



Review

Advances in the Application of Nano-Enzymes in the Electrochemical Detection of Reactive Oxygen Species: A Review

Rongwei Gao and Shujuan Bao *

Institute for Clean Energy and Advanced Materials, School of Materials and Energy, Southwest University, Chongqing 400715, China; rongwei.gao@kuleuven.be

* Correspondence: baoshj@swu.edu.cn; Tel.: +86-23-68254943 or +86-023-68254372

Abstract: Reactive oxygen species (ROS) play an important role in maintaining human health and are recognized as indicators of oxidative stress linked to various conditions such as neurodegenerative and cardiovascular diseases, as well as cancer. Consequently, detecting ROS levels in biological systems is crucial for biomedical and analytical research. Electrochemical approaches offer promising opportunities for ROS determination due to their exceptional sensitivity, speed, and simplicity of equipment. This review covers studies using advanced electrochemical nanozyme sensors for detecting ROS in biological samples that were published over the last ten years, from 2013 to 2023. Emphasis is placed on the sensor materials and different types of modifiers employed for selective ROS detection. Furthermore, a comprehensive analysis of the sensors' selectivity was performed.

Keywords: reactive oxygen species (ROS); nanozymes; electrochemistry sensor; detection; biological objects



Citation: Gao, R.; Bao, S. Advances in the Application of Nano-Enzymes in the Electrochemical Detection of Reactive Oxygen Species: A Review. *Chemosensors* **2023**, *11*, 440. <https://doi.org/10.3390/chemosensors11080440>

Academic Editor: Marcello Mascini

Received: 3 July 2023

Revised: 29 July 2023

Accepted: 2 August 2023

Published: 7 August 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Over the past few decades, substantial progress has been achieved in the study of ROS. Traditionally, ROS have been considered as oxygen derivatives generated through complete or incomplete oxidation processes [1]. Figure 1 illustrates that a significant portion of molecular oxygen utilized by humans is involved in oxidation reactions within mitochondria. In this context, a four-electron transfer to the O₂ molecule yields the formation of two water (H₂O) molecules. However, one-, two-, or three-electron reduction can also generate intermediate ROS, including hydrogen peroxide (H₂O₂), hydroxyl radical (•OH), superoxide anion (O₂^{•−}), singlet oxygen (¹O₂), and others [2]. ROS primarily govern numerous signal transduction pathways within the human body by directly interacting with proteins, transcription factors, and genes, leading to structural modifications and modulation of their functions. Moderate increases in ROS levels typically promote cell metabolism and differentiation, whereas prolonged and excessive ROS production can induce oxidative damage, such as lipid peroxidation, protein degradation, and DNA fragmentation [3,4]. Consequently, the assessment of ROS levels in biological samples such as blood serum is a crucial aspect of the research of medical and analytical fields.

As the field of ROS detection advances, it is essential to address certain limitations. Notably, different ROS possess unique intrinsic properties, such as lifetimes, diffusion rates, and sources of generation, which can introduce challenges and potential inaccuracies in measurements. Moreover, the inherently limited and fluctuating levels of reactive oxygen species (ROS) at their sites of production pose significant challenges for accurate detection methods, particularly when applied to living cells. However, electrochemical methods have emerged as a promising approach for ROS detection in biological samples, offering several advantages, including simplicity of equipment, high sensitivity and selectivity, rapid

response times, and the ability to fabricate micro- and nano-sized sensors for intracellular ROS determination [5,6].

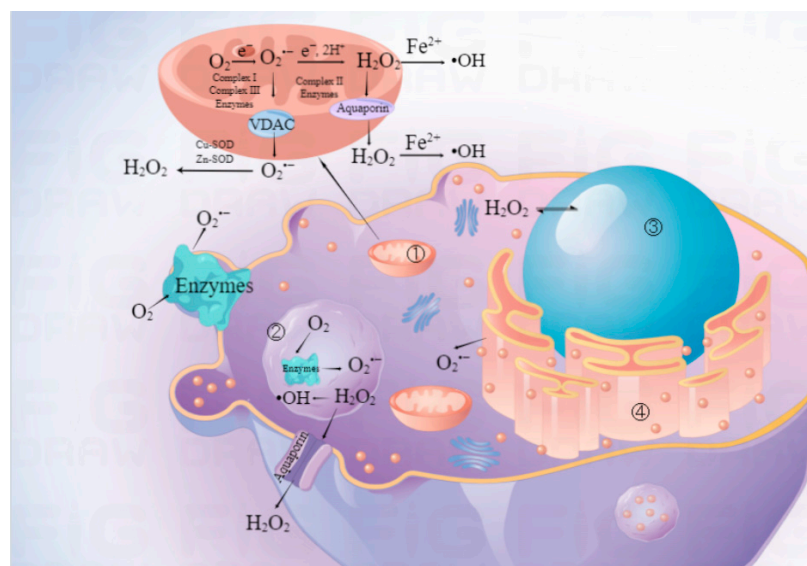


Figure 1. The generation of reactive oxygen species in biological systems. (By Figdraw). ① Mitochondrion; ② cytosol; ③ nuclei; ④ endoplasmic reticulum.

An excellent electrochemical biosensor comprises highly selective and active sites integrated into a stable carrier with excellent electrical conductivity. The integration of natural enzyme-based electrochemical biosensors represent a compelling approach that harnesses the inherent specificity of enzymes alongside the enhanced sensitivity and rapid response characteristics of electrochemical techniques. However, the instability of biological enzymes has hindered their development [7,8]. Since their emergence, nanomaterials have revolutionized the field by providing unprecedented possibilities for designing catalytic interfaces with exceptional activity and selectivity. Moreover, the integration of carbon/metal-based nanozymes, which exhibit high electron conductivity, with catalytic interfaces has demonstrated enhanced interfacial electron-transfer kinetics, thereby enabling the development of high-performance assays. The exceptional conductivity and facile functionalization capabilities of nanozymes, either through conductive matrices or redox-relay units, facilitate efficient electrocatalytic activation. As a result, nanozymes present greater promise for electrochemical sensing applications when compared to many naturally occurring enzymes [9–11]. However, despite the exciting stability and activity demonstrated by nanozymes, achieving selectivity in complex living systems remains a major challenge. This comprehensive review encompasses recent advancements in electrochemical techniques utilized for the detection of reactive oxygen species, including H_2O_2 , $\text{O}_2^{\bullet-}$, and $\bullet\text{OH}$, employing nanozymes as sensing elements. It goes beyond a mere exploration of trends, delving into the selectivity challenges encountered by electrochemical nanozyme sensors. We systematically analyze the challenges that researchers may encounter when employing specific approaches. With ongoing advancements in the sensitivity and selectivity of in vivo ROS biosensors, we hold an optimistic view that the field of ROS research will experience significant advancements.

2. Selection and Construction of Nanozymes with Highly Active Sites for Electrochemical Detection of ROS

From a biological perspective, the active site of a natural enzyme is defined as the region where substrate molecules undergo specific chemical reactions with a significant reduction in activation energy. It serves as the critical component responsible for enzyme function. Consequently, the most effective approach to achieving comparable catalytic performance to natural enzymes is to mimic their active sites. To date, there has been a growing

utilization of diverse nanomaterials in the construction of electrochemical nanozymes for the detection of reactive oxygen species (ROS). This can be attributed to the distinctive physicochemical characteristics exhibited by nanomaterials, including their diminutive size, expansive surface area, and remarkable reactivity. Prominent examples of such nanomaterials encompass noble-metal-based materials, transition-metal-based materials, carbon-based materials, metal–organic framework-based materials, and various other emerging nanomaterials. These materials have garnered significant attention in the field of electrochemical nanozymes due to their unique properties and potential applications in ROS detection.

2.1. Carbon-Based Nanozymes

Nano-carbon materials, including carbon nanotubes, graphene, and other related structures, exhibit exceptional characteristics such as high strength, outstanding electrical conductivity, efficient charge transfer capabilities at high current densities, low resistance, substantial surface area, and remarkable chemical stability. These inherent properties make them highly desirable for various applications, particularly in the field of electrochemical sensing and nanozyme-based systems.

Detection of $O_2^{\bullet-}$. The most commonly used carbon material is graphene. A. Olean-Oliveira et al. recently reported on the development of a nonenzymatic chemiresistor sensor for the detection of superoxide radicals, utilizing an azo-polymer in conjunction with reduced graphene oxide (rGO) employed as the resistive platform for the sensor application [12]. The sensor platform was fabricated by sequentially depositing poly (azo-Bismarck Brown Y) and reduced graphene oxide films using a layer-by-layer assembly technique (Figure 2A). The resulting nanocomposite film demonstrated intriguing synergistic properties, combining the redox properties of the azo-polymer with the excellent electronic conductivity and stability of graphene. Real-time impedance measurements (chrono-impedance) using the poly(azo-BBY)/rGO sensor exhibited a linear relationship between the real impedance and the concentration of superoxide anions (ranging from 0.12 to 2.6 mM), with a detection limit of 81.0 μ M. Xuan Cai et al. further introduced a novel approach for fabricating enzyme-mimicking metal-free catalysts, specifically for the electrochemical detection of $O_2^{\bullet-}$, incorporating phosphate groups into a graphene-based foam [13] (Figure 2B). This was achieved through a template-free hydrothermal process, involving the treatment of graphene oxide (GO) with varying amounts of phytic acid (PA) to obtain a three-dimensional porous graphene-based foam (PAGF). The characterization results confirmed the successful fabrication of the sensors, which were effectively employed for the determination of $O_2^{\bullet-}$ released by cells, showcasing exceptional performance in the dynamic monitoring of cellular $O_2^{\bullet-}$ levels.

Mesoporous carbon materials possess a significant number of edge-plane-like defective sites, which effectively facilitate electron transfer to analytes and enhance the electrochemical activity at the electrode interfaces. The presence of mesoporous channels within the carbon shells of Hollow Mesoporous Carbon Spheres (HMCSs) offers advantageous mass transport and/or charge transfer properties between the sensors and analytes. Li Liu et al. successfully developed an enzyme- and metal-free electrochemical method with remarkable sensitivity for detecting $O_2^{\bullet-}$. This method utilized a screen-printed carbon electrode (SPCE) that was modified with nitrogen-doped hollow mesoporous carbon spheres (N-HMCSs) [14]. As shown in Figure 2C, the electrochemical reduction of $O_2^{\bullet-}$ that takes place on the surface of the modified electrodes is represented by the following equation:



The chronoamperometric responses of the N-HMCSs/SPCE towards $O_2^{\bullet-}$ at -0.15 V were recorded. The current exhibited distinct variations upon the addition of $O_2^{\bullet-}$, demonstrating a proportional relationship with the concentration of $O_2^{\bullet-}$ up to 480 mM. Based on the working area of the electrodes (0.071 cm²), the sensitivity of the N-HMCSs/SPCE was calculated to be 1493.2 mA·cm⁻² mM⁻¹. Furthermore, the limit of detection (LOD) for $O_2^{\bullet-}$ was determined to be 2.2 μ M.

