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Biomimetic Design of Tunable Nanozymes for Microbial Detection: A Review

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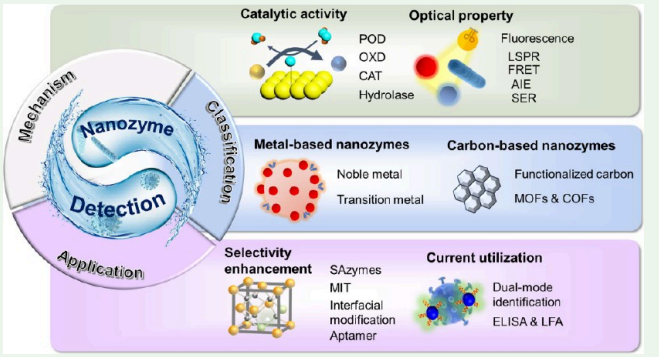
ABSTRACT: The exploration and utilization of microbial resources have spurred the development of high-throughput detection technologies. Nevertheless, conventional methods remain constrained by limited sensitivity, long procedures, and operational complexity. Nanozymes, as artificial enzyme mimics, offer compelling advantages over natural enzymes, including cost efficiency, robust stability, scalable production, and retained catalytic activity under extreme conditions—properties that render them highly attractive for microbial detection. Central to their utility is their capacity for signal amplification, a critical feature that significantly enhances detection sensitivity and accuracy. Hence, we systematically outline the fundamental mechanisms of nanozyme-based detection, including enzyme-like activity and optical properties. We introduce the main categories of these materials and highlight their cutting-edge applications in microbial sensing, with a particular focus on innovative signal amplification strategies that optimize performance. By synthesizing these advances, we aim to provide a roadmap for future research in this dynamic field, underscoring the transformative potential of nanozymes in next-generation microbial diagnostics.



KEYWORDS: *biomimetic nanozymes, selective regulation, interface catalysis, visual inspection, microbial detection*

INTRODUCTION

Microbial resource exploitation has driven advances in high-throughput detection technologies. Visual detection is critical for ensuring high-throughput screening efficiency and accuracy, providing a powerful tool for solving complex biological challenges.[1−4] First, natural microorganisms as cell factories have limitations such as low yield and poor industrial tolerance. Physical, chemical, and other methods are necessary for strain improvement and selection. Second, the primary challenge in strain domestication lies in the labor-intensive nature of current detection techniques. Identification of pathogens in a rapid and accurate way is the most critical step in disease control and prevention. Conventional pathogen detection methods suffer from significant limitations: complexity, time constraints, and restricted sensitivity. These shortcomings substantially increase the costs for large-scale mutant screening. Furthermore, they require intricate sample preparation, trained personnel, and specialized equipment, rendering them impractical for routine clinical diagnostics.[5] Third, infection and contamination continue to pose significant public health challenges. Outbreaks of HIV, Zika virus, Ebola virus, dengue virus, and SARS-CoV-2 have severely threatened global health security.[6] Compounding this issue, the overuse of antibiotics has precipitated widespread antimicrobial resistance, making it become a critical global health crisis.[7] In addition, the contamination of food by foodborne pathogens and toxins is a major hidden danger to food safety. This situation has emerged as a critical global health challenge, affecting millions of individuals annually. In order to overcome this circumstance, enhancement of microbial detection methods and metabolite analysis capabilities is critically needed. Such advancements are indispensable not only for effective disease prevention and containment but also for safeguarding global health security.[8,9]

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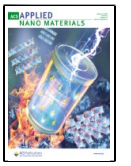

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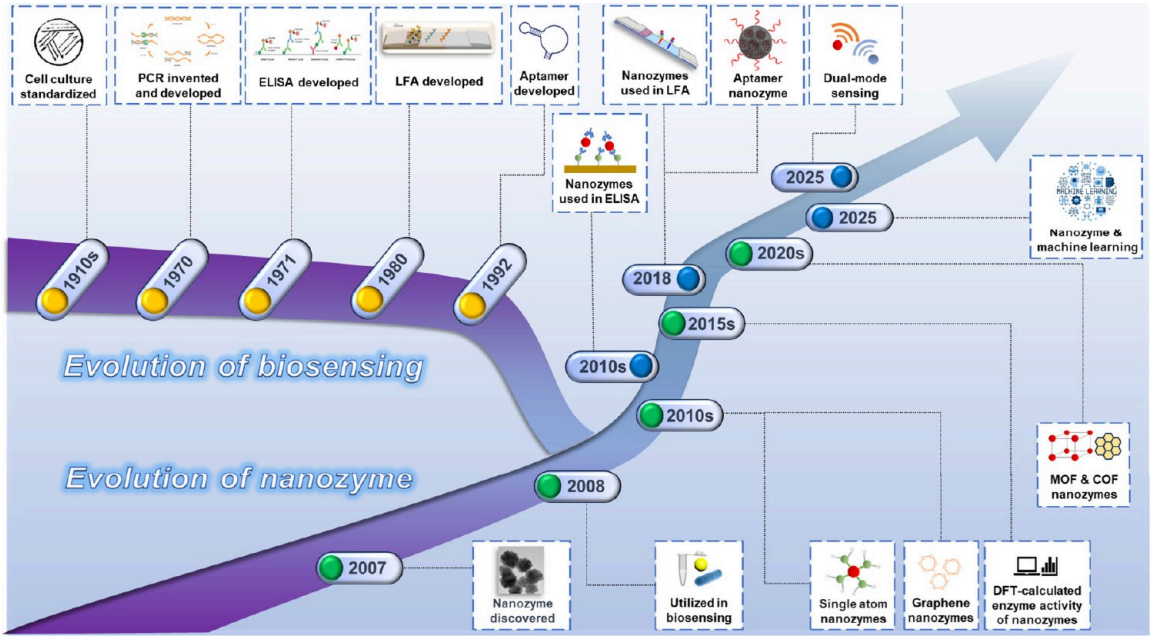

Figure 1. Development of biosensing (from cell culture to nanozymes as biosensors).

