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Application of nanozyme-based biosensors in pesticide residue detection: a review

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Today, one of the important topics related to the health of humans and animals is the effective detection and monitoring of pesticide residues in environmental, food, and agricultural samples. This is because these chemical residues undergo ongoing eco- and bio-accumulation and can cause a variety of cellular toxicities in living organisms. Biosensor platforms are being developed and improved to detect pesticide residues in various sample matrices. The latest biosensors used in this field are nanozyme-based biosensors that operate based on nanomaterials' natural enzyme-mimicking catalytic activity, more commonly called nanozyme activity. In this review article, pesticides, their types, and their detection methods are first mentioned. Then, nanozymes, their classification, and their catalytic mechanisms are described. The last part also explains the use of nanozyme-based biosensors in detecting and identifying various pesticides and their advantages, and multiple examples are provided in this regard. Almost all articles of the last few years on detecting various pesticides using nanozyme-based biosensors have been reviewed in our paper.

1. Introduction

Agricultural pests include insects, plants, fungi, and bacteria that result in crop loss or reduced crop yield. Pests can also reduce crop quality and cause blemishes on agricultural products that reduce their value. Pest damage has plagued farmers since the beginning of agriculture, and efforts to reduce their damage have also begun and continue to this day. In the past century, the production of pesticides that kill pests has been the most critical human effort in the fight against agricultural pests.[1] The use of pesticides is a vital component of modern agriculture, significantly contributing to crop protection and food security.[2–4] In recent decades, using these chemicals has been the main and fundamental method of pest control, which has successfully reduced crop losses.[1] Nevertheless, pesticide residues in food and the environment pose serious risks to human health and ecological systems. Pesticides can contaminate soil, water, and air, adversely affecting biodiversity and ecosystem health. A substantial portion of applied pesticides does not reach the target pests. It disperses into the environment, leading to water contamination, soil fertility reduction, and harm to non-target organisms. Exposure to pesticide residues can lead to acute symptoms like headaches, dizziness, nausea, and skin irritations, and chronic health issues such as cancer, neurological disorders, and endocrine disruption. Organophosphate pesticides, for instance, are associated with neurodevelopmental disorders in children, while certain herbicides and fungicides have been linked to endocrine and reproductive issues.[5–8] Ensuring food products are free from harmful pesticide residues is crucial for consumer safety. Regulatory agencies set maximum residue limits (MRLs) to ensure safety, but violations can occur. Accurate detection and monitoring of pesticide residues help minimize their harmful impacts, promoting a balanced ecosystem, secure food supplies, and human well-being. Continuous research and technological advancements enhance detection capabilities, underscoring the vital role of pesticide detection in protecting our environment and health.[9] Thus, detecting these residues is crucial for safeguarding both ecological and human health, and advanced detection methods, such as biosensors and chromatography, are increasingly used to ensure food safety and maintain public health.[10–12] Gas chromatography (GC), high-performance liquid chromatography (HPLC), and their combination with mass spectrometry (MS) are among the most important analytical methods for pesticide detection,[13–15] which are not capable of rapid detection due to complex pretreatment and long

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specialized operations. Nowadays, biosensors are an excellent alternative to the aforementioned methods to detect pesticides more quickly, efficiently, and easily. Biosensors are sensitive, simple, and portable devices that can convert biochemical signals from the interaction between target molecules and biological recognition elements into optical or electronic outputs,[16–18] rapidly detecting different target molecules *in situ*.[19] In published research, various optical and electrochemical biosensors have been reported for pesticide detection.[20] Bioidentification elements, such as biological enzymes, are the mainstay of traditional biosensors. These biosensors have disadvantages such as high cost and instability, and do not operate in variable environments in the field.[21] Today, the use of nanomaterials to manufacture biosensors has expanded to overcome these disadvantages.[22] Among these nanomaterials, nanozymes or artificial enzymes with enzyme-like properties have an important place.[23,24] The synthesis of these nanomaterials is easy and inexpensive. They are also highly stable, and their catalytic activity can be designed by controlling their shape and size.[25,26] Nanozymes have wide applications in various fields, including the environment, diagnosis, and treatment of diseases, and one of their applications is the construction of sensors for accurate and rapid detection of pesticide residues.[27–32] Pesticide residues are an increasing concern for ecosystems and human health due to the widespread use of synthetic pesticides in modern agriculture. Conventional laboratory methods such as GC-MS and LC-MS, while highly accurate, are limited by long analysis times, high costs, and the need for specialized operators, making them impractical for routine or on-site detection. To address these drawbacks, electrochemical sensors and biosensors have emerged as effective alternatives, offering portability, ease of use, high sensitivity, and strong selectivity. Recent advances have further enabled integration with portable and smartphone-based platforms for real time, point of care monitoring of pesticide residues. For example, silver nanoparticle coated polydopamine copper phosphate hybrid nanoflowers have been reported as highly sensitive SERS probes for detecting thiol containing pesticides at picomolar levels. Such innovations highlight the growing scientific and industrial interest in portable biosensing devices that can deliver rapid, cost effective, and reliable pesticide detection. Within this context, nanozyme based biosensors are especially promising because they combine the catalytic efficiency of natural enzymes with the durability, stability, and affordability of nanomaterials, positioning them as next generation tools for food safety and environmental protection.[33–36] In recent years, nanozyme-based biosensors have been widely used for pesticide residue analysis, and their results have been published. So far, a few limited review articles have been written on using nanozymes in pesticide detection. However, there has been a great lack of space for a complete review article that introduces the types of pests, pesticides, old and new methods for their detection, and nanozymes, their types, and their applications in pesticide detection. In this review paper, while explaining all these cases, we have classified pesticides, and by reviewing the articles from recent years, we have introduced the types of nanozyme based biosensors that have been used for their detection. The key concepts of this review are summarized in the graphical abstract. It illustrates the central problem of pesticide residues in the environment affecting human health, presents nanozyme-based biosensors as a fast and sensitive solution, and details the common detection strategies (*e.g.*, colorimetric, fluorescent) and catalytic mechanisms (*e.g.*, POD, OXD) used to target various classes of pesticides.

2. Types of pesticides

Insecticides are chemicals used to control or eliminate insects, protecting crops from damage that can significantly affect yield and quality.[37] In this category, organophosphates such as malathion and chlorpyrifos are effective against a wide range of insects by inhibiting the enzyme acetylcholinesterase.[38,39] Carbamates such as carbaryl and aldicarb are similar to organophosphates but less persistent in the environment.[40,41] Also, pyrethroids such as permethrin and cypermethrin are synthetic versions of natural pyrethrins from chrysanthemums, known for their quick action.[42,43]

Herbicides control unwanted plants or weeds that compete with crops for nutrients, water, and sunlight. They can be selective (targeting specific weeds) or non-selective (killing all vegetation).[44] In this category, glyphosate is a non-selective herbicide inhibiting a plant enzyme involved in synthesizing essential amino acids, which is widely used in agriculture and horticulture.[45,46] Atrazine is a selective herbicide primarily controlling broadleaf and grassy weeds in crops like corn and sugarcane by inhibiting photosynthesis.[47]

Fungicides are chemicals that prevent or eliminate fungal infections in plants, which can cause significant crop losses and affect food quality.[48] Chlorothalonil is a broad-spectrum fungicide controlling fungal diseases in vegetables, fruits, and ornamental plants by inhibiting spore germination and mycelial growth.[49] Mancozeb is a multi-site fungicide interfering with multiple enzyme systems in fungi, reducing resistance development, and used on crops like potatoes, tomatoes, and grapes.[50]

Rodenticides manage rodent populations that can damage crops, stored food, and property. They are often formulated as baits to attract rodents.[51,52] In this category, warfarin is an anticoagulant rodenticide causing internal bleeding in rodents, now less common due to resistance.[53] Bromadiolone is a more potent, second-generation anticoagulant effective against resistant rodents by inhibiting vitamin K synthesis, essential for blood clotting.[54]

3. Pesticides analytical detection methods

Ensuring the detection of pesticide residues is vital for safeguarding food supplies, protecting ecosystems, and preventing harmful health effects in humans. Chromatography techniques are widely used for their accuracy and reliability in detecting pesticide residues. Gas chromatography (GC) separates volatile compounds based on boiling points and interactions with the column's stationary phase. This technique is effective for detecting organochlorine and organophosphate pesticides, often coupled with detectors like flame ionization detector (FID) or electron capture detector (ECD).[55] HPLC is used for non-volatile and thermally unstable compounds. It separates compounds based on interactions with the stationary and mobile phases, commonly used for detecting herbicides and fungicides. It can be coupled with detectors like UV-vis, diode array detector (DAD), and MS for enhanced detection.[56] MS is often coupled with chromatography techniques like GC and HPLC for enhanced sensitivity and specificity. GC-MS allows for precise identification and quantification of pesticide residues. GC separates compounds, while MS provides detailed molecular information.[57] LC-MS is used for detecting a wide range of polar and non-volatile pesticides, offering high sensitivity and specificity.[58] Although chromatography based methods (*e.g.*, GC-MS, LC-MS) remain the gold standard for pesticide residue analysis due to their precision and reliability, they are constrained by several limitations. These techniques require laborious sample pretreatment, sophisticated instrumentation, and trained personnel, which restrict their application in on site or large-scale monitoring. Additionally, analysis times can be lengthy, limiting their use in situations that demand rapid decision making. These drawbacks underscore the need for alternative platforms, such as nanozyme-based biosensors, which offer speed, portability, and cost effectiveness while maintaining high sensitivity.

Electrochemical methods utilize changes in electrical properties to detect pesticide residues.[59] For instance, voltammetry measures current as a function of applied voltage, which is used for pesticides that undergo redox reactions. Differential pulse voltammetry (DPV) detects organophosphate pesticides by measuring oxidation or reduction currents.[60] Impedance spectroscopy measures system impedance over a frequency range, detecting changes in sensor surface electrical properties upon pesticide molecule binding.[61] Also, conductometric sensors measure changes in electrical conductivity due to pesticides' presence, suitable for on-site applications.[62]

Biosensors are emerging as rapid, cost effective alternatives for on site detection of pesticide residues.[63] Aptamer based biosensors use nucleic acid sequences that bind specifically to target molecules, offering high selectivity and sensitivity for various pesticides.[64,65] Also, enzyme-based biosensors use enzymes that react with specific pesticides, producing measurable signals. For example, acetylcholinesterase-based biosensors detect organophosphate and carbamate pesticides.[66,67]

Nanomaterial-based biosensors are another group of biosensors that enhance sensitivity and stability using nanomaterials like gold nanoparticles or carbon nanotubes, detecting pesticides at very low concentrations.[68]

Nanozymes are nanomaterials with intrinsic enzyme-like activities. They can mimic the catalytic functions of natural enzymes, such as oxidases, peroxidases, and catalases.[69] Nanozyme-based biosensors offer several unique advantages over traditional enzymes for pesticide detection. Their stability, cost-effectiveness, ease of production, and enhanced detection capabilities position them as a superior choice compared to conventional methods.[70]

4. Nanozymes

As previously mentioned, nanozymes are nanomaterials with intrinsic enzyme-like properties that offer a fascinating alternative to natural enzymes in various applications due to their stability, ease of production, and robustness. In this section, we describe the types of structures and morphologies of nanozymes, their classification, catalytic mechanisms, and factors affecting them.