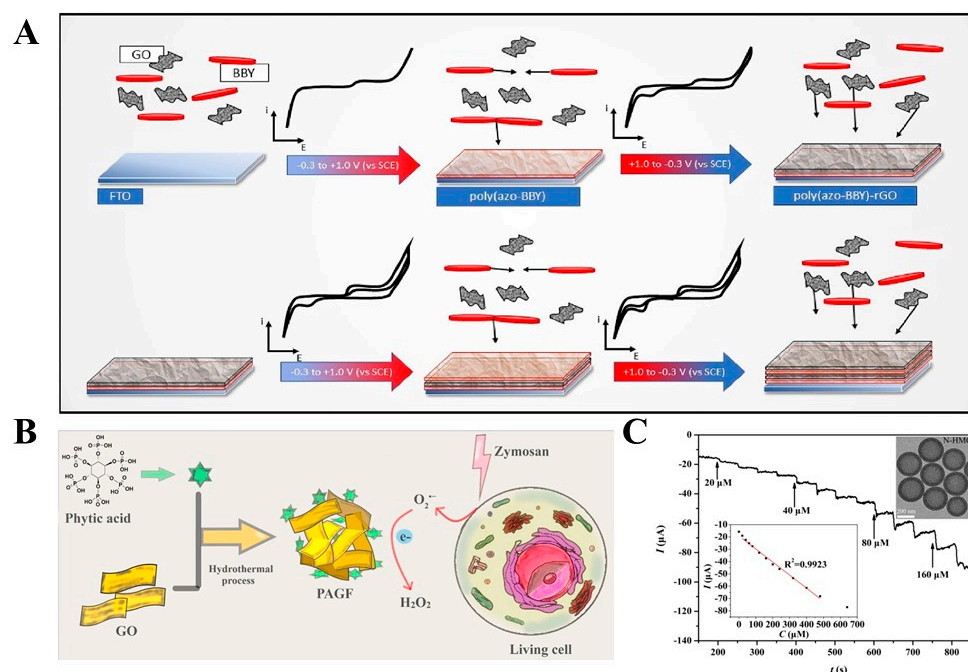


Figure 2. (A) Schematic illustration of the construction of a sensor utilizing a poly(azo-BBY)-rGO film [12], Copyright: Elsevier B.V. (B) Illustration of the synthesis of PAGF materials and their application in detecting cell-released superoxide anions [13], Copyright: Elsevier B.V. (C) Current-time response of N-HMCS/SPCE with successive injection of O₂^{•-} into 0.1 M deoxidized PBS (pH 7.4) at −0.15 V [14], Copyright: Elsevier B.V.

Detection of H₂O₂. The generation of H₂O₂ was first discovered in 1966, and since then, a great deal of work has been performed to investigate the production of hydrogen peroxide and its important role in the body. According to some works, H₂O₂, an essential and potent oxidant, plays a pivotal role in various biological processes, encompassing intercellular signaling, immune cell recruitment, and modulation of cellular morphology and differentiation. Its significance lies in its simplicity, importance, and remarkable oxidative capabilities, which contribute to the intricate mechanisms underlying fundamental biological functions. Despite its lower oxidant power compared to O₂^{•-}, H₂O₂ is recognized as an extremely potent cytotoxic agent. However, in a groundbreaking study conducted by J. Q. Tian et al. in 2013, it was demonstrated for the first time that ultrathin graphitic carbon nitride (g-C₃N₄) nanosheets possess exceptional electrocatalytic properties, making them a cost-effective, environmentally friendly, and highly efficient catalyst for the reduction of hydrogen peroxide [15]. Another noteworthy contribution in this field was made by the research group led by J. Bai [16] who developed H₂O₂ sensors utilizing carbon dots (CDs) and multi-walled carbon nanotubes (MWCNTs). Notably, the CDs/MWCNTs/GCE sensor exhibited a significant synergistic effect, leading to enhanced performance, including an improved LOD of 0.25 μM.

H₂O₂ detection using carbon-based nanomaterials often relies on the electrochemical sensing principle, where H₂O₂ undergoes a redox reaction at the surface of the carbon nanomaterial, leading to measurable electrical signals. As represented by the following equation:

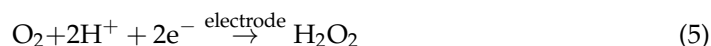


2.2. Noble-Metal-Based Nanozymes

Noble metal nanomaterials, such as gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs), have captured significant interest within the scientific community due to their remarkable stability, high conductivity, facile preparation, expansive specific surface area, and outstanding biocompatibility. Notably, emerging research has highlighted the crucial

influence of nanoparticle size, shape, and distribution on their electrocatalytic activity. These findings underscore the significance of tailoring these parameters to optimize the electrochemical performance of noble metal nanomaterials.

Detection of $\text{O}_2^{\bullet-}$. W. Z. Fan. et al. successfully obtained uniformly dispersed silver nanoparticles (AgNPs) by pyrolyzing a novel silver-based metal–organic framework, as reported in their study [17]. Specifically, the AgNPs@C nanocomposites were synthesized through thermal treatment of the Ag-based metal–organic frameworks, utilizing silver as the metal center and benzimidazole as the organic ligand. An electrochemical response towards the reduction of $\text{O}_2^{\bullet-}$ was observed in the obtained AgNPs@C nanocomposites, exhibiting an ultra-wide linear range (3.032×10^{-13} to 5.719×10^{-5} M) and an exceptionally low detection limit (1.011×10^{-13} M), as depicted in Figure 3A. Furthermore, the fabricated sensor was successfully employed for real-time detection of $\text{O}_2^{\bullet-}$ released from HeLa cells under both normal and oxidative stress conditions. Based on the experimental results, the authors presented the reduction mechanism of $\text{O}_2^{\bullet-}$ on the AgNPs@C/GCE as:



As shown in Equation (3), $\text{O}_2^{\bullet-}$ rapidly decomposes into H_2O_2 and O_2 in aqueous solution due to its inherent instability. It is well-known that Ag nanoparticles possess catalytic activity towards the decomposition of H_2O_2 [18]. Consequently, it was observed that AgNPs exhibit enhanced catalytic efficacy in the decomposition of H_2O_2 as depicted in Equation (4). Subsequently, the liberated oxygen originating from Equations (3) and (4) undergoes diffusion in the vicinity of the electrode, allowing for its detection through reduction on the modified electrode, as illustrated in Equation (5). Other common methods involve the combination of nanoparticles and carbon materials. For instance, T. D. Wu. et al. fabricated a nanocomposite (NCF-Ag) comprising nitrogen-doped cotton carbon fiber and silver nanoparticles (Figure 3B). The fabricated sensor exhibited remarkable electrochemical performance in the identification of $\text{O}_2^{\bullet-}$, demonstrating notable attributes such as a low limit of detection (LOD) of 2.53×10^{-14} M, an extensive linear detection range spanning from 7.59×10^{-14} to 7.22×10^{-5} M, and exceptional selectivity [19]. In a separate investigation, a nanocomposite comprising silver nanoparticles and multi-walled carbon nanotubes (AgNPs/MWNTs) was employed as an efficient electrode material, enabling sensitive detection of superoxide anions.

Detection of H_2O_2 . D. Z. Zhu [20] drew inspiration from self-assembling peptide nanofibers (PNFs) and successfully designed and synthesized a novel hybrid material called PtNWs-PNFs/GO, which consists of biomimetic graphene-supported ultrafine platinum nanowires (PtNWs) integrated with PNFs. The controllable self-assembly process allowed the PNFs to act as a bridge between the GO nanosheets and PtNWs. The presence of PtNWs, known for their high catalytic activity, imparts remarkable electrochemical properties to the PtNWs-PNFs/GO hybrid-based sensor. The sensor demonstrates an extended linear detection range of 0.05 mM to 15 mM and a low detection limit of 0.0206 mM. Euna Ko et al. [21] immobilized bimetallic Au and Pt nanoparticles on the surface of agarose microbeads through chemical means, resulting in a hybrid nanostructure termed Au@PtNP/GO (Figure 4). The synergistic effect between the bimetallic nanoparticles and GO conferred strong peroxidase-like catalytic activity to the hybrid nanostructure, particularly towards the 3,3',5,5'-tetramethylbenzidine (TMB) substrate in the presence of H_2O_2 . This hybrid nanostructure enables dual applications of colorimetric and electrochemical detection. Upon the introduction of the TMB substrate solution containing H_2O_2 , the catalytic oxidation of TMB takes place. Consequently, on the electrode surfaces, the oxidized TMB is subsequently subjected to electrochemical reduction, thereby leading to the

achievement of an expanded detection range for H_2O_2 spanning from 1 μM to 3 mM, along with a reduced lower limit of detection quantified as 1.62 μM . Furthermore, the developed point-of-care (POC) devices exhibited accurate determination of H_2O_2 , demonstrating strong repeatability and reproducibility in real sample testing using artificial urine.

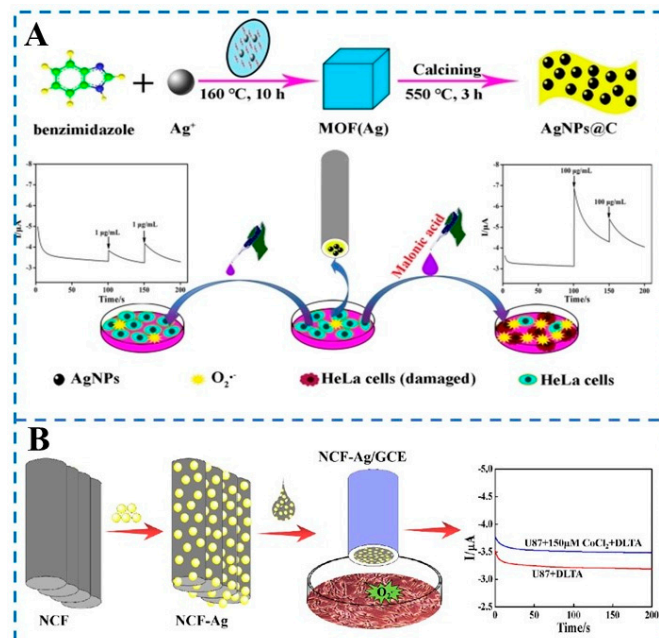


Figure 3. (A) A sensitive superoxide anion sensor constructed with Ag-based metal–organic frameworks, which can directly detect $\text{O}_2^{\bullet-}$ released from cells [17]. Copyright: Wiley Analytical Science. (B) Schematic illustration of the preparation and application of the NCF-Ag/GCE [19]. Copyright: Elsevier B.V.

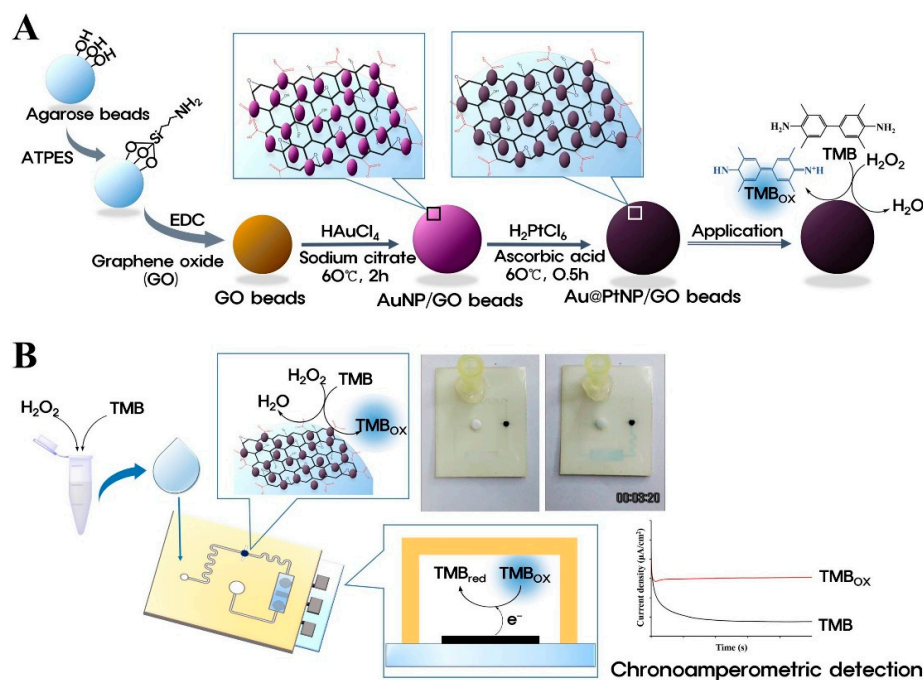


Figure 4. Schematic diagram of (A) the preparation of Au@PtNP/GO microbeads and (B) H_2O_2 detection on electrochemical POC devices with Au@PtNP/GO nanozymes [21]. Copyright: Elsevier B.V.

Detection of $\bullet\text{OH}$. As is commonly known, among all the free radicals, hydroxyl radicals ($\bullet\text{OH}$) exhibit exceptional reactivity and pose significant dangers due to their

nanosecond-scale lifetime. Consequently, the electrochemical detection of hydroxyl radicals presents a formidable challenge, necessitating rapid response times, ultra-high sensitivity, and low detection limits. Regrettably, the current literature offers limited insight into the electrochemical detection of hydroxyl radicals, and the majority of developed sensors have not been employed for the analysis of biological samples. In this regard, two groups of modifier materials have emerged as potential candidates for $\bullet\text{OH}$ detection: thiol self-assembled monolayers (SAM) and CeO_x nanoclusters.

