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Nanozymes: From New Concepts, Mechanisms, and Standards to Applications

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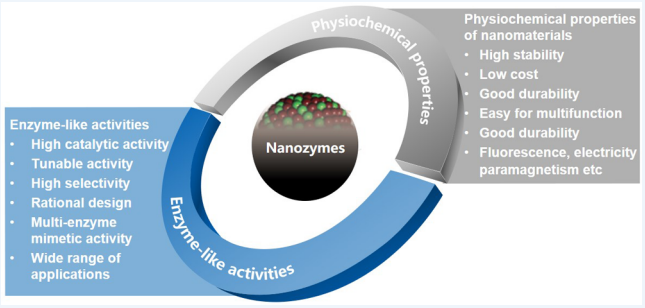
CONSPECTUS: Nanozymes are nanomaterials with intrinsic enzyme-like characteristics that have been booming over the past decade because of their capability to address the limitations of natural enzymes such as low stability, high cost, and difficult storage. Along with the rapid development and ever-deepening understanding of nanoscience and nanotechnology, nanozymes hold promise to serve as direct surrogates of traditional enzymes by mimicking and further engineering the active centers of natural enzymes. In 2007, we reported the first evidence that Fe_3O_4 nanoparticles (NPs) have intrinsic peroxidase-mimicking activity, and since that time, hundreds of nanomaterials have been found to mimic the catalytic activity of peroxidase, oxidase, catalase, haloperoxidase, glutathione peroxidase, uricase, methane monooxygenase, hydrolase, and superoxide dismutase. Uniquely, a broad variety of nanomaterials have been reported to simultaneously exhibit dual- or multienzyme mimetic activity. For example, Fe_3O_4 NPs show pH-dependent peroxidase-like and catalase-like activities; Prussian blue NPs simultaneously possess peroxidase-, catalase-, and superoxide dismutase-like activity; and Mn_3O_4 NPs mimic all three cellular antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase. Taking advantage of the physiochemical properties of nanomaterials, nanozymes have shown a broad range of applications from in vitro detection to replacing specific enzymes in living systems. With the emergence of the new concept of "nanozymology", nanozymes have now become an emerging new field connecting nanotechnology and biology.



Since the landmark paper on nanozymes was published in 2007, we have extensively explored their catalytic mechanism, established the corresponding standards to quantitatively determine their catalytic activities, and opened up a broad range of applications from biological detection and environmental monitoring to disease diagnosis and biomedicine development. Here we mainly focus on our progress in the systematic design and construction of functionally specific nanozymes, the standardization of nanozyme research, and the exploration of their applications for replacing natural enzymes in living systems. We also show that, by combining the unique physicochemical properties and enzyme-like catalytic activities, nanozymes can offer a variety of multifunctional platforms with a broad of applications from in vitro detection to in vivo monitoring and therapy. For instance, targeting antibody-conjugated ferromagnetic nanozymes simultaneously provide three functions: target capture, magnetic separation, and nanozyme color development for target detection. We finally will address the prospect of nanozyme research to become "nanozymology". We expect that nanozymes with unique physicochemical properties and intrinsic enzyme-mimicking catalytic properties will attract broad interest in both fundamental research and practical applications and offer new opportunities for traditional enzymology.

1. INTRODUCTION

Enzymes are biocatalysts that facilitate a majority of biological reactions that occur in living systems.[1] However, the intrinsic drawbacks of natural enzymes, such as ease of denaturation, high cost, laborious preparation, and difficulties in recycling, greatly limit their practical applications.[2] To overcome these limitations, artificial enzymes have been developed as stable, low-cost alternatives to natural enzymes since the 1950s.[3] Nanozymes, as a new type of promising artificial enzyme, have attracted considerable interest over the past decade owing to their obvious advantages over natural enzymes and conventional artificial enzymes, such as high and tunable catalytic activities, low cost, easy large-scale production, and high stability (Table 1).[4,5] Given these advantages, nanozymes have attracted widespread interest. In very recent years, along with significant advances in nanotechnology, biotechnology, and nanomaterial science, great progress has been achieved in the field of nanozymes.[6,7]

In this account, we will focus on our endeavors on developing promising nanozymes for applications from biological detection and imaging to disease diagnosis and biomedicine development. Importantly, we will discuss the prospect of nanozyme research to become "nanozymology"

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Table 1. Comparison among Nanozymes, Natural Enzymes, and Nanomaterial-Based Catalysts

	advantages	challenges
nanozymes	(1) high catalytic activity	(1) limited types of nanozymes
	(2) tunable catalytic activity and types	(2) limited substrate selectivity
	(3) multienzyme mimetic activity	(3) the ambiguous mechanism
	(4) high stability	(4) size-, shape-, structure-, and composition-dependent catalytic properties
	(5) recyclable utilization	(5) lack of standards and reference materials
	(6) easy to mass produce	(6) potential nanotoxicity
	(7) low cost	
	(8) long-term storage	
	(9) robustness to harsh environments	
	(10) Easy to multifunctionalize (large surface area for bioconjugation)	
	(11) controllable catalytic activity and types via external stimuli (such as light, ultrasound, heat, magnetic field, etc.)	
	(12) unique physicochemical properties (such as fluorescence, electricity, paramagnetic properties, etc.)	
natural enzymes	(1) high catalytic activity	(1) high cost
	(2) high substrate selectivity	(2) limited stability
	(3) good biocompatibility	(3) hard to store long term
	(4) wide range of biocatalysis	(4) hard to mass produce
	(5) wide range of applications	(5) time-consuming separation and purification
	(6) rational design via gene and protein engineering	(6) hard to use in a harsh environment (such as heat, extreme pH, salinity, UV irradiation, etc.)
nanomaterial-based catalysts	(1) tunable catalytic activity	(1) low specificity
	(2) low cost	(2) low selectivity
	(3) easy to mass produce	(3) poor biocompatibility
	(4) high stability	(4) size-, shape-, structure-, and composition-dependent catalytic properties
	(5) robustness to harsh environments	
	(6) long-term storage	
	(7) exact catalytic mechanism	
	(8) atomically precise structural information	

based on the established theoretical mechanisms and kinetics, nanozymatic concepts, and the corresponding standards for measuring nanozyme performance.