4.1. Morphology and structure

Nanozymes come in various structural forms, such as nanoparticles that are small particles ranging in size from 1 to 100 nanometers, often composed of metals like gold, silver, or iron.[71] Another form of nanozymes is nanotubes, which are cylindrical nanostructures, usually made of carbon, that can mimic enzyme activity.[72] Another category is nanowires which are wire-like structures at the nanoscale, which are used due to their high aspect ratio and catalytic properties.[73] Also, nanocomposites are hybrid materials combining different types of nanomaterials to enhance the enzymatic activity of nanozymes.[74] The morphology of nanozymes plays a decisive role in their catalytic performance. Nanoparticles, with their high surface-to-volume ratio, expose more active sites and generally exhibit stronger peroxidase-like activity. Nanotubes and nanowires, due to their elongated one-dimensional structures, enable efficient electron transfer along their axes, which is highly advantageous for redox-driven reactions. In contrast, nanocomposites, which integrate multiple nanomaterials, generate synergistic effects that enhance catalytic efficiency, stability, and selectivity. For example, porous frameworks provide greater access to active sites, while core shell architectures improve substrate affinity and overall stability. In Fig. 1, schematic overview linking nanozyme morphology to catalytic performance and illustrating their representative catalytic pathways. Different morphologies such as nanoparticles, nanotubes, nanowires, and nanocomposites influence surface area, electron transport, and synergistic interactions, thereby shaping catalytic efficiency. In parallel, nanozyme mechanisms including peroxidase like catalysis by Fe_3O_4 and Prussian blue systems, as well as the $Fe^{IV}{=}O$ intermediate pathway of HRP highlight how structural and electronic features govern activity and stability in biosensing applications. These mechanistic insights demonstrate how morphology and electronic structure govern the catalytic efficiency of nanozymes, directly impacting their biosensing performance.[75]

4.2. Classification and catalytic mechanisms

There are different classifications of nanozymes based on their catalytic activities. Their catalytic mechanisms can mimic several natural enzymes, including peroxidases (POD), superoxide dismutases (SOD), oxidases (OXD), and catalases (CAT).[76,77] POD-mimicking nanozymes catalyze the reduction of hydrogen peroxide (H_2O_2) to water, resembling the function of natural peroxidases.[78,79] Iron oxide nanoparticles (Fe_3O_4) are a prime example, capable of oxidizing substrates like 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of H_2O_2,

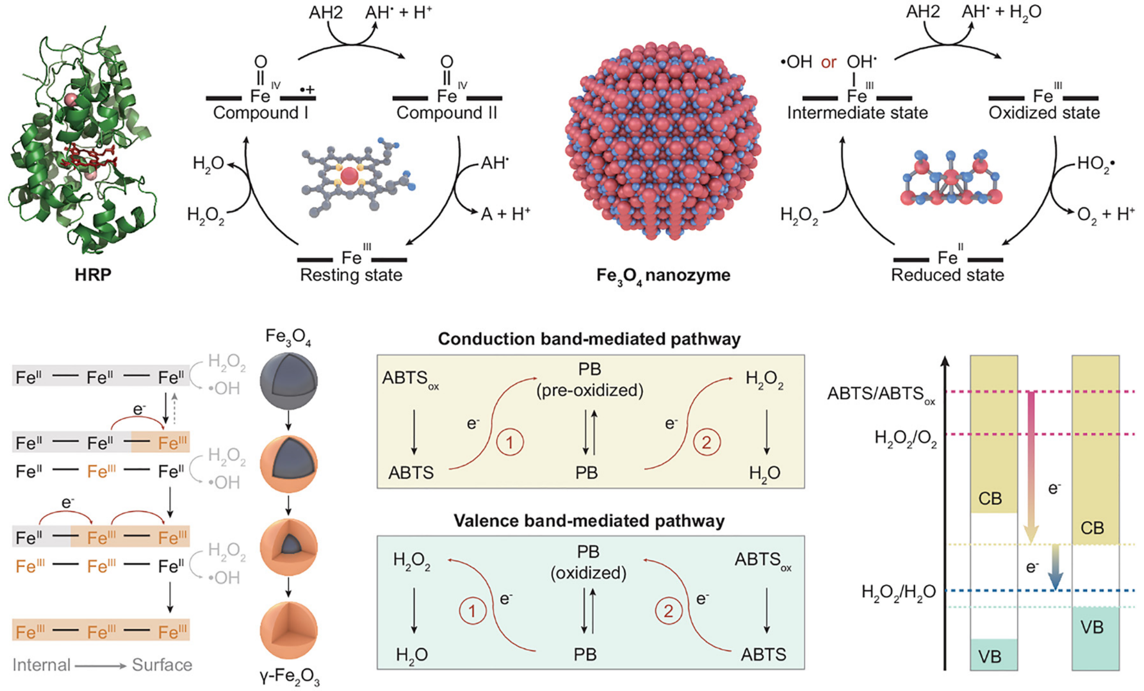

Fig. 1 A schematic illustration showing the relationship between nanozyme morphology and catalytic performance[75] [Licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License].

producing a color change detectable by spectroscopy. This activity has significant applications in biosensing and environmental monitoring.[80] SOD-mimicking nanozymes catalyze the dismutation of superoxide radicals (O^{2-}) into less reactive molecules, namely oxygen and hydrogen peroxide.[81,82] Cerium oxide nanoparticles (CeO_2), for example, can mimic SOD activity due to the reversible redox cycling between Ce^{3+} and Ce^{4+}, providing antioxidant properties beneficial for therapeutic applications.[83] In this classification, oxidase-mimicking nanozymes catalyze oxidation reactions like natural oxidases.[84] Also, catalase-mimicking nanozymes catalyze the decomposition of hydrogen peroxide into water and oxygen, similar to natural catalases, (Fig. 2).[85,86]

4.3. Factors influencing catalytic efficiency

Several factors influence the catalytic efficiency of nanozymes. The elemental composition and structural characteristics of nanozymes significantly impact their catalytic performance. For example, the specific surface area, particle size, and crystallinity determine the active sites available for catalysis. Optimizing these parameters can enhance the interaction between nanozymes and substrates, improving catalytic efficiency.[87] Surface functionalization of nanozymes with organic or inorganic molecules can enhance their catalytic activity and selectivity. Modifying the surface with polymers, ligands, or biomolecules can improve substrate affinity and increase stability in biological environments.[88] For instance, coating iron oxide nanoparticles with dextran enhances their peroxidase activity and biocompatibility.[89] The catalytic efficiency of nanozymes is influenced by environmental conditions such as pH, temperature, and ionic strength of the reaction environment. Nanozymes often exhibit optimal activity within specific pH and temperature ranges. For instance, iron oxide nanozymes show maximum peroxidase activity at acidic pH, while gold nanozymes perform best in neutral to slightly basic conditions.[90] The concentration of substrates affects the catalytic efficiency of nanozymes. At low substrate concentrations, the reaction rate increases with an increase in substrate concentration until a saturation point is reached. Beyond this point, additional substrate does not increase the reaction rate, a behavior similar to Michaelis–Menten kinetics observed in natural enzymes.[91] Co-factors, such as metal ions or organic molecules, can enhance or inhibit the catalytic activity of nanozymes. For example, the addition of ascorbic acid can improve the oxidase-mimicking activity of gold nanoparticles, while specific metal ions can inhibit their activity.[92] The aggregation state of nanozymes can affect their catalytic properties. Dispersed nanozymes have higher surface area and accessibility to substrates, leading to improved catalytic efficiency. Conversely, agglomeration can reduce active surface area and hinder substrate interaction, decreasing catalytic performance.[93] Moreover, by precisely tuning their composition, morphology, and surface chemistry, nanozymes have proven extraordinarily versatile—enabling ultrasensitive electrochemical sensing of chemotherapeutics,[94] green extraction of bioactive natural products,[95] plant-inspired therapeutic nanoparticle synthesis for psoriasis,[96] peroxidase-mimetic glucose assays,[97] environmental biomarker monitoring for biodiversity conservation,[98] and industrial dye quantification *via* DNA-based nano-biocomposite electrodes.[99]

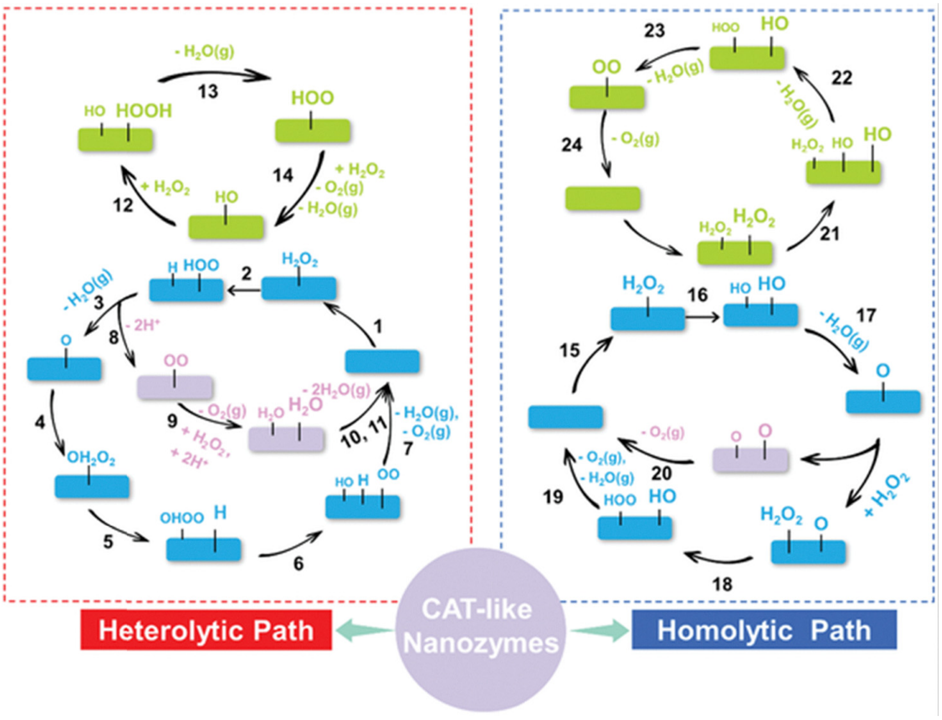

Fig. 2 Schematic representation of the catalytic mechanism of CAT-like nanozymes; the single ring on the left denotes the heterolytic reaction pathway, whereas the double ring on the right corresponds to the homolytic pathway (Licensed under ref. 86).

