) [101]. This enhanced activity was thought to have resulted from the interface of the dumbbell-like Pt8Pd32-Fe3O4 nanoparticles by which an excellent peroxidase activity higher than that of HRP was achieved under biologically relevant conditions [102].

The surface modification of iron oxide nanoparticles can also contribute to improving the catalytic performance. Recently, Fan et al. reported that grafting histidine molecules onto the surface of Fe3O4 nanoparticles can increase their affinity toward H2O2 and hence the catalytic efficiency [103]. In resemblance to the hydrogen bond formation at the catalytic site of HRP, the H2O2 actively bound to the imidazole group of histidine on the Fe3O4 nanoparticle surface, resulting in the enhancement in both peroxidase- and catalase-mimetic activities. On the other hand, Zhang et al. developed Fe3O4 nanoparticles encapsulated with a molecularly imprinted polymer (MIP) blend for improved selectivity as well as catalytic activity [104]. The synthetic method involved an encapsulation of the nanoparticles conjugated with target molecules (e.g., TMB) via polymerization after which the target molecules were removed to generate cavities for that specific molecule. Remarkably, the peroxidase-mimetic activity of the MIP-Fe3O4 nanoparticles was enhanced 100-fold compared with that of bare Fe3O4 nanoparticles (Fig. 4E).

Integration with carbon-based nanomaterials to form nanocomposites has been actively investigated to synergize the catalytic effects of both materials. As part of such efforts, Dong et al. fabricated graphene oxide (GO)-Fe3O4 magnetic nanocomposites and demonstrated their intrinsic peroxidase-mimetic activity with higher affinity for H2O2 (Fig. 4F) [105]. Zubir et al. discovered that the synergistic effect in GO-Fe3O4 nanocomposites derived from the strong interactions between the Fe3O4 nanoparticles and GO via the Fe-O-C bonds at the interface [106].

### 3.2. Biomedical applications

The catalytic properties of iron oxide nanoparticle-based nanozymes in addition to their biocompatibility, versatility in surface modification, and magnetic separation capability could find use in many areas of biomedical applications. Some recent progress in niche application as well as biosensing and disease treatment are briefly reviewed in this section.

First of all, the excellent stability and performance in the catalytic activity of iron oxide nanoparticles have enabled the development of advanced biosensors [107–112]. For example, Vallabhan et al. reported enhanced peroxidase-mimetic activity of Fe3O4 nanoparticles over wide ranges of pH and temperature in the presence of adenosine triphosphate (ATP) [113]. It was suggested that the complexation between ATP and Fe3O4 nanoparticles could contribute to the production of OH at physiological pH via electron transfer reactions. They also demonstrated that the interaction between ATP and Fe3O4 nanoparticles can be applied to the single-step detection of glucose in human blood serum.

Duan et al. reported a novel type of immunochromatographic strip that uses Fe3O4 nanoparticles for rapid and highly sensitive diagnosis of Ebola virus (EBOV) [114]. They utilized the anti-EBOV antibody-functionalized Fe3O4 nanoparticles as nanzyme probes for recognition, separation, and naked-eye visualization of EBOV on the strip. The peroxidase-mimetic activity of the Fe3O4 nanoparticles could lower the detection limit by 100-fold for the detection of the glycoprotein of EBOV compared with that of the standard colloidal gold strip. Moreover, they showed that the Fe3O4 nanoparticle-based strips can detect other infectious viruses if paired with corresponding antibodies, demonstrating the versatility of the strips as a rapid, simple, and accurate diagnostic tool (Fig. 5A).

In place of antibodies, DNAs or aptamers can also be combined with the catalytic activities of iron oxide nanoparticles for biodetection [115,116]. For instance, Thiramanas et al. conjugated iron oxide nanoparticles with aminated forward primers specific to a gene of Vibrio cholerae for polymerase chain reaction (PCR)-amplified colorimetric detection [117]. After the PCR amplification of the target gene and the binding of biotin-modified reverse primers, the iron oxide nanoparticles were separated by a magnet
and incubated in a streptavidin-coated microwell for subsequent detection of the target gene. The peroxidase-mimetic activity of the iron oxide nanoparticles with the addition of H2O2 and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) enabled the colorimetric detection since the remaining nanoparticles in the microwell were proportional to the amount of the target gene.

Disease treatment is another area that has attracted increasing attention. Especially, chemodynamic therapy (CDT) using inorganic nanoparticle-based nanozymes is an emerging cancer treatment approach in which ROS are produced via chemical reactions to damage DNAs and proteins at tumor sites [118]. For example, iron oxide nanoparticles can produce toxic hydroxyl radicals via Fenton or Fenton-like reactions, which may be accommodated by the mild acidity and the high level of H2O2 at tumor microenvironment to selectively trigger the generation of hydroxyl radicals [119,120]. This kind of stimuli-responsiveness is particularly advantageous for biosafety because the Fenton reaction is suppressed in non-tumoral microenvironment with slight basicity and insufficient H2O2 concentration.

Recently, several groups have focused on improving the efficiency of CDT by utilizing a broad range of nanomaterials, modulating tumor microenvironments, and introducing external triggers [121–125]. For example, Huo et al. integrated both glucose oxidase enzymes and ultrasmall Fe3O4 nanoparticles into the large pores of biodegradable dendritic silica nanoparticles (MFMSNs) for efficient PDT of hypoxic cancer (a). O2 generation by MFMSNs with H2O2 in PBS (b). The change in tumor volume after PDT treatment with different types of nanoparticles in vivo (c). Reproduced with permission from Ref. [128]. Copyright 2017 American Chemical Society.