2. NANOZYME: DISCOVERY AND DEVELOPMENT

Nanozymes are nanomaterials exhibiting intrinsic enzyme-like characteristics, which are distinct from the "nanozymes" with natural enzymes or catalytic ligand immobilization on nanomaterials. The term nanozymes was first coined by Pasquato, Scrimin, and co-workers in 2004 to describe the transphosphorylation reactivity of triazacyclononane-functionalized gold NPs.[8] Since the intrinsic peroxidase-like property of Fe_3O_4 NPs was reported in 2007,[4] nanozymes has specifically referred to nanomaterials with intrinsic enzyme-like characteristics. We demonstrated that Fe_3O_4 NPs could catalyze the oxidation of peroxidase substrates (i.e., 3,3,5,5-tetramethylbenzidine (TMB), diazo-aminobenzene (DAB), and *o*-phenylenediamine (OPD)) in the presence of H_2O_2 to produce colorimetric reactions (Figure 1), showing the peroxidase-like activity of Fe_3O_4 NPs toward typical peroxidase substrates. Kinetics studies indicated a ping-pong catalytic mechanism of the Fe_3O_4 nanozyme-based catalytic reaction, and the measured Michaelis–Menten kinetic parameters exhibited higher catalytic activity (k_{cat} = 8.58 × 10^4 s^{-1}) but lower substrate affinity (K_M = 154 mM) than did those of horseradish peroxidase (HRP). After that, we defined the catalytic activity units and further established series standards by which the catalytic activities and kinetics of various nanozymes can be quantitatively compared.[9]

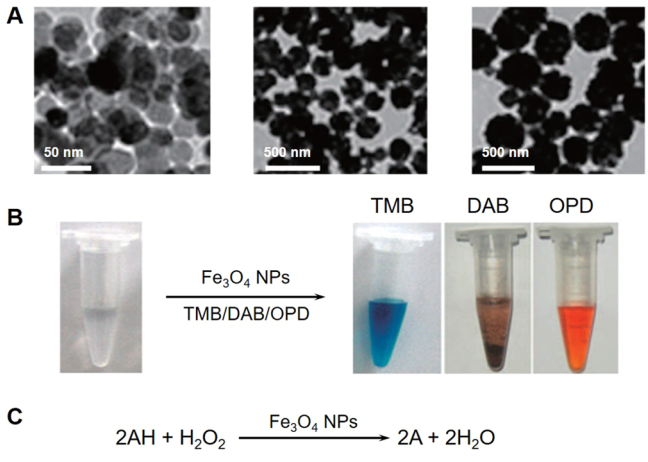

Figure 1. Fe_3O_4 NPs show peroxidase-mimicking activity. (A) TEM images of Fe_3O_4 NPs of different sizes. (B) Fe_3O_4 NPs catalyze the oxidation of various peroxidase substrates (TMB, DAB, and OPD) in the presence of H_2O_2 to produce different color reactions. (C) Scheme of the mechanism of catalysis by Fe_3O_4 NPs. AH represents the peroxidase substrate, which is a hydrogen donor. Reproduced with permission from ref 4. Copyright 2007, Nature Publishing Group.

After we uncovered the intrinsic peroxidase-like activity of Fe_3O_4 NPs in 2007,[4] there have been more than 200 research groups around the world working on nanozyme research, and dozens of nanozymes have been developed covering hundreds of nanomaterials.[6,7] As shown in Figure 2, incredible growth has been witnessed in the field of nanozyme research by the

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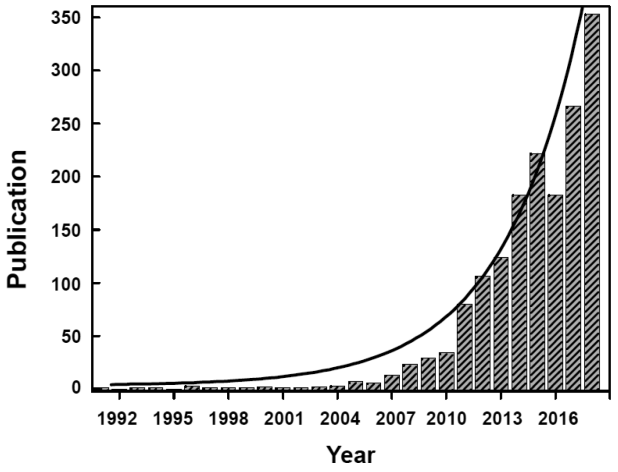

Figure 2. Number of published papers on nanozyme research by the end of 2018. The data is based on the Web of Science. Reproduced and modified with permission from ref 6. Copyright 2018, Royal Society of Chemistry.

exponential number of publications, suggesting their scientific significance and broad application prospect. With the emergence of the new term "nanozymology",[9] nanozymes have now developed from a concept to a new research field connecting nanotechnology and biology.

3. NANOZYME: CONSTRUCTION AND ENGINEERING

Unlike the natural enzymes that typically exhibit high catalytic activity and substrate selectivity, nanozymes usually have relatively low activity and poor specificity and mimic only limited types of enzymes, which severely limit their practical application.[6,7] To make nanozymes better alternatives to natural enzymes, much effort has been expended to improve the performance of nanozymes, including regulating their substrate selectivity, improving their catalytic activity, and developing their multienzyme mimetic activity. So far, iron-based and carbon-based nanozymes have been the most studied and reconstructed because of their good biocompatibility and great potential for biomedical applications. Herein, we introduced a series of work on how to improve the selectivity and catalytic activity of iron-based nanozymes by mimicking the active central structure of natural enzymes. We also introduced how to develop the multifunctionalities and multienzyme activities of carbon-based nanozymes by heteroatomic doping, which have been the most reported currently.

3.1. Iron-Based Nanozymes

Fe_3O_4 NPs show peroxidase-like activity because of the large area of ferric and ferrous iron available on their surfaces, which catalyze the oxidation of peroxidase substrates in a manner similar to that of the iron active sites within the heme group of natural enzyme HRP.[4] To improve the performance of the Fe_3O_4 nanozyme, we introduced histidine residues onto the surface of Fe_3O_4NPs to mimic the architecture of the iron active center within HRP.[10] The introduced histidine residues assisted the location of H_2O_2 substrates into their active sites and thus enhanced their substrate binding affinity and catalytic activity. We show that the histidine-modified Fe_3O_4 NPs exhibited more than a 10-fold higher binding affinity (K_M) for H_2O_2 and a 20-fold higher catalytic efficiency (k_{cat}/K_M) than did naked Fe_3O_4 NPs.