5. Application of nanozymes in pesticide detection

Excessive pesticide application in agriculture has raised significant concern because of their environmental persistence, bioaccumulation, and potential health hazards. Challenges arising from such facts make nanotechnology provide alternative methods for developing nanozymes as efficient, cost-effective, and sustainable alternatives for conventional pesticide detection. Nanozymes have become highly active nanomaterials possessing enzyme-like properties, becoming prospective candidates for pesticide detection by biosensors.[100] The various attempts at using nanozyme-based biosensors in detecting pesticides have been successfully demonstrated in recent years, with massive improvements regarding sensitivity and selectivity. Nanozymes applications in biosensors showed promising results in real-time monitoring of pesticides, thus contributing to more sustainable agricultural practices.[101] Their high sensitivity and specificity make them suitable for rapid detection methods.[102] In this section, we have categorized the main types of pesticides and explained each one. Then, we have described some examples of studies conducted in pesticide detection using nanozyme-based biosensors. Finally, we have provided a table in which other studies conducted in this field are listed.

5.1. Detection of organophosphorus pesticides

Organophosphate (OPs) pesticides are among the most widely used highly toxic pesticides and threaten food safety. They are commonly used in agricultural production due to their high efficiency and positive characteristics.[103,104] Improper use of this group of pesticides causes their residues to enter crops, water, and soil, and creates irreversible risks to human health,[105,106] including inhibiting cholinesterase (ChE) activity in the human body and causing neurological symptoms.[107,108] International regulatory bodies have imposed restrictions on the use of OPs, so that their use has been banned or restricted in many countries.[109] Accordingly, detecting OPs residues is vital for human health and the environment. As you read in the previous sections, using nanozyme-based biosensors is one of the newest diagnostic methods in this field.

In 2023, Wang *et al.* introduced a method for detecting OPs by using a POD based on the photothermal effect. The device was designed with a gold nanobipyramid (Au NBP) core, which was coated by a thin layer of MIL-101–NH_2Fe, creating a hybrid nanozyme with a core–shell structure. This structure played a crucial role in enhancing both sensitivity and specificity. The synthesis involved growing a MIL-101–NH_2Fe shell around the Au NBP core, forming a hybrid nanozyme. The Au NBP, due to its photothermal properties, efficiently absorbs near-infrared (NIR) light, generating localized heat under laser irradiation, which in turn boosts the nanozyme's peroxidase-like (POD) activity. This leads to a remarkable 100-fold increase in sensitivity for detecting OPs, such as paraoxon-ethyl. The development of this hybrid nanozyme also enables the creation of a colorimetric detection platform. The platform works by combining acetylcholinesterase (AChE) inhibition with the enhanced catalytic activity of the nanozyme, allowing for a clear visual signal in the presence of OPs. This approach was further incorporated self-validating test strips, which enable real-time, sensitive visual detection of OPs, providing significant potential for practical applications in environmental monitoring and food safety. These test strips provide an easy and effective solution for detecting organophosphorus pesticides, such as paraoxon-ethyl, in various sample types. A significant advancement is their ability to perform real-time detection with high sensitivity, which enhances practical applications compared to earlier, more complicated methods.[110]

Li *et al.* synthesized $PtCu_3$ alloy nanocrystals (NCs) using a one-step reduction method, which provides a straightforward and scalable approach. This synthesis technique enhances the catalytic efficiency of the $PtCu_3$ NCs, which exhibit peroxidase-like activity for identifying organophosphorus pesticides. These nanoparticles catalyzed the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB), resulting in a color change from colorless to blue, serving as the detection signal. The method also incorporates the enzyme AChE, which hydrolyzes acetylthiocholine into thiocholine, inhibiting TMB oxidation and causing a decrease in blue color intensity. However, the presence of OPs inhibited AChE activity, leading to a stronger blue color.[111]

Wanqi *et al.* introduced a dual-mode sensing system for detecting organophosphorus pesticides, utilizing platinum–nickel nanoparticles (Pt–Ni NPs) with inherent oxidase-like (OXD) activity. These nanoparticles were synthesized through a straightforward one-step reduction process, producing highly active Pt–Ni NPs with optimized size and composition for superior catalytic efficiency. The resulting nanozymes catalyzed the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB), which produced a distinct blue oxidized form (oxTMB) while also generating heat under near-infrared irradiation. This enabled simultaneous colorimetric and photothermal detection within a single platform. The sensing mechanism is based on the hydrolysis product of acid phosphatase (ACP) with L-ascorbic acid 2-phosphate, which typically suppresses TMB oxidation. When OPs are present, they deactivate ACP, lifting this inhibition and allowing Pt–Ni NPs to drive the catalytic oxidation of TMB. This produces measurable changes in both solution color and temperature. In addition, trisodium phosphate was found to compete with the OXD-like activity of Pt–Ni NPs, further confirming the validity of the proposed reaction pathway. The developed dual-mode sensor exhibited a clear linear response to chlorpyrifos, achieving detection limits of 1.2 ng mL^{-1} for colorimetric analysis and 1.66 ng mL^{-1} for photothermal analysis. Importantly, the combination of two complementary readouts addresses the susceptibility of single-mode sensors to environmental disturbances, thereby improving both sensitivity and accuracy. This work presents a practical, rapid, and reliable strategy for OP detection and demonstrates strong potential for applications in food safety monitoring and environmental protection, (Fig. 3).[112]

Zeng *et al.* developed an approach to overcome the challenges associated with traditional oxidase-like nanozymes, which often struggle with fluctuating oxygen levels during catalysis, affecting detection accuracy. To address this, they synthesized $Au@MnO_{2-X}$ nanozymes utilizing a core–shell structure, where the inner gold (Au) core significantly enhances electron transfer to the thin manganese dioxide (MnO_{2-X}) layer (see Fig. 4). This innovative design promotes catalytic oxidation that is independent of oxygen, relying instead on efficient electron transfer. As a result, a novel nanozyme-based sensor that operates independently of oxygen was developed through homogeneous electrochemistry, demonstrating an outstanding detection limit of 0.039 ng mL^{-1} for organophosphorus pesticides. Moreover, the integration of UV-vis spectroscopy with 3,3′,5,5′-TMB and the use of linear discriminant analysis allowed the sensor to effectively distinguish among five different pesticides: diazinon, dipterex, ethion, chlorpyrifos methyl, and omethoate. The $Au@MnO_{2-X}$ nanozyme system also successfully mitigates interference from dissolved oxygen and

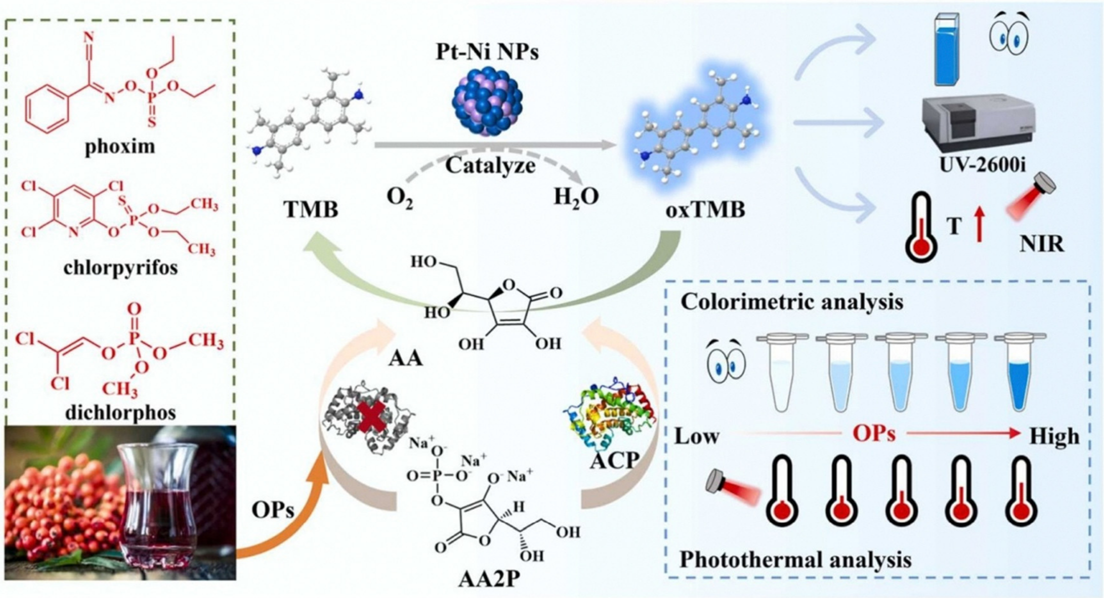

Fig. 3 Diagram illustrating the probe designed for dual-mode colorimetric and photothermal sensing of organophosphate residues, which is based on the oxidase-like (OXD) activity of platinum–nickel nanoparticles (Pt–Ni NPs) (Licensed under ref. 112).