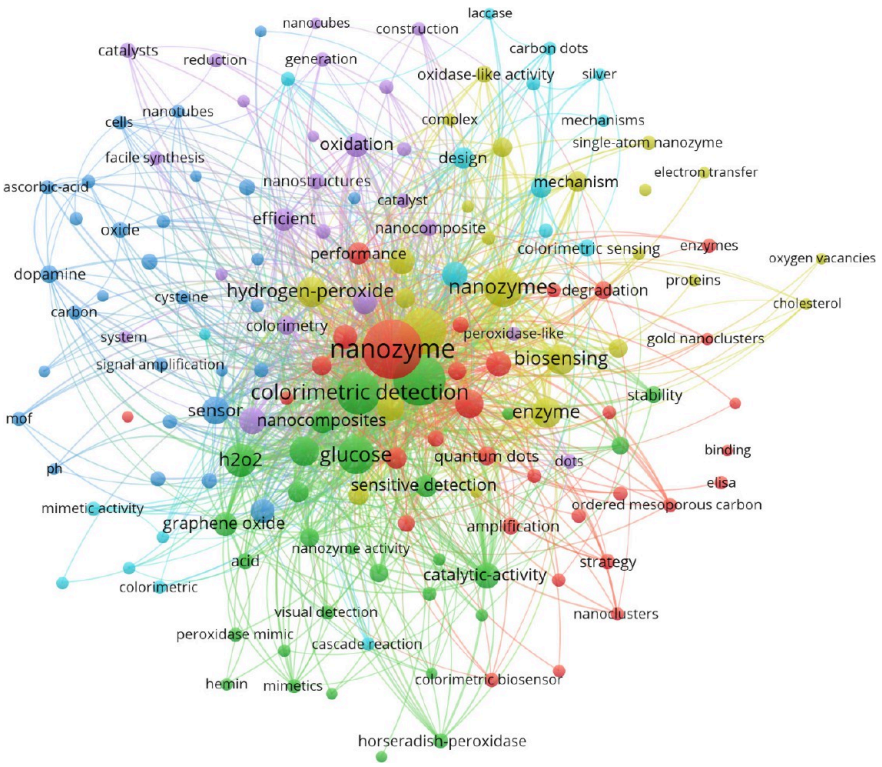

Figure 2. Research trends in biosensing by nanozymes (generated by VOSviewer, data sourced from Web of Science).

For decades, biosensing technology has advanced significantly in response to the growing demand for microbial detection. These notable developments are highlighted in Figure 1. As a foundational approach in biosensing innovation, standard microbial detection remains indispensable. This methodology primarily encompasses culture-based techniques, molecular analyses, and immunoassays.[10−12] Among these, microbial culture remains a cornerstone for pathogen identification.[13] While offering high specificity, this approach is labor-intensive and time-consuming, requires significant expertise, and is prone to contamination factors that can impede target pathogen detection.[12] Molecular methods, notably polymerase chain reaction (PCR) and its derivatives, enzymatically amplify specific microbial DNA/RNA fragments in vitro.[14] However, PCR-based detection presents inherent limitations, including false positives/negatives, stringent template quality requirements, inability to differentiate viable from nonviable bacteria, quantitative constraints, and operational complexity. Immunological methods, such as enzyme immunoassays (EIA/ELISA), flow injection immunoassays,

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immunochromatographic lateral flow assays, and immunomagnetic separation, are widely employed for pathogen detection.[15] Although they enable rapid analysis, these techniques suffer from relatively low sensitivity (typically 10^3−10^4 CFU/mL), proneness to matrix interference in complex samples, and variable antibody−antigen binding affinities, limiting detection accuracy. Collectively, these constraints underscore the critical need for novel detection platforms with enhanced rapidity, sensitivity, and specificity to overcome current limitations.

High-throughput detection offers distinct advantages for industrial strain breeding and harmful microorganism screening, driving an escalating demand for precision diagnostics compatible with such systems. Within these platforms, biosensors enable multiplexed detection through nucleotide hybridization analysis, microbial metabolic profiling, pathogen−eukaryotic cell interaction monitoring, and antibody-mediated capture of signature biomarkers.[8] Advances in nanomaterials have revealed the unique properties and functions of nanozymes,[16−18] with significant interest in their enzyme-mimetic catalysis and biological applications since Yan et al.'s seminal 2007 discovery of Fe_3O_4 nanoparticles' horseradish peroxidase (HRP)-like activity.[19] Nanozyme activity originates from nanomaterial crystal structures and interfacial reactions, where structural engineering, elemental hybridization, and surface modification modulate the catalytic performance to control detection signals. For microbial sensing, nanozymes leverage their enzyme-mimetic properties and distinctive optical characteristics to efficiently identify microorganisms and secreted metabolites.[17,20−23] Compared to natural enzymes, nanozymes present compelling practical advantages, including scalable low-cost production,[24] enhanced long-term stability, and remarkable tolerance to harsh environments. Despite these strengths, relatively low selectivity currently constrains broader implementation, prompting the active development of enhancement strategies. Today, research on nanozymes has expanded to encompass their catalytic activities, structural properties, diverse applications, and strategies for performance enhancements (Figure 2), significantly improving the efficiency and reliability of materials.

In this review, we systematically examine the fundamental detection mechanisms of nanozyme-based biosensing platforms for the detection of microorganisms and their metabolites and consolidate major nanozyme types and novel applications documented in the literature. We further analyze researcher-developed strategies for enhancing nanozyme selectivity. Our critical analysis focuses on four pivotal advances: (1) rational engineering of catalytic selectivity through structural design and interfacial modification; (2) sensitivity enhancement via signal amplification and dual-recognition systems; (3) functionalization with high-affinity aptamers to augment target specificity; and (4) development of portable platforms for point-of-care diagnostic applications.

2. DETECTION MECHANISM OF NANOZYMES

Nanozymes leverage their enzyme-mimetic catalytic activity and intrinsic optical properties to concurrently generate, amplify, and visualize detection signals. These multifunctional capabilities—rooted in catalytic mechanisms, interfacial reactions, and photonic characteristics—are strategically exploited in biosensing platforms to enable precise microbial detection.[25]

2.1. Catalytic Functions. Visualization of signals primarily employs the enzyme-mimetic functions of nanozymes, including peroxidase (POD)-like, oxidase (OXD)-like, catalase (CAT)-like, and alkaline phosphatase (ALP)-like activity. Most nanozymes exhibit POD-like activity (Figure 3a). This activity

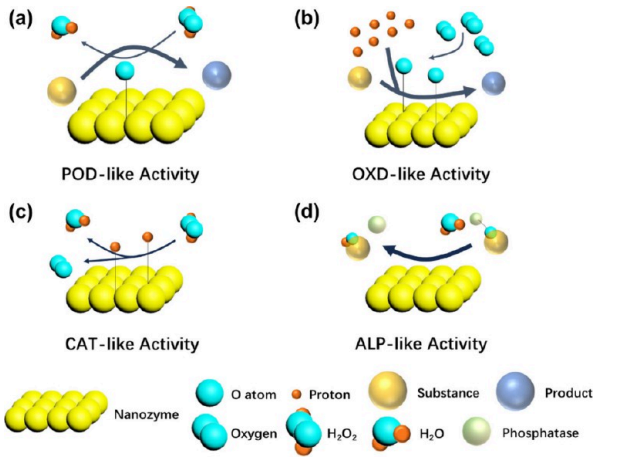

Figure 3. Schematics of enzyme-mimetic activities of nanozymes: (a) POD-like activity, (b) OXD-like activity, (c) CAT-like activity, and (d) ALP-like activity.

