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Colorimetric detection of hydrogen peroxide using nanozymes

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13.1 Introduction

Following the discovery of the first enzyme, diastase, in the year 1833, the discovery of other hydrolytic enzymes took place. Then, in 1836, after the concept of catalysts was introduced in the literature, Wilhelm Kühne named these structures as enzymes in 1877 (Heckmann and Paradisi, 2020). Over time, the industry has progressively adopted enzymatic approaches in chemical synthesis due to their ecofriendly characteristics, remarkable efficiency, and increasing popularity compared with traditional methods (Wu et al., 2021). However, laborious downstream processes, low stabilities, and high costs of enzymes triggered the emergence of enzyme-like alternative structures. Over the years, Nobel laureate scientists have developed molecules that possess highly selective structure-specific interactions and can "recognize" each other to form complexes, as well as RNA-based biocatalysts, not only serve as molecules of heredity but also could act as biocatalysts (Gao and Yan, 2016). However, despite being groundbreaking discoveries, the limitations in their usage have sparked new quests and searches. In 2007, Gao et al. discovered magnetic Fe_3O_4 nanoparticles (NPs) with peroxidase-like activities and nanomaterials with enzyme-like characteristics, which are called "nanozymes," have been defined in the literature (Gao et al., 2007; Gao and Yan, 2016). Combining nanotechnology and enzymatic activity to enable rapid and sensitive detection of certain biomarkers for disease diagnosis, nanozymes are fascinating tools made of nanomaterials with enzyme-like properties. By mimicking the catalytic activity of natural enzymes, they provide several applications in medical sciences, bioimaging, and environmental remediation, offering several advantages over traditional enzyme-based methods (e.g., improved stability, cost-effectiveness, and tunable catalytic properties) (Huang et al., 2019). Moreover, leveraging the constructed catalytic functions of natural enzymes, they simulate the biosystem-inspired

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behaviors in biological processes or living organisms (Zhang et al., 2023). As artificial enzymes, nanozymes have stimulated the oxidoreductase enzymes often used in biomedical applications, proving their potential to be a good candidate for point-of-care (POC) sensing platforms. In this chapter, we summarize the state of the art for the nanozymes in colorimetric detection of hydrogen peroxide (H_2O_2), of which detection has crucial importance in many fields of bioanalyses and pharmaceutical assays.

13.2 The importance of H_2O_2 detection for life sciences

Belonging to the family of reactive oxygen species (ROS), H_2O_2 is essential as a signaling molecule in metabolic functions (Woolley et al., 2013). Abnormal changes in H_2O_2 serve as a critical messenger in biological systems and have been associated with various diseases such as atherosclerosis, chronic inflammation, and cancer (Coyle and Kader, 2007; Houstis et al., 2006; Wittmann et al., 2012). Hence, selectivity and sensitivity are of great importance in its detection. Considering not only biomedical but also environmental applications, monitoring the concentration of H_2O_2 is a critical parameter in water and air pollution control and water treatment processes (Deo, 1988). Although enzymatic methods usually diagnosed it, these methods have some disadvantages of low stability of enzymes, high cost, and difficult downstream processes (Liu et al., 2022a). Therefore, nanozymes, the intersection of nanotechnology and nanomaterials, offered a new approach to H_2O_2 detection, and various conjugate systems have been used along with electrochemical and colorimetric sensors. Considering the highly active nature of H_2O_2, detection mechanisms show variety due to the transduction elements. As such, electrochemical sensing mechanisms are based on the oxidation of mediator molecules, whereas the formation/degradation of the dye results in the colorimetric detection of H_2O_2 (Giaretta et al., 2022).

Although the underlying mechanisms of both methods are different, some chromogenic reagents such as 3,3′,5,5′-tetramethylbenzidine (TMB) reacting with peroxidase enzymes can be used both in the singular and dual modes in the presence of H_2O_2. For instance, Gu et al. developed a dual-mode biosensing platform for intra/extracellular colorimetric and homogeneous electrochemical detection of H_2O_2 by using FeSx/SiO_2 NPs (Fig. 13.1A) (Gu et al., 2023). Similarly, Xu et al. used TMB with a novel MoS_2 and gold (Au) NPs integrated Fe_3O_4 nanocomposite having intense peroxidase activity for high-sensitivity detection of H_2O_2 (Fig. 13.1B) (Xu et al., 2022). On the other hand, Ko et al. used bimetallic NPs and Au NPs in a microfluidic device for dual quantification of H_2O_2 (Fig. 13.1C) (Ko et al., 2019).