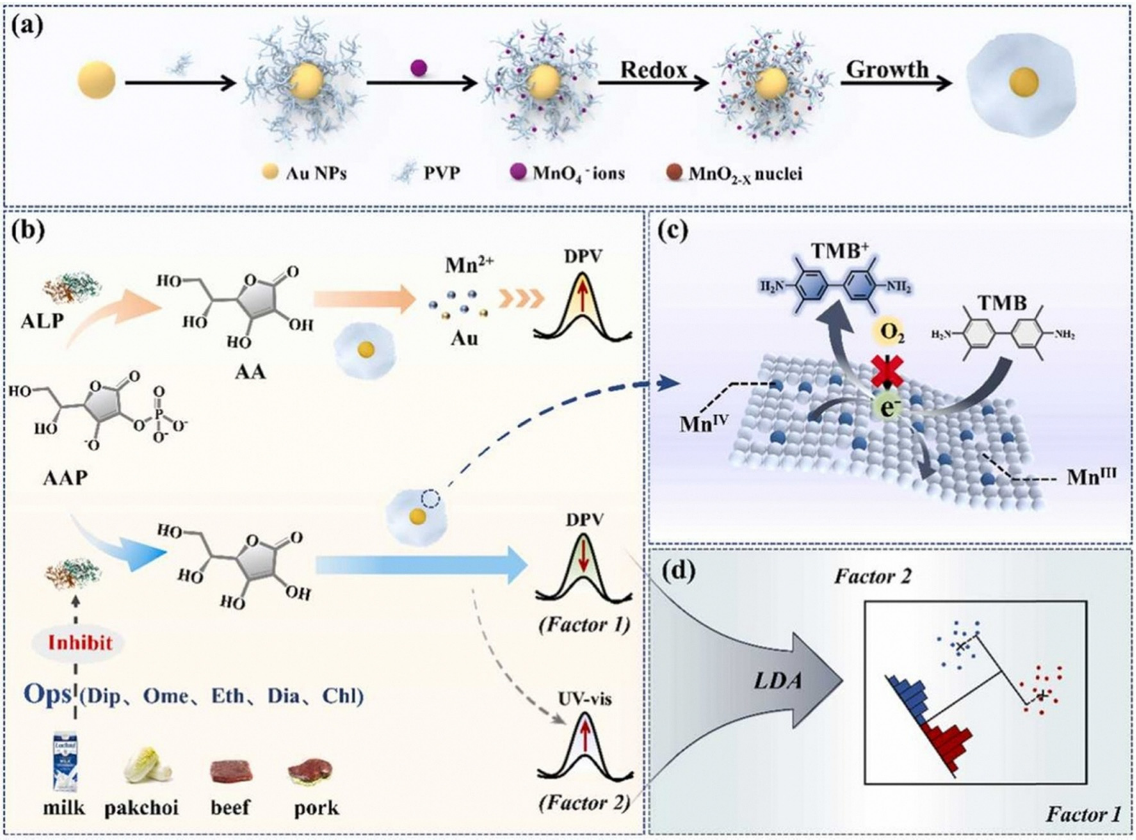

Fig. 4 Diagram illustrating the synthesis, detection, and discrimination sensor for recognizing organophosphorus pesticides (OPs) using Au@MnO_{2-X} nanosheets (NSs). The diagram includes: (a) the synthesis process of Au@MnO_{2-X} NSs, (b) the design of the HEC sensor based on Au@MnO_{2-X} NSs for detecting OPs, (c) the catalytic mechanism of Au@MnO_{2-X} NSs with TMB, and (d) the application of linear discriminant analysis (LDA) for distinguishing five different OPs (Licensed under ref. 113).

color, common challenges in detection methods. The study further examined the inhibition mechanism of organophosphorus pesticides (OPs) on alkaline phosphatase (ALP), leading to the design of a dual-signal sensor array that combines ALP and Au@MnO_{2-X} nanozymes for ultrasensitive detection and differentiation of OPs.[113]

Shen's research group has proposed a dual-mode sensor for detecting organophosphorus pesticides (OPs) using oxidase-like 2D fluorescent nanozymes based on ultrathin C_3N_4 nanosheets. Unlike traditional approaches relying on peroxidase-like activity with hazardous H_2O_2, their approach utilizes PtPd nanoparticles grown on ultrathin 2D graphitic carbon nitride (g-C_3N_4) nanosheets. This nanozyme exhibits oxidase-like activity, facilitating the removal of $O_2^{-1\bullet}$ from dissolved O_2 during acetylcholinesterase hydrolysis of acetylthiocholine to thiocholine, which inhibits the oxidation of *o*-phenylenediamine to 2,3-diaminophenothiazine (DAP). In the presence of OPs, which inhibit AChE and reduce TCh production, the unblocked PtPdNPs@g-C_3N_4 nanozyme enhances DAP production, leading to noticeable color changes and dual-color ratiometric fluorescence in the sensing system. Integrated with a smartphone, this H_2O_2-free nanozyme-based 2D sensor offers colorimetric visual imaging and dual-mode fluorescence capabilities for OPs detection in real samples. This sensor represents a significant advancement for potential commercial applications in early warning systems and environmental food and health safety management, effectively reducing risks associated with OPs pollution.[114]

5.1.1. Malathion.

The most widely used insecticide among organophosphate pesticides is malathion, which is used to repel a wide range of insects in different environments.[115] Today, more than 700 malathion-based pesticides are used on crops such as peanuts, rice, and wheat.[116] The Environmental Protection Agency has classified this substance as a group III carcinogen. Therefore, excessive and long-term use of this chemical poses a serious risk to human health.[117] For this reason, countries and international organizations have set strict limits on the use of malathion in food.[118] Accordingly, accurate, rapid, and efficient detection of malathion is crucial for maintaining food safety and, consequently, human health.[119] Nanozyme-based biosensors are the best option for this task.

In the study by Huang *et al.*, a stable colorimetric biosensor selective detection of malathion residue in food is developed based on aptamer-regulated laccase-mimic activity. This innovative biosensor integrates smartphone technology to facilitate rapid and user-friendly detection. The biosensor employs aptamer M17-F, which enhances the affinity of Ag_2O nanoparticles for the substrate 2,4-dichlorophenol, thus significantly improving their laccase-mimicking abilities. The synthesis of the biosensor involves several critical steps. Initially, Ag_2O nanoparticles are synthesized through a chemical reduction method, followed by their functionalization with the M17-F aptamer. This modification ensures that the nanoparticles exhibit a higher binding affinity for the target malathion, enhancing their catalytic efficiency. During the catalytic process, the laccase-mimic activity generates an abundance of semiquinone radicals that react with a chromogenic agent, forming dark red products. This color change can be easily captured by smartphone cameras, which measure the RGB values in the solution, facilitating quick detection of malathion residues. The biosensor demonstrates a low detection limit of 5.85 nmol L^{-1} and shows selectivity over other competing pesticides, highlighting its effectiveness for real sample applications. The innovative aspect of this research lies in integrating aptamer technology with laccase-mimicking activity, offering a significant advancement over previous detection methods. This approach enhances sensitivity and selectivity and streamlines the detection process, making it suitable for point-of-care testing in food safety applications. This biosensor showcases a promising solution for detecting malathion residues, contributing to food safety and quality assurance.[120]

In another study, the detection of malathion based on the enhanced oxidase-mimetic activity of polydopamine-coated palladium nanocubes (PDA-Pd/NC) constructed an efficient and sensitive colorimetric detection platform. The synthesis of PDA-Pd/NCs involves several key steps. Initially, palladium (Pd) nanocubes are synthesized through a chemical reduction, typically using a palladium precursor such as $PdCl_2$. The nanocubes are then coated with polydopamine through the oxidative polymerization of dopamine, which significantly enhances their catalytic properties. This coating increases substrate accumulation and accelerates electron transfer, producing remarkable oxidase-like activity. The researchers utilize TMB as a chromogenic substrate to successfully detect acid phosphatase (ACP) activity, relying on the vigorous oxidase activity of PDA-Pd/NCs. When malathion is present, it inhibits ACP activity, resulting in decreased production of the chromogenic product. Consequently, a colorimetric assay based on the PDA-Pd/NCs, TMB, and ACP system is developed. The method exhibits a broad linear detection range of 0–8 μM. It achieves a low detection limit of 0.023 μM, indicating exceptional analytical performance surpassing many previously reported malathion analysis methods. This research offers a novel approach to enhancing the catalytic activity of dopamine-coated nanoenzymes, presenting a significant advancement in pesticide detection strategies, particularly for malathion. The pioneering integration of PDA-Pd/NCs not only improves sensitivity but also provides a practical framework for real-time monitoring of pesticide residues, underscoring the importance of establishing efficient methods to ensure food safety.[121]

5.1.2. Dichlorvos. One of the most common and effective insecticides used in various countries is dichlorvos (DDVP), the type of OPs.[21] According to the World Health Organization (WHO) classification, this chemical is in class 1B of highly hazardous chemicals.[122] DDVP is highly toxic and, even at low concentrations, can inhibit the activity of ChE and increase the amount of the neurotransmitter acetylcholine. This causes excessive stimulation of synaptic transmission and ultimately cholinergic toxicity, paralysis, and even death.[123] International organizations have imposed strict restrictions on the use of DDVP, but unfortunately, today, excessive and non-standard use of this substance has caused food contamination. Therefore, detecting DDVP in food and monitoring its amount is very important. Simple, rapid, and efficient detection techniques, such as using nanozyme-based biosensors, are among the latest methods for identifying this chemical.[124]

In 2024, Liu *et al.* developed a portable colorimetric sensing platform to quickly and precisely quantify DDVP pesticide. This innovative platform employs a bimetallic oxide nanozyme, made from iron and manganese ($FeMnO_x$), to facilitate highly efficient chromogenic catalysis (Fig. 5). The synthesis process for the $FeMnO_x$ nanozyme included a co-precipitation technique followed by thermal treatment to enhance its catalytic effectiveness. With impressive oxidase-like activity, the nanozyme successfully oxidizes 3,3′,5,5′-TMB to produce a blue-colored compound, showcasing a low Michaelis–Menten constant (K_m) of 0.0522 mM, which reflects its catalytic performance. The presence of DDVP influences the chromogenic reaction by inhibiting the activity of acetylcholinesterase, resulting in noticeable color changes. These alterations can be measured spectrophotometrically, directly linking to DDVP concentration. To improve usability, the researchers incorporated a 3D-printed mini lightbox with a smartphone to capture and analyze the colorimetric signals, enabling field-based detection. This platform demonstrated a broad detection range of 1 to 3000 ng mL^{-1}, with a detection limit of 0.267 ng mL^{-1}. Its simplicity, sensitivity, and cost-effectiveness distinguish it from earlier DDVP detection methods. By integrating advancements in nanozyme technology, 3D printing, and digital information processing, this portable sensing platform presents a fresh approach to monitoring pesticide residues in food products, addressing vital issues related to food safety and environmental health.[124]

5.1.3. Diazinon. Diazinon is one of the most famous organophosphate insecticides widely used for various crops. Diazinon is used to control and repel a wide range of pests,[125] but excessive amounts of it are hazardous for human health and can cause side effects such as muscle tremors, blurred vision, nausea, and difficulty breathing. It has been proven that this chemical can be mutagenic and cause various types of cancer in humans.[126,127] Accordingly, rapid and accurate detection of diazinon residual amounts in water and food is crucial for maintaining human health. One of the best methods for measuring this substance is using nanozyme-based biosensors.