enables nanozymes to utilize H_2O_2 as an electron acceptor for catalyzing substrate oxidation, thereby achieving the oxidation of hydrogen donors, such as colorimetric indicators. This process mainly involves three stages: the dissociation of hydrogen peroxide, the formation of adsorption intermediates, and the transfer of H atoms.[17] OXD-like activity, such as displayed by Fe, Cu, Ce, and Mn, can directly mediate the oxidation of hydrogen donors in an oxygen atmosphere without the assistance of H_2O_2 (Figure 3b). The process of this change can be clarified based on DFT simulations of the color change reaction of 3,3′,5,5′-tetramethylbenzidine (TMB).[23] CAT-like activity allows H_2O_2 molecules to rearrange their atoms on the tiny surfaces of noble metals, CeO_2, and other minerals, producing H_2O and O_2, which completes a catalytic cycle like that of catalase (Figure 3c). The oxygen produced can create a pressure difference with the environment, thereby achieving displacement of the dying material and reaching the indicated effect.

Currently, over 90% of nanozymes exhibit redox enzyme activity, while only a limited number demonstrate hydrolase activity. For instance, CeO_2 and MnO_2-x nanozymes exhibit alkaline phosphatase activity.[26] Phosphatase-mimicking nanozymes catalyze phosphate ester bond hydrolysis via P═O cleavage, exhibiting ALP-like activity (Figure 3d). Generally speaking, these materials can efficiently hydrolyze substrates with a phosphate monoester structure. However, for triesters of phosphate, although materials such as CeO_2, Ce-MOF, and Co_3O_4/rGO have corresponding catalytic activities, due to the influence of the steric hindrance of the phosphate substrate, their hydrolysis ability for triesters of phosphate is greatly limited.[27] Therefore, some researchers have attempted to improve the catalytic performance of these materials by taking advantage of the substrate synergy effect, and these attempts have also been proven to achieve effective adsorption between the substrate and the materials. The ALP-like activity is currently effective for treating substrates with phosphate ester structures, presenting significant potential for applications in cell membrane detection and sensing.

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Table 1. Application of Nanozymes in Visual Sensing for Detection

structure	analyte	enzyme-like activity	LOD	detection range	ref
Fe_{AC}/Fe_{SA}-NC	organophosphorus pesticides	OXD	1.9 pg/mL	0.005−50 ng/mL	43
Fe−N/C	ACP	OXD	0.048 U/L	−	44
Fe-N-C/Fe-N-C-urea	oral cariogenic bacteria	OXD	10^2 CFU/mL	10^2−10^7 CFU/mL	45
Cu NPs-N-C	organophosphorus pesticides	POD	0.60 ng/mL	1−300 ng/mL	46
Mn/PSAE	tumor cells	CAT, OXD, POD	−	−	47
SA-Fe/NG	Cr(VI)	POD	3 nM	30 nM to 3 μM	48
H-Co SAC	NSE	OXD	5.19 pg/mL	−	49
Co-N-C	thiol	OXD	0.07 μM (GSH); 0.06 μM (Cys)	−	50
MnO_2QDs@Lip	SARS-CoV-2 antigen	OXD	65 fg/mL	0.1 pg/mL to 100 ng/mL	51
MnO_2 nanosheets	acid phosphatase	OXD	0.046 mU/mL	0.075−0.45 mU/mL	52
AgPt-Fe_3O_4	CO	POD	5.6 ppb	−	53
R-Fe_3O_4/Au	GSH	POD	0.10 μM	1−150 μM	54
	cholesterol		0.08 μM	1−100 μM	
Fe_3O_4@COF@Os	prostate-specific antigen	POD	3.83 pg/mL	−	55
man-PB	*E. coli* O157:H7	POD	10^2 CFU/mL	10^2−10^8 CFU/mL	56
PB NPs	glycocholic acid	POD	10 ng/mL	29−1200 ng/mL	57
Fe_3O_4 NPs	clenbuterol	POD	0.2 ng/mL	−	58
Fe_3O_4 NPs	Ebola virus	POD	1 ng/mL	−	59
DPA-Ce-GMP	GSH	OXD	17.1 nM	0.05−10 μM and 10−40 μM	60
CuO NPs	human serum and fruits	OXD	0.16 μM	−	61
CuS HNCs	TA	POD	0.13 μM	1−10 μM	62
gold nanorods	malathion	POD	1.78 μg/mL	−	63
AuNPs	*E. coli* O157:H7	−	10^5 CFU/mL	−	64
AuNPs	protease	POD	44 ng/mL	−	65
AuNPs	CEA	OXD	0.66 pg/mL	0.001−100 ng/mL	66
	AFP	POD	9.5 pg/mL	0.011000 ng/mL	
Ag@GQD	GSH	OXD	0.59 μM	0−20 μM	67
CuTA@Ag	GSH	POD	10^{-7} M	1−100 μM	68
PVP-PtNPs	H_2S	CAT	0.17 μM	1−250 μM	69
PtGNPs	SARS-CoV-2	POD	0.1 ng/mL	−	70
PtGNPs	*E. coli* O157:H7	POD	3.3×10^4 CFU/mL	−	71
AuNP-ICA	*E. coli* O157:H7	POD	1.25×10^1 CFU/mL	−	72
Au@Pt	potato virus X	POD	4 and 8 pg/mL	−	73
Au@Pt NRs	measles	POD	10 ng/mL	−	74
CeO_2@NC	paraoxon	ALP	−	3.0−100.0 μM	27
CeO_2/L-g-C_3N_4	HQ	OXD	0.094 μM	0.454−37 μM	75
Asp-Fe-CDs	GSH	POD	0.296 μM	−	76
FeS_2@C NSs	glucose	POD	0.19 μM	0.5−50 μM	77
His-Fe_3O_4	cholesterol	POD	0.446 μM	−	78
GO@H-Fe_3O_4	uric acid	POD	1.5 μM	5−800 μM	79
GPF-mGOx@MOF	glucose	OXD	0.009 mM	0.2−11 mM	80
hemin@Ftn	tumor cells	POD	−	−	81
anti-CD44 mAbs	MDA-MB-231 cells	OXD	186 cells	−	82
Fe@hemin-peroxidase	SARS-CoV-2	POD	0.1 ng/mL	0.2−100 ng/mL	83
MoCu-2MI	cholesterol	POD	1.2 μM	2−140 μM	84
FeS_2@SNW-1	GSH	POD	1.61 μM	4.46 μM to 1.14 mM	85
TpDA-Cu	Tr	OXD	0.013 μg/mL	0.038−7.6 μg/mL	86

