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Nanozyme: An emerging alternative to natural enzyme for biosensing and immunoassay

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ABSTRACT

Nanozyme, a term defined for nanomaterial with enzyme-like properties, has attracted significant research attention owing to its striking merits. Recently, a surge of nanozymes have been demonstrated to catalyze some typical enzymatic reactions mimicking oxidase, peroxidase and catalase. Especially, nanozymes with peroxidase-like activity have grown into a big family due to their broad range of applications in the field of biosensing and immunoassay. Since inorganic nanoparticles possess the advantages of high stability and easy surface modification, nanozymes have been emerging alternatives to natural enzymes to some extent. In this Review, we briefly summarize several typical nanozymes and then focus our attention on their enormous applications with respect to analytical chemistry. Representative examples would be discussed in detail from the literatures of last 10 years. Additionally, the current challenges and future directions about nanozymes are speculated at the end of this review.

1. Introduction

Natural enzymes, which are mostly proteins, have significant applications in medicine, agriculture, chemical industry, and food processing because of their high substrate specificity and catalytic efficiency for biological reactions. However, after exposure to extreme pH and high temperature, the catalytic activity of enzymes is usually lost. Enzymes are also susceptible to digestion by proteases. These intrinsic drawbacks dramatically hinder the practical applications of enzymes [1]. Thus, enormous efforts have been devoted to the development of new and efficient artificial enzymes. In the beginning, researchers paid their attentions to cyclodextrins, porphyrins, polymers, and supramolecules, which mimicked the structures and enzyme-like catalytic activities of enzymes.

The situations have been changed since the first exciting discovery of ferromagnetic nanoparticles (Fe_3O_4 MNPs) with unexpected peroxidase-like catalytic activity in 2007 [2]. Yan et al. showed that the inorganic nanoparticles could work like an enzyme to catalyze the oxidation of typical peroxidase substrates with H_2O_2. Fe_3O_4 MNPs instead of horseradish peroxidase (HRP) were used in immunoassay [2] and colorimetric analysis [3]. Later, the term "nanozyme" was defined by Wei and Wang, representing the nanomaterial-based artificial enzyme [4]. As inorganic nanoparticles possess the advantages (such as simple preparation, high stability, easy surface modification, and low-cost), the concept of nanozyme immediately attracted the enormous interest of the scientists from various fields including analytical chemistry and nanomedicine. Currently, enormous nanomaterials have been found with enzyme-like catalytic activity, mainly belonging to oxidoreductase and hydrolases. Especially, nanozymes with oxidoreductase-like activity, such as peroxidase, oxidase and catalase, have been extensively applied in biosensors, immunoassay, and therapy.

In this review, we first introduce the development of representative nanozymes, and then highlight the recent outstanding progress of nanozymes in the field of analytical chemistry. At the end of the article, future challenges and perspectives towards nanozymes are discussed in detail. The analytical methods used here ranges from electrochemistry, colorimetry, luminescence, and fluorescence to enzyme-linked immunosorbent assay (ELISA). The targets could be small molecules, biomacromolecules and even the specific cells, most of which are detected in vitro, and the methods

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have showed potential applications in practical samples. Specifically, based on the integrated nanozymes, cerebral glucose could be analyzed online in the living rats' brain by combining with the microfluidic chip, making a big progress in the in vivo detection technology.

2. Representative nanozymes

In this section, we introduce a few representative nanozymes.

(a) Peroxidase-like nanozymes. Peroxidases are a family of enzymes that catalyze the oxidation of the substrates by a peroxide, such as H_2O_2 [Equations (1) and (2)]. HRP, cytochrome *c* peroxidase, and streptavidin peroxidase are the common peroxidases which have been widely used in bioanalytical and clinical chemistry. Early studies from Yan group have reported that Fe_3O_4 MNPs could mimic the activity of HRP [2]. Then, sensitive colorimetric or electrochemical biosensors based on Fe_3O_4 MNPs have been fabricated for H_2O_2 detection [3,5]. To date, various nanomaterials have been demonstrated to possess the peroxidase-like activity, ranging from metals [6,7], metal oxides [3,8], and metal-organic frameworks (MOFs) [9,10] to carbon-based nanomaterials [11,12]. Peroxidase mimics have grown into a big family and attracted immense attention due to their wide applications in biosensing and immunoassay.

