Recent Advances on Nanozyme-based Electrochemical Biosensors

Xiaoyu Wang,[a] Shaojun Dong,[b] and Hui Wei*[a]

Abstract: Nanozymes are nanomaterials with enzyme-like catalytic activities. The unique features of nanozymes (such as high stability, low cost, large surface area for bioconjugation, ease of storage, and multi-functionalities) offer unprecedented opportunities for designing electrochemical biosensors. Recent years have witnessed the rapid development of nanozyme-based electrochemical biosensors. To highlight these achievements, this review first discusses the representative nanozymes including peroxidase mimics, oxidase mimics, hydrolase mimics, and superoxide dismutase mimics used in electrochemical biosensors. Then, it summarizes the bioanalytical applications for the detection of various analytes. Finally, current challenges and future research directions are summarized.

Keywords: Nanozymes · Electrochemical detection · Biosensors · Minireview

1 Introduction

As a subclass of chemical sensors, electrochemical biosensors are devices consisting of a biological target recognition element and an electrochemical signal transduction element [1–2]. In the presence of a target analyte, the biological recognition element (e. g., enzymes, antibodies, or aptamers) of electrochemical biosensors first selectively reacts with the specific analyte, and the devices subsequently transduce the biological recognition events into detectable electrical signals [2–5]. Electrochemical biosensors play crucial roles in bioanalysis because of their distinct advantages, including high sensitivity and selectivity, ease of miniaturization for point-of-care diagnostics, simplicity of construction, etc. [2–5]. According to the different biological recognition processes, electrochemical biosensors can be mainly classified into two categories [2]: (1) biocatalytic sensors and (2) affinity sensors. Biocatalytic sensors primarily use enzymes as biological recognition elements to react with a target analyte and then generate electroactive species for signal amplification. Electrochemical affinity sensors are based on the specific affinity reactions between the target analyte and biological component (e. g., DNA hybridization, or antibody-antigen complexation), which will result in the changes in electrochemical signals. In affinity sensors, enzymes are usually used as catalytic tags for signal amplification. Therefore, enzymes have played vital roles in both biocatalytic sensors and affinity sensors. However, the intrinsic disadvantages (such as low operational stability, high production cost, low tolerance to harsh conditions, and difficulty in storage) of enzymes have significantly limited their further use for electrochemical biosensors [3].

To address these limitations of natural enzymes, artificial enzymes using alternative materials to imitate the unique catalytic activities of enzymes have been developed [6]. Among different artificial enzymes, nano-materials with enzyme-like characteristics (i. e., nanozymes) are regarded as next-generation artificial enzymes due to their unique advantages (e. g., low cost, high stability, and ease of storage) over natural enzymes and conventional molecular or polymeric enzyme mimics [7–15]. In addition, emerging from their nanoscale sizes, nanozymes are endowed with unique features in terms of large surface area for bioconjugation, self-assembly abilities, and multi-functionalities (e. g., magnetic properties and surface plasmon resonance) beyond catalysis [16]. Therefore, using nanozymes as the substitutes for natural enzymes would significantly promote the sensing performance of electrochemical biosensors. First, nanozymes can be used as electrode modifiers and catalytic labels to replace natural enzymes, which would improve the stability and lower the cost of electrochemical biosensors. Second, some unique physicochemical properties of nanozymes beyond catalysis would also significantly benefit the sensing performance of electrochemical biosensors. For example, a large surface area of nanozymes can be used for bioconjugation with biological recognition elements (e. g., antibodies or aptamers). The excellent

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electron conductivity of carbon- and metal-based nanozymes would significantly improve the interfacial electron-transfer kinetics. Moreover, the magnetic properties of some iron-based nanozymes offer great opportunities for analytes’ separation and purification. Along with the significant advances made in the field of nanozymes, electrochemical biosensors based on nanozymes have been extensively studied for various analytes ranging from ions and small molecules to nucleic acids, proteins, exosomes, cells, etc. [17–20].