(D) Photo images of hearts from rats of sham, the coronary artery ligature (CAL) model, and the CAL model after treatment with DMSA-modified Fe2O3 nanoparticles (a). Photo images of a Guinea pig Langerdorff heart (left) and the hearts after ischemia and reperfusion (IR) without (middle) or with (right) DMSA-modified Fe2O3 nanoparticle treatment (b). Reproduced with permission from Ref. [132]. Copyright 2015 Springer Nature.
reactions [126]. The integrated glucose oxidase facilitated the generation of intratumoral H$_2$O$_2$ from glucose and O$_2$, while the catalytic nanocomposites responsive to the acidic tumor microenvironment produced ·OH radicals to induce cell apoptosis. Moreover, the subsequent depletion of intracellular glucose could starve cancer cells, leading to efficient tumor cell apoptosis as its therapeutic efficacy was confirmed in vivo. It was later reported that glucose oxidase could be substituted with gold nanoparticles to construct an advanced nanzyme system that bypasses the intrinsic drawbacks of natural enzymes [127].

On the other hand, Kim et al. reported another notable scheme for hypoxic cancer treatment in which the enhanced catalytic activity of MnFe$_2$O$_4$ nanoparticles improved the therapeutic efficacy of photodynamic therapy (PDT) [128]. Tumor hypoxia, a distinctive environmental characteristic of many solid tumors, deters the effectiveness of PDT because PDT agents rely on the presence of O$_2$ to generate singlet oxygen to kill tumor cells. Although direct delivery or light-induced generation of O$_2$ seems ideal to alleviate the hypoxic condition, achieving high O$_2$ delivery efficiency or low light stimulation threshold for efficient O$_2$ generation may not be a trivial task. Accordingly, the authors used bio-compatible MnFe$_2$O$_4$ nanoparticles to generate O$_2$ onsite by their catalytic activity without any external stimulation. For PDT, nanocomposites were prepared by anchoring MnFe$_2$O$_4$ nanoparticles onto the surface of mesoporous silica nanoparticles loaded with photosensitizers (e.g., Chlorin e6). The nanocomposite with endogenous H$_2$O$_2$ in the tumor could continuously generate O$_2$ by which enhanced PDT efficacy was achieved as demonstrated with in vivo tumor-bearing mouse experiments. Moreover, a significant enhancement in T$_2$ MRI contrast could be observed due to the magnetic moment of the MnFe$_2$O$_4$ nanoparticles (Fig. 5B).

The peroxidase-mimetic activity of iron oxide nanoparticles was also shown to be useful for the degradation of the protective biofilms of bacterial origin. Gao et al. revealed that the cleavage of biofilm components, including nucleic acids, proteins, and oligosaccharides, by H$_2$O$_2$ could be more effective with the assistance of Fe$_3$O$_4$ nanoparticles [129]. Such enhancement was attributed to the generation of oxidative free radicals by the peroxidase-mimetic activity of the Fe$_3$O$_4$ nanoparticles, suggesting the competency of the Fe$_3$O$_4$-H$_2$O$_2$ system as an improved disinfectant for the destruction of preformed biofilms and killing of bacteria. Further application in vivo was carried out for the degradation of dental biofilms either to prevent the onset or to suppress the severity of dental caries [130]. The peroxidase-mimetic activity of the Fe$_3$O$_4$ nanoparticles was more pronounced in the acidic microenvironment of dental caries so that highly efficient biofilm degradation and rapid killing of Streptococcus mutans bacteria could be achieved simultaneously. Additionally, these nanoparticles could reduce the demineralization of hydroxyapatite under acidic conditions, revealing another asset of using iron oxide nanoparticles for treatment (Fig. 5C).

While most of the reports on iron oxide nanoparticle-based nanozymes rely on their peroxidase-mimetic activity to produce oxidative radicals, the catalase-like activity of iron oxide nanoparticles to scavenge H$_2$O$_2$ are reported as well. Hu et al. synthesized fibrous gel by electrospinning hematite nanoparticles in a poly (vinyl alcohol) solution and crosslinking the resultant mat for effective wound healing [131]. The obtained membrane possessed high water permeability and catalase-mimetic activity to convert H$_2$O$_2$ into O$_2$ and water, providing a suitable condition for cell proliferation. In cell studies, the membrane accelerated the growth of the fibroblasts even in 50 μM of H$_2$O$_2$ as the catalase-mimetic membrane reduced the total H$_2$O$_2$ concentration and thereby could be rendered as an advanced dressing for wound healing.

Xiong et al. reported the cardioprotective effect of DMSA-modified Fe$_3$O$_4$ nanoparticles [132]. It was observed that the nanoparticles could inhibit the calcium influx to cells, suppress the intracellular ROS, and decrease the oxidative damages to the membrane lipids. Although the exact mechanism was not fully elucidated, the Fe$_3$O$_4$ nanoparticles exhibited better cardioprotection than conventional drugs, and their efficacy was verified in vitro and in vivo (Fig. 5D).

Zhang et al. also reported the use of the catalase-mimetic activity of Fe$_3$O$_4$ nanoparticles for neuroprotection [133]. In the cellular study, it was observed that the Fe$_3$O$_4$ nanoparticles internalized and protected the cells against H$_2$O$_2$-induced oxidative damages. The neuroprotective effect of the Fe$_3$O$_4$ nanoparticles was further validated in Parkinson’s disease cell model, as demonstrated by the reduced level of α-synuclein and inhibited activation of caspase-3. Aged drosophilae for in vivo studies showed enhanced climbing ability and extended life span along with the reduced ROS levels after dietary intake of the Fe$_3$O$_4$ nanoparticles. Additionally, the dietary intake could ameliorate the neurodegeneration in a drosophila model for Alzheimer’s disease.

4. Other metal oxide-based nanomaterials

Various types of metal oxide nanomaterials other than iron oxide and ceria have also been utilized to mimic enzymatic properties. In this section, we highlight a few of them used in biomedical applications.