As shown in Figure 5, X. W. Xu et al. [22] have successfully developed a novel electrochemical sensor utilizing a self-assembled nanoporous gold layer (NPGL) modified with 6-(Ferrocenyl) hexanethiol (6-FcHT) on a glassy carbon electrode (6-FcHT/NPGL/GE). This sensor demonstrates remarkable sensitivity and selectivity in detecting the release of $\bullet\text{OH}$ from living cells. The enhanced sensitivity can be attributed to the unique porous architecture of NPGL, which significantly increases the electrode's surface area and facilitates rapid electron transport during electrochemical reactions. Moreover, NPGL offers abundant active binding sites for the efficient assembly of the $\bullet\text{OH}$ capture agent (6-FcHT), ensuring excellent selectivity.

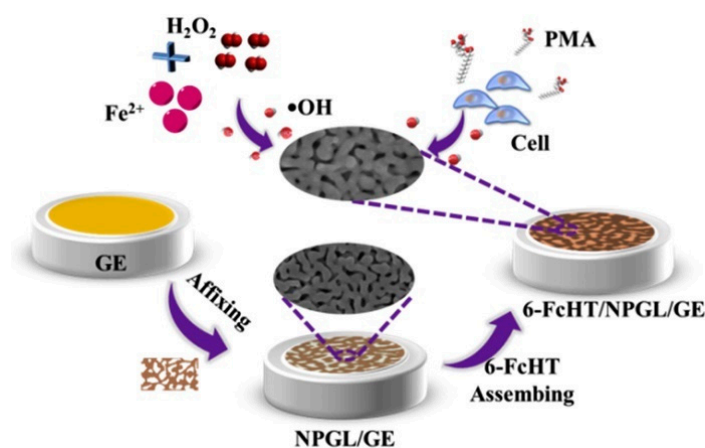


Figure 5. The schematic for preparing 6-FcHT/NPGL/GE [22]. Copyright: Elsevier B.V.

In comparison, when 6-FcHT was solely immobilized on a glassy carbon electrode (6-FcHT/GE), the sensitivity for $\bullet\text{OH}$ detection was measured at $0.0305 \text{ mA nM}^{-1}$ with a detection limit of 0.133 nM within the linear range of 0.4 nM to 70 nM . After implementing the NPGL modification, the sensitivity of 6-FcHT/NPGL/GE towards $\bullet\text{OH}$ increased substantially to $0.1364 \text{ mA nM}^{-1}$, while the detection limit significantly decreased to 0.316 pM . Furthermore, the linear detection range was effectively extended from 1 pM to 100 nM . Importantly, the sensor exhibits additional merits in terms of reproducibility, repeatability, and stability, making it suitable for direct electrochemical detection of $\bullet\text{OH}$ in HepG2 cells.

2.3. Transition-Metal-Based Nanozymes

2.3.1. Metallic Oxides

Metal oxides are considered to be highly significant and extensively studied solid catalysts due to their exceptional structural characteristics, including a large surface area, semiconducting behavior, and facile synthesis. These unique properties make metal oxides attractive candidates for the development of enzyme-less sensors with enhanced effectiveness. Extensive research has been conducted to explore the potential of metal oxides in this regard.

Detection of $\text{O}_2^{\bullet-}$. Y. L. Liu et al. [23] synthesized Co_3O_4 nanoparticles (NPs) with an average diameter of about 30 nm by utilizing ZIF-9 as a template (Figure 6A). The resulting electrode exhibited excellent electrochemical activity for the determination of $\text{O}_2^{\bullet-}$. This enhancement in performance can be attributed to the introduction of multi-walled carbon

nanotubes (MWCNTs) as a substrate, which enhances the electron-conductive property. The three-dimensional architectures of metal–organic frameworks (MOFs) utilized here served as a template material and underwent a conversion process to yield Co_3O_4 @CMWCNTs. These hybrid structures possess a high specific surface area and well-defined mesopores, facilitating enhanced contact with $\text{O}_2^{\bullet-}$ species. A. L. Ding and F. Liu et al. [24] conducted an investigation into the synthesis of MnO nanocomposites integrated within cellulose nanofiber (CNF) aerogels for the purpose of determining $\text{O}_2^{\bullet-}$. The process involved the synthesis of MnO nanoparticles through the calcination of potassium permanganate (KMnO_4) embedded within bacterial cellulose (BC) hydrogels, followed by the carbonization of these hydrogels to yield CNF aerogels with embedded MnO nanoparticles. The resulting sensor demonstrated a linear amperometric response characterized by a considerable sensitivity of $76.2 \times 10^{-3} \mu\text{A cm}^{-2} \text{M}^{-1}$ and a low detection limit of $1.2 \times 10^{-9} \text{M}$ within a concentration range of $5.0 \times 10^{-9} \text{M}$ to $2.5 \times 10^{-6} \text{M}$. This novel approach provides a promising strategy for $\text{O}_2^{\bullet-}$ sensing, utilizing well-designed nanocomposite structures embedded within CNF aerogels.

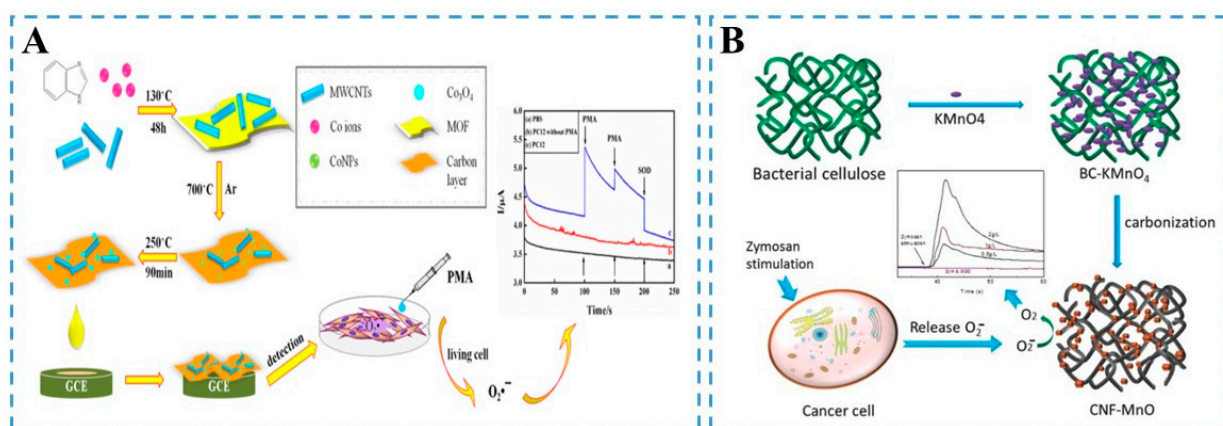


Figure 6. (A) Schematic illustration of preparation and application of Co_3O_4 nanocrystals [23]. Copyright: Elsevier B.V. (B) Schematic illustration of the preparation of 3D MnO–CNF nanocomposites [24]. Copyright: Wiley.

Detection of H_2O_2 . Gaseous hydrogen peroxide plays a significant role as a biomarker linked to severe medical conditions like lung cancer and asthma. The conventional approach for monitoring exhaled breath biomarkers involves the utilization of exhaled breath condensate along with standard analytical techniques. In this case, Ursa Klun et al. [25] presented a novel H_2O_2 -gas-sensing approach, depicted in Figure 7. The aqueous polyacrylate gel electrolyte containing Cu (II) ions served as a sensing material that facilitated the accumulation and stabilization of the gaseous analyte. Through redox interaction with Cu (II) ions, the H_2O_2 was rapidly and sensitively detected. Notably, the gas sensor developed by Klun et al. demonstrated successful and rapid detection of gaseous H_2O_2 within only 2 min of accumulation under ambient conditions. The sensor demonstrated favorable sensitivity in the lower concentration range, and it exhibited a broad linear response across the investigated concentration range of $10\text{--}100 \text{ mg m}^{-3}$. This research breakthrough not only advances the field of H_2O_2 detection but also expands the potential applications in emerging fields such as explosive detection, environmental monitoring, and occupational health and safety. By providing a fast, sensitive, and reliable method for H_2O_2 gas sensing, this work opens doors for various practical applications and contributes to the advancement of detection techniques in diverse fields.

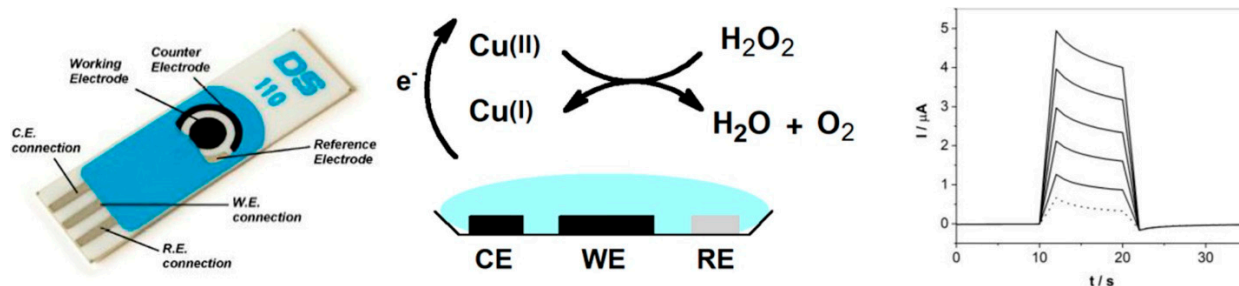


Figure 7. Drop Sens screen-printed carbon electrode and schematic presentation of sensor processes [25]. Copyright: Elsevier B.V.

Detection of $\bullet\text{OH}$. The prevailing approach for the catalytic reduction of hydroxyl radicals ($\bullet\text{OH}$) involves the utilization of cerium oxide (CeOx) nanoclusters. H. Ghaedamini's group [26] proposed a novel electrochemical sensor that incorporated a composite of cerium oxide nanoclusters, gold nanoparticles (AuNPs), and a highly conductive carbon material for the detection of $\bullet\text{OH}$. The fabrication process involved the deposition of AuNPs onto carbon (Au/Carbon), followed by a controlled surface reaction to decorate the AuNP surface with CeOx nanoclusters to scavenge $\bullet\text{OH}$. The synergistic effect of CeOx and AuNPs resulted in enhanced electrochemical signals and excellent $\bullet\text{OH}$ detection capability of the sensor. Duanghathaipornsuk et al. [27] conducted a research study to examine the impact of size and content variations of cerium oxide nanoparticles (CeNPs) on a composite sensor developed for the detection of hydroxyl radicals ($\bullet\text{OH}$). Their findings revealed that composite sensors containing 50 wt% CeNPs displayed the most significant responses in the detection of $\bullet\text{OH}$.

In summary, although CeOx -based electrochemical sensors display robust catalytic activity, their electrical conductivity is limited and often necessitates the incorporation of carbon materials with superior electrical conductivity. Additionally, achieving selectivity in practical testing remains a significant challenge. We posit that the issue of accurately determining $\bullet\text{OH}$ in biological samples should receive heightened attention in future investigations. Furthermore, in our perspective, an area of great interest lies in the development of non-toxic and readily available modifiers that exhibit high selectivity toward the hydroxyl radical.

2.3.2. Transition Metal Phosphates

Transition metal phosphates have garnered significant attention as a class of noble-metal-free materials investigated for use in electrochemical sensors. These materials are particularly appealing due to several advantageous characteristics. Firstly, transition metal phosphates are low-cost, making them economically viable for large-scale production and widespread application. Additionally, they are abundantly available in the Earth's crust, ensuring a sustainable and readily accessible supply.