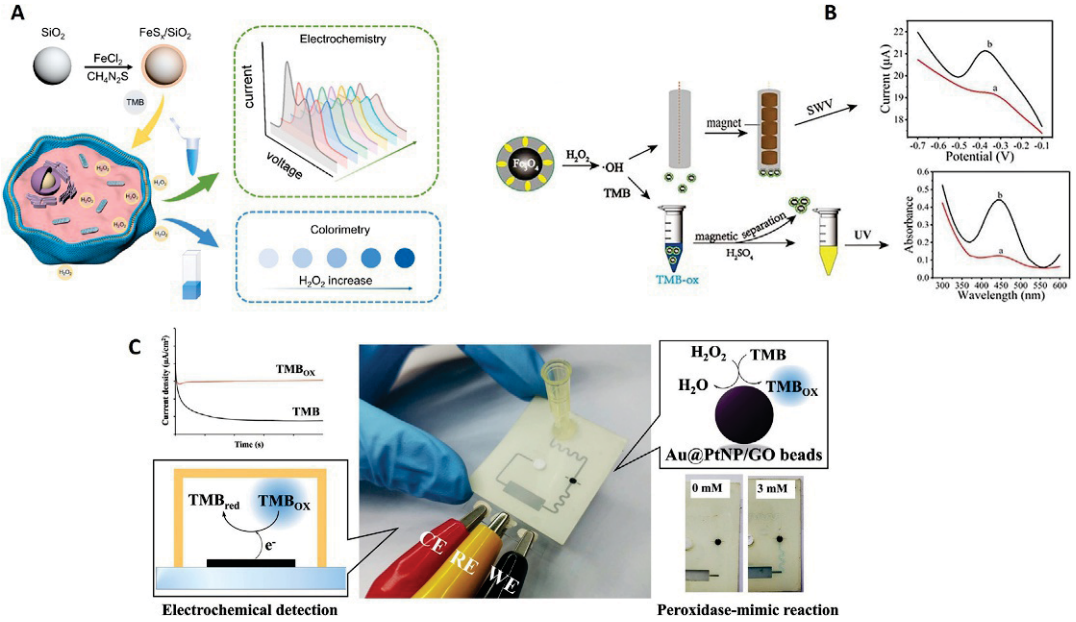

FIG. 13.1 Schematic illustration of dual mode detection of H_2O_2. (A) Detection through the FeSx/SiO_2 in an intra/extracellular environment. (B) Electrochemical response and UV–vis absorption spectra caused by Fe_3O_4@MoS_2-Au nanocomposites in the absence and presence of H_2O_2. (C) Electrochemical POC devices with Au@PtNP/GO nanozymes. *Panel (A) Reprinted with permission from Gu, C., Bai, L., Hou, T., Zhang, L., Gai, P., Li, F., 2023. Dual-mode colorimetric and homogeneous electrochemical detection of intracellular/extracellular H_2O_2 based on FeSx/SiO_2 NPs with high peroxidase-like activity. Anal. Chim. Acta 1265, 341332. https://doi.org/10.1016/J.ACA.2023.341332. Panel (B) Reprinted with permission from Xu, W., Fei, J., Yang, W., Zheng, Y., Dai, Y., Sakran, M., Zhang, J., Zhu, W., Hong, J., Zhou, X., 2022. A colorimetric/electrochemical dual-mode sensor based on Fe_3O_4@MoS_2-Au NPs for high-sensitivity detection of hydrogen peroxide. Microchem. J. 181. https://doi.org/10.1016/j.microc.2022.107825. Panel (C) Reprinted with permission from Ko, E., Tran, V.K., Son, S.E., Hur, W., Choi, H., Seong, G.H., 2019. Characterization of Au@PtNP/GO nanozyme and its application to electrochemical microfluidic devices for quantification of hydrogen peroxide. Sens. Actuators B Chem. 294, 166–176. https://doi.org/10.1016/j.snb.2019.05.051.*

13.3 Nanozymes for colorimetric hydrogen peroxide detection

We categorized nanozymes into three main groups in terms of their composition: inorganic-based, carbon-based, and hybrid/organic-based nanozymes.

13.3.1 Inorganic-based nanozymes

Inorganic-based nanozymes comprise inorganic materials, such as metal NPs (e.g., gold, silver, platinum, palladium, iridium), metal oxides (e.g., cerium oxide, chromium oxide, cobalt oxide, iron oxide, manganese oxide, titanium oxide, tungsten oxide, vanadium

oxide), metal sulfides (cobalt sulfide, copper sulfide, iron sulfide, molybdenum sulfide, nickel sulfide, silver sulfide), and metal nitrides (iron nitride, platinum nitride) were listed in Table 13.1. They often exhibit excellent catalytic activity and stability, making them useful for various applications, including sensing, bioimaging, and water treatments.

The choice of synthesis method and the resulting nanostructure is critical for the specific requirements of the H_2O_2 detection application, including sensitivity, selectivity, and stability. Common chemical synthesis methods for the nanozymes include hydrothermal (Chen et al., 2021; Jamil et al., 2021; Niu et al., 2016; Sun et al., 2016; Vinothkumar et al., 2019; Xia et al., 2020), coprecipitation (Hormozi Jangi et al., 2020), pyrolysis (Wang et al., 2022b), atomic coating (He et al., 2022), chemical reduction (Uzunboy et al., 2022; Xu et al., 2022), solvothermal (Li et al., 2020; Lian et al., 2021a; Peng et al., 2023), and alcohol thermal reaction (Liu et al., 2019a). In a divergent way, Fan et al. obtained bimetallic PtPd nanoflowers using the surfactant directing method followed by molecularly imprinted polymer (MIP) modification (Fan et al., 2019). The successful integration of MIP and nanozymes led to enhanced selectivity and activity. Moreover, solvothermally synthesized Fe_3O_4 NPs are firstly converted into FeS_2 nanozymes through sulfidation (Song et al., 2020). Similarly, Xu et al. synthesized Fe_3O_4@MoS_2-Au NPs by a multistep process (Xu et al., 2022). Au NPs, which were obtained by chemical reduction, were doped in MoS_2 to enhance dispersibility and stability. Then, Fe_3O_4@MoS_2-Au nanocomposites were synthesized by hydrothermal method.