The goal of this review is to highlight recent advances in nanozyme-based electrochemical biosensors. We will first introduce the representative nanozymes (i.e., peroxidase mimics, oxidase mimics, hydrolase mimics, and superoxide dismutase mimics) used in electrochemical biosensors. Then, we will focus on the recent advances of applications in nanozyme-based electrochemical biosensors for detecting various target analytes. Finally, we highlight the challenges facing nanozyme-based electrochemical biosensors and discuss the future research directions to further advance the field.

2 Representative Nanozymes used in Electrochemical Biosensors

2.1 Peroxidase Mimics

We first introduce the peroxidase-like nanozymes because most of electrochemical biosensors are constructed by using peroxidase mimics. As shown in Scheme 1, peroxidase can catalyze the oxidation of reducing substrates with the assistance of peroxides (e.g., hydrogen peroxide, \( \text{H}_2\text{O}_2 \)) [21]. Peroxidase-like nanozymes have attracted widespread attention since Yan et al. first discovered the unexpected peroxidase-like activity of \( \text{Fe}_3\text{O}_4 \) magnetic nanoparticles (MNP) [22]. To date, hundreds of nanomaterials, including metal oxide-based (e.g., \( \text{Fe}_2\text{O}_3 \), \( \text{Co}_3\text{O}_4 \), and \( \text{V}_2\text{O}_5 \)), metal-based (e.g., Pt, Ru, Pd, and Ir), carbon-based (e.g., graphene oxides, graphene quantum dots, and carbon nitride), metal-organic framework (MOF)-based (e.g., two-dimensional (2D) M-TCPP(Fe) nanosheet MOFs, M=Co, Zn, and Cu), and single atom-based (e.g., ZIF-8 derived carbon nanospheres with a zinc-centered porphyrin-like structure) have been discovered with peroxidase-like catalytic activities [7, 9, 23]. These peroxidase mimics have been applied in various fields, such as biomedical detection, therapy, and environmental protection [7, 9]. However, most of peroxidase mimics possessed moderate catalytic activity, which was generally inferior to their natural counterparts [7, 9]. The catalytic activities of peroxidase mimics directly affect the detection sensitivity of nanozyme-based electrochemical biosensors. To improve the detection performance of electrochemical biosensors, great efforts have been devoted to engineering the nanozymes’ catalytic activities. Several factors, including composition, size, morphology, and facet, have been demonstrated to modulate the peroxidase-like activities of nanozymes [24]. For example, Xia et al. obtained nickel-platinum nanoparticles (Ni-Pt NPs) with a record high peroxidase-like catalytic efficiency by doping nickel into platinum to form nickel-rich cores and platinum-rich shells [25]. Yan et al. found that the smaller the size of \( \text{Fe}_3\text{O}_4 \) MNPs (30 nm, 150 nm, and 300 nm), the higher

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\text{AH}_2 + \text{ROOH} \xrightarrow{\text{Peroxidase}} \text{A} + \text{ROH} + \text{H}_2\text{O}
\]

Scheme 1. The reaction catalyzed by peroxidase.

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the peroxidase-like activities [22]. By using density functional theory (DFT) calculation, Gao et al. demonstrated that Au with [211] facets exhibited the highest peroxidase-like activity because it possessed the lowest activation energy barriers of rate-determining step [26]. In addition to composition and structure factors, surface modifications also played important roles in improving nanozyme’s catalytic activity [27–29].

Even though various strategies were used for regulating the peroxidase-like activities of nanozymes, the relationships between these factors and catalytic activities were ambiguous. To rationally design the peroxidase-like nanozyme’s catalytic activity [27–29].