In 2023, Abdolmohammad-Zadeh *et al.* introduced a chemiluminescence biosensor based on a molybdenum disulfide and zirconium metal–organic framework nanocomposite (MoS_2@-MIP-202(Zr)) for the indirect monitoring of diazinon. This novel nanocomposite showcased peroxidase-like activity within a $NaHCO_3$–H_2O_2 chemiluminescence system, utilizing the inhibitory effect of diazinon on acetylcholinesterase to facilitate detection. The synthesis involved the hydrothermal creation of MoS_2 and the solvothermal preparation of the zirconium metal–organic framework, followed by their integration to form

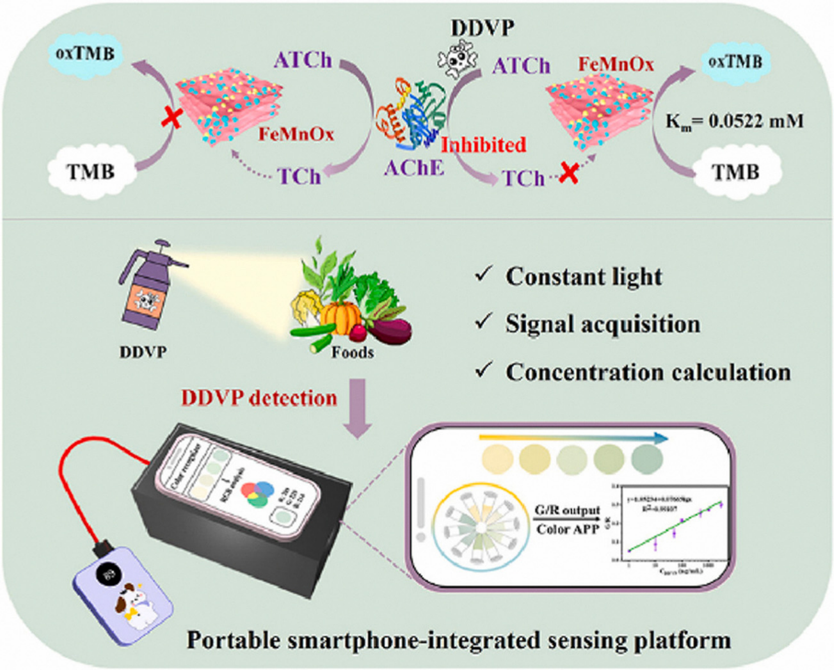

Fig. 5 Illustration of a smartphone-integrated portable colorimetric detection platform using FeMnO$_x$ nanozyme for quantifying DDVP (Licensed under ref. 124).

a stable composite. The resulting biosensor demonstrated impressive performance, with a linear calibration range of 0.5 to 300.0 nmol L^{-1}, a detection limit of 0.12 nmol L^{-1}, and a quantification limit of 0.40 nmol L^{-1}. Its relative standard deviations (% RSD, n = 5) were 3.66% for within-day and 1.35% for between-day measurements at a diazinon concentration of 100 nmol L^{-1}, indicating high precision. The biosensor also achieved excellent recovery rates for spiked water samples, with tests on certified reference materials confirming its accuracy and absence of matrix interference. This innovative biosensor signifies a noteworthy improvement in the sensitive and accurate detection of diazinon in environmental monitoring systems.[128]

5.1.4. Acetamiprid.

5.1.4. Acetamiprid. Acetamiprid is a neonicotinoid insecticide widely used in agricultural production.[129] This chemical can contaminate soil and water and pose significant risks to human health and the environment.[130,131] Neurotoxic effects, infertility, and adverse effects on fetal development are among the most important adverse effects of acetaminophen on human health.[132,133] Accordingly, it is essential to design and develop accurate and reliable methods for detecting this chemical, one of the most recent of which is nanozyme-based biosensors.

In the research conducted by Yu *et al.*, a novel aptasensor was developed to detect acetamiprid (Ace) in environmental water samples. This study presented an innovative approach using Fe–N–C single-atom nanozymes (SAzymes) to enhance the monitoring of Ace in water. The Fe–N–C SAzymes were synthesized using a simple method that ensured optimal atom utilization and uniform distribution. These nanozymes demonstrated exceptional catalytic activity and stability, essential for reliable detection. The researchers thoroughly examined the oxidase-like properties of the SAzymes, revealing their effectiveness in catalyzing reactions with chromogenic indicators like (TMB). This interaction enabled efficient signal transduction during the detection process. In addition, using thiol-modified aptamers was significant in modulating the oxidase-like activity of the Fe–N–C SAzymes, thus enhancing the system's specificity and sensitivity. The study achieved an impressive limit of detection of 16.9 nM for Ace, demonstrating the efficacy of this cutting-edge approach. The key advantages of this research over previous methods stem from the utilization of single-atom nanozymes, which enhance catalytic performance and allow for greater control over enzymatic activity through aptamer regulation. This results in a detection platform that is both highly sensitive and selective, offering promising applications for biological research and environmental monitoring, thereby highlighting the potential of multifunctional Fe–N–C SAzymes in advancing biosensor technologies.[134]

5.1.5. Dimethoate. Dimethoate (DMT) is a highly effective acaricide that is widely used for cash crops.[135] Excessive use of this pesticide causes it to enter plant tissues, soil, and water,[136] and can pose risks to human health. One of the dangers of this chemical is the accumulation of acetylcholine,[137] which causes excessive stimulation of the central nervous system and cholinergic nerves, and ultimately, abnormalities in the nervous system, reproductive system, and liver.[138] Accordingly, designing a sensitive, easy, and rapid method for detecting dimethoate residues is essential. The use of nanozyme-based biosensors is one of these methods.

Xia *et al.* (2024) developed a smartphone-assisted colorimetric sensor and enzyme sheets for the swift detection of dimethoate residues in vegetables, leveraging the inhibitory effect of dimethoate on the laccase-like activity of coral-like silver citrate (AgCit). It was found that dimethoate attaches to the AgCit surface, decreasing semiquinone radical production and lowering the laccase activity. This inhibition results in a noticeable color shift from red to light pink or colorless, with a detection limit as low as 1.0 μg L^{-1}. The enzyme sheets demonstrated excellent selectivity and rapid response, providing results in just 5 minutes. Furthermore, AgCit catalyzes the conversion of 2,4-dichlorophenol, producing quinones that subsequently react with 4-aminoantipyrine to yield a red compound that peaks at an absorption wavelength of 510 nm. The adsorption of dimethoate on AgCit obstructs its active sites, further diminishing the catalytic activity and leading to a linear response in absorbance. By immobilizing AgCit on a membrane, the system's visual detection capabilities were enhanced, offering an innovative approach to detecting pesticide residues while expanding the use of laccase-like activity for other analytes.[139]

5.2. Detection of herbicides

5.2.1. Atrazine. Atrazine is one of the S-triazines that are the most widely used herbicides in the world. This chemical is widely used to control broadleaf weeds and annual grasses.[140] After application, atrazine remains in the soil for about 100 days and can therefore contaminate surface and groundwater sources for drinking.[141] Even low concentrations of this chemical in drinking or recreational water pose a risk to human health and can disrupt the endocrine glands.[142] Some studies have also shown that atrazine causes premature and small babies to be born.[143] For this reason, detecting and determining the amount of atrazine in human health is very important. Using biosensors based on nanozymes is one of this field's most accurate analytical methods.

Du *et al.* developed a self-powered sensor that operates without an external power source, showcasing its potential for portable detection of environmental contaminants (Fig. 6). This sensor was designed explicitly for the ultra-sensitive detection of atrazine, an endocrine disruptor. An essential innovation of this study is using a cobalt-metal–organic framework (Co-MOF) nanozyme, miming glucose oxidase (GOD) activity to provide continuous energy through glucose oxidation. The Co-MOF nanozyme was synthesized *via* a solvothermal process and optimized for size and morphology, demonstrating high catalytic efficiency (K_m = 15.8 mM). The sensor's unique design separates anodic energy generation from cathodic recognition, enhancing both selectivity and sensitivity. It achieves a wide detection range of 1 pM to 100 nM, with an exceptionally low detection limit of 0.65 pM. These features indicate that the sensor has promising selectivity and stability compared to existing sensors.[144]

5.2.2. Glyphosate. Glyphosate is an effective and widely used herbicide, the use of which is increasing worldwide.[145] Despite this, excessive and prolonged use of this pesticide leads to contamination of water and soil resources and, consequently, the food chain, and can enter the human body and threaten their health.[146,147] Heart damage,[148] disruption of lipid metabolism,[148] and increased oxidative stress[149] are among the most critical risks of glyphosate on human health. Therefore, the design and development of rapid, efficient, and convenient glyphosate detection methods, such as nanozyme-based biosensors, is important.

In 2024, Huang *et al.* underscored the pressing need to address public concerns regarding food safety and environmental protection by emphasizing the importance of sensitive glyphosate detection. Their study identified glyphosate's unique ability to interact with nanozymes derived from iron organic frameworks (Fe-MOFs), enabling selective detection of this herbicide. To tackle this challenge, the researchers developed a novel approach for dual-mode glyphosate detection

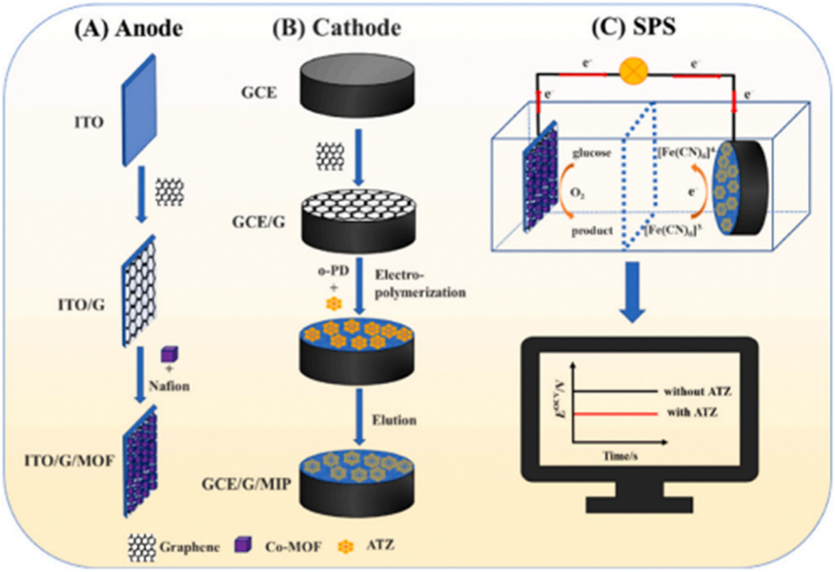

Fig. 6 The process involves preparing the MOF- customized anode (A), cathode (B), setup and detection steps for self-powered (C) (Licensed under ref. 144).