4.1. Manganese oxide nanoparticles

As an established nanzyme, manganese oxide nanoparticles possess multiple enzyme-mimetic activities that can be exploited for many applications such as cytoprotection, biosensing, and disease therapy. For example, a recent study by Li et al. described the use of MnO$_2$ nanoparticles to encapsulate yeast cells as an intelligent and protective shell [134]. The MnO$_2$ nanoparticles were precipitated on the surface of the cells to form a sufficiently thick coating by which superoxide dismutase- and catalase-mimetic activities to scavenge ROS was granted. While maintaining their viability and metabolic activities, the protected cells showed an improved tolerance against physical stress factors such as dehydration and lytic enzymes. They maintained high survival rates even after exposure to H$_2$O$_2$ at high levels. Subsequently, glutathione was employed to dissolve MnO$_2$ nanoparticles shell through the reduction of Mn$^{4+}$ to Mn$^{2+}$, allowing the recovery of the cellular function of the yeast cells. Their results offered an intriguing strategy to use nanozymes for cell-based applications (Fig. 6A).

In addition to superoxide dismutase- and catalase-mimetic activities, glutathione peroxidase-mimetic activity can be provided by manganese oxide nanomaterials. Singh et al. reported the multi-enzymatic properties of flower-shaped Mn$_3$O$_4$ nanoparticles [135]. Glutathione peroxidase is known to scavenge ROS by using glutathione as an electron donor. These nanoflowers had a larger surface area compared to those of cubes, polyhedron and hexagonal plates, and exhibited higher catalase-, glutathione peroxidase- and superoxide dismutase-like activities. Such enhanced catalytic activities were ascribed to their large pore size (8.9 nm) as well as the large surface area of the nanoflowers. Their potential as an antioxidant to relieve the oxidative stress was demonstrated by in vitro experiments in which cell the viability of SHSY-5Y cell line was reserved after the co-treatment of the neurotoxin 1-methyl-4-phenylpyridinium (MPP+) and the nanoflowers.

The 2-dimensional MnO$_2$ nanoflakes with peroxidase- and glucose oxidase-mimetic activities were reported by Han et al. [136]. Typically, different pH condition is required for different enzymatic activities. However, the nanoflakes synthesized by the bovine...
serum albumin (BSA)-templated biomineralization method exhibited optimized dual enzyme-mimetic activities in a coinciding pH range. The oxidation of glucose and the colorimetric detection of \( \text{H}_2\text{O}_2 \) were carried out solely with \( \text{MnO}_2 \) nanoflakes, enabling rapid and simple glucose sensing in a one-pot reaction. This colorimetric detection method showed high glucose detection sensitivity by the oxidation of TMB as a substrate for blood samples (Fig. 6B).

Based on the glucose oxidase-mimetic activity and strong near-infrared light absorption property of \( \text{MnO}_2 \) nanosheets, Tang et al. recently developed an advanced photothermal therapy for tumors \([137]\). First, the intracellular glucose consumption after the cellular uptake of the nanosheets could starve the cancer cells. Second, the near-infrared light irradiation could induce the photoablation of the tumors. The sensitized cancer cells with glucose starvation enabled a significant increase in the efficiency of photothermal therapy. Moreover, the nanosheets enabled photoacoustic imaging as a guide for photothermal therapy as confirmed in vitro and in vivo.

4.2. Vanadium oxide nanowires

Even though vanadium oxide nanomaterials are one of the earliest nanozymes that have been investigated, the synthetic methods are not well developed compared to other materials. Consequently, most of the current reports on their enzyme-mimetic activities are based on using \( \text{V}_2\text{O}_5 \) nanowires.

A study by Andre et al. described the intrinsic peroxidase-mimetic activity of \( \text{V}_2\text{O}_5 \) nanowires in the presence of \( \text{H}_2\text{O}_2 \) \([138]\). The nanowires were synthesized using a hydrothermal method and showed optimal reactivity at pH 4.0 in a concentration-dependent manner. They reported that their affinities to ABTS and \( \text{H}_2\text{O}_2 \) were higher than those of HRP and vanadium-dependent haloperoxidase (V-HPO), their natural counterparts. The proposed mechanism is that the exposed lattice plane of nanowires initially forms metastable peroxo complexes with \( \text{H}_2\text{O}_2 \) in acidic pH as an intermediate. The subsequent nucleophilic attack of ABTS to the peroxo complex facilitates the oxidation of ABTS. Another ABTS molecule is oxidized at the site, and the nanowires regain their peroxidase-like property (Fig. 7A). Such peroxidase-mimetic activity of the \( \text{V}_2\text{O}_5 \) nanowires was employed in anti-biofouling as demonstrated by Natalio et al. \([139]\).

Vernekar et al. reported the glutathione peroxidase-mimetic activity of \( \text{V}_2\text{O}_5 \) nanowires against \( \text{H}_2\text{O}_2 \) \([140]\). \( \text{V}_2\text{O}_5 \) nanowires served as an antioxidant to reduce oxidative stress-induced intrinsically and extrinsically with the assistance of glutathione. Other intracellular factors like NADPH and glutathione reductase were required for the repeated catalytic cycle (Fig. 7B). Effective protection of the cells was observed against ROS-induced damages including protein carbonylation, lipid peroxidation, and DNA strand breaks. The therapeutic potential of the \( \text{V}_2\text{O}_5 \) nanowires for the prevention of disorders associated with increased levels of ROS was demonstrated.

Huang et al. developed nanocomposites of \( \text{V}_2\text{O}_5 \) nanowires, \( \text{MnO}_2 \) nanoparticles, and polydopamine to achieve the synergistic antioxidant effect \([141]\). The nanocomposites served as an intracellular antioxidant defense system in which \( \text{V}_2\text{O}_5 \) nanowires mimicked glutathione peroxidase while \( \text{MnO}_2 \) nanoparticles mimicked superoxide dismutase and catalase. Polydopamine, with its intrinsic superoxide dismutase-mimetic properties, was used for the growth of \( \text{MnO}_2 \) nanoparticles on \( \text{V}_2\text{O}_5 \) nanowires. When these multi-enzyme-mimetic nanocomposites were tested in vitro, a reduction in oxidative stress by the excellent ROS-scavenging activities was observed. In vivo experiments using an ear inflammation mouse model further confirmed their effectiveness as an antioxidant defense system (Fig. 7C).