With an average diameter of 3.5 nm exhibited unexpected glucose oxidase-like activity [33]. Dong et al. demonstrated that the process of glucose oxidation catalyzed by Au NPs is the same as that of their natural counterpart (i.e., a two-step reaction involving the dehydrogenation of glucose and the subsequent two electrons reduction of O₂ to H₂O₂) [34]. In addition, crown-jewel-structured Au/Pd nanoclusters (NCs) with active Au atoms decorated on Pd NCs possessed enhanced catalytic activity for catalyzing glucose oxidation to generate gluconic acids and H₂O₂ [35]. In addition to Au-based nanomaterials, modified carbon nitride and V₂O₅ nanobelts were discovered to have glucose oxidase-like activities [9,36–37]. Several laccase substrates (e.g., phenol, hydroquinone, naphthol, catechol, and epinephrine) could be catalytically oxidized by multicopper laccase mimicking nanozymes. Multicopper laccase can be mimicked not only by copper-containing nanomaterials (e.g., copper-containing carbon dot and copper coordinated nucleotides) but also by some non-copper nanomaterials (e.g., nanoceria and Pt NPs) [9]. MoO₃ NPs were found to have sulfite oxidase (SuOx)-like activity, which could convert sulfite to sulfate under physiological conditions [38]. Further studies suggested that MoO₃ NPs could serve as SuOx alternatives for SuOx knockdown liver cells.

A strategy for rational design of oxidase mimics was also developed [39]. Based on a protein engineering inspired strategy, a Hammett-type structure-activity linear free energy relationship was revealed for MOF-based oxidase mimics by combining experimental results with DFT calculations (Figure 2A) [39]. The oxidase-like activities of MOF-based nanozymes increased with the increasing Hammett 𝜎 value with electron-withdrawing ligands. MIL-53(Fe)-NO₂ with the strongest electron-withdrawing NO₂ substituent exhibited the highest

![Image](https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/elan.202100684)
oxidase-like activity (Figure 2). Moreover, the Hammett-
type structure-activity linear free energy relationship was
also extended to other MOF types.

2.3 Hydrolase Mimics

Hydrolases can catalyze the hydrolysis of chemical bonds
with various substrates, including esters, phosphoesters,
amides, carbohydrates, etc. For example, CeO$_2$
with deoxyribonuclease (DNase) I-like catalytic activity could
cleave single stranded oligonucleotides into shorter frag-
ments [40]. Zr$_6$-based MOF NU-1000 could catalyze the
hydrolysis of phosphate ester bonds, which was subse-
quently used for the destruction of the nerve agent
simulant (i.e., dimethyl 4-nitrophenyl phosphate, DMNP)
and the highly toxic chemical warfare agent (i.e., O-
pinacolyl methylphosphonofluoridate, also known as So-
man) [41].

A strategy for data-informed discovery of highly active
hydrolase mimics was developed [42]. Two key factors
(i.e., Lewis acidity of the metal clusters and the length of
ligands) for design of MOF-based hydrolase mimics were
identified by data-informed analysis. Based on these two
key factors, Ce-FMA-MOF-based hydrolase mimics with
excellent phosphatase-, protease-, and glycosidase-like
activities were constructed [42].

2.4 Superoxide Dismutase Mimics

Superoxide dismutase (SOD) can catalyze the dismuta-
tion reaction of O$_2^*$ to H$_2$O and O$_2$ (Scheme 3).
Representative nanomaterials, including carbon-based,
cerium-based, and melanin-based nanozymes, have been
demonstrated to act as SOD mimics.