$$2AH + H_2O_2 \xrightarrow{\text{peroxidase}} 2A + 2H_2O \quad (1)$$

$$2AH + ROOH \xrightarrow{\text{peroxidase}} 2A + ROH + H_2O \quad (2)$$

(b) Oxidase-like nanozymes. Oxidases catalyze the oxidation of substrates with molecular oxygen (O_2) [Equations (3) and (4)]. In generally, a specific name of oxidase is given according to the oxidized biological substrates. For example, glucose oxidase (GOx), alcohol oxidase (AOx), lactate oxidase (LOx), cholesterol oxidase (COx), and uric acid oxidase (UOx) are the specific oxidases that catalyze the oxidation of glucose, ethanol, lactate, cholesterol, and uric acid, respectively. Nowadays, several nanoparticles have been found to exhibit oxidase-like activity, such as Au NPs [13,14], MnO_2 [15] and CeO_2 [16]. However, most of oxidase mimics could not selectively catalyze the oxidation of a given substrate like protein enzymes. Improving the selectivity of oxidase mimics will be a great challenge to be tackled in the future.

$$AH + O_2 \xrightarrow{\text{oxidase}} A + H_2O \quad (3)$$

$$AH + O_2 + H_2O \xrightarrow{\text{oxidase}} A + H_2O_2 \quad (4)$$

(c) Antioxidant nanozymes. Reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet -}$), hydroxyl radical (•OH) as well as H_2O_2 are present in numerous biological processes. However, the excessive generation of ROS would cause tissue injury and associated inflammation. Superoxide dismutase (SOD) and catalase are the important antioxidant enzymes in healthy cells to remove excess ROS [Equations (5) and (6a)]. Early studies have shown that fullerene and its derivatives [17–19] as SOD mimics possessed good neuroprotective effects. Besides, CeO_2 nanozymes exhibited attractive applications in anti-oxidation, anti-inflammatory, as well as promoting the growth of stem cell [4]. Inspired by the potential biomedical applications of nanozymes, researchers continued to design various types of antioxidant nanozymes, promoting the development of nanomedicine [20] such as efficient cytoprotection [21–23] and cancer therapy [24].

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2 \quad (5)$$

$$H_2O_2 \xrightarrow{\text{catalase}} O_2 + 2H_2O \quad (6a)$$

Although various types of nanozymes have been reported that could be applied in biosensors and therapeutics, there are still some gaps between nanozymes and natural enzymes [25]. For example, the selectivity of many nanozyme based biosensors is dependent on the specificity of the coadjutant enzymes. Improving the specificity of nanozymes and enhancing the catalytic activity become scientific and technological challenges in the future. Recently, Liu et al. have created substrate binding pockets on the Fe_3O_4 nanozymes by modifying them with molecularly imprinted polymers, opening a new gate to improve the specificity and catalytic activity of nanozymes [26].

3. Applications in analytical chemistry

Nanozymes have shown broad range of applications in the field of biosensing and immunoassay. According to the targets of interest and the different detecting strategies, this part was divided into the following five subsections: (1) Determination of H_2O_2. Peroxidase mimics could catalyze the reaction of H_2O_2 and a series of organic substrates, such as 3,3′,5,5′-tetramethylbenzidine (TMB), 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) and *o*-phenylenediamine (OPD), to give blue, green, and orange colors, respectively. Since the colorimetric reaction rate relies on the concentration of H_2O_2, nanozymes with peroxidase-like activity could be applied in the direct determination of H_2O_2. Also, analytes could be detected based on nanozymes by electrochemistry, luminescence, or other methods if the signals were modulated directly by the analytes. (2) Determination of glucose. The H_2O_2-producing targets could be detected indirectly by peroxidase mimics. For example, H_2O_2 is the common end product of the oxidase (GOx, AOx, LOx, COx, UOx and so on) catalyzed reactions, the corresponding specific substrates could be quantified by the production of H_2O_2. As the living cells could release H_2O_2, nanozyme based biosensors possess great potential applications in real-time tracking of the secretion of H_2O_2 in living human cells [27]. (3) Nanozyme based biosensors to evaluate antioxidants. On the other hand, molecules, which can consume H_2O_2 such as antioxidants, would also be evaluated by the sensors [28–30]. (4) Analysis by regulating the catalytic activities of nanozymes. When the surface of nanozymes are modified with biomacromolecules, small molecules or ions, the catalytic ability would be modulated because the chemical reactions happened mainly on the surface of nanoparticles [31]. Inhibition or promotion would result in the changes of signal, indicating the different concentrations of the target. (5) Nanozyme based immunoassay. Peroxidases such as HRP are widely applied in immunoassay as a label to trace the antigen, antibody, virus or cell. Due to the high stability and easy surface modification, nanozymes with peroxidase-like activity have been emerging alternatives to HRP in immunoassay [32]. Detailed discussions of each part is presented as following.