Recently, researchers have aimed to minimize toxic chemicals, reduce waste, and utilize sustainable materials and methods, developing green-synthesized nanozymes for colorimetric H_2O_2 detection. Most of these approaches involve using plant extracts to reduce metal salts into nanostructures. For instance, Pd and Ag nanoclusters were obtained from *Erigeron canadensis* L. (Tripathi and Chung, 2020) and cinnamon (Elgamouz et al., 2022) extracts, respectively. In another study, Fe_3O_4 nanocomposites were synthesized under low temperature in the presence of *Musa paradisiaca* L. (Rasheed et al., 2022) peels. Phytochemicals present in plant extracts and peels such as polyphenols, terpenoids, alkaloids, and saponins serve as reducing and stabilizing agents.

Various inorganic nanostructures have been used for nanozymes, and they are typically classified based on their composition and synthesis methods. Some common nanostructures used are nanowires (Sun et al., 2016), nanobranch-based clews (Niu et al., 2016), nanorods (Vinothkumar et al., 2019), nanoflowers (Fan et al., 2019; Lian et al., 2021a), NPs (Chen et al., 2019; Fan et al., 2019; Lian et al., 2021a; Rasheed et al., 2022; Song et al., 2020; Uzunboy et al., 2022; Wang et al., 2022b), nanoplates (Li et al., 2020), nanoclusters (Elgamouz et al., 2022; Liu et al., 2019a; Tripathi and Chung, 2020), nanosheets (Xia et al., 2020), nanocomposites (Chen et al., 2021; Ding et al., 2019; Hormozi Jangi et al., 2020; Jamil et al., 2021; Xu et al., 2022), and nanofibers (Xu et al., 2022).

The nanozymes are used for colorimetric H_2O_2 detection by mixing them with a suitable substrate that undergoes a color change upon reaction with H_2O_2. Commonly used chromogenic substrates include TMB, o-phenylenediamine (OPD), 3, 3′-diaminobenzidine

Table 13.1 Inorganic-based nanozymes and their applications.

Nanozymes	LOD	Selectivity	Applications	Conditional parameters	Reference
[a]V_2O_5	1.0 μM	Glucose	– Aqueous solution	Acetate buffer solution (pH 4.0)	Sun et al. (2016)
[a]Uncapped nanobranch-based CuS clews	0.063 μM	Glucose	– Aqueous solution	Acetate buffer solution, (pH 4.0)	Niu et al. (2016)
[a]$CePO_4$-CeO_2 composite nanorods	2.9 μM	Glucose	– Aqueous solution – Dual (colorimetric and fluorescence) detection	Citrate buffer solution (pH 4.0)	Vinothkumar et al. (2019)
[a]Cu-$CuFe_2O_4$	0.59 μM	Glutathione	– Aqueous solution – Glutathione detection in human and chicken serum	Acetate buffer solution (pH 4.0)	Xia et al. (2020)
[a]SiO_2@Fe_3O_4/MnO_2	0.26 μM	–	– H_2O_2 detection in milk samples – Organic dye degradation in real water samples	Phosphate buffer solution (pH 7.0),	Hormozi Jangi et al. (2020)
[a]Palygorskite@Co_3O_4 nanocomposites (Pal@Co_3O_4)	1.4 μM	Ascorbic acid, cysteine	– Aqueous solution – Ascorbic acid detection in vitamin C tablets, orange, and fruit juices	Acetate buffer solution (pH 4.0)	Chen et al. (2021)
[a]Bimetallic sulfide NPs [a]$NiCo_2S_4$ (PVP) and $NiCo_2S_4$ (CTAB)	8 μM	–	– Aqueous solution – Dual (colorimetric and electrochemical) detection – Quantitative monitoring of H_2O_2 produced by MDA-MB-231 cells	Phosphate buffer solution (pH 4.0)	Lian et al. (2021b)
[a]FeN_3/PtN_4-S-Azyme	7.97 μM	Dopamine	– Mouse breast cancer cell lines (4T1 cells)	CPBS buffer solution (pH 4.0)	Wang et al. (2022b)
[a]The urchin-like trimetallic nanozyme Pd-Pt-Ir	3.60 mM	Ascorbic acid	– Aqueous solution – Ascorbic acid detection in the paper strips	Acetate buffer solution (pH 4.0)	He et al. (2022)
[a]Fe_3O_4@MoS_2-Au NPs	0.109 μM	–	– Dual (colorimetric and electrochemical) detection – Normal human serum	Acetate buffer solution (pH 4.0)	Xu et al. (2022)

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Table 13.1 Inorganic-based nanozymes and their applications—cont'd