4.3. Copper oxide nanoparticles

Copper, as one of the most affordable noble metals with a large abundance and low toxicity, has received extensive scientific and industrial attention for its diverse uses including catalytic applications. The enzyme-mimetic activities of \( \text{CuO} \) nanoparticles have
also been studied for biomedical applications. Numerous applications derived from the peroxidase-mimetic activity of CuO nanoparticles, and quantitative detection of cholesterol, glucose, and L-lactate was demonstrated to list a few examples [142,143].

Enhancement in the peroxidase-mimetic activity of CuO nanoparticles could be achieved by doping Zn into the nanoparticles as presented by Nagvenkar et al. [144]. Doped zinc induced unorganized crystal lattice formation with defects that served as active catalytic sites leading to catalytic superiority to the undoped CuO nanoparticles. The glucose detection sensitivity was substantially improved as manifested by the lower limit of detection compared to other contemporary studies in which other pure metal oxides were used.

Hu et al. designed a catalytic cascade system in which CuO nanoparticles served as cysteine oxidase- and peroxidase-mimetic nanozymes simultaneously [145]. In the presence of O2, CuO nanoparticles converted cysteine into cystine with the generation of H2O2. Subsequently, the peroxidase-mimetic activities of CuO nanoparticles, along with H2O2, facilitated the oxidation of terephthalic acid into fluorescent hydroxyterephthalate. The self-cascade catalytic cycle enabled the fluorometric detection of cysteine with high sensitivity in pharmaceuticals and human plasma samples.

4.4. Other metal oxide nanoparticles

Despite the great diversity of nanzymes derived from metal oxide nanoparticles, many of them have similar types of catalytic activity to those mentioned previously. As such, only a few representative examples are introduced herein.

Ragg et al. investigated the applicability of MoO3 nanoparticles as an artificial sulfite oxidase in physiological conditions [146]. Sulfite oxidase is a mitochondria-localized molybdenum-containing enzyme that plays a role in the catabolism of cysteine and methionine, and in the detoxification of sulfite and sulfur dioxide. TPP was conjugated onto the surface for selective targeting of the nanoparticles to mitochondria. When their catalytic activity was tested in vitro with sulfite oxidase knockdown liver cells, recovery in the sulfite oxidase-related cellular activities could be observed, demonstrating their potential for treating sulfite oxidase deficiency (Fig. 8A).

There have been efforts to use Co3O4 nanoparticles in place of Fe3O4 for the enhancement in peroxidase-mimetic activity performance [147]. Among such attempts, the study carried out by Jiang et al. is notable in that not only the catalytic activity but also the size uniformity and selective targeting efficiency were improved [148]. They used ferritin nanocages as a template for the synthesis
of Co₃O₄-based nanozymes for hepatocellular carcinoma (HCC) prognosis. The utilization of ferritins as template provided distinct advantages over typical nanozyme synthesis in that ferritins with size uniformity could be genetically engineered to provide targeting moiety. The Co₃O₄ nanozymes in ferritin modified with SP94 peptides (SFSIIHTPILPL) distinguished HCC tumor tissues from non-tumor liver tissues with high sensitivity of 63.5% and specificity of 79.1% using DAB as a substrate. The results were comparable to those of a clinical HCC-specific marker, α-fetoprotein (Fig. 8B).

As commonly observed for the metal oxides with non-stoichiometric composition, NiO nanoparticles also exhibit peroxidase-mimetic activity because of the coexistence of Ni²⁺ and Ni³⁺ ions that can accommodate the adsorption of various oxygen anions [149]. One of the early reports on the peroxidase-mimetic activity of NiO nanoparticles by Liu et al. described their use in colorimetric detection of glucose [150]. Conjugation of 5,10,15,20-tetakis(4-carboxylphenyl)-porphyrin on the NiO nanoparticle surface increased the generation of photoexcited electrons, and subsequent transfer of the electrons to the conduction band of the nanoparticles enhanced the peroxidase-mimetic activity. NiO nanoparticles with higher peroxidase-mimetic activity than that of ceria nanoparticles were reported as well [151].

5. Noble metal nanoparticles

As can be surmised from their well-established uses in many catalytic reactions, nanoparticles of noble metals have already received tremendous attention as nanozymes for biomedical applications. Similar to many other catalytic nanoparticles, they have often also been reported to exhibit pH-dependent enzyme-mimetic activities. For example, noble metal nanoparticles show peroxidase-mimetic activities in acidic conditions, whereas catalase-mimetic activities dominate in neutral and basic conditions. Li et al. explained such characteristics for nanomaterials of gold, silver, platinum, and palladium by using experimental results and theoretical calculations [152]. The results suggest that preferential pre-adsorption of OH group in basic conditions determined the types of catalytic activities. In other words, the pre-adsorbed OH could promote subsequent adsorption of H₂O₂ on the metal surface, leading to the decomposition of H₂O₂ into H₂O and O₂. On the other hand, the pre-adsorbed protons in acidic conditions weakened the interactions between H₂O₂ and metal surface. The decomposition of H₂O₂ generated .OH radicals, which subsequently oxidized the substrate.

5.1. Gold nanoparticles

The excellent biocompatibility of gold has provided a firm basis for its extensive use in biotechnology. Moreover, the relatively facile functionalization of gold via strong gold-thiol bond can be considered as another merit. What makes it even more fascinating among many types of metallic nanomaterials is its size- and shape-dependent surface plasmon resonance. Wide absorption range spanning from ultraviolet to near-infrared and surface-enhanced Raman scattering effect allows extensive employment of gold nanoparticles in biomedical applications [153]. Based on these properties of gold nanoparticles, biosensing has already been demonstrated for numerous diagnostic targets [154–156]. Apart from these, gold nanoparticles have also received considerable attention as nanozymes [157–165].