2.2. Optical Function. Nanomaterials can amplify generated signals, thereby enhancing the sensitivity of sensing and detection. For some noble metal materials, such as gold and silver, they can achieve color changes by taking advantage of their unique optical properties, thus demonstrating high sensitivity in colorimetric detection.[22]

In comparison to colorimetric detection, fluorescence detection exhibits higher sensitivity.[28] Nanozymes with fluorescence properties are suitable for biosensing and bioanalysis. Förster resonance energy transfer (FRET) is a typical characteristic of fluorescence detection. This electromagnetic effect can transfer energy from a FRET donor to FRET acceptor in a nonradiative way.[29] Because of its short-distance effectiveness, FRET is sensitive in short-range detection. For regular visualized detection, FRET plays an important role in the construction of a dual-mode platform, showing great potential in cell imaging, disease diagnosis, and molecule detection.[28,30,31]

The local surface plasmon resonance (LSPR) phenomenon enables nanozymes to exhibit considerable spectral absorption in the UV−visible light range, altering the material color at the macroscopic scale. The LSPR phenomenon causes corresponding absorption peak shifts due to changes in the shape, structure, size, and composition of the material, which can be

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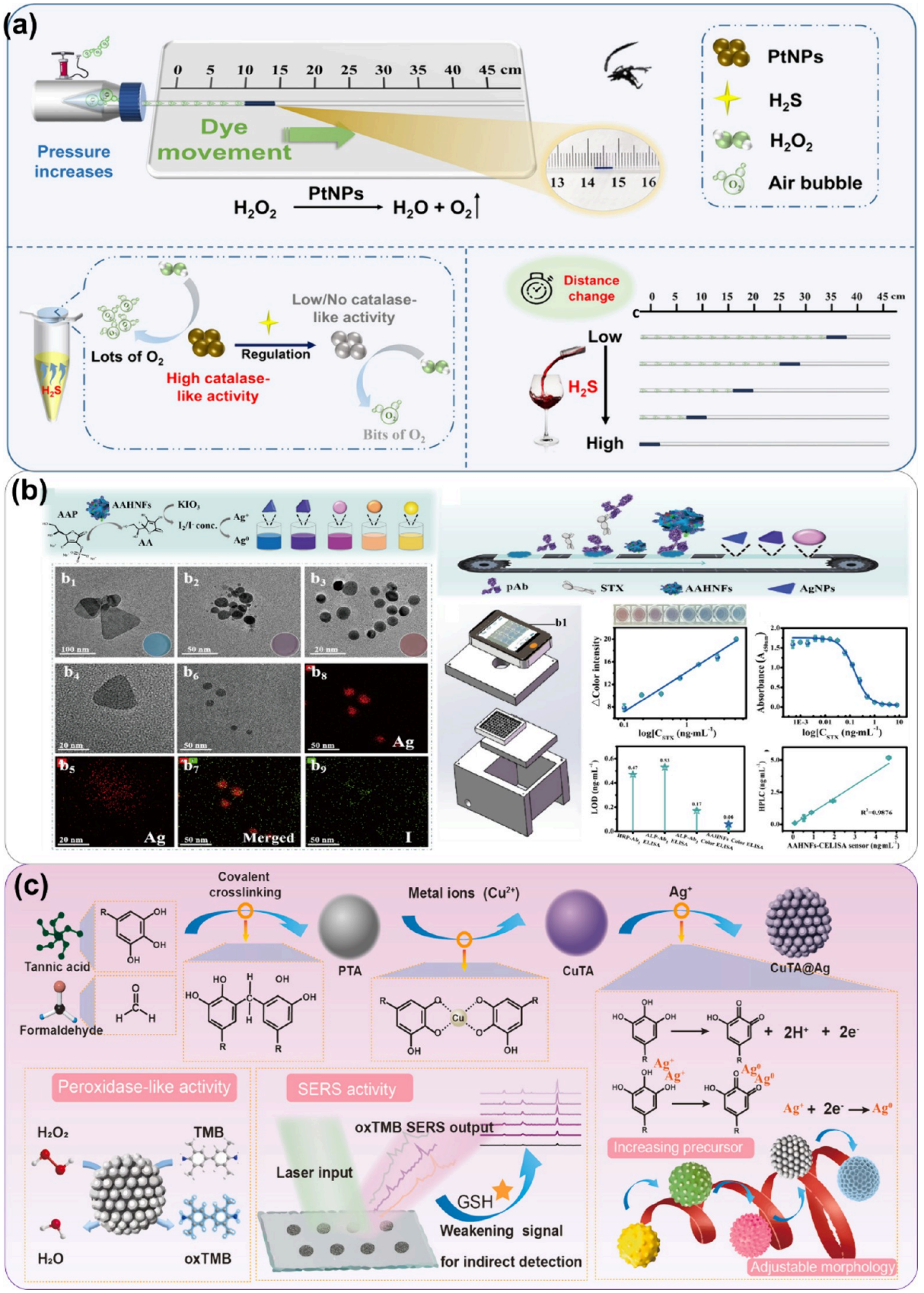

Figure 4. Noble metal nanozymes and their applications. (a) Schematic illustration of the nanozyme-catalyzed pressure-powered distance variation for portable H_2S quantitative detection. Reproduced with permission from ref 69. Copyright 2024 the authors of ref 69, under exclusive license to Springer Nature. (b) Ag nanoplates activate the LSPR effect for determination of STX. Reproduced from ref 87. Copyright 2024 American Chemical Society. (c) Illustration of the CuTA@Ag nanozyme sensor. Reproduced with permission from ref 68. Copyright 2023 Elsevier.

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used to undergo changes in structure and other conditions under specific circumstances, further transforming the color of the detection system and achieving the effect of colorimetric detection. The LSPR−nanozyme hybrids develop a novel paradigm for ultrasensitive biosensing when strategically combined with nanozyme catalytic cascades, where enzymatic processes change local dielectric environments or create plasmonic-active byproducts.[32] Such systems make use of synergistic signal amplification: enzyme-mediated substrate conversion causes nanoscale interfacial changes, which are transduced into colorimetric readouts observable by naked-eye observation.[33−35]

Significantly, certain nanozyme architectures exhibit aggregation-induced emission (AIE) characteristics, wherein restricted intramolecular motion (RIM) upon nanoparticle assembly generates enhanced photoluminescence quantum yields compared with monodisperse states. Tang et al. described the luminescence properties of a hexaphenylsilole derivative exhibiting unusual fluorescence behavior. Though remaining nonemissive in dilute solutions, it shows intense emission in aggregated/solid states.[36] This discovery was coined AIE and soon gained considerable attention. The synergistic suppression of both intramolecular rotation and vibration, collectively termed RIM, has been established as the dominant mechanism of AIE.[37] RIM-based AIEgens provide a versatile platform for diverse applications, spanning chemical sensing, bioimaging, and photodynamic therapy. In the nanomaterial field, nanozymes combined with AIE properties can create colorimetric/fluorescence systems for the detection of organophosphorus pesticides and methyl mercaptan and even cure cancers.[38−40]

3. APPLICATION OF NANOZYMES IN VISUALIZED SENSING FOR DETECTION

Nanozymes in recent studies can be basically classified as metal-based nanozymes and carbon-based nanozymes.[5,41,42] Most nanozymes visualize detection progress through their catalytic activities, while some of them rely on their optical properties. These materials consistently demonstrate broad linear detection ranges and low detection limits, positioning them as promising biosensing platforms. Therefore, they have solid potential for use as biosensors in microbial detection applications. Nanozymes with outstanding visual detection capabilities are summarized in Table 1. These applications span diverse biosensing fields, including microbial detection, metabolite analysis, and target molecular sensing.