Nanozymes	LOD	Selectivity	Applications	Conditional parameters	Reference
[b]Bimetallic oxide nanofibers ($CoMnO_3$ NFs)	0.24 μM	Glucose and uric acid	– Aqueous solution – Milk samples – Visual detection of H_2O_2 using the smartphone-assisted colorimetric sensing system (LOD was 2.1 μM) – Cytotoxicity tests using the two types of cancer cells (B16 and HepG2) and a kind of normal cells (HUVECs)	Acetate buffer solution (pH 4.0)	Peng et al. (2023)
[a]Silver nanoclusters	0.62 μM	Xanthine (TMB required)	– Aqueous solution	Ultrapure water	Elgamouz et al. (2022)
[a]Fe_3O_4 @ *Musa paradisiaca* L. NPs	0.008 μM	–	– Aqueous solution – Tap water and milk samples	PBS buffer solution (pH 7.0)	Rasheed et al. (2022)
[a]Fe-Doped CoO nanocomposites	4.40 μM	Dopamine	– Aqueous solution – Dual (colorimetric and fluorescence) detection – Human urine samples	Buffer solution (pH 4.0)	Lian et al. (2021a)
[a]The molecularly imprinted polymer PtPd nanoflowers (T-MIP-PtPd NFs)	0.005 μM	Glucose	– Aqueous solution	Acetate buffer solution (pH 4.0)	Fan et al. (2019)
Cr_2O_3 NPs	1.5 μM	–	– Aqueous solution – Redox colorimetric detection – Triacetone triperoxide (TATP) samples	Ammonium buffer solution (pH 10.0)	Uzunboy et al. (2022)
[a,c]Pt/$WO_{2.72}$ nanoplates	2.33 μM	Glucose	– Aqueous solution – Dual (colorimetric and fluorescence) detection – Hemolysis activity – Hydroxyl radical elimination	PBS buffer solution (pH 7.4)	Li et al. (2020)
[a]Au NPs encapsulated by Au nanoclusters (AuNP@AuNCs)	10 μM (colorimetric) 0.2 μM (fluorimetric)	Glucose	– Aqueous solution – Dual (colorimetric and fluorescence) detection	Acetate buffer solution (pH 4.0)	Chen et al. (2019)

[a]Fe-doped Ag_2S	7.82 μM	–	– Aqueous solution	Acetate buffer solution (pH 4.0)	Ding et al. (2019)
[a]Cu(II)-coated Fe_3O_4 NPs	0.2 μM	–	– Aqueous solution	Acetate buffer solution (pH 3.0)	Liu et al. (2019a)
[a]FeS_2 NPs	0.91 μM		– Aqueous solution – Dual (colorimetric and fluorescence) detection – Human serum samples – In vitro cytotoxicity in HeLa cells	Acetate buffer solution (pH 4.0)	Song et al. (2020)
[a]Pd nanoclusters	0.0625 μM		– Aqueous solution	Acetate buffer solution (pH 5.0)	Tripathi and Chung (2020)
[a]Cr_2O_3-TiO_2 nanocomposites	0.003 μM	–	– Aqueous solution – Tap water, milk, and fetal bovine serum albumin – Detection using Cr_2O_3-TiO_2-modified filter paper sensor	Distilled deionized water	Jamil et al. (2021)

[a] Peroxidase (POD).
[b] Oxidase (OXD).
[c] Catalase (CAT).

(DAB), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium (ABTS), terephthalic acid, dopamine. The presence of H_2O_2 triggers the catalytic activity of the nanozymes, leading to a color change that can be detected visually or with a spectrophotometer.

The limit of H_2O_2 detection for inorganic-based nanozymes can vary widely depending on several factors, including the type of nanozyme, the assay conditions, and the measurement techniques used. The limit of detection (LOD) for the nanozymes mentioned in Table 13.1 ranges from nanomolar to millimolar concentrations. The lowest LOD values belong to Cr_2O_3-TiO_2 nanocomposites (LOD: 3 nM) (Jamil et al., 2021), PtPd nanoflowers (LOD: 5 nM) (Fan et al., 2019), and Fe_3O_4@*Musa paradisiaca* L. NPs (LOD: 8 nM) (Rasheed et al., 2022). The smaller particle size in nanozymes generally results in a higher surface area, which can lead to more catalytic sites and improved enzyme-like activity (Hormozi Jangi et al., 2020). Additionally, it is essential to note that the catalytic activity can be influenced by its composition, surface functionalization, and the reaction conditions. In the context of measurement techniques, although electrochemical and fluorescence techniques are generally more sensitive, the colorimetric approach has some distinct advantages, such as easy operation and a significantly faster reaction time.

13.3.2 Carbon-based nanozymes

Being able to produce allotropes such as fullerene, carbon nanotubes, or graphene oxide (GO), carbon-derived nanomaterials are considered promising candidates for nanozyme development. The unique surface groups, number of active sites, and electronic structures of carbon enable carbon-based nanozymes to possess OXD, CAT, POD, or superoxide dismutase (SOD) enzymes-like activities (Sun et al., 2023). Moreover, showing high biocompatibility as well as relevant optical and thermal properties, carbon-based nanozymes have recently gained much attention as potential tools for biomedical applications, and the main criteria for their applications can be summarized as follows: catalytic mechanism/efficiency, controllable design (size, geometry, morphology), and controllable biocompatibility (Ding et al., 2021).

This chapter primarily focuses on the colorimetric detection of H_2O_2, specifically nanozymes exhibiting POD catalytic reactions, based on the simultaneous oxidation of an electron-donating substrate and reduction of H_2O_2, used for the colorimetric detection of H_2O_2. Literature findings indicate the use of carbon nanodots (CD) with high surface energy and surface/volume ratios, as well as GO quantum dots, as nanozymes. Additionally, these carbon allotropes, owing to their distinctive properties, can interact strongly with the surrounding environment and are often incorporated into composites with metals (e.g., Pt (Cui et al., 2007; Glebova et al., 2019), Cu (Cantürk and Kováčik, 2022; Sundaram et al., 2018), Co (Chaudhary et al., 2022; Wei et al., 2015; Mayani et al., 2012)), or Au (Lawson et al., 2008; Mayani et al., 2012) NPs/structures. Table 13.2 presents a compilation of composite structures involving a variety of materials and carbon allotropes (such as carbon dots/quantum dots (Guo et al., 2022; Su et al., 2020, 2022),

Table 13.2 Carbon-based nanozymes and their applications as POD.