using colorimetric and fluorescence methods. This innovative strategy not only enhances detection sensitivity but also broadens the applicability of the sensor in various contexts. Fe-MOFs were synthesized under mild conditions, which is a significant advantage over traditional synthesis methods that often require harsh environments. The process involved mixing iron salts with organic linkers to form a stable framework. This careful control over synthesis conditions resulted in Fe-MOFs that exhibited notable peroxidase-like activity. The nanozymes effectively catalyzed the conversion of 3,3′,5,5′-TMB in the presence of H_2O_2, facilitating the colorimetric response necessary for glyphosate detection. One of this approach's key innovations is glyphosate's ability to inhibit the catalytic activity of Fe-MOFs through physical interactions (such as electrostatic and hydrogen bonding) and chemical interactions. This mechanism of interference results in reduced absorbance and increased fluorescence, thereby optimizing detection sensitivity.[150]

In another study in 2024, researchers developed a photoelectrochemical (PEC) sensor utilizing a 3D polymer phenylethnylcopper and nitrogen-doped graphene aerogel (PPhECu/3DNGA) electrode combined with Fe_3O_4 nanozyme for sensitive detection of glyphosate in agricultural matrices. The synthesis of the PPhECu/3DNGA electrode involved a multi-step process. Initially, nitrogen-doped graphene aerogel (NGA) was fabricated by hydrothermal method, where a mixture of graphene oxide and nitrogen-containing precursors was subjected to high temperatures. This process increased the surface area and introduced nitrogen functionalities that enhanced electron transfer properties. Next, phenylethnylcopper was incorporated into the aerogel structure through a simple blending method, allowing for the formation of the 3D composite material. The final step involved the incorporation of Fe_3O_4 nanoparticles, which were synthesized separately and then anchored onto the PPhECu/3DNGA composite. This innovative approach provides significant advantages over previous methods. The three-dimensional structure of the electrode facilitates rapid electron transfer and offers a large surface area for the attachment of the nanozyme, leading to an enhanced signal output and analytical sensitivity. Also, substituting peroxidase-mimicking Fe_3O_4 NPs for natural enzymes enhances the stability of the sensor under varying ambient temperatures, addressing one of the critical limitations faced by conventional enzyme-based sensors. The sensor's design takes advantage of Gly's ability to inhibit the catalytic activity of Fe_3O_4 NPs, allowing for the detection of glyphosate concentrations ranging from 5×10^{-10} to 1×10^{-4} mol L^{-1}. The sensor's effectiveness was additionally confirmed with tests on actual agricultural samples, such as soil, tea, and maize seeds, showcasing its practical applicability in monitoring glyphosate levels in agricultural environments.[151]

5.3. Detection of carbamate pesticides

5.3.1. Carbaryl. Carbaryl is a well-known pesticide commercialized in 1956 under the name carbamate. This organic nitrogen compound has sound control effects against many agricultural pests. However, improper use of this pesticide, like other chemicals, endangers human health.[152] The presence of excessive amounts of carbaryl causes it to enter the body of poultry, livestock, and humans through the respiratory system, skin, or digestive tract, and by inhibiting acetylcholinesterase, it damages the pancreas, brain, nervous system, muscles, and liver.[153] Accordingly, monitoring and controlling carbaryl pesticide residues in food are of great importance for maintaining human health.[154] Today, much research has been conducted in the field of accurate and rapid detection of carbaryl using nanozyme-based biosensors.

Lu *et al.* introduced light-activated nanozymes (adenosine monophosphate–Ce^{3+}–fluorescein) for the colorimetric detection of carbaryl, effectively tackling the challenges associated with pH levels in conventional enzyme-based detection methods. Traditionally, the oxidation of the substrate 3,3′,5,5′-TMB is only effective at pH levels below 5, whereas most enzymes function best at neutral pH. The authors integrated fluorescein into coordination polymers to address this issue to create innovative nanozymes that enable efficient light-activated oxidation. The synthesis of these nanozymes involved a solvothermal method combining adenosine monophosphate (AMP) with cerium ions (Ce^{3+}), which enhanced light sensitivity by incorporating fluorescein. The resulting nanozymes, benefiting from the extended triplet state of Ce^{3+}, allowed for substrate oxidation at neutral pH, overcoming typical pH limitations. This biosensing system achieved a sensitive detection limit of 1.53 μg L^{-1} for carbaryl, significantly below the EU's maximum residue limit of 50 μg L^{-1}. Furthermore, it demonstrated remarkable selectivity for carbaryl compared to other pesticides and endocrine disruptors, effectively identifying the compound in water and food samples.[155]

5.4. Detection of fungicides

5.4.1. Thiram. Thiram is a thiocarbamate fungicide widely used to preserve stored and shipped vegetables and fruits. Studies have shown that this chemical can cause toxicity to the endocrine and nerve systems, which it does by inactivating the transcription factor NF-kB and hypoxia-inducible factors.[156–158] For this reason, international regulatory agencies have set the maximum residue level of thiram in fruits at about 5.0 μg mL^{-1}.[159] Overall, accurate and efficient detection of thiram in agricultural products ensures food safety and human health. One of the newest methods for detecting this chemical is using nanozyme-based biosensors.

In a 2024 study by Yan *et al.*, a colorimetric sensor was developed for the accurate and reliable detection of thiram pesticide in fruit juices, addressing the potential risks thiram poses to food safety. This sensor utilized a hybrid glutathione–iron (GSH–Fe) nanozyme, which exhibited effective peroxidase-mimicking activity, showing a Michaelis constant (K_m) of 0.551 mM, comparable to natural enzymes. The GSH–Fe nanozyme synthesis involved combining glutathione with iron ions, leading to a stable hybrid structure that enhances catalytic performance. The peroxidase-like activity of the GSH–Fe nanozyme is primarily due to the presence of iron ions, which facilitate the catalytic reaction by mimicking the active sites of natural peroxidases. The innovative aspect of this study lies in the specific inhibition of the GSH–Fe nanozyme's catalytic activity by thiram, which occurs through surface passivation. This interaction results in observable changes in the colorimetric signal, allowing for straightforward visual detection of the pesticide. Furthermore, the research culminated in developing a portable hydrogel kit, enabling quick qualitative detection of thiram in fruit juices. By integrating an image-processing algorithm, the colorimetric data from the hydrogel reactor were converted into quantitative results, achieving an impressive detection limit of 0.3 μg mL^{-1}. The sensor demonstrated excellent selectivity and stability, with recovery rates ranging from 92.4% to 106.9% in various fruit juice samples, showcasing its potential for real-world applications in food safety monitoring. This hybrid nanozyme approach not only enhances the sensitivity and reliability of pesticide detection but also sets a precedent for the development of portable sensing technologies in food safety applications.[159]

5.4.2. Thiophanate-methyl.

Thiophanate-methyl (TM) is a benzimidazole fungicide widely used to control pests and pathogens of crops.[160] It is also used for post-harvest food preservation and pre-sowing treatment.[161] This chemical has neurotoxicity, genotoxicity, reproductive toxicity, and teratogenicity, and its long-term association with it is very dangerous for human health.[162,163] So far, many methods have been used to detect TM, one of the most recent is nanozyme-based biosensors.

In the research carried out by Tai *et al.* (Fig. 7), a sensitive platform for the dual-mode detection of thiophanate-methyl (TM) was used, which involved a composite nanozyme made of Fe_3O_4/graphene oxide nanoribbons (Fe_3O_4/GONRs). This nanozyme displays peroxidase and catalase activities, facilitating enzyme-free colorimetric and fluorescent detection of TM, as shown in Fig. 7. The synthesis of the Fe_3O_4/GONRs composite comprises several essential steps. First, Fe_3O_4 nanoparticles are created using a co-precipitation method, which involves combining ferrous and ferric ions in an alkaline environment. Subsequently, graphene oxide nanoribbons (GONRs) are generated by chemically reducing graphene oxide, enhancing their surface area and functional groups for better interaction with the target analyte. Integrating Fe_3O_4 nanoparticles with GONRs results in a composite nanozyme with improved catalytic capabilities. During the detection process, the Fe_3O_4/GONRs composite catalyzes the oxidation of 3,3′,5,5′-TMB to its oxidized form (oxTMB) using hydrogen peroxide (H_2O_2). However, the presence of TM leads to its adsorption onto the composite, which inhibits its catalytic activity. This inhibition reduces the conversion of TMB to oxTMB, resulting in a decline in absorbance and an increase in fluorescence, thereby enabling dual-mode detection. The method achieved detection limits of 28.1 ng mL^{-1} for colorimetric detection and 8.81 ng mL^{-1} for fluorescence detection, demonstrating its effectiveness in identifying pesticide residues in water and food samples, with recovery rates ranging from 94.8% to 100.8%.[164]

In the research by Zhang *et al.*, a colorimetric platform was enhanced for the specific detection of the agricultural fungicide thiophanate-methyl. This method leverages TM's unique ability to inhibit nanozyme activity due to its affinity for metal sites, which arises from its symmetrical ethylenediamine and bisthiourea groups, leading to a notable decrease in catalytic activity. The platform utilizes a copper-doped carbon nanozyme