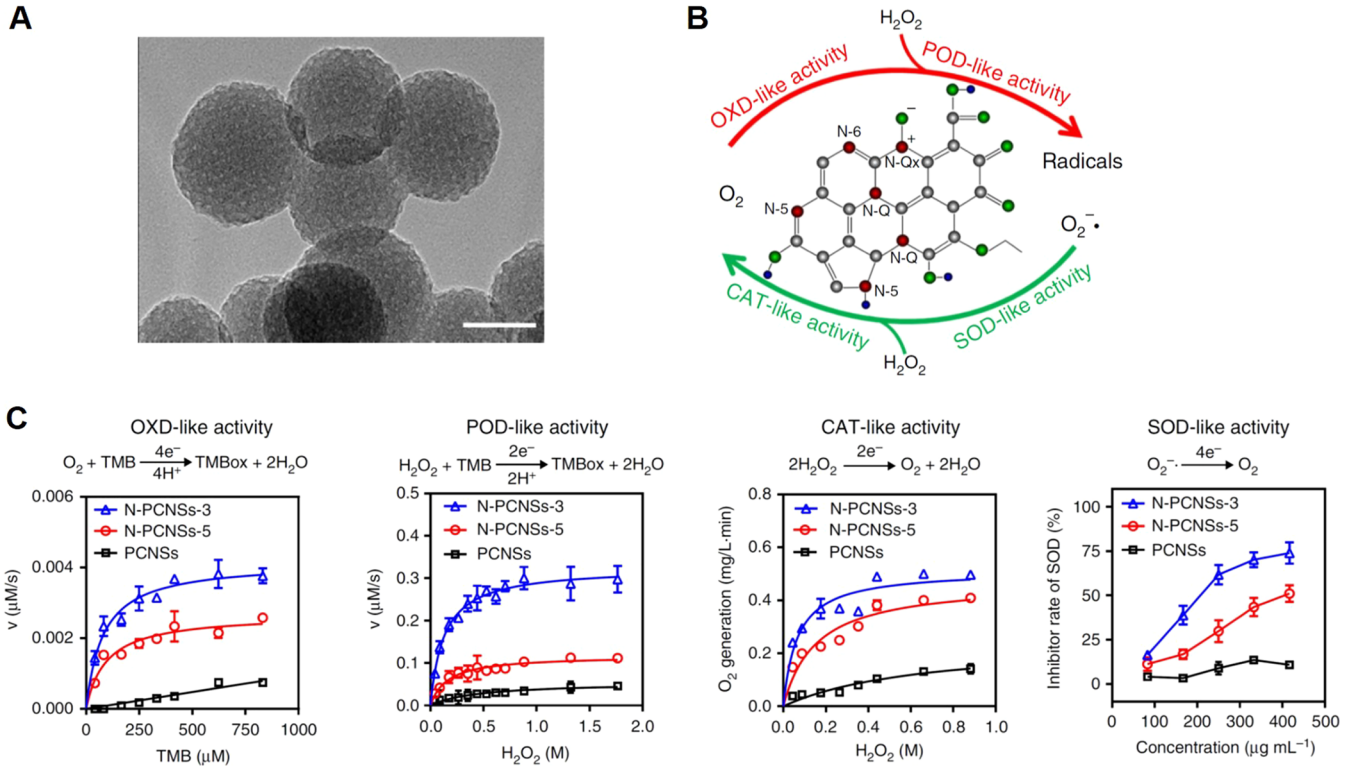

Figure 3. Nitrogen-doped carbon NPs (N-PCNSs) exhibit multienzyme activity. (A) TEM images of N-PCNSs. Scale bar: 100 nm. (B) Schematic presentation of enzyme-like activities of N-PCNSs. (C) Kinetics for oxidase-, peroxidase-, catalase-, and superoxide dismutase-like activity of N-PCNSs. Reproduced with permission from ref 20. Copyright 2018, Nature Publishing Group.

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To further mimic the $M-N_4$ (M = metal, N = nitrogen) porphyrin active centers of the natural heme-containing enzymes, we engineered $Zn-N_4$-centered single-atom nanozymes (SAzymes) with well-defined electronic and geometric structures, which realized for the first time a great improvement in catalytic activity and substrate selectivity.[11] The engineered $Zn-N_4$ SAzymes make nanozymes the better alternative to natural enzymes owing to their maximum atom efficiency, unique quantum size effect, and excellent substrate selectivity. Meanwhile, the clear active center structure of SAzymes makes it easy to gain insight into the catalytic mechanisms of their intrinsic enzyme-like catalytic activity. Likewise, Hartwig and co-workers engineered a CYP119-like nanozyme containing a single-atom iridium porphyrin that exhibited kinetic parameters and catalytic activity similar to those of the natural enzymes.[12] The iridium porphyrin SAzymes achieved the insertion of carbenes into C−H bonds with up to a 98% enantiomeric excess, 35 000 turnovers, and a 2550 h^{-1} turnover frequency. These above works show that the engineered nanozymes catalyzing enzyme-mimicking reactions can possess the fundamental characteristics of natural enzymes: fast kinetics, high productivity, and high selectivity under the same reaction conditions. With the emergence of SAzymes, nanozyme shows promise in realizing its practical applications by replacing natural enzymes in specific enzyme-based applications.

3.2. Carbon-Based Nanozymes

Carbon-based nanomaterials have also been widely discovered to possess enzyme-like activity. For example, carbon nanotubes,[13] graphene oxide,[14] carbon nitride,[15] carbon nanodots,[16] fullerene,[17] and some other forms of carbon nanomaterials[18,19] have been extensively reported to possess intrinsic peroxidase-, oxidase-, catalase-, superoxide dismutase-, or hydrolase-like activity. Our labortory devoted much effort to the heteroatomic-doped carbon nanomaterials to develop their unique multifunctionalities and multienzyme activities.

Heteroatomic doping to nanocarbon is an effective strategy for developing high-performance carbon-based nanozymes. We and other groups showed that the graphene and carbon nanotubes generally exhibited very weak enzyme-like activity with limited catalytic types.[4−6] After nitrogen doping into the carbon framework, we demonstrated that the resulting carbon nanozymes not only exhibited significantly improved catalytic efficiency but also performed four microenvironment-responsive enzyme-like activities under physiological conditions, including peroxidase-, oxidase-, catalase-, and superoxide dismutase-like activities (Figure 3).[20] Intriguingly, the N-doped carbon nanospheres could selectively regulate reactive oxygen species (ROS) generation in an acidic tumor microenvironment and significantly inhibit tumor growth in vivo.

Beside nitrogen, many other elements, such as Fe,[21] Co,[22] Se,[23] and Ni,[24] have also been doped into the framework of carbon nanostructure to mimic the activity of peroxidases, catalase, oxidase, and superoxide dismutase. Thus, we hypothesize that it is possible to make a library of carbon-based nanozymes with regulatable multienzyme activities that can replace natural enzymes in living systems.

4. APPLICATION

Because of the high stability, low cost, large surface area for functionalization, and tunable activity, nanozymes have shown a broad spectrum of applications. In particular, by combining the unique physicochemical properties and catalytic activities of nanozymes, we developed a series of universal platform technologies with applications in bioanalysis, disease diagnosis, and therapy.