Nanozymes	LOD	Selectivity	Applications	Conditional parameters	Reference
CuO-g-C_3N_4 nanocomposites	1.2 μM	–	– Aqueous solution – Dual (electrochemical and fluorescence) detection	Acetate buffer solution (pH 4.2)	Zhu et al. (2018)
PtCNPs	0.15 μM	Glucose	Aqueous solution	Acetate buffer solution (pH 3.6)	Bao et al. (2019)
Fe-N-C SAzymes	–	–	In vitro detection in HeLa cells	Acetate buffer solution (pH 3.0)	Jiao et al. (2019)
Hemin@CD	0.11 μM	Glucose, xanthine	– Aqueous solution – Dual (colorimetric and fluorescence) detection	Deionized water	Su et al. (2020)
GO/AuNPs nanocomposites	0.042 μM	Glucose	– Aqueous solution	Acetate buffer solution (pH 4.0).	Qi et al. (2020)
FeS NPs embedded in 2D carbon nanosheets	0.78 μM	–	– Aqueous solution – Antioxidant capacity – Dual (colorimetric and fluorescence) detection	Acetate buffer solution (pH 4.0)	Song et al. (2022)
Graphdiyne oxide quantum dots	1.5 μM	Cysteine	– Aqueous solution – Sensing biological antioxidants	Acetate buffer solution (pH 4.0)	Guo et al. (2022)
Co- and N-doped CDs	0.15 μM	Xanthine, cholesterol	– Aqueous solution – Dual (colorimetric and fluorescence) detection – In vitro detection in HeLa cells – In vivo detection	Neutral buffer solution	Su et al. (2022)
Fe-N–C SAzymes	4.36 μM	Glutathione	– Aqueous solution – Human serum	Acetate buffer solution (pH 4.0)	Lu et al. (2022)
Pt single atoms modified carbon nitride nanorod	1 μM	–	– Aqueous solution – Antibiotic detection – Antibacterial therapy – In vivo application	Acetate buffer solution (pH 4.0)	Fan et al. (2022)
2D-1D MoS_2-C nanotubes	1.4 μM	–	Food analysis (in soda water)	Acetate buffer solution (pH 4.0)	Zhang et al. (2022)
Fe_3O_4-Fe^0/Fe_3C	67.1 pM	–	– Aqueous solution – Food (in milk samples) – In vitro detection in HepG2 cells	Acetate buffer solution (pH 4.0)	Baye et al. (2023)
Ternary MIL-101(Fe)@ P_2W_{18}@SWNT	0.3 μM	Glucose	Aqueous solution	Acetate buffer solution (pH 3.5)	Liu et al. (2023)

single-atom nanozymes (SAzymes) (Jiao et al., 2019; Lu et al., 2022)) for the purpose of offering insights into the utilization of carbon-based nanozymes and their applications, specifically in colorimetric detection of H_2O_2. As a crucial indicator of disease processes, the detection of H_2O_2 is vital in diagnostic methods. Hence, Jiao et al. conducted a preliminary investigation to evaluate the effectiveness of their newly designed nanozyme framework, Fe-N-C SAzymes, through the in situ detection of H_2O_2 generated by HeLa cells (1.9 mM H_2O_2 for 10^6 cells/plate) (Jiao et al., 2019). Similarly, Baye et al. suggested the use of Fe_3O_4-Fe^0/Fe_3C, for a colorimetric detection of H_2O_2, where they used milk and the HepG2 cells for their detection assay (Baye et al., 2023). Both investigations illustrated the feasibility of Fe-C-based nanozymes for detecting H_2O_2 within living cells. In addition to the live cell applications, the POD-like activities of Fe-embedded carbon-based nanosheets (Song et al., 2022), carbon nanotubes (Liu et al., 2023), or SAzymes (Lu et al., 2022) were tested in buffer solutions. As such, the enzyme-mimicking characteristics of Fe-N-C SANs were thoroughly examined by Lu et al., utilizing their POD-like behavior for the purpose of detecting H_2O_2 (LOD: 4.26 μM) and glutathione (LOD: 100–400 μM) (Lu et al., 2022). Song et al. used FeS NPs embedded in 2D carbon nanosheets as nanozymes, known for their strong POD-like capabilities, to develop a colorimetric (LOD: 0.78 μM) and fluorescence (LOD: 0.86 μM) detection technique. Liu et al., on the other hand, benefited from the single-walled carbon nanotube (SWNT) features for the colorimetric detection of both H_2O_2 (LOD: 0.3 μM) and glucose (LOD: 0.2 μM) (Liu et al., 2023). While carbon-based nanozymes were used in combination with Fe additives, combinations with structures such as Au (Qi et al., 2020), Co (Su et al., 2022), Cu (Zhu et al., 2018), Pt (Bao et al., 2019; Fan et al., 2022), or Mo (Zhang et al., 2022) have shown the ability to further reduce the LOD for H_2O_2. Indeed, Qi et al. demonstrated the POD-mimicking activity of GO/Au NPs nanocomposites for colorimetric detection of H_2O_2 (LOD: 42 nM), where the TMB substrate was oxidized, resulting in a blue product. Additionally, they reported the colorimetric analysis of glucose in human serum by using the same nanozyme combined with the oxidation reaction of glucose oxidase (Qi et al., 2020). In a similar manner, Zhu et al. have described CuO-g-C_3N_4 nanocomposites as nanozymes with POD-like properties. These nanozymes exhibit a dual effect, enabling both electrochemical and colorimetric detection (LOD: 1.2 μM) (Zhu et al., 2018). Notably, another dual (colorimetric (LOD: 0.11 μM) and fluorometric (LOD: 0.15 μM)) sensing system for H_2O_2 based on Hemin@carbon dots was reported, where ABTS, TMB, and OPD were used to prove POD-like activity (Su et al., 2020). In another study, Co- and N-doped carbon dots nanozymes (LOD: 0.15 μM), having enhanced POD-like activity and biocompatibility under neutral pH conditions, were used for monitoring the in vivo levels of endogenous H_2O_2 (Su et al., 2022). Similar LOD was reported with the Pt-doped carbon NPs by Bao et al., where they focused to the sensitive and selective detection of H_2O_2 (LOD: 0.15 μM), and glucose (LOD: 0.3 μM) (Bao et al., 2019).