Detection of $\text{O}_2^{\bullet-}$. Manganese phosphate ($\text{Mn}_3(\text{PO}_4)_2$) has gained considerable attention in the field of electrochemical sensors due to its biocompatible nature, making it suitable for biomedical applications [28]. Moreover, $\text{Mn}_3(\text{PO}_4)_2$ holds pivotal importance in material sciences owing to its unique catalytic and electronic properties. However, the performance of electrochemical sensors based on transition metal phosphate nanozymes is significantly influenced by the microstructure and size of the materials. To address this challenge, various strategies have been explored. M. Q. Wang [29] synthesized nanostructured $\text{Mn}_3(\text{PO}_4)_2$ hollow spheres with tunable pore structures using a micro-emulsion method, as depicted in Figure 8. The resulting nanostructured $\text{Mn}_3(\text{PO}_4)_2$ hollow spheres exhibited a high surface area and porous structure, enabling exceptional catalytic performance towards the detection of $\text{O}_2^{\bullet-}$. In another study, A. L. Ding et al. [30] developed a graphene/DNA/ $\text{Mn}_3(\text{PO}_4)_2$ biomimetic enzyme. The inclusion of graphene as a carrier proved highly beneficial in augmenting the catalytic activity of $\text{Mn}_3(\text{PO}_4)_2$ nanoparticles,

while DNA adsorbed on the graphene surface facilitated the growth of $\text{Mn}_3(\text{PO}_4)_2$. The resulting graphene/DNA/ $\text{Mn}_3(\text{PO}_4)_2$ nanozyme exhibited outstanding electrochemical performance, notably reducing the response time and significantly enhancing the sensor's sensitivity towards $\text{O}_2^{\bullet-}$. The sensor achieved a low detection limit of 1.67 nM and a sensitivity of 3.54 mA mM^{-1} . Similarly, Y. Wang et al. [31] employed chitosan to confine ultra-small $\text{Mn}_3(\text{PO}_4)_2$ particles, demonstrating excellent biocompatibility. They further constructed an in situ detection chip for the electrochemical sensing of $\text{O}_2^{\bullet-}$ from murine breast tumor cells (4T1), achieving a low detection limit of 9.4 nM and good selectivity. X. Cai et al. [32] introduced a carbon-mediated approach for the rapid synthesis of transition metal phosphates with precise control over their shape and size. This strategy encompassed the assembly of melamine, phytic acid, and transition metal ions on the carbon substrate's surface.

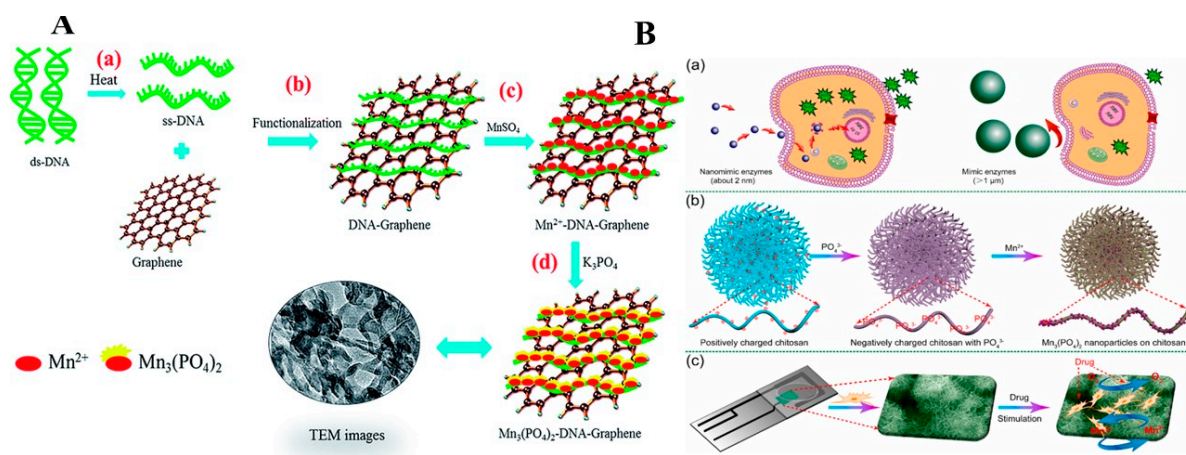


Figure 8. (A) Schematic illustration of the preparation procedure for graphene/DNA/ $\text{Mn}_3(\text{PO}_4)_2$ [30]. (a) The solution was annealed at 95 °C for 15 min to produce single-stranded DNA (ssDNA); (b) ss-DNA mixed with 15 mL of 1 mg mL^{-1} graphene; (c) The mixture was dispersed into 0.1 M MnSO_4 solution; (d) 0.1 M K_3PO_4 solution were dropwisely added under stirring and kept at room temperature for 30 min. Copyright: Royal Society of Chemistry. (B) (a) Nanomimic enzymes in immediate cell environment or inside the cells (left image) induce oxidative-stress-related cytotoxicity and smaller damage of larger mimic enzymes in immediate cell environment (right image); (b) the mechanism of growth of nano- $\text{Mn}_3(\text{PO}_4)_2$ on chitosan; (c) preparation of the chip device in 3D cell adsorption environment and the oxidation mechanism [31]. Copyright: Elsevier B.V.

In summary, manganese phosphate-based biomimetic sensors exhibit high selectivity towards $\text{O}_2^{\bullet-}$, a long shelf-life (approximately 30 days), an easy modification procedure, low cost, and a low nanomolar-level detection limit. However, these sensors suffer from the disadvantage of a large size due to the rapid formation of manganese phosphate, which hinders rapid and sensitive in vivo detection. Additionally, their poor electrical conductivity poses a challenge. In addition, other phosphates commonly used to detect superoxide anions are cobalt phosphate ($\text{Co}_3(\text{PO}_4)_2$) [33], iron phosphate (FePO_4) [34], nickel phosphate (NiPO_4NRs) [35], etc.

Detection of H_2O_2 . The Ag/ FePO_4 nanozymes were prepared by D.J. Rao group [36] using a modified silver mirror reaction at the gas–liquid interface. The FePO_4 nanospheres possessed a high surface-to-volume ratio and displayed a negative surface charge, enabling a large surface area for loading more Ag nanoparticles (NPs) and enhancing the catalytic performance towards the reduction of H_2O_2 . Electrochemical investigations of the sensor demonstrated excellent analytical performance, with a wide linear range from 3.0×10^{-5} to 1.1×10^{-2} mol $\cdot \text{L}^{-1}$ and a low detection limit of 4.7 $\mu\text{mol} \cdot \text{L}^{-1}$. The sensor also exhibited acceptable reproducibility and anti-interference ability. L. J. Peng et al. [37] synthesized the $\text{Co}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ nanozyme in situ through direct reaction between cobalt (II) and phosphate. The researchers confirmed that the $\text{Co}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ nanozyme exhib-

ited peroxidase-like activity. The investigation further revealed that the peroxidase-like activity followed Michaelis–Menten kinetics, with a Michaelis constant (K_m) of 0.073 mM, indicating a higher affinity towards H_2O_2 compared to natural horseradish peroxidase (HRP) and some other peroxidase nanozymes. The mechanistic exploration experiments confirmed that the hydroxyl radical ($\bullet OH$) was the primary active species involved in the catalytic reaction of $Co_3(PO_4)_2 \cdot 8H_2O$. This research introduced a novel nanozyme that can be conveniently synthesized in situ, thereby eliminating the requirement for harsh, laborious, and time-consuming synthesis and purification procedures.

2.4. Metal–Organic-Framework-Based Nanozymes

Metal–organic frameworks (MOFs) have garnered significant attention due to their unique properties, such as a high specific surface area, tunable pore structure, and exposed activity. Comprising metal nodes and organic ligands, MOFs have emerged as versatile materials with diverse applications in the fields of electrochemistry, fluorescence, colorimetry, photo-electrochemistry, and electrochemiluminescence sensing. Their exceptional properties make them well-suited for these applications, enabling the development of advanced sensing platforms and devices. The high specific surface area of MOFs facilitates efficient analyte adsorption and interaction, while the tunable pore structure allows for the selective trapping and recognition of target molecules. Additionally, the exposed activity of MOFs contributes to their enhanced performance in various sensing modalities. As a result, MOFs have demonstrated great potential as promising materials for the development of next-generation sensing technologies.

Detection of $O_2^{\bullet -}$. Y. H. Zhang et al. [38] conducted a study aimed at enhancing the sensing performance of $O_2^{\bullet -}$ by developing a straightforward one-step strategy for the morphology-controllable synthesis of a manganese–organic framework (Mn-MOF) (Figure 9). Interestingly, they achieved the synthesis of Mn-MOF nanoparticles, asymmetric nano-lollipops, and nanorods with homogeneous components by carefully adjusting the solvent ratios and regulating the initial precursor concentrations. Subsequently, the authors found that the Mn-MOF nano-lollipops exhibited superior $O_2^{\bullet -}$ -sensing capabilities compared to other nanostructures due to their larger active surface areas, which can be attributed to the excellent dispersibility provided by the asymmetric structure, as well as the accelerated electron transfer rate facilitated by the stem structure. By utilizing the Mn-MOF nano-lollipops for $O_2^{\bullet -}$ detection, a high sensitivity of $105 \mu A cm^{-2} \cdot \mu M^{-1}$ was achieved, enabling the successful real-time and in situ detection of $O_2^{\bullet -}$ released from living cells. This research not only provides valuable insights into the solvents engineered morphologies of other MOF nanomaterials but also advances the understanding and potential applications of Mn-MOF nanostructures in sensing technologies.

Detection H_2O_2 . MOF-based electrochemical sensors have gained significant attention for the detection of H_2O_2 . Gao et al. [39] synthesized a Pt-nanoparticle-modified metalloporphyrin MOF (Pt@PMOF(Fe)) with multienzyme activity, which was utilized to construct an electrochemical H_2O_2 sensor. Another study by Z. Q. Wei's group [40] involved the synthesis of a 3D Co-based Zeolitic Imidazolate Framework (3D ZIF-67) for H_2O_2 detection. X. Liu's group [41] reported the development of a nickel metal–organic framework nanosheet array on Ti-mesh (Ni-MOF/TM) as an enzyme-free electrochemical sensing platform for H_2O_2 determination. X. L. Yang et al. [42] selected MIL-47(V) as an electrocatalyst to explore the feasibility of electrochemical sensing of H_2O_2 . Nevertheless, these conventional approaches were executed in the single-readout mode, which makes them vulnerable to false-positive or false-negative outcomes arising from external interferences, such as the intricacies of the biological milieu, non-standardized testing protocols, and discrepancies among operators or testing environments. These limitations pose substantial challenges to the accuracy of single-readout analytical methods and impose constraints on their practical applications in disease diagnosis.

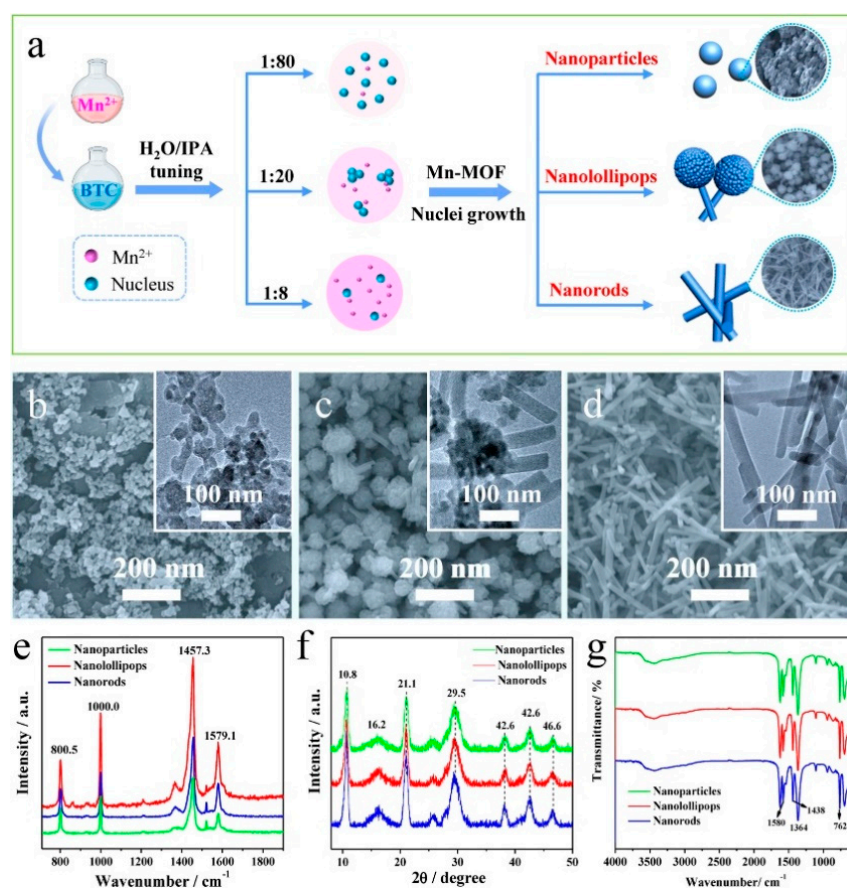


Figure 9. (a) Scheme of solutions of ratio-controlled Mn-MOF nanoparticles, nanoparticles, and nanorods. SEM images of Mn-MOF prepared in ratio of 1:80 (b), 1:20 (c), and 1:8 (d); insets are the corresponding TEM images. (e) Raman spectra and (f) XRD patterns of Mn-MOF nanoparticles, Mn-MOF nanorods, and Mn-MOF nanoparticles. (g) FTIR spectra of different Mn-MOF nanomaterials [38]. Copyright: Elsevier B.V.