3.1. Determination of H_2O_2

H_2O_2 is closely related to cell growth and signal transduction. Overproduction of H_2O_2 is associated with the danger of a variety of

inflammatory-type diseases, such as atherosclerosis, hepatitis, and chronic obstructive pulmonary disease [33,34]. Therefore, H_2O_2 detection is of great interest owing to its important roles in biology and medicine. Wang et al. have reported the first example of H_2O_2 detection by using Fe_3O_4 MNPs as the peroxidase mimic and ABTS as the colorimetric substrate [3]. With the development of nanomaterials, plenty of peroxidase-like nanozymes have been discovered for colorimetric sensing of H_2O_2 [27,35–37]. Sotiriou et al. have rationally designed CeO_2 nanoparticles with antioxidant catalase-like activity, which became luminescent by doping Eu^{3+} [38]. The luminescence of nanozyme was quenched distinctly by interaction with H_2O_2, rendering the label-free and highly sensitive H_2O_2 biosensors down to 0.15 μM in solutions for biological assays. Further, an ethanol sensor was constructed when the designed nanozyme was coupled with AOx. Electrochemical technique is considered to be one of the efficient methods for H_2O_2 analysis due to its simple operation, high sensitivity and good selectivity. Some peroxidase-like nanozymes also exhibit good electrocatalytic capability towards H_2O_2. A great increase of reductant current occurred when H_2O_2 was introduced into the peroxidase mimic modified electrode [5]. Our group discovered that TiO_2 nanotube array (TiO_2 NTA) had the properties of peroxidase mimic, and showed high electrocatalytic activity towards H_2O_2 reduction at the same time [8]. More importantly, TiO_2 NTA itself could be served as the working electrode, making the method for H_2O_2 sensing facile and simple. Besides, TiO_2 NTA showed good stability in wide pH and temperature range over the natural enzyme HRP, extending the field of practical applications.

3.2. Determination of glucose

As an important research area of analytical chemistry, glucose biosensors have received significant attention over the past decades due to their wide applications in biomedical, clinical research, food production, and even ecology [3,39–41]. Glucose and similar analytes that could generate H_2O_2 by the oxidase catalytic reaction can be detected based on the combination of corresponding specific oxidase and peroxidase mimic [12,42].

3.2.1. Electrochemical glucose sensors

As mentioned above, TiO_2 NTA exhibited peroxidase-like catalytic activity and high electrocatalytic activity towards H_2O_2 reduction. Through simple physical adsorption, an electrochemical glucose biosensor was successfully constructed by coupling TiO_2 NTA with GOx. The proposed glucose biosensor showed excellent selectivity and practicability in real sample assays. Similar strategy could be applied in the electrochemical glucose biosensors based on the combination of GOx and peroxidase mimic [54].

3.2.2. Colorimetric glucose sensors

Generally, common colorimetric glucose biosensors based on the combination of GOx and peroxidase mimic were fabricated through a two-step process [3,7,43]: first, glucose was oxidized to produce H_2O_2 in neutral solution; then, a peroxidase mimic catalyzed the oxidation of organic substrates with H_2O_2 to give a colorimetric signal usually in acid solution [Equations (6b) and (7)]. The different reaction conditions for the two-step process of glucose determination make the operation complicated, promoting analysts to find out a new way to construct one-step colorimetric glucose biosensors. Two strategies reported recently are discussed as follows.

$$\text{glucose} + O_2 \xrightarrow{\text{GOx}} \text{gluconic acid} + H_2O_2 \quad (6b)$$

$$2AH + H_2O_2 \xrightarrow{\text{peroxidase mimic}} 2A + 2H_2O \quad (7)$$

Strategy One: exploring nanozymes with good catalytic activity in neutral solutions. A simple strategy was developed for Ni/Co layered double hydroxides as a peroxidase mimic via the co-precipitation method [44]. Importantly, this material exhibited high catalytic activity in neutral pH solutions (phosphate buffer, Tris–HCl buffer, and even water). Therefore, a novel one-step method for the detection of glucose was developed in water, showing shorter reaction times, simpler steps and easier of operations. Furthermore, this material was also applied in detecting acetylcholine (ACh) by the similar one-step strategy as H_2O_2 could be generated in the presence of both acetylcholinesterase and choline oxidase in the solution containing ACh.

Strategy Two: fabricating artificial enzyme systems for tandem catalysis.

(i) Natural enzymes with nanozymes.