3.1. Metal-Based Nanozymes. Metal nanozymes, including noble metal nanozymes and transition metal nanozymes, are widely applied in detection. Since Fe_3O_4 nanoparticles demonstrated outstanding POD-like activity in 2007,[19] large amounts of research on nanozymes have appeared, focusing on their catalytic activity and utility. Today, exceptional enzyme-like characteristics prove that metal nanozymes are competitive with conventional detecting methods.

3.1.1. Noble Metal Nanozymes. Noble metal nanozymes are of importance in detection and antibacterial use.[63,88] Materials such as gold, silver, and platinum have been broadly applied. Noble metal nanozymes have intrinsic catalytic activities as well as unique optical properties. These make them promising in visualized sensing.

As enzyme mimics, noble metal nanozymes are able to identify targets by colorimetric reactions through colorimetric indicators.[63] For example, plasmonic Au NPs obtain POD-like activity, which can be used in the oxidation of TMB. With the assistance of indicators, Au NPs with a casein coat can specifically diagnose protease.[65] A water-soluble silver nanozyme (Ag@GQD) with high OXD-like activity can sense glutathione (GSH) with high affinity.[67] In addition to colorimetric detection via catalytic reactions, some nanozyme-based sensors can convert mechanical stimuli, such as pressure, into visible readouts. Hu et al. proposed a noble metal nanozyme catalytic pressure sensing platform which can be visually quantified with the naked eye (Figure 4a).[69] CAT-like activity of the Pt nanozyme dramatically boosts the pressure within the sealed container due to the decomposition of H_2O_2 to O_2 and accelerates the movement of the indicator dye. With a limit of detection (LOD) of 0.17 μM, the experiment proved the effectiveness of this sensor in evaluating the degree of spoilage of red wine in the H_2S determination.

Unlike other nanozymes, which rely on indicators for sensing, noble metal nanozymes are fascinating because of their special characteristics. LSPR is one of those appealing features, which is promising in environment monitoring, biological marker analysis, and food safety analysis.[89] It has enormous potential in visualization applications of biosensing. Wu et al. established an aptamer sensor based on LSPR of Au NPs.[64] The biosensor uses electrostatic repulsion with the aptamer to prevent Au NPs from aggregating when exposed to salt, which makes it sensitive in detection of *E. coli* O157:H7, with an LOD of 10^5 CFU/mL. Wei et al. utilized Ag nanoplates for their LSPR performance and successfully determined saxitoxin (STX) with an LOD of 0.06 ng/mL (Figure 4b).[87] Surface-enhanced Raman spectroscopy (SERS) is also an important target identification method that can be activated and amplified by noble metal nanozymes. This technique has both excellent selectivity and sensitivity, making detection fast and accurate. Li et al. developed the CuTA@Ag nanozyme with high performance of POD-like activity and SERS activity; the material achieves quantitative determination of GSH with an LOD of 10^{-7} M (Figure 4c).[68] Yang et al. developed an ultrasensitive multiplex SERS immunosensor consisting of Au NPs and encoded SPCB, which has both POD-like activity and OXD-like activity, enhancing detection signals of Raman reporter molecules.[66] The sensor can be used in detection of carcinoembryonic antigen and alpha-fetoprotein (AFP), with LODs of 0.66 and 9.5 pg/mL, respectively. These works demonstrate some new strategies for noble metal nanozymes in biosensing that are promising for diagnosis of disease markers.

3.1.2. Transition Metal Nanozymes. Transition metal atoms serve as active centers (cofactors) in natural enzymes, facilitating their catalytic processes. Transition metal nanozymes pertain to metals, such as Fe, Zn, Cu, Mn, Ce, and Co, among others. Inspired by the catalytic centers of natural enzymes, transition-metal-based nanozymes (frequently comprising oxides or sulfides) have emerged as important enzyme mimics. These nanozymes have been extensively studied and demonstrate substantial potential for the colorimetric detection of microorganisms and their associated metabolites.

Fe-based nanozymes (e.g., γ-Fe_2O_3 and Fe_3O_4) exhibit significant catalase-like activity and have been extensively explored for applications in microbiological detection and foodborne pathogen analysis. Zhang et al. developed a straightforward colorimetric sensor array based on Fe-N-C and Fe-N-C-urea nanozymes. Differential absorbance profiles enabled the identification of oral cariogenic bacteria,[45] while

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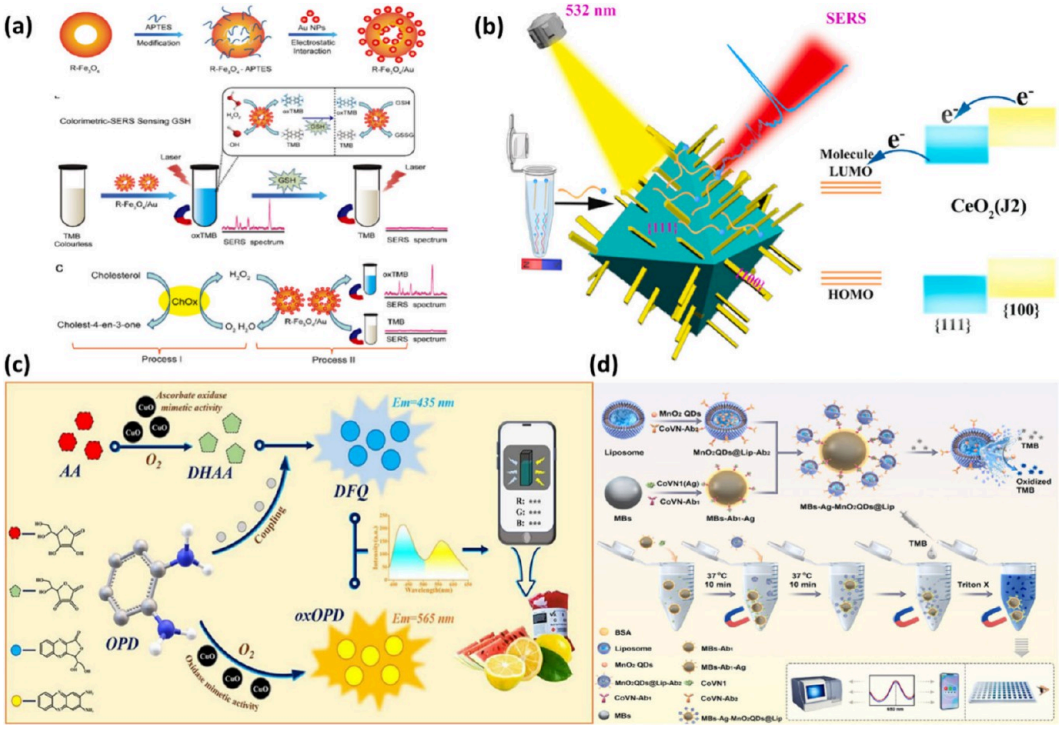