To address this issue, K. Yu et al. [43] proposed a novel approach using a portable colorimetric and electrochemical dual-mode sensor for the detection of cell-secreted H₂O₂ and H₂S, based on the MOF-818 nanozyme. As depicted in Figure 10, the trinuclear copper centers in MOF-818 catalyze the formation of $\bullet OH$ from H₂O₂, leading to the oxidation of the substrate 3,3',5,5'-tetramethylbenzidine (TMB) and the production of blue oxTMB. The authors developed a smartphone sensing system by capturing the Hue-Saturation Value (HSV) of the reaction solution using a “Color Identifier” App. Furthermore, MOF-818 exhibited outstanding electrocatalytic activity in H₂O₂ reduction, as evidenced by its reduction peak potential of 0.08 V vs. Ag/AgCl. This exceptional performance enabled the integration of a smartphone and a mini electrochemical analyzer, leading to the establishment of an ultra-sensitive sensing system for H₂O₂. The inherent advantage of a dual-modal assay lies in the fact that the two signals obtained can be utilized to calibrate the assay results and effectively mitigate false-positive/negative outcomes. Additionally, analytical methods featuring dual readout signals can readily adapt to different analytical conditions, catering to the diverse needs of assay tasks. This innovative dual-mode sensing strategy represents a significant advancement in MOF-based electrochemical sensors and holds great promise for enhancing their accuracy and practical applicability in disease diagnosis and beyond.

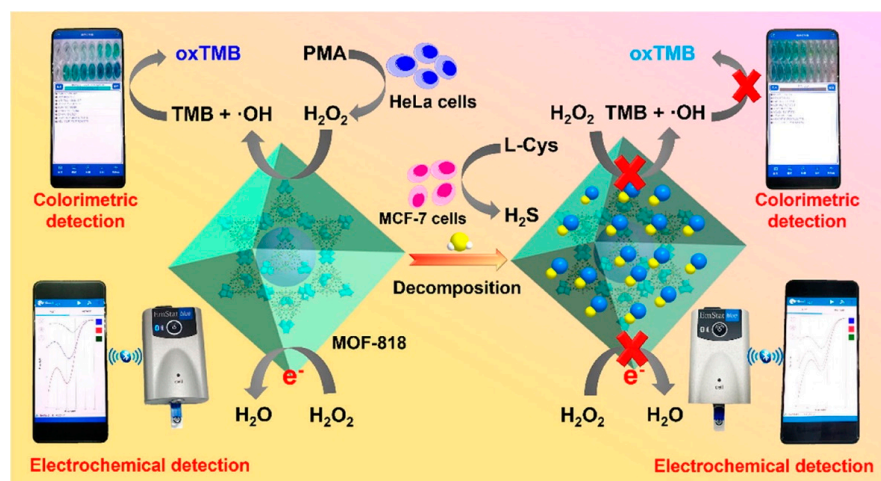


Figure 10. Illustration of MOF-818 nanozyme-based colorimetric and electrochemical dual-mode smartphone sensing platform for in situ detection of H_2O_2 and H_2S released from living cells [43]. Copyright: Elsevier B.V.

To sum up, within recent decades, numerous nanozymes were applied as electrochemical biosensors. However, we also can find that most of studies are focused on the detection of superoxide anions (Table 1) and H_2O_2 (Table 2). The elements commonly used to construct the active sites of nanozymes for detection of ROS are mainly Au, Ag, Pt, Ni, Co, Mn, Fe, Cu, Ti, and Ce. Specially, due to its special chemical properties, CeOx has an excellent antioxidant capacity and catalytic performance, so it has great application potential in detecting ROS. On the one hand, CeOx , as a potent antioxidant, is able to reversibly change its oxidation state in the presence of ROS through the redox cycle, thereby converting excess ROS into harmless substances and protecting cells from oxidative stress. On the other hand, CeOx nanozymes can enhance their selectivity and sensitivity to ROS through surface modifications or composite materials, so as to achieve accurate detection and monitoring of ROS concentration. With the in-depth study of the mechanism of ROS in vivo and the continuous optimization of the properties of nanozyme materials, it is believed that nanozymes will play an important role in the fields of life science, medicine, and environmental studies, providing new solutions for the prevention, diagnosis, and treatment of ROS-related diseases.

Table 1. Main analytical features and application of superoxide anion electrochemical sensors.

Electrode	Linear Range (μM)	LOD (nM)	Sensitivity ($\mu\text{A cm}^{-2} \text{mM}^{-1}$)	Stability (Days)	Application Potential (V)	Ref.
AgNPs@C/GCE	7.422×10^{-4} –0.5719	1.011×10^{-4}	-	7	-0.7	[17]
Co_3O_4 @CMWCNTs/GCE	5×10^{-9} –10	1.6767×10^{-9}	-	-	-	[23]
Mn-MPSA-HCS/SPCE	0–1257.4	1.25	224	-	0.75	[32]
$\text{Ni}(\text{PO}_4)\text{NRs/C-MWCNTs/GCE}$	1–80	97	5.67×10^4	25	-0.3	[35]
2D-mNC@ CeO_2 /SPCEs	8–536	179	401.4	20	-0.5	[44]
PAMAM-Au/GCE	3.69×10^{-5} –37.2	0.0123	-	-	-0.7	[45]
AgNPs-MC/GCE	1.68×10^{-3} –30.6	0.012	-	15	-0.5	[46]
Co-NPs-NG/GCE	1.67×10^{-3} –0.575	1.67	628.86	-	0.9	[47]
$\text{Co}_3(\text{PO}_4)_2/\text{I-rGO/GCE}$	2.4×10^{-3} –2.195	2.4	177.14	30	0.6	[48]
Mn-MPSA-MWCNTs/SPCE	0–1817	127	77.47	30	0.7	[49]

Table 2. Summary of amperometric sensors using nanozymes for the detection of H₂O₂.

Electrode	Linear Range (μM)	LOD (nM)	Sensitivity (μA cm ⁻² mM ⁻¹)	Stability (Days)	Application Potential (V)	Ref.
Fe SAs-N/C/GCE	764–9664	340	22.1	-	−0.05	[50]
Cu@Cu ₂ O/GCE	2–860	460	1855.53	-	−0.5	[51]
rGO/Au-NPs/GCE	25–3000	6.55	0.0641	-	−0.8	[52]
Bi ₂ S ₃ /g-C ₃ N ₄ /GCE	0.5–950	78	1011	7	0.26	[53]
Pt-LEPG/GCE	0.01 × 10 ⁻³ –0.029	0.65	575.75	14	0.5	[54]
CoHCF-NSp's/GCE	2–1130	2.1	329	-	0.8	[55]
Ag/PNA/GCE	1–3000	0.972	1844.76	10	−0.42	[56]
RGO-Pt NPs/GCE	0.5–3475	0.2	459	14	−0.08	[57]
Ag-Au/RGO/TiO ₂ /GCE	10–30,000	3	-	-	-	[58]
MOF-Au@Pt/GCE	0.8–3000	86	24.14	-	−0.12	[59]
LDH/PPy-Ag/GCE	30–800	280	257.64	30	−0.3	[60]
CuO-CeO ₂ /MXene/GCE	5–100	1.67	84.44	-	−0.3	[61]
Ag-Cu nanoalloys/GCE	2000–961,000	152	-	-	−0.07	[62]
AgNPs/2D Zn-MOFs/GCE	5–70,000	1.67 × 10 ³	358.7	6	−0.55	[63]
BiVO ₄ /TiO ₂ /GCE	5–400	5 × 10 ³	3014	90	0.5	[64]
NiCo ₂ S ₄ /rGO/GCE	25–11,250	190	118.5	14	−0.45	[65]
Pt/C-CeO ₂ /GCE	10–30,000	2 × 10 ³	185.4	15	−0.4	[66]
Co ₃ N NW/TM/GCE	2–28	1 × 10 ³	139.9	30	−0.7	[67]
MnO ₂ /Ta/GCE	1–2	60	1111.09	-	−1.21	[68]

3. Selective Challenges of Nanozymes for Electrochemical Detection of ROS

As mentioned in Section 2, with the development of nano-materials, more and more nanozymes have been designed to detection ROS. Although significant progress has been made in the development of biosensors, many of them still face challenges when it comes to practical applications. The selectivity of biosensors becomes a crucial factor when operating in real biological samples, as the presence of various electro-active species, including ascorbate and O₂, can lead to significant interference. Consequently, improving the specificity of nanozymes has been a focus of research efforts. Many researchers have also tried various methods to enhance the specificity of nanozymes, such as the K. L. Fan group [69] who employed a strategy to mimic the enzymatic microenvironment of natural peroxidase enzymes. They achieved this by introducing histidine residues onto the surface of Fe₃O₄ nanoparticles (Figure 11). Compared with unmodified Fe₃O₄, the imidazole of the histidine side chain can form hydrogen bond with hydrogen peroxide, so the modified Fe₃O₄ has an at least 10 times stronger affinity for hydrogen peroxide. However, the selective challenges of nanozymes and the electrochemical method itself still needs to be taken into account. In addition, R. W. Gao et al. also put forward the need to comprehensively consider the view of sensing chips; they believe that only the high activity and selectivity of the recognition factor is not enough, and it is also necessary to consider the adhesion of the recognition factor to the electrode and the rapid transfer and collection of electrical signals.

3.1. Selective Challenge of the Same Nanozymes

First and foremost, despite the significant advancements in the catalytic activity of nanozymes, their selectivity still falls short compared to natural enzymes. As previously discussed, the selectivity of a nanozyme can vary due to factors such as its preparation method, structure, size, and so on (Table 3). For instance, D. Q. Xu's group [70] achieved a rapid and sensitive response to H₂O₂ using a silver-nanoparticle-decorated carbon nanotube (AgNPs-MWCNT) composite, with a linear range of 1 to 1000 μM and LOD of 0.38 μM (S/N = 3). However, X. H. Liu et al. reported a nanocomposite composed of AgNPs/MWNTs utilized as an efficient electrode material for the sensitive detection superoxide anions. Secondly, the utilization of synergistic effects through the combination of different nanomaterials undoubtedly enhances sensor performance. However, it is impor-

tant to consider the inherent variations in nanomaterial production, which can significantly impact the reproducibility of sensors across different laboratories. Incorporating multiple nanomaterials further amplifies these variations, thereby posing challenges to sensor reproducibility. Thirdly, the range of nanomaterial types is vast, and new nanomaterials are constantly being synthesized. The traditional approach of discovering nanocomposites with high catalytic activity by combining various nanomaterials is time-consuming and labor-intensive.