4.1. Immunoassay for Bioanalysis

After uncovering the peroxidase activity of Fe_3O_4 NPs, we immediately developed a nanozyme-based immunoassay composed of capture antibody-coated Fe_3O_4 magnetic NPs. We showed that the prepared nanozyme immunoassay simultaneously performed three functions: target capture, magnetic separation, and nanozyme color development for target detection (Figure 4).[4,9] On the basis of the developed

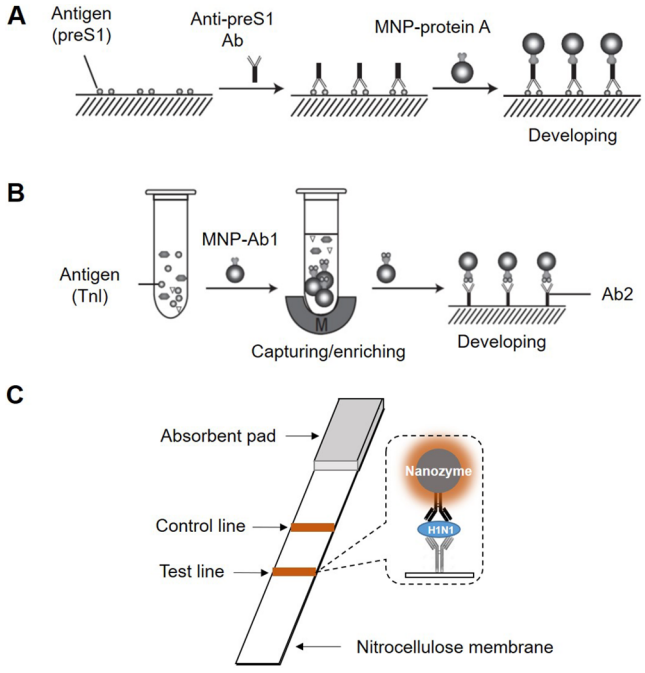

Figure 4. Fe_3O_4 nanozymes for immunoassay. (A) ELISA immunoassay format. The antigens are recognized by the antibodies and then detected by the second antibody-coated Fe_3O_4 NPs which catalyze the oxidation of colorimetric substrates to produce a color reaction to quantitatively determine the antigen concentration. (B) Capture-detection sandwich immunoassay format. Capture antibody-coated Fe_3O_4 NPs simultaneously capture and separate targets from sample solutions and then catalytically use color development for target detection. (C) Strip immunoassay format. Peroxidase nanozymes catalyze the oxidation of colorimetric substrates to produce a color reaction at the test line after binding to the targets. Reproduced with permission from refs 4 and 9. Copyright 2007, 2018, Nature Publishing Group.

nanozyme immunoassay, we achieved rapid, high-throughput detection for biomolecules,[4] influenza and Ebola viruses,[9,25] and chemical contaminants.[26,27] Likewise, Wei and Wang applied the Fe_3O_4 NP-based peroxidase mimics for the detection of H_2O_2 and glucose.[28] Inspired by these pioneering works, thousands of nanozyme assays were developed for the in vitro or in vivo detection of cholesterol,[29] glucose,[28,30] lactate,[31] circulation tumor cells,[32] galactose,[33] and cellobiose[34] by employing the peroxidase mimics or the combined oxidases and peroxidase mimics. In 2018, we developed two clinical nanozyme-based kits approved by the China Food and Drug

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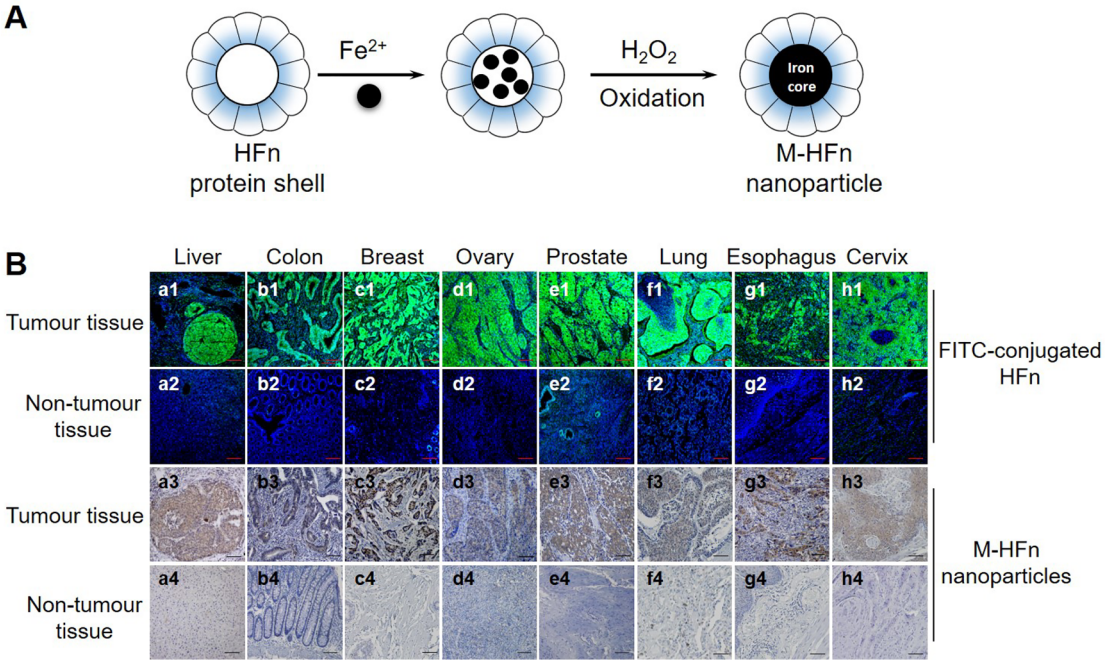

Figure 5. M-HFn NPs as peroxidase mimics for tumor tissue staining. (A) Preparation of M-HFn NPs. (B) M-HFn NPs staining of tumor tissues. Paraffin-embedded clinical tumor tissues and their corresponding normal and lesion tissues were stained with FITC-conjugated HFn protein shells and M-HFn NPs. Tumor tissues showed strong positive staining for M-HFn NPs (brown) and FITC-conjugated HFn protein shells (green fluorescence), whereas the normal and lesion tissue controls were negative for M-HFn NPs and FITC-conjugated HFn. Reproduced with permission from ref 36. Copyright 2012, Nature Publishing Group.

Administration (CFDA),[35] showing the great application prospects of nanozyme immunoassays.

4.2. Immunohistochemical Staining for Disease Diagnosis

Peroxidase nanozymes catalyze the oxidation of colorimetric substrates to give a color reaction that can be used for visualizing the recognized biomarkers within tissue sections for pathological disease diagnosis and therapeutic monitoring. We pioneeringly prepared magnetoferritin nanozymes (M-HFn) by encapsulating iron oxide NPs inside the recombinant human heavy-chain ferritin (HFn) protein nanocages.[36] HFn could specifically identify overexpressed transferring receptor 1 in tumor tissues while the iron oxide nanocores could catalyze the oxidation of peroxidase substrates in the presence of H_2O_2 to produce an intense color reaction for visualizing tumor tissues. We examined 474 clinical specimens from patients with nine types of cancer and demonstrated that the magnetoferritin nanozymes could distinguish cancerous cells from normal cells with 98% sensitivity and 95% specificity (Figure 5). On the basis of the bioengineered M-HFn NPs, we developed a rapid, simple, and economical immunohistochemical kit for cancer diagnosis by simultaneously employing the peroxidase-like activity and tumor-targeting property of M-HFn NPs to achieve tumor targeting and visualization in one step (Figure 6). Compared to traditional immunohistochemistry, the M-HFn nanozyme method is more rapid and simpler, which greatly shortens the diagnostic time and reduces the cost and thus has significant implications for cancer diagnosis.