Recent studies have demonstrated that coating enzyme with zeolitic imidazolate framework 8 (ZIF-8) can protect the biomacromolecules from thermal, biological and chemical degradation with maintenance of bioactivity [45–48]. Ge et al. have synthetized a multi-enzyme-containing MOF nanocrystals, GOx&HRP/ZIF-8 nanocomposites, which showed enhanced catalytic efficiency, selectivity and high stability due to the protecting effect of the framework, making it promising for developing reliable glucose biosensors [49]. Inspired by the merits of ZIF-8, more and more attentions have been paid to biomimetic mineralization of ZIF-8 [50–53]. Our group has fabricated an artificial enzyme system GOx@ZIF-8(NiPd) nanoflower for tandem catalysis as shown in Fig. 1A [54]. Via a facile co-precipitation, nanozyme (NiPd hollow nanoparticles [7]) and natural enzyme (GOx) were simultaneously immobilized on ZIF-8. GOx@ZIF-8 (NiPd) not only exhibited the peroxidase-like activity of NiPd but also maintained the enzymatic activity of GOx. By using OPD as the chromogenic substrate to produce a typical yellow oxidized product 2,3-diaminophenazinc (DAP), the cascade reaction for the visual detection of glucose could be achieved in one step [Equation (8)]. What's more, GOx@ZIF-8 (NiPd) modified glassy carbon electrode (GOx@ZIF-8 (NiPd)/GCE) exhibited good electrochemical performance towards glucose, but the mechanism of the process was quite different from the mentioned glucose electrochemical sensor based on GOx and peroxidase mimics. The reduction peaks were considered to be generated from the high electrocatalytic property towards oxygen reduction reaction instead of peroxidase-like activity [Equations (9)–(11)], achieving an interferent-free amperometric biosensor for the detection of glucose. The proposed strategy builds a potential bridge of cooperation between nanozymes and natural enzymes, combining together their properties and functionalities. Given the variety of enzymes, systems with different functions would be prepared, making efforts to multi-catalysis and tandem reactions.

$$\text{glucose} + \text{OPD} + O_2 \xrightarrow{\text{GOx@ZIF-8(NiPd)}} \text{gluconic acid} + \text{DAP} + H_2O \quad (8)$$

$$\text{glucose} + \text{GOx(FAD)} \rightarrow \text{GOx}(FADH_2) + \text{gluconolactone} \quad (9)$$

$$\text{GOx}(FADH_2) + O_2 \xrightarrow{\text{NiPd}} \text{GOx(FAD)} + H_2O \quad (10)$$

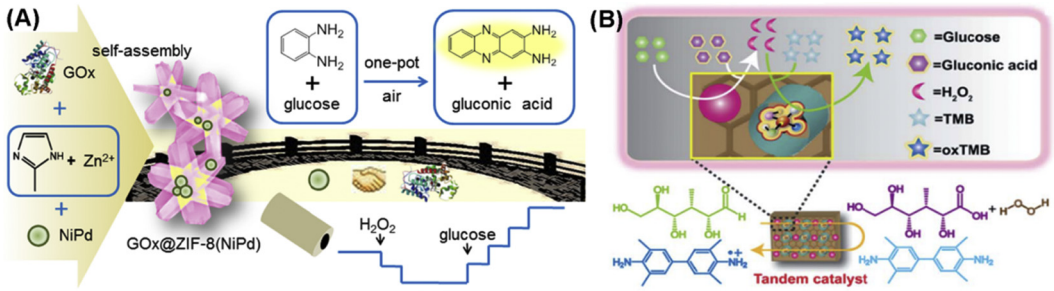

Fig. 1. (A) Schematic presentation of GOx@ZIF-8(NiPd) used as artificial enzyme system for tandem catalysis. (B) Schematic illustration of a tandem reaction taking place in the GSHA system. Reprinted with permission from Refs. [54] and [59]. Copyright (2017) Wiley-VCH and (2015) Royal Society of Chemistry.

Total reaction:

$$\text{glucose} + O_2 \xrightarrow{\text{GOx@ZIF-8(NiPd)}} \text{gluconolactone} + H_2O \qquad (11)$$

(ii) Nanozymes with nanozymes.

Au NPs have been proved to be effective nanozymes with intrinsic GOx-like activity, which could catalyze the oxidation of glucose to produce H_2O_2 [13,14,55,56]. Based on the GOx-like activity of Au NPs, Qu et al. have developed a series of artificial enzyme system for self-activated cascade catalysis [57–59]. Specifically, a graphene-mesoporous silica hybrid (Fig. 1B) was used as a nanocontainer to anchor AuNPs (served as GOx mimic) and hemin (served as peroxidase mimic) at different locations, both of which can work successfully in the tandem catalytic reaction for glucose detection [59].