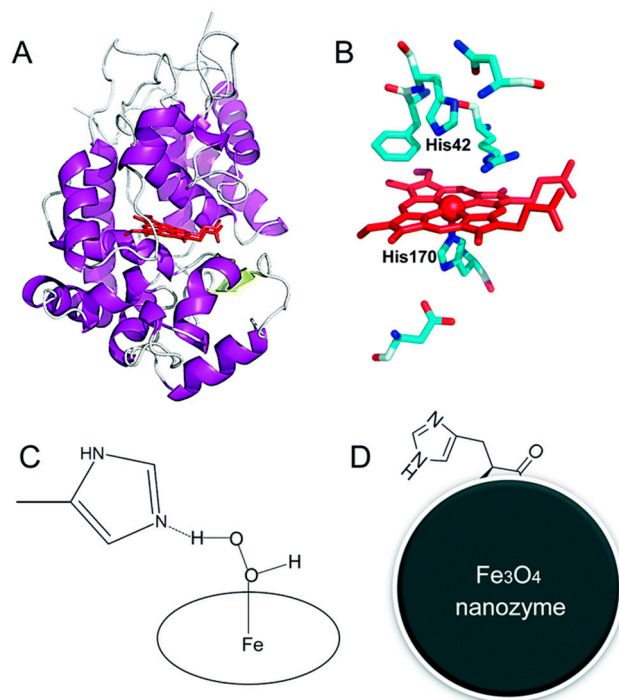


Figure 11. Architecture of the active site in HRP and comparison with the histidine-modified Fe₃O₄ nanozyme. (A) Protein structure of HRP (PDB entry 1HCH); (B) architecture of active site in HRP; (C) H bond between histidine residual and H₂O₂ in the initial state of catalysis of HRP; (D) enhancement of Fe₃O₄ nanozyme activity by histidine modification [69]. Copyright: Royal Society of Chemistry.

Table 3. Summary of electrochemical sensors using the same nanozymes for the detection of different analytes.

Nanozyme	Analyte	Linear Range (μM)	LOD (nM)	Sensitivity (μA cm ⁻² mM ⁻¹)	Ref.
AgNPs-MWCNT	H ₂ O ₂	1–1000	380	2556	[70]
AgNPs-MWCNT	O ₂ ^{•-}	3.65×10^{-7} – 5.59×10^{-4}	0.1192	80.22	[71]
CeO ₂	H ₂ O ₂	0.001–0.125	0.4	141.96	[72]
CeO ₂	O ₂ ^{•-}	8–536	179	401.4	[44]
MnO ₂	H ₂ O ₂	25×10^{-3} –2	5	3261	[73]
MnO ₂	oxalate	7.8–250	910	-	[74]
Pt/TiO ₂ nanotube	H ₂ O ₂	0–20	400	40	[75]
Pt-TiO ₂ NPs	NO	0.01–17,790	2.47	7.81	[76]
PBA/UiO-66/NF	H ₂ O ₂	50–3500	0.02	1903	[77]
PBA/UiO-66/NF	Glucose	200–450	0.28	22,800	[77]

Addressing these challenges requires concerted efforts in the field of nanozyme research. Strategies aimed at improving the selectivity of nanozymes, developing standardized protocols for nanomaterial synthesis, and exploring novel approaches for efficient discovery of high-performance nanocomposites are crucial steps toward overcoming these limitations.

3.2. Selective Challenge of Electrochemical Technology

In recent years, several remarkable electrochemical studies have been published, highlighting the attractiveness of electrochemical technology as a tool for the determination of important small molecules and biomarkers. This technique offers advantages such as rapid detection, small device size, portability, and the ability for continuous monitoring. However, the issue of limited selectivity arises when current signals are mixed, compromising the accuracy of measurements. In an effort to address this challenge, V. Kumar et al. [78] developed a hybrid alginate–polyacrylamide hydrogel through in situ self-assembly of a reduced graphene oxide–cerium oxide nanocomposite (rGO–CeO₂) and cytochrome c (Cyt c) for electrochemical detection of reactive oxygen species (ROS). The hydrogel platform provided a significantly enlarged electroactive surface area, enhancing the reactivity of Cyt c. Moreover, the integration of the rGO–CeO₂ nanocomposite into the hydrogel improved both the electrochemical signals and structural stability of the sensor.

However, it is worth noting from Figure 12 that when the potential exceeds 0.75 V, all three species (H₂O₂, •OH, and O₂^{•−}) undergo oxidation. Since their oxidation potentials are very similar, the resulting current signals become mixed, further emphasizing the necessity of developing sensors with improved sensitivity and selectivity for comprehensive investigations. To overcome these limitations, future research efforts should focus on the development of sensors that can offer enhanced sensitivity and selectivity. Such advancements will enable more precise and accurate studies in the field of electrochemical sensing, paving the way for applications in various areas of science and technology.

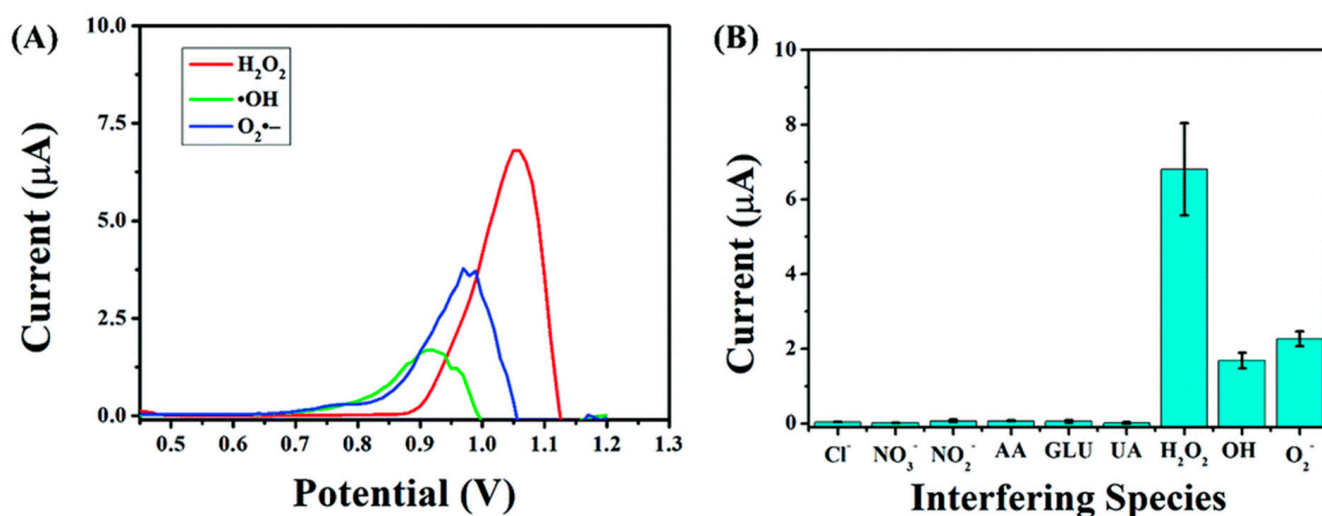


Figure 12. (A) Representative DPV responses after simultaneous addition of H₂O₂, •OH, and O₂^{•−} (30 μM) on rGO–CeO₂@Cyt c hydrogel/SPE. (B) Selective DPV responses of rGO–CeO₂@Cyt c hydrogel/SPE towards ROS over various interfering species, as labeled [78]. Copyright: Royal Society of Chemistry.

4. Conclusions and Future Perspectives

In this comprehensive review, we have discussed the recent advances in the application of nanomaterial-based electrochemical sensors for the detection of reactive oxygen species (ROS), specifically focusing on H₂O₂, •OH, and O₂^{•−}. Despite the concerted efforts of numerous research groups, the field of electrochemical ROS detection and quantification still faces significant challenges that need to be addressed.

Firstly, as this review of recent studies has shown, most electrochemical sensors for detection ROS are focused on H₂O₂ and O₂^{•−}, which is due to their half-life being longer than that of •OH (the half-life of H₂O₂, O₂^{•−}, and •OH are 10–20 h, 6–10 min, and 10 s, respectively). Thus, they are more easily detected by electrochemical methods. At the same time, we also notice that the materials and determination process for the detection of H₂O₂ and O₂^{•−} are similar. However, it is important to note that the determination

of $\bullet\text{OH}$ requires distinct approaches due to the unique characteristics of this free radical. Therefore, the development of sensors with improved sensitivity and selectivity is necessary to investigate the impact of $\bullet\text{OH}$ in mammalian cells and its association with oxidative-stress-induced diseases.

The second crucial issue pertains to enhancing the sensitivity and selectivity of electrochemical sensors for ROS detection, particularly in the context of medical applications. The electrochemical technique shows immense promise for real-time detection utilizing micro- or even nanoscale electrodes, paving the way for the advancement of in vivo sensors capable of real-time monitoring of live cells, irrespective of their type or location. This necessitates sensors with enhanced sensitivity and selectivity. In this regard, nanozymes can greatly benefit from interdisciplinary collaborations, including advanced information technology such as machine learning. Prior to the synthesis of nanomaterials, machine learning algorithms can be employed to simulate and identify candidate materials that meet specific criteria, resulting in the establishment of a library of catalytically active materials with high activity, stability, and repeatability. Furthermore, the application of machine learning algorithms enables training using extracted features from cyclic voltametric curves of known concentrations of target analytes, thus facilitating precise concentration calculations.

Thirdly, when developing nanozyme-based electrochemical sensors, the potential for industrialization and commercialization should be taken into consideration. Emerging technologies like the Internet of Things can be employed for real-time monitoring and automated control of the production process.

In conclusion, the remarkable ability of nanozyme-based electrochemical sensors for ROS detection underscores their potential for occupying a significant market share in the commercial application of sensors as the synthesis of nanozymes continues to mature. With ongoing improvements in the sensitivity and selectivity of in vivo ROS biosensors, the field of ROS research stands to benefit significantly, advancing our understanding of oxidative-stress-related phenomena.

Author Contributions: Writing—original draft preparation: R.G.; Supervision, S.B. All authors have read and agreed to the published version of the manuscript.

Funding: This work is financially supported by National Natural Science Foundation of China (Grant number: 21972111).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sies, H.; Jones, D.P. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 363–383. [[CrossRef](#)] [[PubMed](#)]
2. Halliwell, B. Reactive Species and Antioxidants. Redox Biology Is a Fundamental Theme of Aerobic Life. *Plant Physiol.* **2006**, *141*, 312–322. [[CrossRef](#)] [[PubMed](#)]
3. Rahal, A.; Kumar, A.; Singh, V.; Yadav, B.; Tiwari, R.; Chakraborty, S.; Dhama, K. Oxidative stress, prooxidants, and antioxidants: The interplay. *Biomed. Res. Int.* **2014**, *2014*, 761264. [[CrossRef](#)] [[PubMed](#)]
4. Sayre, L.M.; Perry, G.; Smith, M.A. Oxidative Stress and Neurotoxicity. *Chem. Res. Toxicol.* **2008**, *21*, 172–188. [[CrossRef](#)]
5. Geraskevich, A.V.; Solomonenko, A.N.; Dorozhko, E.V.; Korotkova, E.I.; Barek, J. Electrochemical Sensors for the Detection of Reactive Oxygen Species in Biological Systems: A Critical Review. *Crit. Rev. Anal. Chem.* **2022**, 1–33. [[CrossRef](#)]
6. Duanghathaipornsuk, S.; Farrell, E.J.; Alba-Rubio, A.C.; Zelenay, P.; Kim, D.S. Detection Technologies for Reactive Oxygen Species: Fluorescence and Electrochemical Methods and Their Applications. *Biosensors* **2021**, *11*, 30. [[CrossRef](#)]
7. Gao, R.; Yang, X.; Yang, Q.; Wu, Y.; Wang, F.; Xia, Q.; Bao, S.-J. Design of an amperometric glucose oxidase biosensor with added protective and adhesion layers. *Microchim. Acta* **2021**, *188*, 312. [[CrossRef](#)]
8. Kaur, J.; Choudhary, S.; Chaudhari, R.; Jayant, R.; Joshi, A. *Enzyme-Based Biosensors*; ResearchGate: Berlin, Germany, 2019; pp. 211–240.