Likewise, Gu and Zhang et al. used avastin antibody-functionalized Co_3O_4 NPs as target-specific peroxidase mimics for the immunohistochemical staining of vascular endothelial growth factor (VEGF) in tumor tissue slices.[37] Until now, nanozyme-based staining methods have been developed for the pathological diagnosis of liver cancer,[38] esophageal cancer,[39]

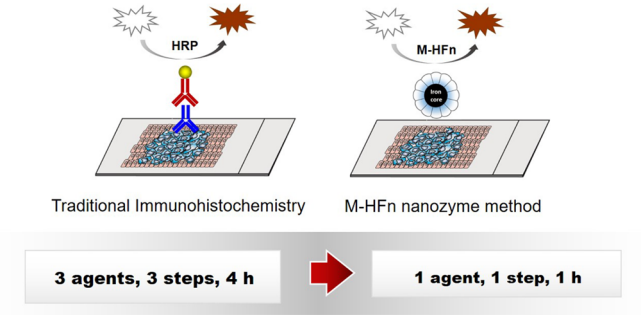

Figure 6. Comparison of traditional immunohistochemistry with the M-HFn nanozyme method for staining tumor tissues.

and oral squamous cancer.[40] Besides the cancer diagnosis, we recently also prepared a magnetoferritin nanozyme for the pathological identification of high-risk and ruptured atherosclerotic plaques from patients with symptomatic carotid disease.[41] We examined 50 carotid endarterectomy specimens and demonstrated that the M-HFn nanozymes could distinguish the infiltrated macrophages within high-risk plaque tissues, and M-HFn staining displayed a significant correlation with plaque vulnerability ($r = 0.89$, $P < 0.0001$) (Figure 7).

4.3. In Vivo Imaging

By employing unique physicochemical properties (such as luminescence, X-ray absorption, and paramagnetic properties), nanozymes have been widely developed for the in vivo imaging of disease. For instance, after we achieved tumor tissue in vitro staining by using the peroxidase-mimicking activity of M-HFn NPs (Figure 5),[36] we further explored the high r_2 relaxivity of M-HFn NPs and achieved tumor in vivo magnetic resonance imaging (MRI).[42] In addition, our groups also developed a strategy for tracking the in vivo behaviors of NPs with

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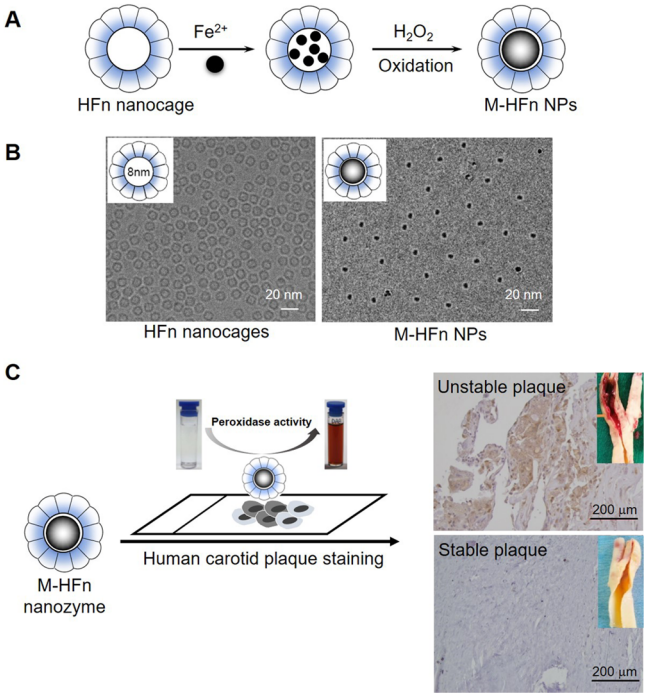

Figure 7. M-HFn nanozymes for the pathological identification of unstable atherosclerotic plaques. (A) Schematic illustration of the preparation of M-HFn NPs. (B) Cryo-EM image of HFn nanocages (left) and M-HFn NPs (right). (C) M-HFn nanozymes specifically stain the unstable plaques. Reproduced with permission from ref 41. Copyright 2019, Nature Publishing Group.

peroxidase-mimicking activity after intravenous injection into mice.[43] Very recently, we further prepared a graphene quantum-dot-based nanozyme for H_2O_2-responsive catalytic photoacoustic imaging of a xenograft nasopharyngeal carcinoma.[44] In this work, the peroxidase activity of graphene quantum dots could effectively convert peroxidase substrate 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) into its oxidized form, which exhibited strong near-infrared absorbance, causing it to be an ideal contrast agent for photoacoustic imaging (Figure 8).

Recently, nanozyme-based multimodality imaging probes have also been widely developed, benefiting from their explored multifunctional properties of nanomaterials. Intriguingly, Tian et al. developed iridium oxide NPs with catalase-like activity, extraordinary photothermal conversion efficiency, and a high X-ray absorption coefficient, which achieved tumor phototherapy and simultaneous photoacoustic/thermal imaging and computed tomography.[45] This thus opens novel avenues to nanomedical applications in which nanozymes could lead to a better understanding of nanomedicine uptake, biodistribution, and pharmacokinetics.

4.4. Disease Therapy

By modulating ROS production, nanozymes have been broadly exploited for disease therapeutics. For example, by catalytically generating abundant ROS selectively in a tumor microenvironment, nanozymes with peroxidase or oxidase-like activity can be used for antitumor therapeutics. By using the ROS scavenging capability, nanozymes with catalase or superoxide dismutase-like activity can help to detoxify ROS and thus prevent inflammatory and aging-related diseases.

Taking advantage of the ROS-generating capability of nanozymes, we developed a microenvironmentally responsive carbon-based nanozyme for tumor suppression.[20] In this work, the developed carbon nanozyme performed oxidase-like and peroxidase-like activities selectively in a tumor acidic microenvironment and substantiality increased the ROS concentration by simultaneously converting both O_2 and H_2O_2 to free radicals, which effectively suppressed tumor growth. On the basis of a similar idea, several other nanomaterials (such as Fe_3O_4, platinum, and BSA-IrO_2 nanomaterials) have also demonstrated their applications in tumor treatment.