Figure 5. Transition metal nanozymes and their applications. (a) Illustration of the preparation of R-Fe_3O_4/Au for detection of GSH and cholesterol. Reproduced with permission from ref 54. Copyright 2022 Elsevier. (b) Mechanism of facet junction of CeO_2 for sensitive detection of ATP. Reproduced with permission from ref 90. Copyright 2022 Elsevier. (c) Ratiometric fluorescence system constructed through the dual functionality of CuO NPs and its applications. Reproduced with permission from ref 61. Copyright 2021 Elsevier. (d) Ultrasensitive colorimetric immunosensor based on MnO_2QDs@Lip for SARS-CoV-2 antigen detection. Reproduced with permission from ref 51. Copyright 2023 Elsevier.

machine learning facilitated the discrimination of diverse cariogenic bacteria across different genera. The system makes it possible to distinguish various cariogenic bacteria and bacteria of different genera assisted by machine learning. Duan et al. designed a new kind of hydrophilic AgPt-Fe_3O_4 nanozyme that has a special two-part structure and can work like a peroxidase. By taking advantage of the strong toxicity of CO to the material, this material can achieve colorimetric detection of CO in solution with an LOD as low as 5.6 ppb.[53] Huang et al. utilized gold nanoparticles to alter magnetic cyclic Fe_3O_4 (R-Fe_3O_4/Au), resulting in the synthesis of a new POD-like nanozyme with a dual-channel detection platform for colorimetric detection and Raman detection (Figure 5a).[54] The combination of colorimetric reaction and SERS makes GSH analysis effective and sensitive, with an LOD as low as 0.10 μM. These effective improvements fully utilize the superior characteristics of iron-based materials and provide some creative methods for the advancement of nanozymes with elevated precision and sensitivity.

Ce-based nanozymes are impressive for their intrinsic multienzyme activity. The presence of mixed Ce^{4+}/Ce^{3+} in cerium ions enables them to show POD-, OXD-, SOD-, CAT-, and ALP-like catalytic performance. Wang et al. developed DPA-Ce-GMP nanomaterials, which can efficiently catalyze TMB oxidation and can be used for the detection of glutathione with an LOD of 17.1 nM.[60] Li et al. produced CeO_2/L-g-C_3N_4, demonstrating remarkable OXD-like activity in the detection of hydroquinone (HQ). The system demonstrates a linear detection range of 0.454−37 μM and an LOD of 0.094 μM.[75] Furthermore, the Ce^{4+}/Ce^{3+} active center endows the cerium nanozyme with phosphohydrolase activity. Gai et al. developed a simple colorimetric method for the rapid and selective detection of oxygen and phosphorus by constructing a unique CeO_2@NC nanozyme.[27] Through reaction, increasing concentrations of oxygen and phosphorus result in a more noticeable yellow color via the naked eye. During the application process, visible results can be obtained within the linear range of 3.0−100.0 μM, which is conducive to the rapid detection of organophosphorus pesticides. Besides its excellent catalytic ability, CeO_2 also shows a strong SERS effect, enabling sensitive detection of ATP (Figure 5b).[90] Moreover, by using defect engineering to create heterojunctions, CeO_2 can possess superior photocatalytic activity.[91] These examples demonstrate the versatility of cerium-based nanozymes in biosensing,[91] highlighting their intrinsic capacity for the optical detection of microbes.

Cu-based nanozymes can be used as excellent nanomaterials for colorimetric detection. Their enzyme-like activity is shown in the form of both atoms and compounds.[92,93] Wu et al. synthesized Cu-N-C nanomaterials for the detection of organophosphorus pesticides.[46] By integrating natural enzymes, this cascade reaction system achieves detection with an LOD as low as 0.60 ng/mL. Wang et al. designed an effective fluorescence detection system for ascorbic acid (AA) ratios using CuO NPs (Figure 5c).[61] The system can catalyze the rapid conversion of AA to dehydrogenous ascorbic acid, which then interacts with nonfluorescent *o*-phenylenediamine (OPD) to produce quinoxaline-1-one (DFQ), exhibiting different fluorescence. With an LOD of 0.16 μM, the CuO NP system has been successfully applied to the detection of AA in both serum and fruit targets. Zhang et al. prepared CuS HNCs with high POD-like activity and photothermal effect.[62] Through the LSPR effect and creation of reactive oxygen species (ROS), the

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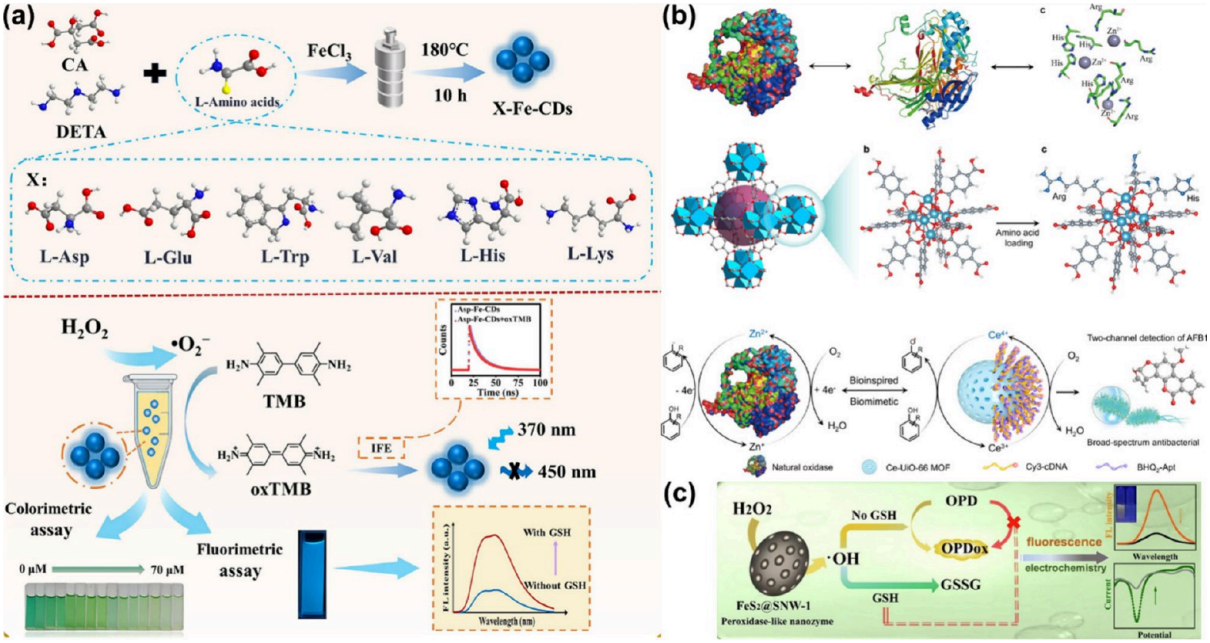