For example, Weerathunge et al. developed a pesticide acetamiprid detection assay by combining the peroxidase-mimetic activity of gold nanoparticles and the specific targeting ability of S–18 aptamer [158]. In their design, the enzyme-mimetic activity of the gold nanoparticles was initially inhibited by the aptamer coating. The
aptamers were detached in the presence of the target molecule, and the gold nanoparticles regain their catalytic properties. The process was reversible and enabled the visual determination of the catalytic activity by color change.

Wang et al. incorporated gold nanoparticles on peroxidase-mimetic ultrathin graphitic carbon nitride (g-C3N4) nanosheets to improve its catalytic activity and minimize the concentration of H2O2 required for bacteria killing and wound disinfection [159]. The peroxidase-mimetic activity increased as the amount of gold nanoparticles on the g-C3N4 nanosheets increased compared with bare gold nanoparticles and g-C3N4 nanosheets. In contrast to other peroxidase-mimetic systems that often require a high concentration of H2O2 and cause other unwanted side effects, the reported hybrid nanocomposites could effectively decompose H2O2 at biologically relevant concentration (50–100 μM) into OH to kill drug-resistant bacteria in a lung infection model and accelerate the rate of wound healing.

In addition to peroxidase-mimetic activity, gold nanoparticles mimic glucose oxidase as indirectly monitored by Li et al. [160]. They used complementary sequences of thiolated oligonucleotides to couple plasmonic 50 nm- and catalytic 13 nm-sized gold nanoparticles in proximity forming halo-like structure. The density of the nucleotides on the nanoparticles was controlled to allow the catalytic activity exclusively on the smaller-sized gold nanoparticles. Oxidation of the adsorbed glucose changed the permittivity of the catalytic gold nanoparticles, and the subsequent variation in the plasmonic resonance property of the halo-like system indicated the glucose oxidase-mimetic activity of gold nanoparticles. This study provides a novel methodology to observe the catalytic activity of the gold nanoparticles by plasmonic imaging.

Studies on the multi-enzyme-mimetic activities of gold nanoparticles were also conducted. Gold nanoparticles exhibit pH-dependent catalytic activity like other noble metal nanoparticles, as mentioned earlier in this section. Peroxidase-mimetic activity originates from the decomposition of H2O2 into OH radicals at low pH, and catalase-mimetic activity arises from the decomposition of H2O2 into O2 at basic pH. He et al. reported that gold nanoparticles have intrinsic superoxide dismutase-mimetic activity, although the mechanism was not elucidated in detail [166].

A combination of peroxidase- and oxidase-mimetic activities was reported for the gold nanoparticles supported on mesoporous silica by Tao et al. [167]. It was shown that the composite material facilitated the decomposition of H2O2 into hydroxyl radical as well as the generation of other ROS such as superoxide anion. An outstanding antibacterial capacity, together with the removal of biofilms, was achieved.

Interestingly, the surface-enhanced Raman scattering (SERS) can be combined with gold nanozymes for bioassays, as demonstrated by Hu et al. [168]. Gold nanoparticles were grown in a porous metal-organic framework, and their peroxidase-mimetic activity could convert the Raman-inactive leucomalachite green molecules caged inside the metal-organic framework into the Raman-active malachite green molecules. The nanocomposite itself served as a SERS substrate so that the Raman signal from the product molecules could greatly be enhanced. The system was versatile enough to detect various target molecules. For instance, by assembling glucose oxidase or lactate oxidase onto the nanocomposites, glucose or lactate could be detected, respectively. The effectiveness of this system was verified by in vitro experiments (Fig. 9A).

Gao et al. devised an efficient analytical technique for the quantification of membrane protein expression on cells [169]. The critical component in their design was the antibody-conjugated

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**Fig. 9.** (A) Schematic illustration of gold nanoparticles@metal-organic framework@oxidase (AuNPs@MIL-101@oxidase) for efficient enzymatic cascade reactions and enhanced Raman scattering signal (a). Concentration changes in lactate and glucose levels in an ischemia and reperfusion mouse model with or without astaxanthin (ATX) pretreatment (b). Reproduced with permission from Ref. [168]. Copyright 2017 American Chemical Society. (B) Schematic illustration of peptide-conjugated gold nanoparticles (peptide-AuNPs) for the quantification of GPIIb/IIa expression levels (a). Schematic illustration of microplate-based ELISA using peptide-AuNPs (left), and the optical density at 652 nm depending on the concentration of integrin (right) (b). Schematic illustration of cancer cell immunoassay using peptide-AuNPs (left), and Au concentration (red) and optical density (blue) depending on the number of human erythroleukemia (HEL) cells (right) (c). Reproduced with permission from Ref. [169]. Copyright 2015 American Chemical Society.
gold nanoparticles with the peroxidase-mimetic activity. The quantification was done by performing a colorimetry assay using integrin as the model protein and integrin-specific gold nanoparticles to oxidize TMB substrate. Subsequent two-photon photoluminescence imaging provided information about the spatial distribution of the target proteins. The overall process was rapid and facile because cell lysis or protein extraction steps were not necessary. Inductively coupled plasma mass spectroscopy (ICP-MS) analysis of the gold nanoparticles and integrin levels further validated the accuracy of the method (Fig. 9B).