3.2.3. Determination of glucose in vivo

As discussed above, most of the targets are detected in vitro. In this section, the in vivo detection methods are highlighted. Fabricating the artificial enzyme systems for tandem catalysis made a big progress for the determination of glucose in vivo. Wei et al. have designed integrated nanozymes (INAzymes) by simultaneously embedding hemin and GOx in ZIF-8 nanostructures [52]. The tandem reaction for glucose analysis occurred in close (nanoscale) proximity to each other, overcoming the problems of diffusion-limited kinetics and product instability. The INAzymes with improved catalytic activity and stability were successfully used for facile colorimetric visualization of cerebral glucose in the living rats. Moreover, they have constructed an integrative INAzyme-based online analytical platform through further combination with a microfluidic chip, which was used to continuously monitor the dynamic changes of striatum glucose in living rats' brain following ischemia/reperfusion. Recently, Wei et al. designed another type of integrated nanozymes to determine glucose and lactate metabolism in tumors, which first combined the peroxidase-like activity and the surface-enhanced Raman scattering (SERS) activity of AuNPs [60]. AuNPs were in situ grown in MIL-101, a kind of porous and thermally stable MOF. The obtained AuNPs@MIL-101 nanozymes worked as peroxidase mimics to oxidize Raman-inactive reporter leucomalachite green (LMG) into the active malachite green (MG), simultaneously enhancing the Raman signals of MG. When assembled the oxidase (GOx and LOx) onto AuNPs@MIL-101 to form a new artificial enzyme system, the corresponding specific substrate (glucose and lactate) could be detected through the tandem reactions in vitro via SERS. Furthermore, the therapeutic efficacy of potential drugs were evaluated in living rats. As a result, nanozyme based biosensors have showed great potential practical applications not only in real biological samples but also in vivo.

3.2.4. Colorimetric bioactive paper

Using colorimetric bioactive paper to detect the target by the visible color change, have attracted great research efforts in last decade due to its convenience and portability. A bioactive lateral-flow paper for multianalyte detection based on the peroxidase-like activity of graphene oxide@SiO_2@CeO_2 nanosheets (GSCs) would be presented in this part [12]. The combination of graphene oxide and CeO_2 contributed to the high peroxidase-like activity of GSCs. Then, a multiplex bioactive paper was fabricated as shown in Fig. 2. The GSC bioactive paper showed a linear color changed response to the concentrations of H_2O_2 by using OPD as the dye indicator. When the oxidase enzymes, such as GOx, UOx, LOx and COx, were immobilized in the different test zones of GSC paper, respectively, these corresponding substrates could be quantitative detected simultaneously. Also, the bioactive paper showed an excellent analytical performance in human serum or urine, exhibiting the potential practical applications of nanozymes.

3.3. Nanozyme based biosensors to evaluate antioxidants

Antioxidants, a group of molecules that scavenge the reactive oxygen species, or the free radicals produced in biological systems, have been used as conventional additives in food products, cosmetics, and dietary supplements owing to their specific protection functions. They can be evaluated by the sensors based on the nanozymes according to the consume of H_2O_2. He et al. synthesized Co_3O_4 NPs with peroxidase-like activity via a hydrothermal method [61]. Then, the antioxidant activities of ascorbic acid, tartaric acid, and tannic acid were evaluated by Co_3O_4 NPs, among which tartaric acid exhibited the highest antioxidant activity. Nanozymes can be used to evaluate antioxidant capabilities and screen enzyme inhibitors.

3.4. Analysis by regulating the catalytic activities of nanozymes

Through regulating the peroxidase-like activity of Fe_3O_4 NPs, a novel nanoreactor was synthesized by coating Fe_3O_4 NPs with mesoporous SiO_2 shell to colorimetric detection of protein (Fig. 3A) [62]. When a modified thrombin aptamer probe (aptamers: singled stranded oligonucleotides possess high recognition ability to specific targets) was immobilized on the nanoreactor surface, the etched Fe_3O_4 NPs inside the layer could efficiently catalyze TMB to produce a strong absorbance signal. Upon addition of thrombin, the catalytic activity of Fe_3O_4 NPs was greatly inhibited by the bounding block protein layer with the aptamer on the surface of nanoreactor. Based on this assay, the proposed strategy exhibited