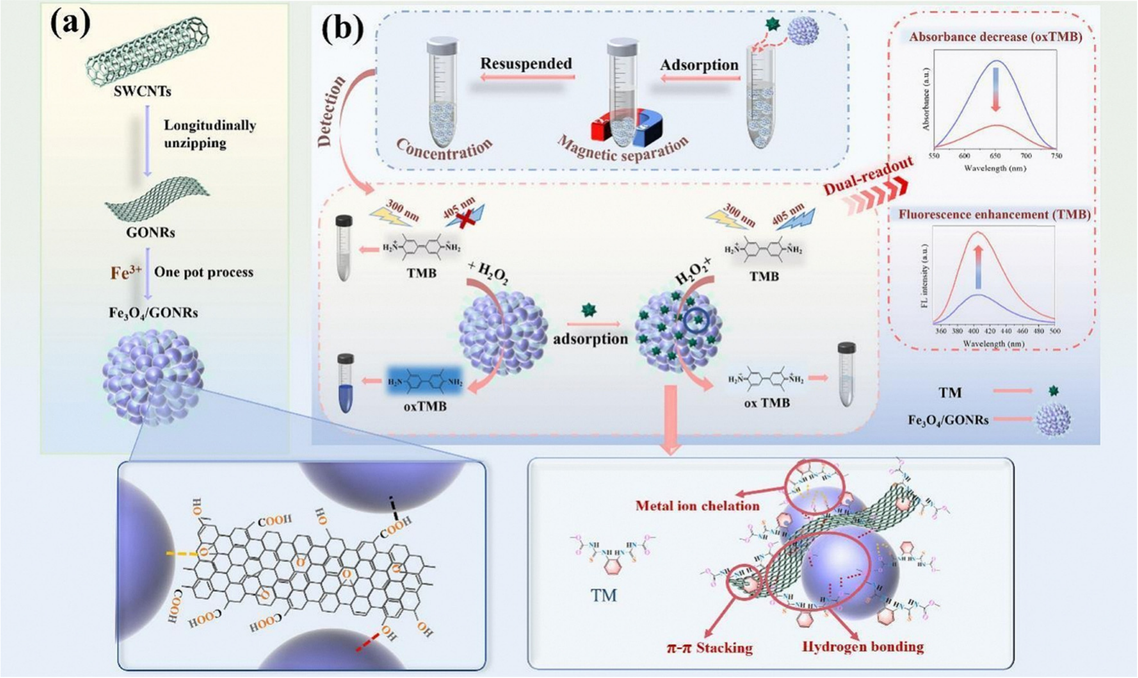

Fig. 7 (a) The synthesis procedure of Fe_3O_4/GONRs (b) schematic representation of the dual-mode detection of TM using fluorescence and colorimetric methods (Licensed under ref. 164).

(Cu–CN) synthesized to enhance its oxidase-like properties. This enhancement is achieved by incorporating copper ions into the carbon structure, which promotes the production of reactive oxygen species (ROS) necessary for chromogenic oxidation of the substrate. A significant benefit of this detection method is its specificity; TM selectively inhibits the nanozyme's activity more than other potential pesticides, allowing for a clear response. The process has a linear detection range from 0.2 to 15 μg mL^{-1}, with a low detection limit of 0.04 μg mL^{-1} (S/N = 3). Also, the study integrates smartphone technology for quick detection, enabling the analysis of RGB values from the colorimetric system. This offers a novel and efficient approach to detecting TM in environmental samples, marking an advancement over traditional methods. Overall, this work emphasizes the potential of Cu-doped carbon nanozymes for pesticide detection and the importance of developing practical, user-friendly methods for monitoring agricultural residues to ensure food safety and environmental health.[165]

5.4.3. Thiabendazole. One of the pesticides used to prevent post-harvest fruit spoilage is thiabendazole (TBZ).[166] It has been proven that prolonged exposure to this chemical can cause cancer in humans.[167] Accordingly, the permissible amount of TBZ for different types of fruit ranges from 0.05 to 10 mg kg^{-1}, as set by the European Union.[168] Various methods have been reported to detect this chemical in agricultural products. One of the fastest and most efficient methods is using nanozyme-based biosensors.

In a recent study, Ariti *et al.* developed a magnetite chitosan hydrogel (MCH) that is an effective peroxidase-like nanozyme for smartphone-assisted colorimetric detection of thiabendazole. The unique polycationic properties of chitosan create a microenvironment that mimics the amino acids found in horseradish peroxidase (HRP) enzymes, enhancing catalytic activity. The intrinsic peroxidase activity of the MCH nanozyme was established using H_2O_2 and 3,3′,5,5′-TMB as substrates, producing an absorption peak at 652 nm. Notably, the MCH exhibited a 24% increase in peroxidase activity under acidic conditions compared to pure Fe_3O_4, demonstrating the significant role of chitosan in enhancing electron transfer processes. Kinetic studies aligned with the Michaelis–Menten model, revealing V_{max} and K_m values comparable to natural HRP enzymes. Furthermore, the MCH nanozyme displayed upgraded thermal and long-term stability, maintaining 80% of its activity after four reuse cycles. The presence of TBZ resulted in a concentration-dependent inhibition of peroxidase activity, with a linear response between 0.1 and 100 μM (R^2 = 0.998). This characteristic allowed for detection limits of 0.73 μM using a spectrometer and 1.84 μM with smartphone-based analysis, underscoring the nanozyme's practicality as a sensor for food safety monitoring. Compared to existing methods, this study highlights the innovative use of magnetite chitosan hydrogels, which not only boost sensitivity and stability but also offer a more user-friendly approach to real-time monitoring of pesticide residues in food samples. By integrating smartphone technology, this research paves the way for accessible and effective detection of harmful substances in agricultural products, addressing growing concerns over food safety.[168]

5.5. Detection of phenolic and other compounds

5.5.1. Dihydroxybenzene isomers. Dihydroxybenzene isomers include catechol (CT), hydroquinone (HQ), and resorcinol (RC), which are widely found in pesticides and other industrial products such as pharmaceuticals, dyes, and petrochemicals.[169–171] International agencies have listed these chemicals as priority pollutants and hazardous pollutants because they are toxic even at low concentrations and can negatively affect human health.[172] Accordingly, their accurate and rapid measurement of agricultural products and water is critical. The best option for this issue is nanozyme-based biosensors.

Due to the significant environmental and human health risks associated with dihydroxybenzene isomers, Kong *et al.* developed straightforward and rapid detection strategies for distinguishing them. Traditional methods often only target individual dihydroxybenzene isomers, limiting their effectiveness in analyzing complex samples. This research developed a colorimetric sensing array using a double-shell hollow $FeCoO_x$ nanozyme with peroxidase-like properties. The unique catalytic interactions of $FeCoO_x$ with various phenolic compounds enabled the identification of three different dihydroxybenzene isomers through principal component analysis. This array effectively distinguished different mixtures of dihydroxybenzene isomers within 30 minutes in both tap and environmental water samples. The method exhibited a linear response to resorcinol, hydroquinone, and catechol within specified concentration ranges, with corresponding detection limits. Additionally, a portable, smartphone-based platform was created for real-time analysis of mixed dihydroxybenzene isomers in water. This research presents a new technique for differentiating phenolic compounds with similar structures and offers potential for detecting and monitoring phenolic pollutants in industrial wastewater.[173]

Another study investigated the application of silver nanozyme (Ag-nanozyme) functions as a peroxidase mimic for the colorimetric detection of dihydroxybenzene isomers and the quantification of hydroquinone in actual samples. The synthesis of the Ag-nanozyme involved a reduction method utilizing an extract from *Piper pedicellatum* (PP), which contains catechin as its primary component. This process included the extraction of active components from PP, followed by the reduction of silver nitrate in an aqueous solution with the PP extract, leading to the formation of silver nanoparticles (Ag NPs) and the subsequent creation of the PP-AgNZ. This nanozyme demonstrated significant oxidase-like activity by catalyzing the oxidation of TMB in the presence of hydrogen peroxide (H_2O_2), resulting in an optimized color reaction after 30 minutes at 45 °C, and exhibiting a notable K_m value of 0.0636 mM for TMB. The interference of dihydroxybenzene isomers with TMB oxidation causes observable color intensity changes for each isomer (Resorcinol, HQ, Catechol). The detection ranges were established as 10 to 270 nM for Catechol and RE, and 10 to 390 nM for HQ, with low detection limits of 32.38 nM for Catechol, 30.64 nM for HQ, and 37.5 nM for resorcinol. Additionally, evaluating smartphone-assisted detection enhances the method's accessibility and practicality, effectively identifying HQ. The validation of PP-AgNZ performance with real environmental samples yielded excellent recoveries between 91.4% and 108.6%, emphasizing its strong potential as a nanozyme for use in environmental chemical applications.[174]

5.5.2. Catechol. Catechol is a phenolic compound, and various derivatives of it exist in nature. This chemical is widely used in pesticides and other industries. During industrial production processes, a small amount of unreacted catechol in the reaction medium enters the environment along with water and other organic solvents and contaminates it. Catechol is a highly toxic organic pollutant that is very difficult to decompose and can endanger human health.[175,176] Accordingly, detecting and removing catechol from agricultural products and the natural environment is critical. Nanozyme-based biosensors are among the newest diagnostic methods in this field.

In 2024, Zhou *et al.* aimed to design a Janus micro-composite system to enhance the detection and degradation of catechol, a pollutant found in water. This innovative approach centers around copper sulfide (CuS) micro-flowers embedded with carbon dots and the enzyme laccase. The result is a multifunctional platform, the Lac-MnO_2@C@CuS composite, equipped with manganese dioxide nanoparticles (MnO_2 NPs) on one side. This configuration empowers the structure with dual capabilities, functioning as a detection and degradation tool. The MnO_2 nanoparticles play a critical role by producing oxygen, which drives the composite's autonomous motion in a hydrogen peroxide (H_2O_2) solution. When immersed in a 5 wt% H_2O_2 environment, the micro-composite moves directionally at 178.83 ± 2.07 μm s^{-1}. This motion amplifies its catalytic efficiency, allowing for the detection of catechol at concentrations as low as 0.49 μM across a range of 0–100 μM. Furthermore, the composite decomposes H_2O_2 through the Fenton reaction, producing reactive oxygen species that achieve a catechol removal efficiency of up to 96.6%. The Lac-MnO_2@C@CuS Janus micro-composite is a promising advancement in environmental water treatment technology, with potential applications as a biosensor. Its dual functionality makes it effective for detecting catechol and enables it to actively reduce pollutant levels, thereby contributing to better water quality management.[177]

5.5.3. Hydroquinone. Hydroquinone is a water-soluble phenolic compound that is known as an industrial raw material and a vital chemical synthesis intermediate.[178] This chemical is used in various industries, including pharmaceuticals and cosmetics, so that the use of small amounts of it in creams and ointments can be used to treat freckles and skin diseases.[179] Hydroquinone is also widely present in the composition of pesticides, and its high concentration causes serious problems for human health.[180] The entry of this chemical through the skin, nose, and mouth of humans causes tachycardia, kidney failure, severe headache, and even possibly death.[181] Hydroquinone contamination in agricultural products and water resources is a serious threat to human health.[182] Therefore, designing and developing accurate and rapid methods for detecting this pollutant (including nanozyme-based biosensors) is essential for protecting the environment and human health.