3 Nanozymes-based Electrochemical Assays

3.1 Ions

Hg$^{2+}$ and Pb$^{2+}$ were detected with nanozyme-based
electrochemical biosensors by exploring their effects on
the DNA hybridization reactions [43–44]. As shown in
Figure 3A, DNA functionalized iron-porphyrinic MOF
(Gr-$5/(Fe-P)n$-MOF) can serve as a nanoprobe for Pb$^{2+}$
detection [44]. Gr-$5$ in the nanoprobe could be specifi-
cally cleaved by Pb$^{2+}$ at the ribonucleotide (rA) site to
expose the short (Fe-P)$n$-MOF-linked oligonucleotide
fragment. Then, the peroxidase-like (Fe-P)$n$-MOF was
brought to the electrode surface by a hybridization
reaction between a short oligonucleotide fragment with
hairpin DNA pre-immobilized on the surface of a screen-
printed carbon electrode (Figure 3B). Electrochemical
sensing signals were obtained by using (Fe-P)$n$-MOF to
catalyze the oxidation of TMB in the presence of H$_2$O
(Scheme 3) [44]. In addition to metal ions, As(V) could
be detected by inhibiting the peroxidase-like activity of
cobalt oxyhydroxide (CoOOH) nanoflakes directly [45].
Qiu et al. found that CoOOH nanoflakes could catalyze
the oxidation of ABTS with assistance of H$_2$O. However,
As(V) could specifically bind onto CoOOH nanoflakes
via electrostatic attraction and As–O bond interaction,
resulting in the decreased catalytic activity of CoOOH
nanoflakes. By using CoOOH nanoflakes as electrode
modifiers, electrochemical detection of As(V) was
achieved [45]. Based on the inhibitory effect on oxidase-
like histidine-capped gold nanoclusters, nitrite was detected by using electrochemical sensors [46]. In addition, electrochemical determination of superoxide anions was accomplished by using SOD-like nanozymes (e.g., Mn$_3$P$_2$O$_7$, Mn$_3$(PO$_4$)$_2$, Co$_4$(PO$_4$)$_2$, and FePO$_4$) [47–51]. Because the electrochemical biosensors possessed high sensitivity and provided a reliable platform to adhere living cells directly on the modified electrode surface, in situ monitoring superoxide anions released from living cells was successfully achieved (Figure 3C) [48].

### 3.2 Small Molecules

Peroxidase-like nanozymes can be used as electrode modifiers for the determination of H$_2$O$_2$ because they can catalyze the reduction of H$_2$O$_2$ [52–57]. For example, electrochemical detection of H$_2$O$_2$ was achieved by using Prussian Blue as an artificial peroxidase to modify a glassy carbon electrode (GCE) [53]. The H$_2$O$_2$ could be reduced by Prussian Blue to generate cathodic current for signaling. Likewise, Dong et al. fabricated an electrochemical H$_2$O$_2$ sensor by layer-by-layer assembling Fe$_3$O$_4$ NPs and poly(diallyldimethylammonium chloride) (PDDA) on the electrode surface [58]. Compared with enzymatic biosensors, the Fe$_3$O$_4$-based sensor exhibited outstanding stability [58]. Moreover, because of the high sensitivity of electrochemical biosensors, extracellular H$_2$O$_2$ with a low concentration released from cells under stimulation was successfully monitored [59–62]. For example, electrochemical sensing of H$_2$O$_2$ was performed by using Au nanoflowers/Fe$_3$O$_4$@ZIF-8-MoS$_2$ nanocomposites as peroxidase mimics and H$_2$O$_2$ concentration released from H9C2 cardiac cells under ascorbic acid (AA) stimulation was successfully determined (Figure 4A and 4B) [59]. By coupling the oxidation of the substrate with their corresponding oxidases (or oxidase mimics) to generate H$_2$O$_2$, some oxidase substrates (e.g., glucose and lactate) can be detected by the aforementioned nanozyme-based electrochemical H$_2$O$_2$ sensors [63–65]. Mao et al. demonstrated that simultaneous and selective online detection of glucose and lactate could be achieved by combining glucose oxidase (GOx)/lactate oxidase (LOx) with peroxidase-like Prussian Blue (Figure 4C) [65]. GOx/Prussian Blue and LOx/Prussian Blue were modified on a dual GCE. On the electrode, glucose or lactate was first oxidized by their corresponding oxidase to generate H$_2$O$_2$, and then the produced H$_2$O$_2$ was immediately reduced by Prussian Blue for signaling (Figure 4C). By integrating a continuous-flow electrochemical cell with the modified dual electrode, glucose and lactate in the rat microdialysate from the striatum were measured simultaneously (Figure 4C) [65].