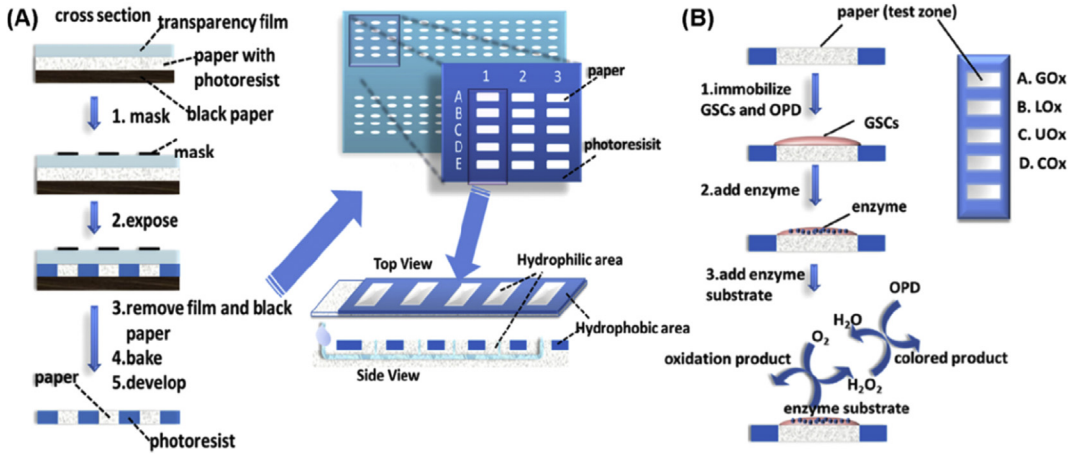

Fig. 2. (A) Schematic illustration of the preparation principle of colorimetric bioactive paper. (B) Schematic illustration of the colorimetric bioactive paper based on GSCs for multiplexed substrate. Reprinted with permission from Ref. [12]. Copyright (2014) Elsevier.