In a study conducted in 2023 by Deng *et al.*, a colorimetric sensor for detecting hydroquinone (HQ) was developed using a composite of manganese and iron metal–organic frameworks (Mn/Fe-MOF) doped with palladium nanoparticles (Pd NPs), termed Mn/Mn/Fe-MOF@Pd1.0. This sensor exhibited enhanced peroxidase-like activity, providing a highly sensitive method for HQ detection in real-world samples such as water and whitening creams. The synthesis involved the creation of Fe-MOF *via* solvothermal methods, followed by manganese doping to improve catalytic activity by adding active sites. Palladium nanoparticles were introduced to enhance the sensor's stability and acid resistance. The Mn/Fe-MOF@Pd1.0 composite accelerates the decomposition of H_2O_2, generating reactive oxygen species that oxidize chromogenic substrates, producing a color change that forms the basis of HQ detection. This approach achieved a detection limit of 0.09 μM within a linear range of 0.3–30 μM, surpassing previous single-mode sensors in sensitivity, stability, and acid resistance. The innovation of this method lies in the synergistic effect of bimetallic active sites and Pd nanoparticle incorporation, leading to enhanced catalytic performance and broader applicability for environmental and industrial HQ detection.[183]

Other studies related to the use of nanozyme-based biosensors in pesticide detection can be seen in Table 1.

6. Conclusion

Nanozyme based biosensors mark a significant leap in pesticide residue detection, surpassing traditional methods like gas chromatography and high-performance liquid chromatography. While conventional techniques offer accuracy, they demand lengthy sample preparation and costly equipment. In contrast, nanozyme based biosensors utilize the enzyme-like catalytic abilities of nanomaterials mimicking peroxidase, oxidase, or catalase activities to deliver rapid, sensitive, and cost effective results. These biosensors have proven effective in detecting various pesticides, including organophosphorus compounds (*e.g.*, malathion, dichlorvos), herbicides (*e.g.*, glyphosate, atrazine), and fungicides (*e.g.*, thiram), with impressive detection limits (*e.g.*, 0.12 nmol L^{-1} for diazinon) and high selectivity.

Their integration with portable technologies, such as smartphone assisted platforms, makes them ideal for monitoring real time food and environmental samples. This practicality enhances their potential to safeguard public health and ecosystems by efficiently addressing pesticide related risks. While smartphone integrated biosensors show great promise for on-site pesticide detection, scaling them for practical field use is hindered by several key challenges. The primary hurdles involve ensuring sensor accuracy and stability amidst variable environmental conditions and within complex sample matrices like soil and water. For successful deployment, the technology must not only be physically robust and durable but also simple enough for non-experts to use reliably. Furthermore, overcoming logistical barriers such as cost effective mass production, data connectivity in remote areas, and securing regulatory approval is essential. Successfully navigating these technical

Table 1 Summary of nanozyme-based methods for pesticide detection

Nanozyme	Synthesis method	Activity	Detection method	Target	LOD	Analysis time	Ref.
AgPd bimetallic nanoflowers	Co-precipitation	Fluorescence species formation	Fluorescence detection	Organophosphoru	0.046 μM (for methyl parathion)	25 min	184
Lignin-based iron single-atom nanozyme	Pyrolysis	Oxidative catalysis	AChE reaction,		10 ng mL^{-1} (for chlorpyrifos)	Not specified	185
Algae-derived biochar	Carbonization	Detection of multiple pesticides	Discrimination and detection in soil, water, and food		1 μM	Not specified	186
Single-atom Fe	Atomic layer deposition	Multi-pesticide detection in vegetables	Dual-mode biosensor		0.01–0.1 ng mL^{-1}	Not specified	187
Nanoceria crosslinked and heteroatom-doped graphene oxide nanoribbons	Hydrothermal	Inhibition-based detection	Colorimetric sensor array		Not specified	Not specified	188
PANI-MnO_2	Chemical polymerization	Colorimetric sensing platform	Colorimetric sensing platform with smartphone integration for detection		0.25 μM (for malathion)	20 min	189
Metal-pyrimidine nanocubes	Solvothermal	Used metal-pyrimidine nanocubes	pH-Switchable multienzyme-like activity		0.015 nM (acidic), 0.023 nM (alkaline)	30 min	190
Fluorescent nanozyme	Precipitation	Involving catalytic or fluorescence changes	Multi-signal sensor array		Not specified	Not specified	191
Fe–N/C single-atom	Atomic layer deposition	Involving catalytic activity and colorimetric response	Smartphone-integrated colorimetric sensor		0.012 μg mL^{-1} (for profenofos)	15 min	192
Fe_7S_8 nanoflakes	Solvothermal	Acetylcholine-triggered enzymatic cascade reaction	Visual detection		0.05 ng mL^{-1} (for phorate)	20 min	193
Glutathione-stabilized gold nanoclusters	Reduction	Enzyme-catalyzed reaction leading to fluorescence	Fluorescent sensor		2.1 ng mL^{-1} (for chlorpyrifos)	30 min	194
Metal–organic framework (MOF)	Solvothermal	Histidine modification for optimizing enzymatic activity	Simultaneous pesticide detection		Not specified	Not specified	195
UiO-66-NH_2 metal–organic frameworks (MOFs) incorporated with tris(2,2′-bipyridyl) ruthenium(II)	Hydrothermal	Bioenzyme-free dual-mode sensing	Dual-mode sensing		0.81 ng mL^{-1} (colorimetric), 0.47 ng mL^{-1} (ratiometric fluorescence)	20 min	196
COF-OMe@Valine-CeO_2	Solvothermal	Phosphatase-like catalytic activity	Ultrasensitive electrochemical		0.011 μmol L^{-1} (for methyl paraoxon)	Not specified	197
Fe/C/Bi_2O_3 nanozymes derived from MOFs	Thermal decomposition	Peroxidase-like catalytic activity	Peroxidase-like detection		0.05 μM (for methyl parathion)	Not specified	198
Nanozyme with multienzyme-like activities	Sol–gel	Concentration-independent model	Identification of pesticides		Not specified	Not specified	199
Single-atom Fe	Atomic layer deposition	Oxidase mimicking	Ratiometric fluorescence		10 ng mL^{-1} (for malathion)	Not specified	200
Copper-based laccase-like nanozymes	Chemical synthesis	Laccase-like activity	Smartphone-assisted sensor array		Not specified	Not specified	201
Copper oxide nanoparticles (CuONPs)	Precipitation	Oxidase activity	Paper-based sensor	Detect some pesticides using a portable paper-based device	0.08 mg L^{-1} (for malathion)	10 min	202
Octahedral Ag_2O particles	Chemical precipitation	Oxidase activity	Facile colorimetric smartphone-based biosensor	Organophosphorus	10 ng mL^{-1}	Not specified	203
Transition metal-doped germanium oxide	Sol–gel	Enhanced enzyme-like activity	Rapid detection	Pesticide residues in water samples	0.1 nM	<10 min	204
Germanium oxide (GeO_2)	Sol–gel	Enzyme-like activity	Pesticide residue detection	Water samples	Not specified	Not specified	205

Table 1 (*continued*)

Nanozyme	Synthesis method	Activity	Detection method	Target	LOD	Analysis time	Ref.
Metal–organic framework (MOF)	Hydrothermal	Dual-mode sensing	Colorimetric/fluorescent detection	Organophosphoru	1.57 ng mL^{-1} (colorimetric), 2.33 ng mL^{-1} (fluorescent)	Not specified	206
Prussian blue analogues of Ni–Co–MoS$_2$	Co-precipitation	Peroxidase-like activity	Utilizing the peroxidase-like activity of the nanozymes to sensitively detect glyphosate and copper	Detection glyphosate and copper	3 nM (glyphosate), 3.8 nM (copper)	Not specified	207
Fe$_3$O$_4$@Cu	Co-precipitation	Peroxidase-like activity	Dual-mode colorimetric-chemiluminescent	Glyphosate	0.086 μg mL^{-1} (colorimetric), 0.019 μg mL^{-1} (chemiluminescent)	Not specified	208
Dendritic-like MXene quantum dots@CuNi.	Hydrothermal	Peroxidase-like activity	Colorimetric detection		1.13 μM	Not specified	209
2D Cu-TCPP(Fe) nanosheets (2D Cu-TCPP(Fe) NSs)	Solvothermal	Electrochemical sensing	Peroxidase-like activity inhibition		0.28 nM (electrochemical), 5.7 nM (colorimetric)	Not specified	210
ZnTCPP@ZIF-90	Hydrothermal	Photoresponsive properties	Colorimetric/fluorescent dual-mode sensing		0.031 μM (colorimetric), 0.024 μM (fluorescent)	30 min	211
Flower-like Ni-MOF@NiV-layered double hydroxides	Hydrothermal	Peroxidase mimetics	Colorimetric detection	Hydroquinone	0.12 μM	10 min	212
Magnetic nanoparticles encapsulated metal–organic framework	Solvothermal	Catalytic activity inhibition	Electrochemical detection	Thiram (THR)	0.2 pM	Not specified	213
WO$_{3-x}$ dots	Hydrothermal	Electrochemiluminescence	Aptasensor for diazinon	Diazinon detection in vegetables	0.17 pg mL^{-1}	Not specified	214
Cu–BDC–NH$_2$	Solvothermal	Catechol oxidase activity	Selective sensing	Catechol detection	0.17 μM	10 min	215

and practical obstacles is critical to transitioning this technology from a laboratory concept into a viable tool for real-world food safety and environmental monitoring. Ongoing research is crucial to refine nanozyme materials, such as single-atom catalysts, and optimize biosensor designs for multiplexed detection and reliability in complex matrices like soil or water. Despite their many advantages, nanozyme based biosensors also face certain limitations compared to conventional analytical techniques. For example, their catalytic activity, while tunable, can sometimes be lower than that of natural enzymes, leading to reduced sensitivity in complex biological matrices. Selectivity can also be an issue, as nanozymes may catalyze multiple substrates, causing potential cross reactivity. Furthermore, reproducibility in large-scale synthesis of nanozymes remains a challenge, which can hinder standardization and commercialization. In contrast, traditional chromatographic methods, though more resource-intensive, provide well established reliability and accuracy. Addressing these limitations through rational nanozyme design, surface functionalization, and integration with selective recognition elements is a critical step toward practical field applications.

In summary, nanozyme-based biosensors offer a transformative approach to pesticide detection, combining speed, sensitivity, and affordability. With continued validation and development, they are poised to become a vital tool for ensuring food safety and environmental protection, mitigating the limitations of traditional analytical methods.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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