Some small molecules, such as dopamine, uric acid, 3,4-dihydroxyphenylacetic acid (DOPAC), norepinephrine, and catechol, could be directly oxidized by using oxidase-like nanozymes to produce electroactive species for signaling [66–70]. Electrochemical detection of dopamine in human serum and artificial cerebrospinal fluid samples was successfully performed by using oxidase-like Co, N co-doped three-dimensional porous carbon composite [66]. Lin et al. synthesized ZIF-67/Cu$_{0.76}$Co$_{2.24}$O$_4$ nanospheres with excellent laccase-like activity (Figure 5) [68]. An online electrochemical system based on ZIF-67/Cu$_{0.76}$Co$_{2.24}$O$_4$ nanospheres was constructed for continuous monitoring of DOPAC. And the change of DOPAC in the brain microdialysate before and after ischemia of the rats’ brain was successfully monitored (Figure 5) [68]. In addition to oxidizing the target analytes, oxidase-like nanozymes can improve the selectivity of electrochemical biosensors by oxidizing some potential interference species [71]. Co-containing zeolitic imidazolate frameworks were developed with excellent ascorbate oxidase-
laccase-like activity. By incorporating the Co-containing zeolitic imidazolate frameworks into an online electrochemical system in the upstream of the detector, ascorbic acid, dopamine, and DOPAC could be eliminated, which enabled the selective detection of uric acid [71].

Other small molecules, including 8-hydroxy-20-deoxy-guanosine, pesticides, antibiotics (e.g., kanamycin), and sulfonamide, were also detected by nanozyme-based electrochemical assays [72–77].

3.3 Nucleic Acids

Several nanozyme-based electrochemical affinity biosensors for nucleic acid detection were developed [78–84]. These detection strategies were mainly classified into two categories. The first type was nanozymes as catalytic labels conjugated with biological recognition elements to form signaling nanoprobes. The second type was that nanozymes acted as electrode modifiers and their
peroxidase-like activity could be regulated by the interaction between the target analytes with their corresponding biological elements. As shown in Figure 6A and 6B, Lei et al. designed a sensitive electrochemical DNA detection platform by combining the peroxidase-like HKUST-1(Cu) with an allosteric switch of hairpin DNA [82]. Signaling nanoprobes were constructed by conjugating HKUST-1(Cu) nanozymes with streptavidin. In the presence of target DNA, the hairpin DNA bound to the target to induce the allosteric switch to form the streptavidin aptamer. Then, signaling nanoprobes could be adsorbed on the electrode surface by the specific interaction between streptavidin and the streptavidin aptamer. Finally, peroxidase-like HKUST-1(Cu) in signaling nanoprobes could catalyze the oxidation of OPD in the presence of H$_2$O$_2$, which in turn generated a detectable electrical signal for quantification of target DNA concentration (Figure 6A and 6B) [82]. A similar target DNA sensing principle was also developed by coupling peroxidase-like zirconium hexacyanoferrate (ZrHCF) MNPs with a reporter DNA [85]. Pre-adsorbed capture DNA on the electrode and reporter DNA could be complementary to the entire target DNA by the hybridization reaction, which allowed reporter DNA to be adsorbed on the electrode surface. Then, zirconium hexacyanoferrate could catalyze the reduction of H$_2$O$_2$ for signaling. Similarly, detection of global DNA methylation in colorectal cancer cell lines was successfully achieved by the conjugate of peroxidase-like mesoporous iron oxide and a 5-methylcytosine antibody (Figure 6C) [84]. The target DNA was first extracted and denatured to get single-stranded DNA (ssDNA) and was subsequently adsorbed onto the surface of a bare screen-printed gold electrode. Mesoporous iron oxide could be brought to the electrode surface because 5-methylcytosine antibody could specifically recognize the methylcytosine groups in ssDNA [84]. Mesoporous iron oxide could catalyze the TMB oxidation with the assistance of H$_2$O$_2$ to give an electrochemical signal for quantification of DNA methylation level (Figure 6C) [84].

In addition to DNA, microRNA could be detected by using nanozyme-based electrochemical affinity biosensors.

**Fig. 6.** (A) Synthesis of FeTCPP@MOF-SA composite and (B) Electrochemical DNA sensing via allosteric switch of hairpin DNA. (C) Schematic representation of the assay for electrochemical detection of global DNA methylation by combining mesoporous Fe$_3$O$_4$ nanozymes with 5-methylcytosine antibodies. (A) and (B) reprinted with permission from Ref. [82]. Copyright (2015) American Chemical Society. (C) Adapted with permission from Ref. [84]. Copyright (2018) Royal Society of Chemistry.