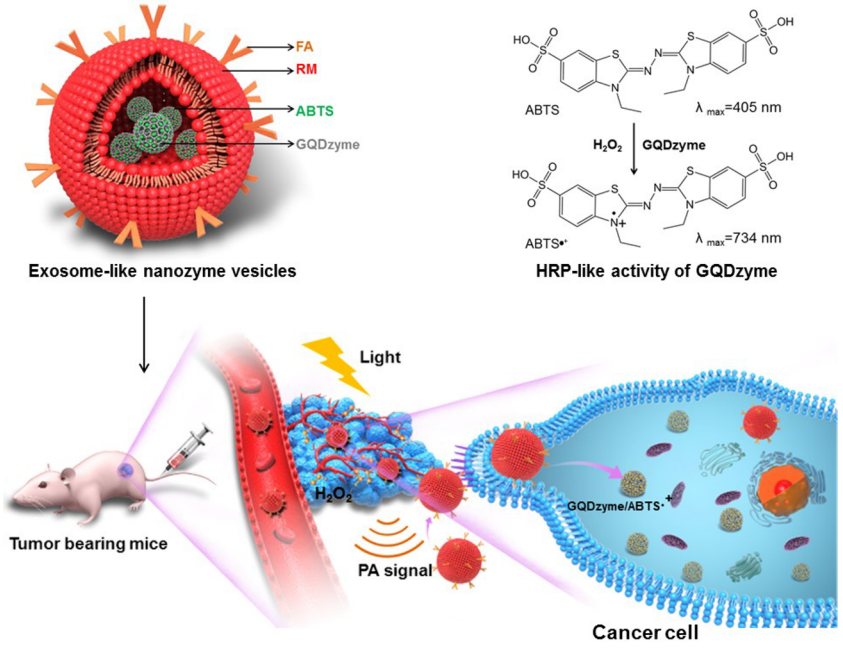

Figure 8. Schematic illustration of exosome-like nanozyme vesicles for the H_2O_2-responsive catalytic photoacoustic imaging of tumors. Reproduced with permission from ref 44. Copyright 2019, American Chemical Society.

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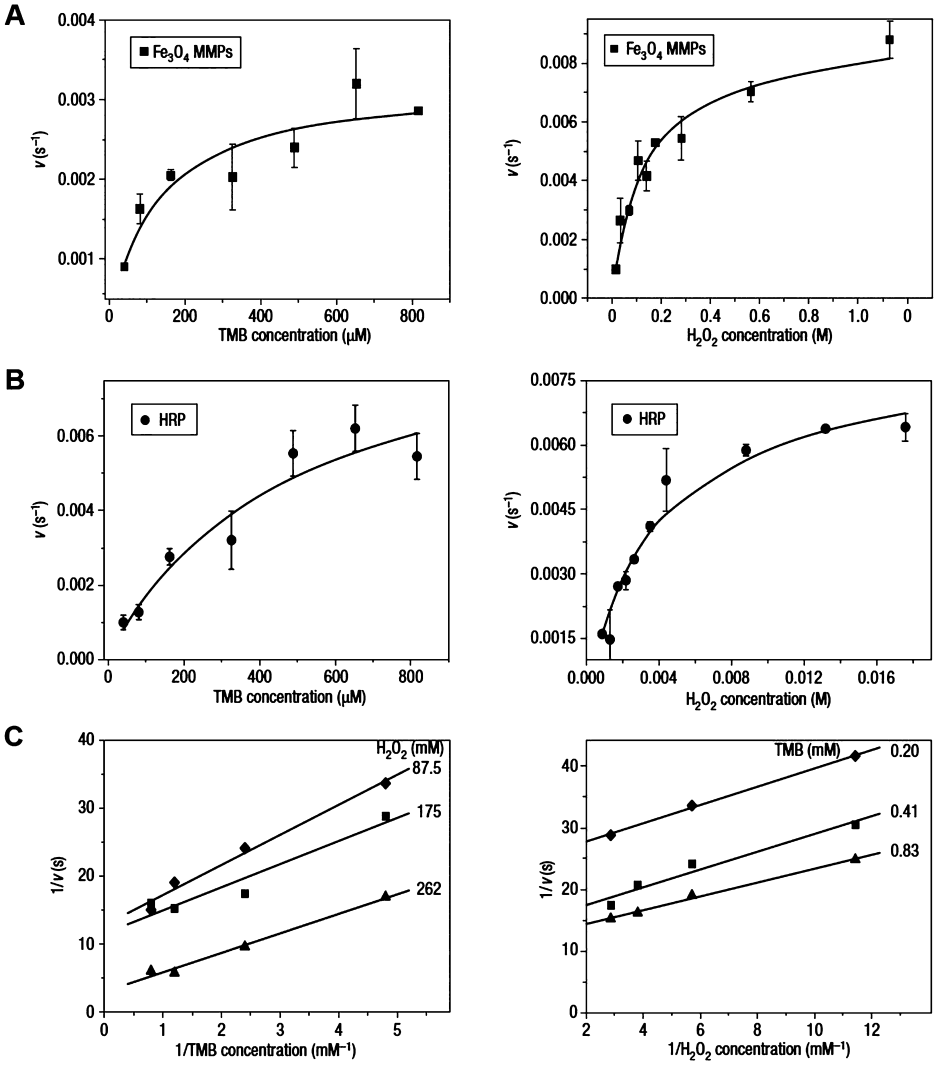

Figure 9. (A, B) Steady-state kinetic assay and catalytic mechanism of the Fe_3O_4 peroxidase nanozyme and HRP. (C) Double-reciprocal plots of the activity of Fe_3O_4 nanozymes at a fixed concentration of one substrate versus the varying concentration of the second substrate for H_2O_2 and TMB. Reproduced with permission from ref 4. Copyright 2007, Nature Publishing Group.

The ROS scavenging abilities of nanozymes largely originate from their superoxide dismutase and catalase mimicking activities, which convert superoxide to H_2O_2 and subsequently to O_2. By scavenging ROS, the antioxidant effects of nanozymes have been extensively explored for antiaging, anti-inflammatory, antioxidation, and neuroprotection functions and the treatment of Alzheimer's and Parkinson's diseases. For example, Mugesh and co-workers discovered that V_2O_5 nanowires possessed remarkable glutathione peroxidase-like antioxidant activity, which could catalyze the decomposition of intracellular overexpressed H_2O_2 in the presence of glutathione and protect cellular components against oxidative damage.[46] Hyeon et al. used nanoceria as a ROS scavenger to protect against ischemic stroke. In their work, the prepared uniform 3 nm nanoceria with optimal doses at 0.5 and 0.7 mg kg^{-1} considerably reduced infarct volumes and the rate of ischemic cell death in vivo.[47] In another interesting study, the prepared nanoceria can also be used for the treatment of Parkinson's disease.[48] Three different types of nanoceria were developed for selectively scavenging intracellular, mitochondrial, and extracellular ROS in Parkinson's disease. Animal experiments showed that scavenging intracellular or mitochondrial ROS could significantly inhibit microglial activation and lipid peroxidation while protecting the tyrosine hydroxylase in the striata of Parkinson's disease model mice. With a deep understanding and precise regulation of their catalytic properties, nanozymes will be applied to more new therapeutic fields.