Figure 6. Carbon-based nanozymes and their applications. (a) L-Asp-modified CDs with POD-like activity and fluorescence. Reproduced with permission from ref 76. Copyright 2025 Elsevier. (b) Structure and mechanism of bioinspired MOF. Reproduced with permission from ref 100. Copyright 2025 Elsevier. (c) FeS_2@SNW-1 with POD-like performance. Reproduced with permission from ref 85. Copyright 2022 Elsevier.

material can diagnose tannic acid using this dual-mode strategy.

Mn-based nanozymes represent prominent nanozyme materials whose versatile enzyme-mimicking activities enable diverse colorimetric reactions. Wang et al. attempted to develop MnO_2 nanosheets for screening acid phosphatase (ACP).[52] These nanosheets exhibited OXD-like activity, catalyzing the oxidation of ABTS to produce a distinct colorimetric signal. The resulting absorbance changes were correlated with ACP concentration, achieving an LOD of 0.046 mU/mL. Chu et. al developed a colorimetric immunosensing system using liposome-encapsulated MnO_2 quantum dot-sized nanozymes (MnO_2QDs@Lip) as a signal reporter for ultrasensitive, rapid detection of SARS-CoV-2 antigen (Figure 5d).[51] Antibody modified on the surface of MnO_2QDs@Lip allows nanozymes to detect targets with high affinity. Achieving an LOD of 65 fg/mL in PBS, this method offers a simple, rapid, and highly sensitive detection platform, demonstrating significant potential for point-of-care testing system development.

3.2. Carbon-Based Nanozymes. Carbon-based nanozymes demonstrate significant potential as enzyme mimics,[94] operating through distinct catalytic mechanisms versus metal-based counterparts, where activity is primarily governed by defect density.[41] This inherent tunability enables diverse applications in rapid microbial detection.

3.2.1. Functionalized Carbon Nanozymes. Various strategies can be used as diagnosis methods for functionalized carbon nanozymes, including colorimetric, fluorescence, and electric.[95−97] Carbon dots (CDs), as a representative category of nanomaterials, are appealing due to their potential for modification. Song et al. modified CDs with L-Asp, providing them with exceptional POD-like activity and fluorescence (Figure 6a).[76] Assisted by TMB, the materials can be used as a dual-mode biosensor for GSH detection with an LOD of 0.189 μM in fluorimetry and 0.296 μM in colorimetry. Carbon nitride and graphite are also important parts of functionalized nanozymes. Hu et al. designed a light-responsive Co/g-C_3N_4 nanostructure for the determination of acetylcholinesterase (AChE) activity.[98] Under exposure to visible light, its enzyme-like property is enhanced, which can trigger a colorimetric reaction with TMB. With a remarkably low LOD of 0.04 mU/mL, this method provides an innovative way for AChE diagnosis. Moreover, hybrid carbon nanomaterials exhibit greater improvement in comparison to single materials. Ding et al. developed FeS_2 encapsulated by carbon nanosheets (FeS_2@C NSs).[77] Hybridization of those two nanomaterials successfully enhances catalytic ability because active sites become more numerous and dispersed. The biosensing platform achieves the determination of glucose with an LOD as low as 0.19 μM. These discoveries provide more inspiration for future advancement of nanozyme design and development.

3.2.2. MOFs and COFs. Metal−organic frameworks (MOFs) have emerged as a type of crystalline coordination polymer formed through the coordination-directed preparation of metal nodes and organic linkers, creating extended 2D or 3D structures with periodic porosity.[42] Kulandaivel et al. successfully constructed Fe−Cu bimetallic nanozymes with both OXD and peroxidase activities using MOF materials. This material achieves colorimetric biosensing of adrenaline through colorimetric reactions, presenting promising prospects for medical diagnostic applications.[99] Wang et al. developed a bioinspired MOF modified by double amino acids with excellent OXD-like activity over a wide pH range (Figure 6b).[100] The Ce MOF@$Arg_{0.25}$@$His_{0.25}$ has a unique energy-regulated substrate lock to assist in selective regulation of OXD-like catalysis. It allows the MOF nanozyme to be used to build a dual-mode sensing platform for detection of AFB1, collectively contributing to future agro-food safety innovations with improved performance. Furthermore, Mo-doped MoCu-2MI demonstrates superior POD-like activity compared to conventional Cu-2MI nanomaterials.[84] This enhancement