**Fig. 7.** Sensing principle of Plet-7a/MnO$_2$-based homogeneous electrochemical biosensor for miRNA assay. Adapted with permission from Ref. [81]. Copyright (2021) Elsevier.
Li et al. demonstrated that MnO$_2$ nanoflakes with oxidase-like activity could serve as electrode modifiers for catalyzing the oxidation of methylene blue (Figure 7) [81]. ssDNA (i.e., P$_{let-7a}$) would shield the active sites and thus decrease the oxidase-like catalytic activity after adsorbing onto the surface of MnO$_2$ nanoflakes. In the presence of microRNA (i.e., let-7a), P$_{let-7a}$ would be taken away from the surface by the hybridization reaction, resulting in the restored oxidase-like activity of MnO$_2$ nanoflakes (Figure 7) [81].

3.4 Proteins

Electrochemical immunosensors have been constructed by peroxidase- or hydrolase-like nanozymes for detecting proteins (e.g., thyroid-stimulating hormone, α-fetoprotein, squamous cell carcinoma antigen, Hepatitis B surface antigen, protein tyrosine kinase-7, cardiac troponin I, telomerase, p53 specific autoantibody, HER2, apolipoprotein E4, and prostate specific antigen) [86–97]. Shiddiky et al. developed electrochemical immunosensors by using peroxidase-like gold-loaded nanoporous ferric oxide nanocubes (Au-NPFe$_2$O$_3$NC) for detection of p53-specific autoantibody [94]. The autoantibody in plasma obtained from patients with epithelial ovarian cancer high-grade serous subtype was also successfully detected, demonstrating the clinical applicability of the electrochemical immunosensors [94]. It is worth to mention that Au-NPFe$_2$O$_3$NC nanozymes were also used for exosome detection, which will be discussed in the next section. In addition to peroxidase-like nanozymes, hydrolase mimics were also used for constructing sandwich-type immunoassay. Yang et al. found that the ester hydrolysis activity of Pt NPs could be activated in the presence of redox H$_3$N-BH$_3$ (Figure 8) [86]. The activation mechanism of...
hydrolysis activity was that metal hydrides are readily generated in the presence of H$_3$N-BH$_3$ to make Pt nanoparticle electron-rich, which might benefit nucleophilic attack of H$_2$O on the carbonyl group of an ester. An immunoassay for electrochemical detection of thyroid-stimulating hormone was performed by using ester hydrolase-like Pt NPs in the presence of H$_3$N-BH$_3$ (Figure 8) [86]. As a catalytic tag, the Pt nanozyme could catalyze the hydrolysis of 4-aminonaphthalene-1-yl acetate to produce electroactive 4-amino-1-naphthol on indium tin oxide (ITO) electrodes for signaling. As shown in Figure 8, due to the rapid ester hydrolysis and two redox cycling involving the 4-amino-1-naphthol, the immunosensors exhibited a superior electrochemical signal-to-background ratio even to natural enzymes, and as low as 0.3 pg/mL of thyroid-stimulating hormone could be successfully detected [86].

In addition to immunoensors, other electrochemical detection strategies were also developed for protein detection (e.g., uracil-DNA glycosylase, matrix metalloproteinase 2, urease, glycated albumin, and telomeres) [98–102]. Zhu et al. demonstrated that the peroxidase- and oxidase-like activities of trimetallic AuPtPd nanospheres could be regulated by urease-mediated proton-consuming enzymatic reactions [100]. Based on this principle, a self-regulated electrochemical bioassay for urease activity was developed and urease activity in spiked human saliva samples was successfully detected. In another study, determination of matrix metalloproteinase 2 was achieved by using oxidase-like Au@Pt bimetallic nanorods [99]. Matrix metalloproteinase 2 specifically
recognized peptides were first assembled on a GCE by an amide reaction and then Au@Pt bimetallic nanorods were immobilized through Au–S bonds. In the presence of matrix metalloproteinase 2, the peptides were cleaved to release the Au@Pt bimetallic nanorods from electrode surface, resulting in a sharply declined electrochemical signal [99].