4.5. Properties of Nanozymes for Biomedical Applications

By combining the unique physicochemical properties and enzyme-like catalytic activities, nanozymes hold promise as in vivo diagnostics and therapeutics. However, the key for the clinical translation of nanozymes hinges on a detailed understanding of their in vivo behavior after administration. The nanozyme biodistribution, pharmacokinetics, target binding, and clearance from the body are complex functions of physicochemical properties such as their composition, size, morphology, and surface chemistry. Moreover, clinical applications of nanozymes will require them to have the lowest possible likelihood of toxicity and can be cleared out of the body on a reasonable time scale. Herein, we discuss the following physicochemical properties that mediate nanozyme behavior in the body, and the fundamental principles that govern the further clinical translation of nanozymes.:(1)

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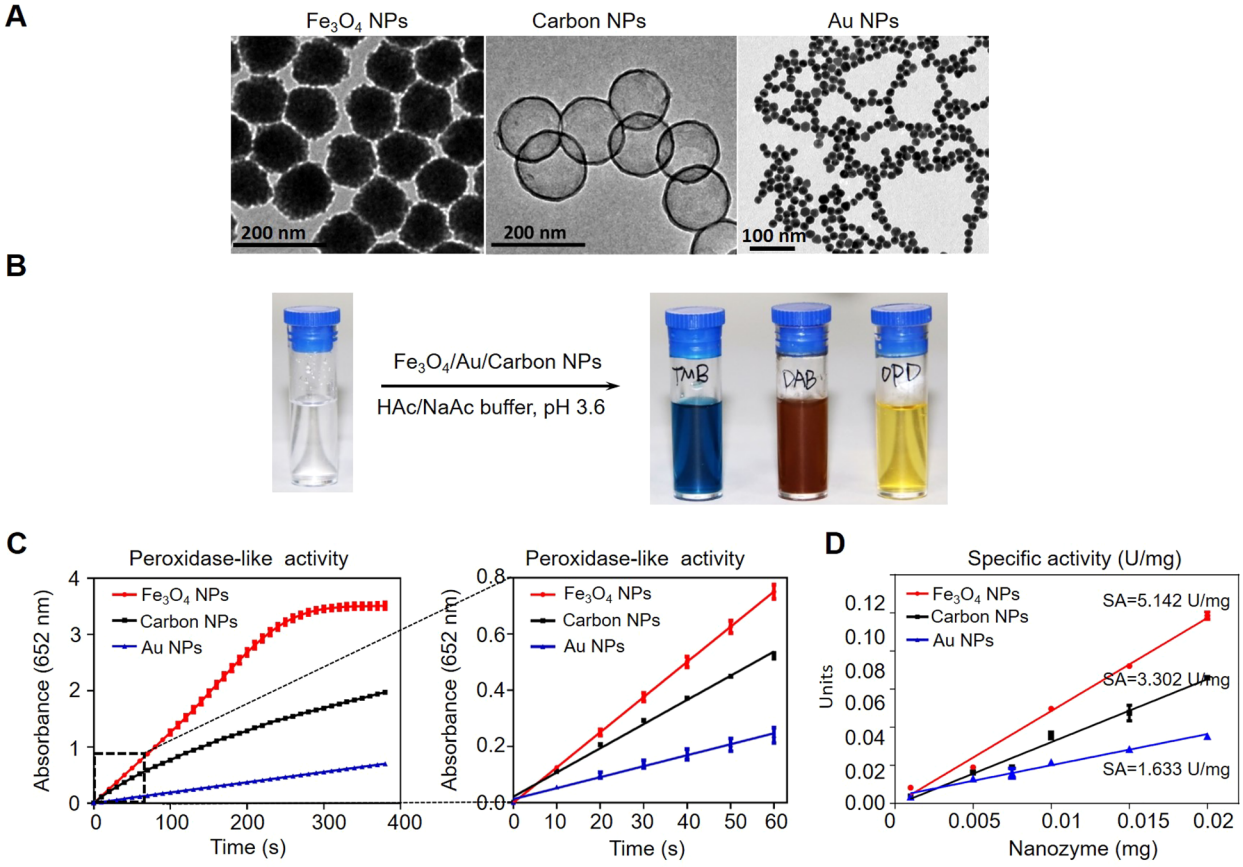

Figure 10. Standardization of nanozyme peroxidase-like catalytic activity. (A) TEM images of three typical peroxidase nanozymes (Fe_3O_4, carbon, and Au NPs). (B) Fe_3O_4, carbon, and Au NPs show peroxidase-like activity and catalyze the oxidation of peroxidase substrates to produce colorimetric reactions. (C) Reaction−time curves of the TMB colorimetric reaction catalyzed by Fe_3O_4 (red), carbon (black), and Au (blue) nanozymes. (D) Specific activities (U/mg) of Fe_3O_4, carbon, and Au nanozymes. Reproduced with permission from ref 9. Copyright 2018, Nature Publishing Group.

composition − biocompatible and completely nontoxic elements or biodegradable to clearable components without adverse toxicity; (2) size and shape − small-enough hydrodynamic diameter for complete renal elimination from the body and to avoid high background retention in the reticuloendothelial system (RES); (3) surface charge − zwitterionic or neutral surface coatings to minimize nonspecific tissue/organ uptake; (4) stability − high chemical stability in aqueous solvents and serum; (5) favorable pharmacokinetics and targeting accumulation − be capable of targeting a disease state efficiently after intravenous injection while being eliminated completely from the body in a reasonable amount of time; and (6) simplicity − easy of scale up and manufacture with robust and reproducible procedures. Although many classes of biocompatible, multifunctional nanozymes have been developed for medical diagnostics and therapeutics, most do not satisfy these criteria, which are critical to moving these potential nanozymes into clinical practice. Therefore, these fundamental characteristics which determine how nanozymes are distributed and eliminated in the body and how they affect body functions should be well understood before they are evaluated fully in humans.

5. KINETICS AND MECHANISM RESEARCH

The catalytic performance of nanozymes is dependent on their kinetic characterization. We first investigated the catalytic mechanism of Fe_3O_4NPs as peroxidase nanozymes and determined their catalytic kinetic parameters because ferromagnetic NPs were the first discovered and, to date, have been the most widely used nanozymes.[4] The Fe_3O_4 nanozyme was used to carry out catalysis according to Michaelis−Menten kinetics and had a similar K_m value and significantly higher k_{cat} and k_{cat}/K_m values compared to those of natural HRP (Figure 9). Because the Michaelis−Menten mechanism is quite classic in enzymology, the catalytic properties of nanozymes and enzymes thus can be quantitively compared in terms of the catalytic efficiency (k_{cat}/K_m), substrate specificity (K_m), and catalytic rate constant (k_{cat}) for specific substrates.