Addressing both environmental and biological applications, Fan et al. presented a study that examined the colorimetric detection of H_2O_2 (LOD: 1 μM) as well as antibiotic detection and antibacterial therapy. Researchers introduced novel viewpoints on the development of highly efficient nanozymes, using SAzyme, which based on Pt single

atoms modified carbon nitride nanorods (Fan et al., 2022). Notably, in addition to its applications in the environment and biology, H_2O_2 is widely utilized in the agricultural and food industries, and it is commonly used in beverage production as a stabilizer. Therefore, it is crucial to detect any residual H_2O_2 in food and beverages. To evaluate the analytical performance of their colorimetric detection platform, Zhang et al. chose to use MoS_2-CNT, for H_2O_2 (LOD: 1.4 μM) which has a layered structure like graphite, as a nanozyme example that could be applied in food analysis (Zhang et al., 2022). Overall, it is important to emphasize that carbon-based nanozymes are rapidly evolving into versatile nanoplatforms that harness the inherent enzyme-like capabilities and distinctive physicochemical characteristics of carbon-based materials. Overall, it is obvious that the use of carbon-based nanozymes has garnered substantial attention in biomedical applications, stretching beyond detection to encompass areas such as environmental applications, imaging/targeting, and therapeutic interventions.

13.3.3 Hybrid/organic nanozymes

The catalytic activity of nanozymes can be enhanced by the hybridization of metal nanocomposites with organic molecules including porous organic polymers, fused aromatic dyes, heteroatom-containing natural ligands and biomolecules. Table 13.3 summarizes

Table 13.3 Hybrid/organic-based nanozymes and their applications.

Nanozymes	LOD	Selectivity	Applications	Conditional parameters	Reference
[a]MIL-53(Fe)	0.13 μM	Ascorbic acid	Aqueous solution	Acetate buffer solution (pH 4.0)	Ai et al. (2013)
[a]FePPOP-1	6.5 μM	Glucose, ascorbic acid, gallic acid, tannic acid	Aqueous solution	Acetate buffer solution (pH 3.8)	Cui et al. (2018)
[a,b]Fe_3O_4-SL	0.139 μM	Glutathione	Aqueous solution	Acetate buffer solution (pH 4.0)	Liu et al. (2021)
[a]FeCu@2-DG	20 cell	–	– Aqueous solution – In vitro detection of exogenous and endogenous H_2O_2 in HepG2 and LO2	Acetate buffer solution	Singh et al. (2022)
[a]SiO_2 @TiO_2/PDI-OH	0.076 μM	Sarcosine	Aqueous solution	Acetate buffer solution (pH 4.0)	Liu et al. (2022b)
[a]2D CycLoDextrin-MOF	60.0 μM	Glucose	– Aqueous solution – In vitro detection in A549, MLE-12, and HepG2 cells	Acetate buffer solution (pH 4.0)	Tan et al. (2022)
[a]CCNPs	54.4 μM	Glucose	Aqueous solution	DI water	Lee et al. (2022)

Continued

Table 13.3 Hybrid/organic-based nanozymes and their applications—cont'd

Nanozymes	LOD	Selectivity	Applications	Conditional parameters	Reference
[a]Zr-MOF-PVP	2.76 μM	Phenol	Aqueous solution	PBS buffer solution (pH 4.0)	Wang et al. (2022a)
[a]CuPd@MIL-101	0.043 μM	Glucose	Aqueous solution	Acetate buffer solution (pH 4.0)	Yang et al. (2021)
[a]HSA@PDA/Fe NCs	0.062 μM	–	– Aqueous solution – In vitro detection in MCF-7 and SMCC-7721 cells	Acetate buffer solution (pH 4.5)	Liu et al. (2020)
[a]Hb-AuNP	1.95 μM	Glucose and ascorbic acid	Aqueous solution	PBS buffer solution (pH 4.0)	Kanwal et al. (2023)
[a]FeP-pSC4-AuNPs	25 μM	–	– Aqueous solution – In vitro detection in HeLa Cells	Acetate buffer solution (pH 4.0)	Hu et al. (2021)
[a]Fe-AL	54 μM	–	Aqueous solution	Acetate buffer solution (pH 5.0)	Li et al. (2021)
[a]FePPOPs-SO3H	26.7 μM	Glucose	Aqueous solution	Acetate buffer solution (pH 3.8)	Liu et al. (2019b)
[a]Ni_xFe-MOF*	0.59 μM	Glutathione	Aqueous solution	Acetate buffer solution (pH 3.6)	Cheng et al. (2022)
[c]Ce-MOF (MVCM)	3.25 μM	–	– Aqueous solution – Commercial disinfectants (AL), tap water, milk, and contact lens solutions	Acetate buffer solution (pH 4.5)	Cheng et al. (2021)
[a,b]MOF(Co/2Fe)	5 μM	–	Aqueous solution	Acetate buffer solution (pH 3.5)	Yang et al. (2017)
[a,b]Pt-CMP	0.54 μM	Glucose	Aqueous solution	Phosphate buffer solution (pH 4.0).	Wang et al. (2019)
[a]c-myc TBA Cu/Ag NCs	7.42 μM	Glucose	Aqueous solution	Acetate buffer solution (pH 4.5)	Du and Wei (2020)
[a]PB/Au fibers	3.4 μM	Uric acid	Aqueous solution	Acetate buffer solution (pH 4.0)	Li et al. (2022)