3.5 Exosomes, Cells, and Bacteria

Exosomes, cells, and bacteria could be detected by combining a biological recognition element (e.g., an antibody or a ligand) with nanozymes. Biological recognition elements could be used for specifically recognizing the over-expressed receptors on exosomes, cells, and bacteria, while nanozymes could serve as catalytic tags for transducing electrochemical signals. For example, Au-NPFe₃O₄NC with peroxidase-like activity and superparamagnetic property was synthesized for exosome detection (Figure 9) [103]. Au-NPFe₃O₄NC was initially functionalized with CD63 antibody to form nanoparticles. Then, the nanoprobe was used for capturing the bulk population of exosomes in cell culture media. It is worth to mention that the superparamagnetic property of the nanoparticle can better help separate and purify the Au-NPFe₃O₄NC/CD63/exosome conjugates by using a facile magnetic washing procedure. Then, the immunosensors were constructed by incubating Au-NPFe₃O₄NC/CD63/exosome conjugates with a facile magnetic modified screen-printed electrode and Au-NPFe₃O₄NC adsorbed on the electrode surface could catalyze the oxidation of TMB with H₂O₂ to give an electrochemical signal (Figure 9) [103].

Folic acid and aptamers were usually used as biological recognition elements for cancer cell detection [104–106]. As shown in Figure 10, Wang et al. developed an electrochemical cytosensor for highly sensitive detection of MCF-7 circulating tumor cells (CTCs) by using the mucin 1 protein aptamer for targeting CTCs and CuO nanozymes as catalytic tags [104]. With the as-developed electrochemical cytosensor, as few as 27 cells per mL could be successfully detected [104].

Likewise, an immunosensor was also constructed for bacteria sensing assay [107–108]. As shown in Figure 11, ultrasensitive detection of Staphylococcus aureus (S. aureus) was achieved by using peroxidase-like 2D CuTCPP(Fe) MOF and anti-S. aureus antibody [107]. The vancomycin pre-adsorbed on the electrode surface could specifically bind with bacteria. Then, sandwich immunoassays were constructed by incubating 2D MOF-anti-S. aureus antibody conjugates with electrode (Figure 11) [107]. The electrochemical biosensors can selectively and accurately identify S. aureus even in the presence of other bacteria.

4 Conclusions, Challenges, and Perspective

In this review, we highlighted the recent advances on nanozyme-based electrochemical biosensors. Representative nanozymes (i.e., peroxidase mimics, oxidase mimics, hydrolase mimics, and superoxide dismutase mimics) used in electrochemical biosensors were overviewed. Moreover, representative examples for in vitro and in vivo bioanalytical applications for ions, small molecules, nucleic acids, proteins, and cells were discussed. Despite the remarkable progress, several challenges faced by nanozyme-based electrochemical biosensors remain to be addressed.

1) Improving nanozymes’ catalytic activities. The catalytic activities of nanozymes are crucial for improving the sensing performance of electrochemical biosensors. Even though several strategies were developed for rational design of nanozymes, the catalytic efficiency of current nanozymes was generally lower than that of natural enzymes. To accelerate the discovery of nanozymes with high catalytic activity, combining machine learning with high-throughput experimentation may be a future development direction.

2) Developing nanozymes with high specificity. Most of nanozymes generally have multi-enzymatic catalytic activities, which may affect the detection accuracy of electrochemical biosensors. For example, when nanozymes with both peroxidase- and catalase-like activities are used as catalytic labels for H₂O₂ reduction reaction, the decreasing electronic signal may be obtained because H₂O₂ can be degraded by their catalase-like activities. Therefore, nanomaterials that can specifically mimic natural enzymes are desired.

3) Developing the alternatives to biological components in electrochemical biosensors. The use of natural enzymes (e.g., glucose oxidase) and bio-ligands (e.g., antibody and aptamer) in electrochemical biosensors compromises the high stability and low cost of nanozymes. The alternatives to biological components in electrochemical biosensors should be developed for further improving their stability and lowering their cost. For example, molecularly imprinted polymers were synthesized on Fe₃O₄ nanozymes and used as plastic antibodies for targets.

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Minireview


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