After that, the catalytic mechanisms and kinetics of the oxidase-,[49] hydrolase-,[50] superoxide dismutase-,[49,51] and catalase-mimicking nanozymes[52] have also been broadly investigated using the classic theoretical system used in enzymology. However, several different kinetics parameters have been reported for one type of nanomaterial owing to the complex interdependence of the physicochemical properties and catalytic characteristics of nanomaterials. Therefore, it is critical to establish universal standards for quantitatively determining their catalytic mechanisms and kinetics, which will enable unbiased data comparisons across independent studies.

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6. STANDARDIZATION OF NANOZYME PERFORMANCE

The enzyme-like activity of nanozymes is highly dependent on their physicochemical properties (such as size, composition, morphology, and surface chemistry). With more and more broad applications of nanozymes, it is urgent to establish standards by which the catalytic activities and kinetics of various nanozymes can be quantitatively compared and that will benefit the development of nanozyme-based analysis and detection technologies.

We first defined the catalytic activity units of peroxidase nanozymes, the most widely used type of nanozymes, and established standards to quantitatively compare the activity level for a broad range of peroxidase nanozymes of interest (Figure 10).[9] Peroxidase nanozymes catalyze the oxidation of commonly used peroxidase colorimetric substrates TMB, DAB, and OPD in the presence of H_2O_2 to give a color reaction. On the basis of the peroxidase nanozyme-catalyzed colorimetric reaction, we measured the catalytic activity of some specific peroxidase nanozymes and determined their activity units and specific activity values. Specifically, one nanozyme activity unit (U) was defined as the amount of nanozyme that catalyzes 1 μmol of product per minute. The specific activity was defined as activity units per milligram of nanozyme. We further standardized the catalytic kinetic constants of peroxidase nanozymes. With the standard catalytic kinetics, nanozymes could be quantitively compared in terms of the catalytic efficiency (k_{cat}/K_m), substrate specificity (K_m), catalytic rate constant (k_{cat}), and maximal reaction rate (ν_{max}) for specific substrates.

Owing to the complex interdependence of physicochemical properties and catalytic characteristics, nanozyme-based detection applications are severely limited by inferior repeatability and reliability. We demonstrated that nanozyme-based detection became repeatable and reliable while under the guidelines of the established standards. Additionally, in disease diagnosis and treatment, the standardization of nanozyme activity units is expected to lead to better dosage control while mediating redox-active biological processes (such as those regulating ROS in the cancer microenvironment, anti-inflammatory processes, neuroprotection, promotion of stem cell growth, and antiaging processes) using redox-based nanozymes.

Although it is urgent to establish nanozyme standards for a fair evaluation of various nanozymes, until now only the standardized assays for peroxidase nanozymes have been proposed as described above.[9] The systematic standards for other types of nanozymes should be set as well in a future study to scientifically characterize nanozyme performance.

7. NANOZYMOLOGY AND PERSPECTIVE

Over the past decade, there have been more than 200 research groups around the world working on nanozymes, and dozens of nanozymes have been developed covering hundreds of nanomaterials. Along with the remarkable achievements made in nanozyme research, their own theoretical mechanisms and kinetics, basic concept definitions, and standards for measuring their catalytic performance have been fully built up. In particular, through the connection of unique physicochemical properties and enzyme-mimicking catalytic activities of nanozymes, their applications have been exploding from in vitro to in vivo. To further drive the rapid development of nanozyme research, it is urgent to put forward a new concept of nanozymology and thereby develop nanozymes in nanozymological methods.

Although nanozyme research has been booming over the past decade, nanozymology is still a young discipline which is full of challenges to be addressed.

(1) Developing nanozymological terms and standards. Although several basic concepts and standards on nanozyme research have been defined, until now nanozyme performance could not be fully characterized with nanozymological terms and standards. Thus, systematic terms and standards should be developed to better characterize nanozyme performance.

(2) Establishing fundamental principles and mechanisms of nanozymology. A deep revealing of the fundamental principles and mechanisms of nanozymes will benefit the understanding of the structure−activity relationship and will guide the rational design of nanozymes with desired properties. For example, although the Michaelis−Menten mechanism is quite common in enzymology, nanozymology differs from enzymes that occur through a heterogeneous mechanism on the surface of nanomaterials instead of the homogeneous reaction in enzymology. Thus, fundamental principles and mechanisms specific to nanozymology should be systematically established and described.

(3) Evaluating bioeffects of nanozymes. A systematic evaluation of the bioeffects of nanozymes, including cytotoxicity, in vivo uptake, biodistribution, immunogenicity, and the pharmacokinetics of nanozymes should be achieved to advance nanozymes for in vivo translational applications.

(4) Clinical translation potential of nanozymes. The advantages of nanozymes in terms of multifunctionality, high stability, and low cost will make them competent for in vivo applications compared to natural enzymes which are easily denatured and degraded after entering the circulation system. However, clinical practical nanozymes should also have two general characteristics of targetability and controllability. Thus, nanozymes should be precisely localized at the diseased sites and delivered to a proper microenvironment which allows them to perform the desired behavior effectively.

(5) Multifunction of nanozymes. By combining unique physiochemical properties and enzyme-mimicking catalytic activities, nanozymes would offer a variety of facile but highly effective and multifunctional platforms, allowing broad applications from in vitro detection to in vivo monitoring and therapy.

Taken together, we believe that nanozymology will have great potential from in vitro detection to in vivo monitoring and catalytic therapy in the future. These above unsolved issues will be the next frontier for further nanozyme research and will accelerate the development of nanozymology in basic research and practical applications.

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Minmin Liang received her Ph.D. in chemistry from the Chinese Academy of Sciences under the guidance of Professor Liang-hong Guo. She then carried out postdoctoral research work with Professor Donald J. Hnatowich at the University of Massachusetts Medical School in the field of radiochemistry. In 2010, she joined the Institute of Biophysics, Chinese Academy of Sciences, as an associate professor and was appointed full professor in 2014. She moved to Beijing Institute of Technology (BIT) as a distinguished professor in 2019. Her current research interests are focused on the synthesis, structural, and performance characterization and application exploitation of new materials.

Xiyun Yan is a member of the Chinese Academy of Sciences, the president of Asian Biophysics Association, and the secretary-general of the Biophysical Society of China. She received her M.D. in immunology from Heidelberg University, Germany in 1993. After postdoctoral research at Memorial Sloan Kettering Cancer Center, she joined the Chinese Academy of Sciences as a professor in 1997. Her current research interests include the discovery of novel tumor biomarkers and the development of novel materials and methods for tumor diagnosis and therapy. She received the 2012 National Prize for Natural Science and the 2015 Atlas Award from Elsevier.

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