[a] Peroxidase (POD).
[b] Oxidase (OXD).
[c] Haloperoxidase (HPOD).

recently reported hybrid/organic-based nanozymes and their applications. The construction of hybrid nanozymes provides relatively simple, low-cost, and robust approaches for mimicking natural enzymes. Metal–organic frameworks (MOFs)-based hybrid nanozymes are important materials consisting of metal ions or clusters coordinated with organic ligands. These molecules have gained significant attention due to their high surface area, well-defined coordination template, and tunable porosity that make them suitable for various applications, including catalysis, gas storage, drug delivery, and sensing. Ai et al. have demonstrated the synthesis of MIL-53(Fe) nanozyme in which MOF is constructed by the coordination of terephthalate to Fe ions. MIL-53(Fe) has exhibited POD-like activity and successfully oxidized the TMB as well as OPD and 1,2,3-trihydroxybenzene (THB) in the presence of H_2O_2 (Ai et al., 2013). Similarly, Wang et al. showed the enhanced POD-like activity of polyvinylpyrrolidone-capped Zr(IV)-based MOFs (Zr-MOF-PVP) as a simple tool for H_2O_2 and phenol detection in solution (Wang et al., 2022a). In addition to single metallic MOFs-based nanozymes, bimetallic derivatives (CuPd@MIL-101, Ni_xFe-MOF*, MOF(Co/2Fe)) have also demonstrated good POD-like activity for colorimetric detection of H_2O_2 in buffer systems (Yang et al., 2017, 2021; Cheng et al., 2022). Additionally, it is possible to use MOF-based nanozymes to monitor intracellular H_2O_2. Tan et al. developed the green CycloDextrin–MOF-based nanozymes (CD-MOF) that enable the detection of H_2O_2 in acetate buffer as well as A549, MLE-12, and HepG2 cells (Tan et al., 2022). In addition to POD-like activities of MOF nanozymes, Cheng et al. developed a halo-POD-mimicking nanozyme, Ce-MOF (MVCM), which catalyzes the oxidation of phenol red (PR) to produce a ratiometric colorimetric signal (from yellow to blue) in the presence of both H_2O_2 and Br ions (Cheng et al., 2021). The system detects H_2O_2 in real samples including commercial disinfectants, tap water, milk, and contact lens solutions. Similar to MOF nanozymes, organic polymer-based nanozymes offer many advantages such as porosity, high surface area, and high biocompatibility. Cui et al. demonstrated the synthesis and POD-like activity of porphyrin-based porous organic polymer, FePPOP-1, which allows the detection of H_2O_2, glucose, and three antioxidants (AA, GA, and TA) in aqueous solutions (Cui et al., 2018). Liu et al. benefited from the hydrophilic porous structure of a metalloporphyrin-based polymer that enhanced the stability and provided increased catalytic recyclability of nanozyme FePPOPs-SO_3H (Liu et al., 2019b). In another study, Lee et al. developed a strategy to enhance the catalytic activity of the multibranched Au-Ag NPs by coating them with Pt and a natural organic polymer named chitosan. The synthesized chitosan-capped multibranched Au-Ag-Pt NPs (CCNPs) were revealed to mimic POD-like activity to detect H_2O_2 through TMB oxidation at μM level (LOD: 54 nM). In addition, CCNPs were also able to detect glucose in buffer solution and human serum samples (Lee et al., 2022). Another natural polymer sodium lignosulfonate (SL) was used as a surfactant to improve the shortcomings of Fe_3O_4 NPs including water solubility and catalytic activity. Fe_3O_4-SL NPs were synthesized through the solvothermal method in which the use of SL allows control of size and morphology. The developed catalytic system enables to detection of H_2O_2 through the formation of blue-colored oxTMB in a buffer solution (Liu et al., 2021). In another study, Li et al. first