A recent study by Xu et al. described the advanced use of multi-enzyme-mimetic gold nanoparticles and ATP synthase in series to drive the enzymatic cascade reactions for producing ATP in resemblance to the oxidative phosphorylation in natural mitochondria [170]. Hollow silica microspheres, on which gold nanoparticles were immobilized, were fused inside the proteoliposomes containing ATP synthase. They acted as glucose oxidase-mimetic nanzymes, accompanied by a decrease in pH upon the oxidative conversion of glucose to gluconic acid. The resulting concentration gradient of protons across the outer membrane coating of proteoliposome could activate the ATP synthase on the exterior of the system to produce ATP. Meanwhile, the generated H₂O₂ along the glacial acid was scavenged by the peroxidase-mimetic activity of the gold nanoparticles to prevent damage to ATP synthase.

5.2. Platinum nanoparticles

Enzymatic properties of platinum nanoparticles that have been reported include catalase-, superoxide dismutase-, peroxidase-, and oxidase-mimetic activities with which the pH-dependency may be involved [171–174]. These activities can likewise be utilized for applications such as biosensing and ROS-scavenging. Below are some of the examples.

Li et al. synthesized 2 nm-sized platinum nanoparticles with peroxidase-mimetic activity for colorimetric detection of Hg²⁺ in drinking water [175]. The synthesized nanoparticle consisted of ~43% Pt⁰ and ~57% Pt²⁺, and the content of the latter decreased upon the addition of Hg²⁺. The peroxidase-mimetic activity of the platinum nanoparticles originates from Pt²⁺, as observed for the DNA-templated platinum nanoparticles [176]. As a consequence, less color change in TMB substrate was observed with a higher concentration of Hg²⁺. Colorimetric detection and quantification of Hg²⁺ with high sensitivity and selectivity could be achieved even in the presence of other metal ions.

The ROS-scavenging property of platinum nanoparticles has led to some interesting applications. Hosaka et al. reported on the platinum nanoparticle-incorporated hemoglobin-albumin cluster that acted as an artificial carrier for O₂ [177]. Hemoglobin itself lacks the countermeasure for ROS-induced oxidative stress while red blood cells contain antioxidant enzymes for secured transport of O₂. The platinum nanoparticles inside the cluster served as superoxide dismutase- and catalase-mimics to promote hemoglobin from ROS, and the resulting hemoglobin-based artificial O₂ carrier showed outstanding stability against oxidative damages.

Moglianetti et al. conducted a systematic assessment on the cellular uptake, cytotoxicity, and catalytic activities of well-purified platinum nanoparticles of two different sizes (5 and 20 nm) [178]. While both nanoparticles were highly biocompatible, the 5 nm-sized platinum nanoparticles showed higher catalytic activity compared to those of the 20 nm-sized nanoparticles, superoxide dismutase, catalase, and peroxidase. Both nanoparticles helped restore intracellular ROS homeostasis as validated by using an in vitro cellular model of oxidative stress-related human cerebral cavernous malformation.

An enzyme replacement therapy for hyperuricemia was carried out using platinum nanoparticles incorporated along with uricase into MSNs by Liu et al. [179]. The prepared artificial metalloenzyme served as a tandem catalyst: the uric acid was degraded by the uricase, and the resulting toxic side product, H₂O₂, was decomposed by the platinum nanoparticles into H₂O and O₂, which further accelerated the degradation of the remaining uric acid. Experimental data revealed that this artificial metalloenzyme possessed not only a strong resistance against changes in pH, temperature, and protease amount in vitro but also a prominent therapeutic effect in vivo as shown in a hyperuricemia mouse model.

For enhanced cancer radiotherapy, Li et al. used porous platinum nanoparticles to increase the delivery efficiency of radiation energy to tumors and to relieve the hypoxic condition that contributed to the radio-resistance of the tumors [180]. The enhanced radiation energy delivery was achieved due to the high atomic number of platinum which can emit electron radiation after interacting with X-ray while the tumor oxygenation was facilitated by the conversion of H₂O₂ into O₂ using the catalase activity of the platinum nanoparticles. The synergistic effect for cancer radiotherapy was evidenced by an in vivo lung cancer mouse model (Fig. 10A).

5.3. Palladium nanoparticles

Although the correlation between crystallographic planes and their corresponding catalytic activities have long been explored, only a few investigations have been carried out for nanozymes. In that sense, a study conducted by Ge et al. using palladium nanoparticles can provide useful guidance in designing enzyme-mimetic nanoparticles [181]. They applied ESR analysis to compare the superoxide dismutase- and catalase-like activities of nanocubes and nanoctahedra that were enclosed by {1 1 1} and {1 0 0} facets, respectively. Although the nanocubes possessed higher surface energy and higher affinity to H₂O₂ than the nanocohedra, the catalytic efficiency of nanoctahedra turned out to be higher. The in vitro cell experimental data showed that the nanoctahedra significantly reduced mitochondrial oxidative stress induced by H₂O₂. Such a result was attributed to the smaller catalytic reaction energy required for {1 1 1} facets than for {1 0 0} facets as supported by the computational analysis.

Wang et al. adopted techniques of bioorthogonal chemistry to construct an external stimulus-responsive catalyst system that can be activated on demand by light [182]. The system comprised mesoporous silica nanoparticles, palladium nanoparticles, azobenzene, and β-cyclodextrin. There was a reversible host-guest interaction, induced by light, between azobenzene and β-cyclodextrin on the surface of mesoporous silica nano particle. Light-induced isomeric transformation of azobenzene led to the subsequent release of β-cyclodextrin from the surface, resulting in the exposure of the palladium nanoparticles embedded inside the mesopores to initiate the catalytic reactions. The validity of this design was confirmed by in situ synthesis of fluorescent probes and mitochondria-targeting by catalyzing cross-coupling reactions (Fig. 10B).

5.4. Multimetallic nanoparticles

The potential of multimetallic nanomaterials to improve catalytic activities has aroused great interest in their use as nanozymes. We can think about two categories of multimetallic nanoparticles for the sake of simplicity.