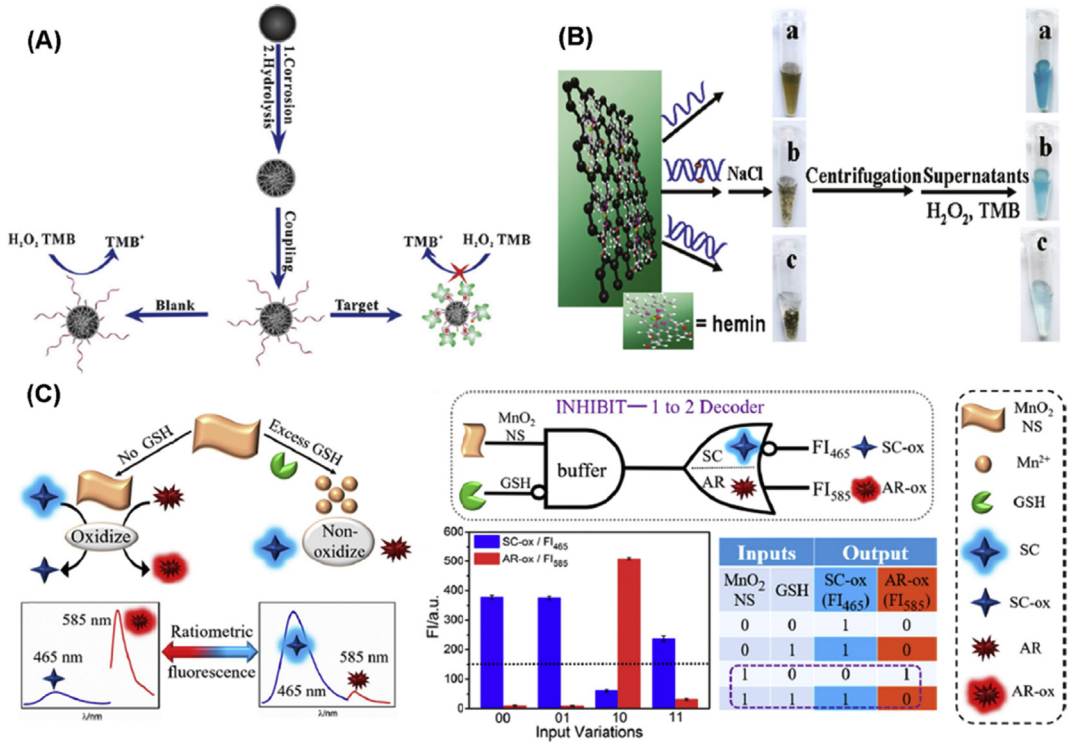

Fig. 3. (A) Schematic illustrating the principle mechanism involved in the colorimetric assay for thrombin. (B) Protocol for SNPs detection. (a) ssDNA (T1) (no precipitation, dark blue), (b) single mismatched duplex DNA (T2/T3) (small amount of precipitation, blue), (c) complementary duplex DNA (T1/T2) (much precipitation, light blue). (C) Schematic operations of the MnO_2 NS-based ratiometric fluorescent sensor for GSH based on two fluorescent substrates. Equivalent logic symbol of the cascade logic circuit, an INHIBIT gate cascade with a 1 to 2 decoder. Reprinted with permission from Refs. [62], [11], and [15]. Copyright (2013) Royal Society of Chemistry, (2011) American Chemical Society and (2017) American Chemical Society.

good selectivity to thrombin and potential practical applications in human serum.

Hemin-graphene hybrid nanosheets (H-GNs) synthesized by a simple wet-chemical strategy exhibited good peroxidase-like catalytic activity and possessed the ability of graphene to distinguish ss- and ds-DNA in the optimum electrolyte concentration [11]. As presented in Fig. 3B, the solution contained ss-DNA/H-GNs were well suspended, however, when the complementary target DNA was added, the forming complementary ds-DNA/H-GNs were coagulated visually. Simultaneously, the peroxidase-like activity of H-GNs was reduced obviously. A label-free colorimetric detection method for DNA sequence was developed. This DNA sensor was of sufficient selectivity to differentiate single mismatches, providing a sensitive and visual method to determinate single nucleotide polymorphisms.

MnO_2 nanosheet (NS) was reported to possess intrinsic oxidase-like activity, which could directly catalyze the oxidation of TMB by molecular oxygen. A novel ratiometric fluorescent assay for ultrasensitive glutathione (GSH) detection was achieved by regulating the oxidase-like property of MnO_2 NS [15]. As shown in Fig. 3C,

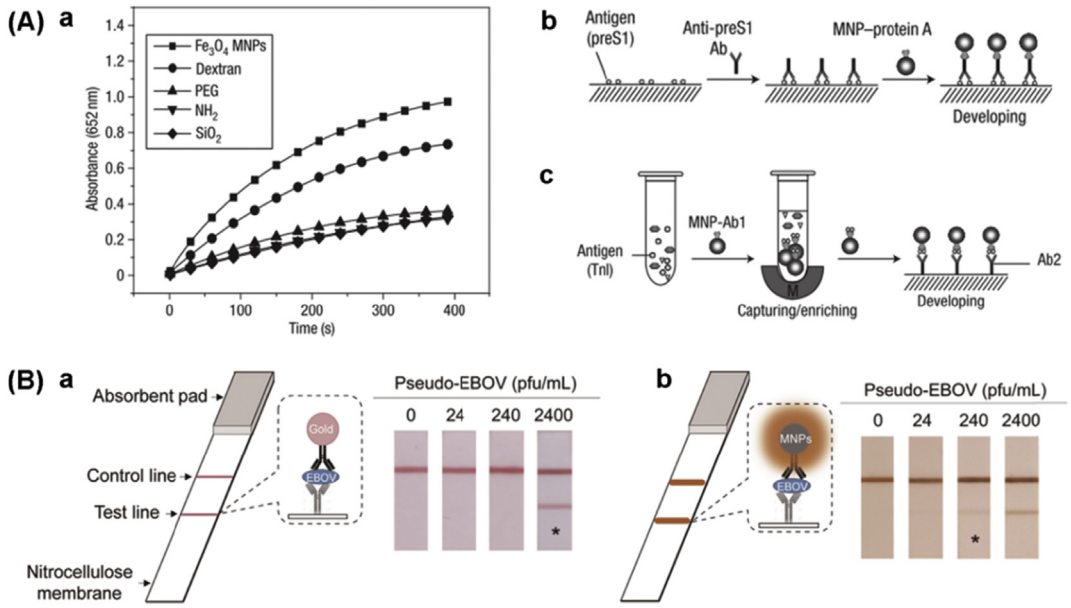

Fig. 4. (A) The catalytic activity of Fe_3O_4 MNPs modified with different coatings (a). Fe_3O_4 MNPs-based immunoassay (b). A capture-detection immunoassay (c). (B) Standard colloidal gold strip (a) and nanozyme-strip employing MNPs in place of colloidal gold to form a novel nanozyme probe (b) for the detection of pseudo-EBOV in human serum in the presence of 1×10^5 pfu/mL influenza A virus. The asterisk (*) indicates the limit of visual detection. Reprinted with permission from Refs. [2] and [65]. Copyright (2010) Nature Publishing Group and (2015) Elsevier.