9. Wang, X.; Dong, S.; Wei, H. Recent Advances on Nanozyme-based Electrochemical Biosensors. *Electroanalysis* **2022**, *35*, 38–39. [\[CrossRef\]](#)
10. Wang, H.; Wan, K.; Shi, X. Recent Advances in Nanozyme Research. *Adv. Mater.* **2019**, *31*, e1805368. [\[CrossRef\]](#)
11. Campuzano, S.; Pedrero, M.; Yáñez-Sedeño, P.; Pingarrón, J.M. Nanozymes in electrochemical affinity biosensing. *Microchim. Acta* **2020**, *187*, 423. [\[CrossRef\]](#)
12. Olean-Oliveira, A.; Pacheco, J.C.; Seraphim, P.M.; Teixeira, M.F.S. Synergistic effect of reduced graphene oxide/azo-polymer layers on electrochemical performance and application as nonenzymatic chemiresistor sensors for detecting superoxide anion radicals. *J. Electroanal. Chem.* **2019**, *852*, 113520. [\[CrossRef\]](#)
13. Cai, X.; Chen, H.L.; Wang, Z.X.; Sun, W.Q.; Shi, L.B.; Zhao, H.L.; Lan, M.B. 3D graphene-based foam induced by phytic acid: An effective enzyme-mimic catalyst for electrochemical detection of cell-released superoxide anion. *Biosens. Bioelectron.* **2019**, *123*, 101–107. [\[CrossRef\]](#)
14. Liu, L.; Zhao, H.L.; Shi, L.B.; Lan, M.B.; Zhang, H.W.; Yu, C.Z. Enzyme- and metal-free electrochemical sensor for highly sensitive superoxide anion detection based on nitrogen doped hollow mesoporous carbon spheres. *Electrochim. Acta* **2017**, *227*, 69–76. [\[CrossRef\]](#)
15. Tian, J.Q.; Liu, Q.; Ge, C.J.; Xing, Z.C.; Asiri, A.M.; Al-Youbi, A.O.; Sun, X.P. Ultrathin graphitic carbon nitride nanosheets: A low-cost, green, and highly efficient electrocatalyst toward the reduction of hydrogen peroxide and its glucose biosensing application. *Nanoscale* **2013**, *5*, 8921–8924. [\[CrossRef\]](#)
16. Bai, J.; Sun, C.H.; Jiang, X.E. Carbon dots-decorated multiwalled carbon nanotubes nanocomposites as a high-performance electrochemical sensor for detection of H₂O₂ in living cells. *Anal. Bioanal. Chem.* **2016**, *408*, 4705–4714. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Fan, W.Z.; Liu, X.H.; Wu, J.S.; Liu, Q.; Ding, L.; Liu, X.H. Development of a Novel Silver-based Sensing Platform for Detecting Superoxide Anion Released from HeLa Cells Directly. *Electroanalysis* **2022**, *34*, 987–994. [\[CrossRef\]](#)
18. Welch, C.M.; Banks, C.E.; Simm, A.O.; Compton, R.G. Silver nanoparticle assemblies supported on glassy-carbon electrodes for the electro-analytical detection of hydrogen peroxide. *Anal. Bioanal. Chem.* **2005**, *382*, 12–21. [\[CrossRef\]](#)
19. Wu, T.D.; Li, L.; Jiang, X.C.; Liu, F.X.; Liu, Q.; Liu, X.H. Construction of silver-cotton carbon fiber sensing interface and study on the protective effect of antioxidants on hypoxia-induced cell damage. *Microchem. J.* **2020**, *159*, 105345. [\[CrossRef\]](#)
20. Zhu, D.Z.; He, P.; Kong, H.; Yang, G.Z.; Luan, X.; Wei, G. Biomimetic graphene-supported ultrafine platinum nanowires for colorimetric and electrochemical detection of hydrogen peroxide. *J. Mater. Chem. B* **2022**, *10*, 9216–9225. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Ko, E.; Van-Khue, T.; Son, S.E.; Hur, W.; Choi, H.; Seong, G.H. Characterization of Au@PtNP/GO nanozyme and its application to electrochemical microfluidic devices for quantification of hydrogen peroxide. *Sens. Actuators B-Chem.* **2019**, *294*, 166–176. [\[CrossRef\]](#)
22. Xu, Y.W.; Wang, D.Y.; Zhang, Y.; Zhang, J.J.; Jiang, S.Y.; Liang, W.C.; Zhu, T.T.; Ye, B.C. A novel electrochemical sensor for determination of hydroxyl radicals in living cells by coupling nanoporous gold layer with self-assembled 6-(Ferrocenyl) hexanethiol. *Anal. Chim. Acta* **2020**, *1096*, 69–75. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Liu, Y.L.; Wei, H.W.; Jiang, X.C.; Guo, H.X.; Liu, X.H. Synthesis of metal-organic frameworks derived nanocomposites for superoxide anion radical sensing and cell monitoring upon oxidative stress. *J. Electroanal. Chem.* **2018**, *820*, 51–59. [\[CrossRef\]](#)
24. Ding, A.L.; Liu, F.; Zheng, J.S.; Chen, J.C.; Li, C.M.; Wang, B. Synthesis of Manganese Oxide Embedded Carbon Nanofibers as Effective Biomimetic Enzymes for Sensitive Detection of Superoxide Anions Released from Living Cells. *Macromol. Mater. Eng.* **2018**, *303*, 1800079. [\[CrossRef\]](#)
25. Klun, U.; Zorko, D.; Stojanov, L.; Mirceski, V.; Jovanovski, V. Amperometric sensor for gaseous H₂O₂ based on copper redox mediator incorporated electrolyte. *Sens. Actuators Rep.* **2023**, *5*, 100144. [\[CrossRef\]](#)
26. Ghaedamini, H.; Alba-Rubio, A.C.; Kim, D.S. A Novel Electrochemical Sensor Based on a Cerium Oxide/Gold/Carbon Nanocomposite for the Detection of Hydroxyl Free Radicals. *J. Electrochem. Soc.* **2023**, *170*, 047510. [\[CrossRef\]](#)
27. Duanghathaipornsuk, S.; Alateeq, F.A.O.; Kim, S.S.; Kim, D.-S.; Alba-Rubio, A.C. The effects of size and content of cerium oxide nanoparticles on a composite sensor for hydroxyl radicals detection. *Sens. Actuators B Chem.* **2020**, *321*, 128467. [\[CrossRef\]](#)
28. Barnese, K.; Gralla, E.B.; Cabelli, D.E.; Selverstone Valentine, J. Manganous Phosphate Acts as a Superoxide Dismutase. *J. Am. Chem. Soc.* **2008**, *130*, 4604–4606. [\[CrossRef\]](#)
29. Wang, M.Q.; Ye, C.; Bao, S.J.; Xu, M.W. Controlled synthesis of Mn₃(PO₄)₂ hollow spheres as biomimetic enzymes for selective detection of superoxide anions released by living cells. *Microchim. Acta* **2017**, *184*, 1177–1184. [\[CrossRef\]](#)
30. Ding, A.L.; Wang, B.; Ma, X.Q.; Diao, J.L.; Zheng, J.S.; Chen, J.C.; Li, C.M. DNA-induced synthesis of biomimetic enzyme for sensitive detection of superoxide anions released from live cell. *Rsc Adv.* **2018**, *8*, 12354–12359. [\[CrossRef\]](#)
31. Wang, Y.; Wang, D.; Sun, L.H.; Xue, P.; Wang, M.Q.; Lu, Z.S.; Wang, F.; Xia, Q.Y.; Xu, M.W.; Bao, S.J. Constructing high effective nano-Mn₃(PO₄)₂-chitosan in situ electrochemical detection interface for superoxide anions released from living cell. *Biosens. Bioelectron.* **2019**, *133*, 133–140. [\[CrossRef\]](#)
32. Cai, X.; Wang, Z.X.; Zhang, H.H.; Li, Y.F.; Chen, K.C.; Zhao, H.L.; Lan, M.B. Carbon-mediated synthesis of shape-controllable manganese phosphate as nanozymes for modulation of superoxide anions in HeLa cells. *J. Mater. Chem. B* **2019**, *7*, 401–407. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Wang, M.Q.; Ye, C.; Bao, S.J.; Xu, M.W.; Zhang, Y.; Wang, L.; Ma, X.Q.; Guo, J.; Li, C.M. Nanostructured cobalt phosphates as excellent biomimetic enzymes to sensitively detect superoxide anions released from living cells. *Biosens. Bioelectron.* **2017**, *87*, 998–1004. [\[CrossRef\]](#) [\[PubMed\]](#)