The first type consists of heterostructured multimetallic nanoparticles, such as core-shell nanoparticles. For instance, gold nanorods were used as a core, and subsequent coating with platinum, palladium, or silver shell proved to enhance their peroxidase-mimetic activities compared to bare gold nanorods.
Fig. 10. (A) Schematic illustration of porous platinum nanoparticle (PtNP)-based enhanced radiotherapy (a). Quantification data of hypoxia-inducible factor (HIF-1α) after PtNP treatment (left) and graphs for tumor growth after different treatments (right) (b). Reproduced with permission from Ref. [180]. Copyright 2019 Elsevier. (B) Schematic illustration of light-triggered bioorthogonal nanozyme system. The photo-induced isomerization for bonding and dissociation of cyclodextrin (top) and intracellular behavior of the nanozyme system (bottom) (a). Fluorescence images of the mitochondria-localized fluorescent probes generated by the light-triggered catalytic cross-coupling reaction: optical image of Hela cells (i), fluorescence image of the synthesized probes (green) (ii), mitochondria (red) (iii), nucleus (blue) (iv), merged image of (ii) and (iii) (v), merged image of (i) and (iv) (vi), merged image of (iii) and (iv) (vii), and merged image of (i), (iii), and (iv) (viii) (scale bar: 10 μm) (b). Reproduced with permission from Ref. [182]. Copyright 2018 Springer Nature.

Fig. 11. (A) Characterization of palladium-iridium (Pd-Ir) core-shell nanocubes: TEM image of Pd-Ir core-shell nanocubes (i). High-angle annular dark-field STEM image of a nanocube (ii). Magnified view of the yellow box in (ii) (iii). Line-scan energy dispersive X-ray spectroscopy data of a Pd-Ir core-shell nanocube (iv) (a). The catalytic activity of the Pd-Ir nanocubes depending on the molar ratio of Ir to Pd (b). Schematic illustration of PSA detection using Pd-Ir nanocube-based ELISA (Pd-Ir ELISA) and conventional HRP ELISA (c). Reproduced with permission from Ref. [186]. Copyright 2015 American Chemical Society. (B) Schematic illustration of the recovery from brain injury by trimetallic (TriM) nanoparticles (a). Lipid peroxide levels (b) and SOD activity (c) in the brain at 1, 7, 14 days post-treatment in vivo. Reproduced with permission from Ref. [190]. Copyright 2019 American Chemical Society.
Usage of gold nanorods as cores prevented the aggregation of small platinum nanoparticles and provided an ascorbic oxidase-like function for enhanced glucose detection. Palladium coating allowed higher peroxidase-mimetic activity with higher sensitivity toward malathion in a colorimetric assay. Silver coating allowed the nanozyme to function as an enhanced peroxidase-mimic at near neutral pH. The exact mechanism of how these heterostructured nanoenzymes show the enhanced performance is yet to be fully clarified.

Immunoassay application of palladium-iridium core-shell nanocubes was reported by Xia et al. [186]. In this study, they precisely controlled the thickness of the iridium shell deposited onto the palladium core to maximize the catalytic performance. In an optimized form, the nanocubes exhibited peroxidase-mimetic activity that is ~400 times higher than that of HRP. The nanocubes conjugated to antibodies for human prostate-specific antigen (PSA) outperformed the conventional HRP-based enzyme-linked immunosorbent assay (ELISA) in terms of sensitivity (Fig. 11A). Ye et al. further improved the sensitivity of the colorimetric assay by using a signal amplification technique [187]. The main idea was to load as many peroxidase-mimetic palladium-iridium nanoparticles as possible into a large spherical vesicle made of gold nanoparticles for amplification in color signal of TMB. Once the gold vesicles and biomarkers attached to one another, the loaded platinum-iridium nanoparticles were released by the heat-induced decomposition of the vesicle. Each released nanoparticle served as an efficient peroxidase mimic, leading to extremely high sensitivity for the target biomarkers. The detection limit was more than 1000-fold lower than that of conventional ELISA in PSA immunoassay.

Cheng et al. reported the use of mesoporous palladium-platinum core-shell nanoparticles with enhanced peroxidase-mimetic activity for lateral flow immunoassay-based bacterial detection [188]. The nanoparticles were integrated into a dual assay format after modification with antibodies. Then, the signal readout by a smartphone-based device was also demonstrated, which might be useful for in-field simplified analysis of pathogenic bacteria.

The other type of multimetallic nanoparticles is the homogeneously alloyed nanoparticles. The most straightforward case is the usage of bimetallic alloy, as demonstrated by He et al. [189]. In their study, silver was alloyed with gold, palladium, or platinum to produce nanoparticles of various morphologies and compositions. These alloyed nanoparticles showed varying activities toward HRP substrates such as TMB, ABTS, and o-phenylenediamine (OPD) depending on their compositions.

Mu et al. investigated the enzyme-mimetic activities of trimetallic nanoparticles made of platinum, palladium, and molybdenum at an atomic ratio of 6:9:1 [190]. The lattice distortion and increased exposure of active sites by the incorporation of molybdenum resulted in enhanced reactive oxygen and nitrogen species (RONS)-scavenging property. Importantly, the suitable pH conditions for the catalytic activities were near neutral. The nanoparticle could be applied to alleviate the RONS-induced stress, as confirmed by in vitro and in vivo experiments (Fig. 11B).

The demonstration of using multimetallic nanoenzymes together with other enzyme-mimetic nanoparticles to catalyze cascade reactions was reported by Wang et al. [124]. They first synthesized platinum-cobalt bimetallic nanoflowers with the oxidase-like activity, and then assembled MnO2 nanoparticles with the catalase-like activity onto the nanoflower surface. The nanoflowers were deployed to treat hypoxic tumors by the cascade reactions: H2O2 in tumor hypoxia was converted to O2 by the MnO2 nanoparticles, and the generated O2 was converted to O2− by the platinum-cobalt nanoflowers. The hypoxic condition could be overcome, and ROS-mediated cancer cell apoptosis could be initiated without external stimuli. Their concept was further validated using an in vivo tumor-bearing mouse model.