MnO_2 NS could surprisingly enhance the fluorescence of nonfluorescent Amplex Red (AR) through the oxidation reaction. At the same time, it could largely quench the fluorescence of highly fluorescent Scopoletin (SC). When premixed with GSH, MnO_2 NS would be reduced to Mn^{2+} and lose its oxidase-like activity gradually, accompanied with subsequent increase in SC's fluorescence and decrease in AR's. Thus, a sensitive ratiometric fluorescent sensor for GSH detection was achieved with the detection limit as low as 6.7 nM, which provided a more accurate and effective detection assay compared with single-signal steady state fluorescence. Furthermore, an INHIBIT gate cascade with a 1 to 2 decoder was constructed based on the above interaction between GSH and MnO_2 Ns/SC/AR. Molecular logic computation was integrated with ratiometric analysis of GSH for the first time, evidently expanding the applications of oxidase mimics. Besides, the peroxidase-like activity of Pd@AuNR is selectively quenched with increasing concentration of malathion [63]. A simple, selective, label-free colorimetric assay for malathion sensing was fabricated by changing the peroxidase-like property of Pd@AuNRs. As a result, the targets that can change the enzyme-like catalytic activities, could be detected through the nanozyme based biosensors. The strategy have been an attractive direction in analysis of proteins, single nucleotide polymorphisms, small molecules and ions [64].

3.5. Nanozyme based immunoassay

When Fe_3O_4 MNPs were found with peroxidase-like catalytic activity by Yan et al., nanozymes were considered to be applied in immunoassay [2]. In order to make them biocompatible, Fe_3O_4 MNPs were modified with different compounds, including SiO_2, 3-aminopropyltriethoxysilane (APTES), polyethylene glycol (PEG) or dextran. As shown in Fig. 4A-a, the dextran modified Fe_3O_4 MNPs maintained the highest degree of activity and were used to immobilize the antibody or protein in the nanozyme-linked immunosorbent assay (NLISA) instead of HRP. The Fe_3O_4 MNPs based NLISA showed an obvious signal at 652 nm when the hepatitis B virus surface antigen existed (Fig. 4A-b). Another capture-detection immunoassay was designed based on the intrinsic magnetism and peroxidase-like activity of Fe_3O_4 MNPs (Fig. 4A-c). Cardiac troponin I was captured by the antibody-labeled Fe_3O_4 MNPs, and the ELISA reader successfully obtained a signal at 652 nm when the substrate TMB was added with H_2O_2, indicating the potential applications of peroxidase mimics in immunoassay instead of HRP. Afterwards, a novel nanozyme-strip for rapid local diagnosis of Ebola virus (EBOV) based on the peroxidase-like activity of Fe_3O_4 MNPs was developed as shown in Fig. 4B [65]. Compared with traditional ELISA, the nanozyme-strip for EBOV detection is much faster (within 30 min) and simpler (without need of specialist facilities). This outstanding method provides a valuable simple screening tool for diagnosis of infection in Ebola-stricken areas, attracting the attentions around the world.

4. Conclusions and perspective

In this review, we have summarized the recent achievements in developing novel nanozymes and expanding the applications. Due to the simple preparation, easy storage and separation, nanozymes especially peroxidase mimics have replaced the uses of natural enzymes gradually in the field of biosensing and immunoassay. Although a great deal of progress has already been made in nanozymes, several challenges and obstacles still remain at this frontier. (1) Given the variety of natural enzymes, future efforts should be focused on designing nanozymes with new catalytic properties such as hydrolase, synthetase, etc. (2) Most of nanozymes could hardly catalyze one specific substrate like natural enzymes. Improving the selectivity of nanozymes will be a great challenge to be tackled in the future. (3) In generally, natural enzymes are working together as enzyme clusters. To mimicking the complex natural enzyme systems, much effort would be focused on nanozyme assemblies or assemblies both of nanozyme and natural enzyme to combine together their properties and functionalities. (4) It is also important to synergistically combine nanozymes with other functional nanoparticles that possessed special nanoscale properties such as magnetics, optics, electrics and mechanics, to

widen the practical applications. (5) Surface modification of nanozymes appears to be an attractive field to tune their catalytic activities in the applications of biosensing for promoting nanozyme research. (6) Why nanoparticles could imitate natural enzymes, which were quite different in many ways, such as composition, conformation and shape? The detailed catalytic mechanisms and theories behind the interesting phenomena need further studies.

Acknowledgments

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