aminated the lignin biomacromolecule and then doped ferric ions to construct Fe-N-C SAzymes, which aimed to mimic natural enzymes active centers. In addition to amine groups of lignin, the presence of hydroxyl and carboxyl groups provided extra stability for the coordination of Fe^{3+} ions that increased the stability and reusability of nanozyme in catalytic detection of H_2O_2 (Li et al., 2021). Recently, biomacromolecules and organic small molecules have attracted significant interest in constructing hybrid nanozymes for H_2O_2 detection. In 2019, Wang et al. developed ultrasmall Pt-based nanozymes utilizing the coordination ability of four natural nucleotides for the first time. Here, nucleotides acted both as stabilizing and as reducing agents, and their chemical composition plays a crucial role in the catalytic activity of nanozyme. Among the developed nanozymes, Pt-CMP (Pt-5′-cytidine monophosphate) has a high sensitivity for both H_2O_2 and glucose (Wang et al., 2019). In another study, Du and Wei used DNA oligomers as templates for the construction of Ag and Cu/Ag nanocomposites. All DNA-NCs demonstrated POD-like activity, and c-myc TBA Cu/Ag NCs (DNA contains cytosine-rich sequences) were investigated for quantification of H_2O_2 through TMB oxidation (LOD: 7.42 μM) (Du and Wei 2020). Proteins-bearing heteroatoms to be suitable for metal coordination sites can also be used as potential templates in nanozyme design. Liu et al. synthesized hybrid nanozyme system HSA@PDA/Fe NCs that were obtained by anchoring catalytic center (Fe (III)/Fe(II)) on human serum albumin@polydopamine framework. In this nanozyme approach, the decoration of HSA@PDA to the metal center not increased only the catalytic activity with respect to free Fe(III) but also enhanced the stability under robust conditions. The HSA@PDA/Fe NCs exhibited high catalytic activity in converting TMB to oxTMB in the presence of H_2O_2 with an nM detection limit (LOD = 62 nM). Importantly, the HSA@PDA/Fe NCs were able to monitor intracellular H_2O_2 in situ generated by the addition of phorbol-myristate-acetate both in MCF-7 (human breast adenocarcinoma) and SMMC-7721 (the human hepatocellular carcinoma) cell lines (Liu et al., 2020). A significant example of the use of proteins in nanozyme design was demonstrated by Kanwal et al. (2023). In their study, they developed a method for one-step green synthesis of Hb-AuNPs in which hemoglobin acts as a template, reducing agent, and stabilizer. The Hb-AuNPs revealed 12-fold higher POD-like activity than horseradish peroxidase (HRP) with 1.95 μM LOD (Kanwal et al., 2023). In addition to the aforementioned proteins, their active sites can be mimicked by small molecules in nanozyme design. Hu et al. developed a nanozyme assembly (FeP-pSC4-AuNPs) by mixing cationic metalloporphyrin (FeP) and *para*-sulfonatocalix[4]arene (*p*SC4) stabilized AuNPs (Hu et al., 2021). The FeP-pSC4-AuNPs successfully detected the presence of H_2O_2 in solution and cellular media (HeLa cells) both calorimetrically and electrochemically. Singh et al., on the other hand, decorated the iron-copper nano-catalyst with 2-deoxy-D-glucose (2-DG), that targets the overexpressed GLUT receptors in many cancer cells, to generate an effective and targeted nanozyme system for H_2O_2 detection. The H_2O_2 detection performance of nanozyme was demonstrated in an aqueous solution and cellular media (HepG2 and LO2 cell lines). It is important to note that the targeting strategy provided high selectivity toward cancer cells over normal cells and detected H_2O_2 in HepG2 cells with LOD = 20 cells/mL (Singh et al., 2022).

In the abovementioned approaches, developed methods use only hybrid nanozymes to detect the presence of H_2O_2 through the formation of color change after oxidation of TMB, ABTS, and PR. However, in some circumstances, a second reaction parameter is needed to accelerate the catalytic activity and sensing performance of nanozymes. Liu et al. developed a nanozyme structure, SiO_2@TiO_2/PDI-OH, demonstrating significant POD-like activity in the presence of LED light (Liu et al., 2022a,b). They performed DFT calculations for the catalytic activity SiO_2@TiO_2/PDI-OH and revealed the importance of the contribution of the structure of photosensitizer and the yolk-shell structure of nanozyme. Additionally, the designed colorimetric system could sensitively detect the presence of H_2O_2 (LOD = 76 nM) and sarcosine (LOD = 120 nM) under LED light irradiation in real samples. It is also possible to adapt nanozymes to wearable biosensing platforms to real-time monitor the levels of disease-related compounds. In this perspective, Li et al. demonstrated the use of a flexible sensing platform composed of Au nanowires and Prussian blue loaded onto stretchable styrene-ethylene-butylene-styrene fibers. The fiber-based nanozyme system exhibited good POD-like activity and allowed colorimetric detection of both H_2O_2 and uric acid in the buffer system (Li et al., 2022).

13.4 Challenges and future perspective

Generated with the development of nanotechnology and engineered at the nanoscale, nanozymes increase efficiency in various applications by increasing the properties of enzymes, such as activity, through their nano-sized structures. Given the substantial demonstrated potential of nanozymes across various biotechnology and biomedical applications, particularly in POC diagnostics, there is a growing interest in utilizing them for detecting biologically and environmentally significant molecules, such as H_2O_2. Although the general colorimetric detection methodology for H_2O_2 is based on the reaction between TMB and HRP, POD-like nanozymes intensively increased the limitation caused by HRP (e.g., low stability) and provided better solutions and resolved the drawbacks. Indeed, nanozymes offer several advantages in detection by enhancing the reaction rate by swiftly decomposing H_2O_2 due to their remarkable catalytic activity. Moreover, their long-term stability and reusable nature improve their potential in many applications. However, their utilization may be constrained by the intricate and time-consuming process of functionalizing nanozymes due to the materials used for synthesizing, which can limit their scalability and commerciality. Hence, a standardization method should be established to explore the potential of nanomaterials not yet used in nanozyme construction.

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