34. Wang, Y.; Wang, M.Q.; Lei, L.L.; Chen, Z.Y.; Liu, Y.S.; Bao, S.J. FePO₄ embedded in nanofibers consisting of amorphous carbon and reduced graphene oxide as an enzyme mimetic for monitoring superoxide anions released by living cells. *Microchim. Acta* **2018**, *185*, 140. [\[CrossRef\]](#)
35. Cui, M.; Zhao, H.Y.; Wen, X.F.; Li, N.; Ren, J.J.; Zhang, C. Facile synthesis of nickel phosphate nanorods as biomimetic enzyme with excellent electrocatalytic activity for highly sensitive detection of superoxide anion released from living cells. *J. Pharm. Biomed. Anal.* **2022**, *212*, 114653. [\[CrossRef\]](#)
36. Rao, D.J.; Zhang, J.; Zheng, J.B. Synthesis of silver nanoparticles-decorated FePO₄ nanosphere at a gas-liquid interface for the electrochemical detection of Hydrogen peroxide. *J. Chem. Sci.* **2016**, *128*, 839–847. [\[CrossRef\]](#)
37. Peng, L.J.; Zhou, H.Y.; Zhang, C.Y.; Yang, F.Q. Study on the peroxidase-like activity of cobalt phosphate and its application in colorimetric detection of hydrogen peroxide. *Colloids Surf. A-Physicochem. Eng. Asp.* **2022**, *647*, 129031. [\[CrossRef\]](#)
38. Zhang, Y.; Guo, C.X.; Du, H.; Wang, X.; Liu, L.; Li, C.M. Solvent-engineered morphologies of Mn-MOF toward ultrasensitive sensing cell superoxide. *Electrochim. Acta* **2022**, *431*, 141147. [\[CrossRef\]](#)
39. Ling, P.; Cheng, S.; Chen, N.; Qian, C.; Gao, F. Nanozyme-Modified Metal-Organic Frameworks with Multienzymes Activity as Biomimetic Catalysts and Electrocatalytic Interfaces. *ACS Appl. Mater. Interfaces* **2020**, *12*, 17185–17192. [\[CrossRef\]](#)
40. Wei, Z.Q.; Li, W.; Yang, H.; Li, T.; He, S.N.; Wang, Y.; Hu, Y.L. Synthesis of 3D Co-based Zeolitic Imidazolate Framework and Application as Electrochemical Sensor for H₂O₂ Detection. *Int. J. Electrochem. Sci.* **2022**, *17*, 221132. [\[CrossRef\]](#)
41. Liu, X.; Xiang, M.H.; Zhang, X.Y.; Li, Q.; Liu, X.Y.; Zhang, W.J.; Qin, X.; Qu, F.L. An Enzyme-free Electrochemical H₂O₂ Sensor Based on a Nickel Metal-organic Framework Nanosheet Array. *Electroanalysis* **2022**, *34*, 369–374. [\[CrossRef\]](#)
42. Yang, X.; Qiu, W.; Gao, R.; Wang, Y.; Bai, Y.; Xu, Z.; Bao, S.-J. MIL-47(V) catalytic conversion of H₂O₂ for sensitive H₂O₂ detection and tumor cell inhibition. *Sens. Actuators B Chem.* **2022**, *354*, 131201. [\[CrossRef\]](#)
43. Yu, K.; Li, M.J.; Chai, H.N.; Liu, Q.; Hai, X.; Tian, M.W.; Qu, L.J.; Xu, T.L.; Zhang, G.Y.; Zhang, X.J. MOF-818 nanozyme-based colorimetric and electrochemical dual-mode smartphone sensing platform for in situ detection of H₂O₂ and H₂S released from living cells. *Chem. Eng. J.* **2023**, *451*, 138321. [\[CrossRef\]](#)
44. Wang, Z.; Zhao, H.; Chen, K.; Zhou, F.; Magdassi, S.; Lan, M. Two-dimensional mesoporous nitrogen-rich carbon nanosheets loaded with CeO₂ nanoclusters as nanozymes for the electrochemical detection of superoxide anions in HepG2 cells. *Biosens. Bioelectron.* **2022**, *209*, 114229. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Liu, F.X.; Yu, R.; Wei, H.W.; Wu, J.S.; He, N.; Liu, X.H. Construction of a novel electrochemical sensing platform to investigate the effect of temperature on superoxide anions from cells and superoxide dismutase enzyme activity. *Anal. Chim. Acta* **2022**, *1198*, 339561. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Liu, F.X.; Jiang, X.C.; He, N.; Yu, R.; Xue, Z.H.; Liu, X.H. Electrochemical investigation for enhancing cellular antioxidant defense system based on a superoxide anion sensor. *Sens. Actuators B-Chem.* **2022**, *368*, 132190. [\[CrossRef\]](#)
47. Zou, Z.; Shi, Z.Z.; Yuan, C.S.; Tang, C.Y.; Wu, C.; Liang, T.T.; Tang, K.L.; Chen, H.; Yang, H.B.; Li, C.M. Steric shelter-free cobalt nanoparticle-based high-sensitive biomimetic superoxide anion sensor. *Mater. Chem. Front.* **2021**, *5*, 6860–6864. [\[CrossRef\]](#)
48. Zou, Z.; Chen, J.; Shi, Z.Z.; Yuan, C.S.; Zhou, G.D.; Liu, Q.; Chen, H.; Zeng, Q.X.; Liang, T.T.; Tang, K.L.; et al. Cobalt Phosphates Loaded into Iodine-Spaced Reduced Graphene Oxide Nanolayers for Electrochemical Measurement of Superoxide Generated by Cells. *ACS Appl. Nano Mater.* **2021**, *4*, 3631–3638. [\[CrossRef\]](#)
49. Cai, X.; Shi, L.; Sun, W.; Zhao, H.; Li, H.; He, H.; Lan, M. A facile way to fabricate manganese phosphate self-assembled carbon networks as efficient electrochemical catalysts for real-time monitoring of superoxide anions released from HepG2 cells. *Biosens. Bioelectron.* **2018**, *102*, 171–178. [\[CrossRef\]](#)
50. Liang, Y.; Zhao, P.; Zheng, J.L.; Chen, Y.Y.; Liu, Y.Y.; Zheng, J.; Luo, X.G.; Huo, D.Q.; Hou, C.J. Fe Single-Atom Electrochemical Sensors for H₂O₂ Produced by Living Cells. *ACS Appl. Nano Mater.* **2022**, *5*, 11852–11863. [\[CrossRef\]](#)
51. Li, H.X.; Jiang, L.L.; Shao, D.; Wu, C.S.; Gao, Y.J.; Yang, Z.Q.; Yang, Z.J. Facile synthesis of Cu@Cu₂O aerogel for an effective electrochemical hydrogen peroxide sensor. *Chin. J. Anal. Chem.* **2022**, *50*, 100060. [\[CrossRef\]](#)
52. Patella, B.; Buscetta, M.; Di Vincenzo, S.; Ferraro, M.; Aiello, G.; Sunseri, C.; Pace, E.; Inguanta, R.; Cipollina, C. Electrochemical sensor based on rGO/Au nanoparticles for monitoring H₂O₂ released by human macrophages. *Sens. Actuators B-Chem.* **2021**, *327*, 128901. [\[CrossRef\]](#)
53. Othmani, A.; Derbali, M.; Kalfat, R.; Touati, F.; Dhaouadi, H. A novel 1D/2D Bi₂S₃/g-C₃N₄ core-shell nanocomposite as a highly performing H₂O₂ non-enzymatic electrochemical sensor. *J. Mater. Res. Technol. -JmrT* **2021**, *15*, 5762–5775. [\[CrossRef\]](#)
54. Lu, Z.W.; Wu, L.; Dai, X.X.; Wang, Y.Y.; Sun, M.M.; Zhou, C.L.; Du, H.J.; Rao, H.B. Novel flexible bifunctional amperometric biosensor based on laser engraved porous graphene array electrodes: Highly sensitive electrochemical determination of hydrogen peroxide and glucose. *J. Hazard. Mater.* **2021**, *402*, 123774. [\[CrossRef\]](#)
55. Banavath, R.; Srivastava, R.; Bhargava, P. Nanoporous Cobalt Hexacyanoferrate Nanospheres for Screen-Printed H₂O₂ Sensors. *ACS Appl. Nano Mater.* **2021**, *4*, 5564–5576. [\[CrossRef\]](#)
56. Saidu, F.K.; Joseph, A.; Varghese, E.V.; Thomas, G.V. Silver nanoparticles-embedded poly(1-naphthylamine) nanospheres for low-cost non-enzymatic electrochemical H₂O₂ sensor. *Polym. Bull.* **2020**, *77*, 5825–5846. [\[CrossRef\]](#)
57. Zhang, Y.Y.; Bai, X.Y.; Wang, X.M.; Shiu, K.K.; Zhu, Y.L.; Jiang, H. Highly Sensitive Graphene-Pt Nanocomposites Amperometric Biosensor and Its Application in Living Cell H₂O₂ Detection. *Anal. Chem.* **2014**, *86*, 9459–9465. [\[CrossRef\]](#)
58. Han, L.; Cui, S.F.; Deng, D.M.; Li, Y.Y.; Yan, X.X.; He, H.B.; Luo, L.Q. Synthesis of Ag-Au/Reduced Graphene Oxide/TiO₂ Nanocomposites: Application as a Non-enzymatic Amperometric H₂O₂ Sensor. *Curr. Anal. Chem.* **2020**, *16*, 485–492. [\[CrossRef\]](#)

59. Wang, H.F.; Chen, W.X.; Chen, Q.Y.; Liu, N.; Cheng, H.J.; Li, T. Metal-organic framework (MOF)-Au@Pt nanoflowers composite material for electrochemical sensing of H_2O_2 in living cells. *J. Electroanal. Chem.* **2021**, *897*, 115603. [\[CrossRef\]](#)
60. Zhang, K.; Zeng, H.Y.; Wang, M.X.; Li, Z. Non-enzymatic H_2O_2 electrochemical sensor based on NiAl-LDH/PPy-Ag composite. *Ionics* **2022**, *28*, 5561–5570. [\[CrossRef\]](#)
61. Zhou, K.W.; Li, Y.; Zhuang, S.J.; Ren, J.; Tang, F.; Mu, J.L.; Wang, P. A novel electrochemical sensor based on CuO-CeO₂/MXene nanocomposite for quantitative and continuous detection of H_2O_2 . *J. Electroanal. Chem.* **2022**, *921*, 116655. [\[CrossRef\]](#)
62. Shafa, M.; Ahmad, I.; Hussain, S.; Asif, M.; Pan, Y.; Zairov, R.; Alothman, A.A.; Ouladsmene, M.; Ullah, Z.; Ullah, N.; et al. Ag-Cu nanoalloys: An electrochemical sensor for H_2O_2 detection. *Surf. Interfaces* **2023**, *36*, 102616. [\[CrossRef\]](#)
63. Chen, S.Y.; Xie, Y.X.; Guo, X.J.; Sun, D.P. Self-supporting electrochemical sensors for monitoring of cell-released H_2O_2 based on metal nanoparticle/MOF nanozymes. *Microchem. J.* **2022**, *181*, 107715. [\[CrossRef\]](#)
64. Derbali, M.; Othmani, A.; Kouass, S.; Touati, F.; Dhaouadi, H. BiVO₄/TiO₂ nanocomposite: Electrochemical sensor for hydrogen peroxide. *Mater. Res. Bull.* **2020**, *125*, 110771. [\[CrossRef\]](#)
65. Wang, M.; Ma, J.W.; Guan, X.L.; Peng, W.C.; Fan, X.B.; Zhang, G.L.; Zhang, F.B.; Li, Y. A novel H_2O_2 electrochemical sensor based on NiCo₂S₄ functionalized reduced graphene oxide. *J. Alloys Compd.* **2019**, *784*, 827–833. [\[CrossRef\]](#)
66. Uzunoglu, A.; Ipekci, H.H. The use of CeO₂-modified Pt/C catalyst inks for the construction of high-performance enzyme-free H_2O_2 sensors. *J. Electroanal. Chem.* **2019**, *848*, 113302. [\[CrossRef\]](#)
67. Xie, F.Y.; Cao, X.Q.; Qu, F.L.; Asiri, A.M.; Sun, X.P. Cobalt nitride nanowire array as an efficient electrochemical sensor for glucose and H_2O_2 detection. *Sens. Actuators B-Chem.* **2018**, *255*, 1254–1261. [\[CrossRef\]](#)
68. Vijayalakshmi, K.; Renitta, A.; Alagusundaram, K.; Monamary, A. Novel two-step process for the fabrication of MnO₂ nanostructures on tantalum for enhanced electrochemical H_2O_2 detection. *Mater. Chem. Phys.* **2018**, *214*, 431–439. [\[CrossRef\]](#)
69. Fan, K.L.; Wang, H.; Xi, J.Q.; Liu, Q.; Meng, X.Q.; Duan, D.M.; Gao, L.Z.; Yan, X.Y. Optimization of Fe₃O₄ nanozyme activity via single amino acid modification mimicking an enzyme active site. *Chem. Commun.* **2017**, *53*, 424–427. [\[CrossRef\]](#)
70. Xu, D.Q.; Hou, B.B.; Qian, L.S.; Zhang, X.J.; Liu, G.D. Non-Enzymatic Electrochemical Sensor Based on Silver Nanoparticle-Decorated Carbon Nanotubes. *Molecules* **2019**, *24*, 3411. [\[CrossRef\]](#)
71. Liu, X.; Ran, M.; Liu, G.; Liu, X.; Xue, Z.; Lu, X. A sensitively non-enzymatic amperometric sensor and its application in living cell superoxide anion radical detection. *Talanta* **2018**, *186*, 248–255. [\[CrossRef\]](#)
72. Rajendran, S.; Manoj, D.; Suresh, R.; Vasseghian, Y.; Ghfar, A.A.; Sharma, G.; Soto-Moscoso, M. Electrochemical detection of hydrogen peroxide using micro and nanoporous CeO₂ catalysts. *Environ. Res.* **2022**, *214*, 113961. [\[CrossRef\]](#)
73. Shu, Y.; Xu, J.; Chen, J.; Xu, Q.; Xiao, X.; Jin, D.; Pang, H.; Hu, X. Ultrasensitive electrochemical detection of H_2O_2 in living cells based on ultrathin MnO₂ nanosheets. *Sens. Actuators B Chem.* **2017**, *252*, 72–78. [\[CrossRef\]](#)
74. Gan, Y.; Hu, N.; He, C.; Zhou, S.; Tu, J.; Liang, T.; Pan, Y.; Kirsanov, D.; Legin, A.; Wan, H.; et al. MnO₂ nanosheets as the biomimetic oxidase for rapid and sensitive oxalate detection combining with bionic E-eye. *Biosens. Bioelectron.* **2019**, *130*, 254–261. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Leonardi, S.G.; Aloisio, D.; Donato, N.; Russo, P.A.; Ferro, M.C.; Pinna, N.; Neri, G. Amperometric Sensing of H_2O_2 using Pt-TiO₂/Reduced Graphene Oxide Nanocomposites. *Chemelectrochem* **2014**, *1*, 617–624. [\[CrossRef\]](#)
76. Patra, D.C.; Deka, N.; Dash, A.; Khan, S.A.; Misra, T.K.; Mondal, S.P. Noninvasive Electrochemical Nitric Oxide Detection in Human Saliva Using Pt and TiO₂ Nanoparticle Composite Electrodes. *ACS Appl. Electron. Mater.* **2023**, *5*, 832–845. [\[CrossRef\]](#)
77. Jiang, Q.; Wang, J.; Liu, T.; Ying, S.; Kong, Y.; Chai, N.; Yi, F.-Y. UiO-66-Derived PBA Composite as Multifunctional Electrochemical Non-Enzymatic Sensor Realizing High-Performance Detection of Hydrogen Peroxide and Glucose. *Inorg. Chem.* **2023**, *62*, 7014–7023. [\[CrossRef\]](#) [\[PubMed\]](#)
78. Kumar, V.; Sachdev, A.; Matai, I. Self-assembled reduced graphene oxide-cerium oxide nanocomposite@cytochrome c hydrogel as a solid electrochemical reactive oxygen species detection platform. *New J. Chem.* **2020**, *44*, 11248–11255. [\[CrossRef\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.