6. Conclusion and outlook

A variety of inorganic nanoparticle-based nanozymes have been developed and used for biomedical applications including biosensing and disease treatment. Although the results are quite promising, it should be admitted that the enzyme-mimetic nanoparticles are not all-purpose biological tools without any shortcomings. These may include the pH-dependent activity of nanozymes. Nanozymes can catalyze both forward and reverse reactions, often determined by the pH of the solution. In other words, nanozymes can serve as either antioxidants in some or oxidants in other applications [34,35]. The degree of freedom in application design is constrained since controlling pH in the actual physiological condition is not always feasible. Moreover, nanozymes can suffer from the lower activity and less specificity than their natural counterparts despite their usage in wider ranges of pH and thermal stability [30–35].

To overcome such limitations, we can first think about the ways to enhance the catalytic performance of nanozymes. A useful insight may be obtained from the T1-weighted MRI contrast agents developed using iron oxide nanoparticles coated with hydrophobic ligands. T1 MRI enhancement effect of a magnetic nanoparticle arises from the direct interaction between the water protons and the magnetic ions exposed on the nanoparticle surface, rather than the total magnetic moment of the nanoparticle [87]. Therefore, it is helpful to reform the surface of the nanoparticles to allow as many water protons as possible to access the nanoparticle surface. For example, 2 nm-sized iron oxide nanoparticles were shown to exhibit a significantly augmented MRI T1 relaxivity when the hydrophobic oleic acid ligands on their surface were completely replaced with hydrophilic poly(ethylene glycol)-derivated phosphine oxide ligands, compared with the relaxivity without removing the hydrophilic layers [191]. Similarly, surface modification methods that just encapsulate hydrophobic layer-coated inorganic nanozymes with amphiphilic polymers would not provide a good enough accessibility of target substrates to the nanozyme surface for catalytic reactions, despite their capability of enhancing colloidal stability in aqueous solutions. It is also expected that a proper surface modification would minimize the interaction between the nanozymes and non-target molecules/cells to prevent unwanted outcomes, such as the disruption of vascular endothelial cell junctions that can promote the metastasis of cancer cells [192]. Accordingly, tremendous efforts are still needed to develop novel methods to achieve both the colloidal stability in physiological conditions and the adequate accessibility to the target substrates.

Besides doping or alloying that has been tried rather frequently, another possible strategy to improve the catalytic activities is taking advantage of supporting materials. Nanozymes supported on an interacting material can exhibit significantly improved catalytic activities because the energetics of molecular binding, reaction, and dissociation on catalytic nanoparticles are closely related to the surface properties such as electron density, interatomic distance, and coordination number [193]. A noteworthy approach is strain-engineering by which the atomic structure of a catalytic layer can be modified by growing it on a supporting material [194,195]. Specifically, in the case of core-shell or core-island shell nanoparticles, the atomic arrangement at the lattice of the shell layer is affected by the core if the lattice parameter of the shell is different from the primary phase. When the lattice parameter of the shell is larger than that of the core, the shell experiences a compressive strain and vice versa. Not only the lattice strain but also the charge transfer property or electronic structure can be affected.
by the supporting material [196]. However, there are only few reports on the researches on support materials and strains of nanozymes combined with atomic-level characterization of interfacial structures [185, 197, 198].

The poor substrate specificity, which stems from the lack of selective substrate binding sites on nanozymes, also needs to be addressed. The molecular imprinting that involves the introduction of polymeric template sites onto nanozymes for specific molecular recognition is one way to overcome the limit [199]. Polymeric template sites, also known as plastic antibodies, are constructed in a way that only specific molecules can have access to the surface of nanozymes. For instance, Zhang et al. reported a nearly 100-fold increase in substrate specificity of peroxidase-mimetic Fe₉O₄ nanoparticles based on the molecular imprinting technique [104]. Its versatility allowed not only Fe₉O₄ but also nanoparticles of gold and ceria to have high specificity as well. Interestingly, the same research group argued that the molecular imprinting method can increase the catalytic activity of the nanozymes by the local enrichment of substrates at the nanozyme surface and by lowering the activation energy [200]. Some other approaches for enhancing the specificity include the coupling of nanozymes with natural enzymes, antibodies, and aptamers for specific substrates [201–204]. However, such a system cannot take the full advantage of inorganic nanozymes due to the relative fragility of the coupled molecules.

Along with these improvements, future nanozymes should be equipped with multifunctionality. Inorganic nanozymes hold great potential in that respect because many functional features useful for biomedical applications have already been developed based on their high design flexibility [205]. The structure of multifunctional nanozymes can either be single- or multi-component, each of which has its own merits in terms of fabrication and functional expansion. A representative functional combination of the former structure would be the magnetic nanozymes that serve as both MRI contrast enhancement agents and nanozymes [206, 207]. Unfortunately, numerous inorganic nanozymes that have been tested so far for biomedical use do not have any or sufficiently high catalytic performance as nanozymes. As a consequence, novel methods to fabricate multi-component structures in which each component carries out its unique functionality are required in order to obtain the desired multifunctionality. We believe that our understandings in inorganic nanoparticle synthesis have been accumulated over time can provide a firm foundation for building such systems regardless of their complexity.

The advancement in diagnostic and therapeutic tools using inorganic nanoparticle-based nanozymes proceeds in tandem with the development of nanomedicines that rely on the properties other than the catalytic activities. Many of them are still at the stage of proof-of-concept, and their long-term safety concerns remain to be addressed. Nevertheless, the inorganic nanoparticle-based nanozymes, along with the growing interest in nanobiotechnology, will continue to thrive and overcome the limits to find its place at the center of future nanomedicine.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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