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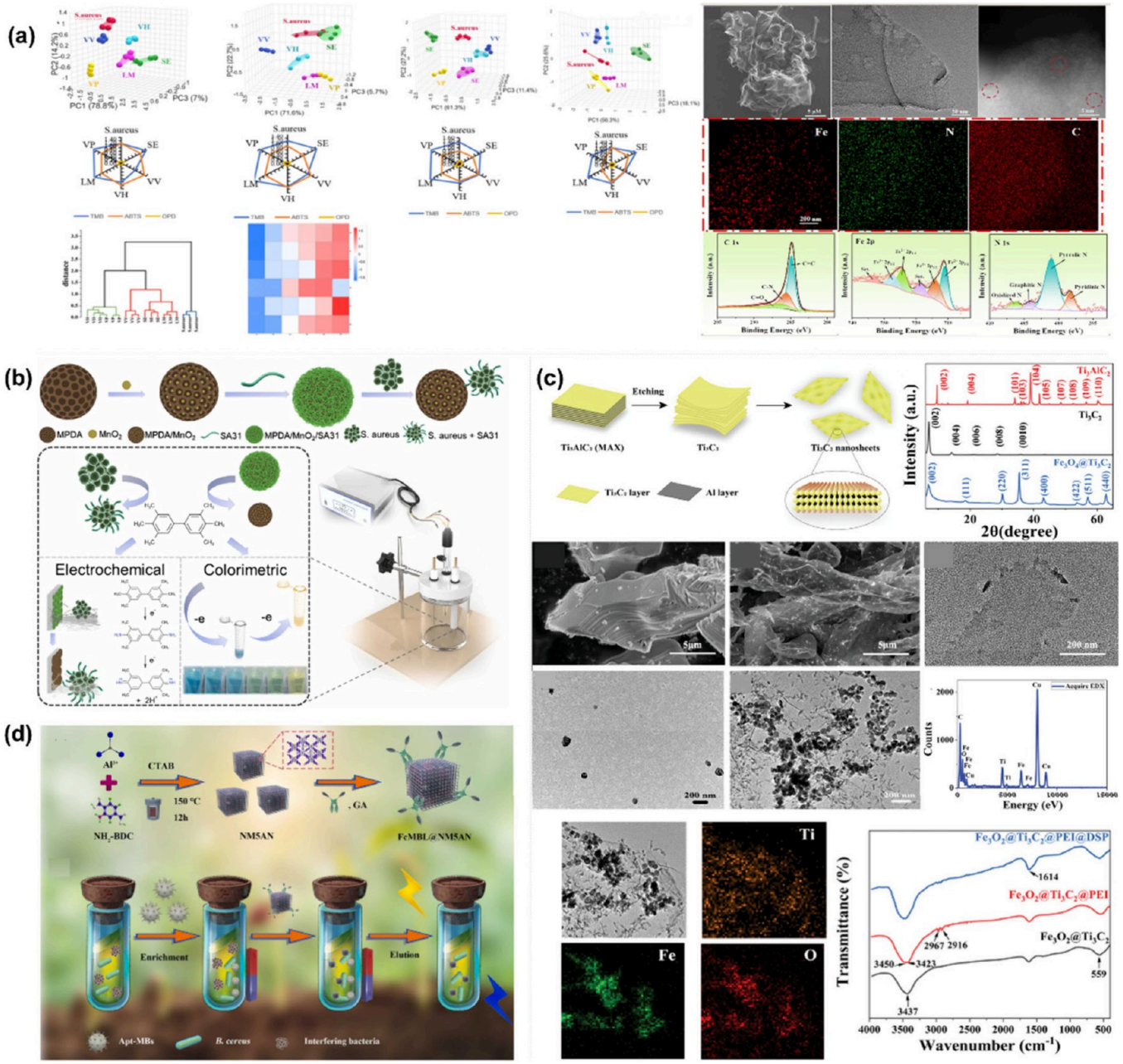

Figure 7. Strategies for catalytic activity improvement. (a) SAzyme colorimetric sensor array assisted by machine learning. Reproduced with permission from ref 108. Copyright 2025 Elsevier. (b) Aptamer colorimetric−electrochemical sensing platform for detection of *S. aureus*. Reproduced with permission from ref 10. Copyright 2023 Elsevier. (c) Aptamer-functionalized Ti_3C_2-based nanoplatform for detection of exosomes. Reproduced with permission from ref 117. Copyright 2024 Wiley-VCH. (d) Selective detection of *B. cereus* in complex matrices by a dual-recognition approach. Reproduced with permission from ref 119. Copyright 2023 Elsevier.

originates from molybdenum-facilitated electron transfer kinetics. The resulting biosensing platform achieves colorimetric cholesterol detection with an LOD of 1.2 μM, exemplifying how MOFs serve as ideal host matrices for encapsulation of functional species in heterogeneous catalysis applications.

Covalent organic frameworks (COFs) are a series of polymers consisting of light elements. Linked by covalent bonds, COFs possess well-shaped structures that can be designed precisely and are promising for further modification.[41] Though COFs are stable enough for catalysis, their limitation in enzyme-like activity requires combination with other nanozymes. He et al. developed an FeS_2-encapsulated COF (FeS_2@SNW-1) as a peroxidase mimic (Figure 6c).[85] It can efficiently analyze GSH in cell lysate; the LOD is as low as 1.61 μM. Besides, a Cu^{2+}-enhanced COF material (TpDA-Cu) improves light-responsive nanozyme activity compared to the unmodified one.[86] It shows a greater V_{max} than before and is suitable for rapid, selective detection of thiram (Tr). Combining Au NPs into COFs makes the SERS effect become more sensitive. Fu et al. prepared Au NPs@COF as an ideal SERS substrate, using 4-nitrophenol (4-NPH) to realize the detection of acetylcholine .[101] It succeeds in sensing with a linear range of 1.0 pM to 10 nM, exhibiting its capacity in bioassay applications. With an adjustable structure and

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appropriate porosity, COFs are becoming increasingly reliable materials for nanozyme enhancement.

4. STRATEGIES FOR IMPROVING NANOZYME SELECTIVITY AND UTILITY

Because of their special physicochemical features and intrinsic enzyme-like activity, nanozymes have become a new option for biosensing. However, in comparison to the evolutionarily refined active-site microenvironments in natural enzymes, nanozymes are compromised by their shortage of catalytic selectivity.[24] To bridge this critical performance gap and enable broader technological implementation, nanozymes require further construction for better applications such as active site advancement, interfacial modification, dual identification strategy construction, and so on.

4.1. Strategies for Improving Selectivity. *4.1.1. Advancement of Active Sites.* It is well-known that natural enzymes exhibit excellent specificity due to their sophisticated structures. As a matter of fact, by simulating active sites from natural enzymes, the catalytic selectivity of nanozymes can be greatly improved.

Molecular imprinting technology (MIT) is the method of producing molecularly imprinted polymers via copolymerization of functional monomers and cross-linkers with template molecules. The MIT nanozyme materials have complementary cavities to improve their substrate recognition capacity. Zhang et al. successfully designed substrate-binding pockets on the interface of Fe_3O_4, Au particles, and CeO_2 NPs through MIT, thereby improving the activity and selectivity of the material and increasing the specificity by 100 times under the optimal conditions.[102] The MIT nanozymes can effectively enrich local substrates and reduce the activation energy of the reaction. MIT confronts persistent challenges, including template leaching, suboptimal binding affinity, and limited compatibility with aqueous systems—critical limitations that constrain the broader implementation of molecularly imprinted polymers in biosensing applications.[103]

Designing single-atom nanozymes (SAzymes) is another way to advance active sites. Created by anchoring isolated metal atoms onto appropriate support substrates at the atomic scale, SAzymes demonstrate exceptional catalytic activity, robust stability, homogeneous active sites, maximum metal atom utilization efficiency, and unique geometric configuration.[104] As kinds of mimicry of the active sites, simulation such as modifying SAzymes into the M−N−C structure endows them with ultrahigh metal atom utilization and unique geometric structure.[105,106] Yang et al. synthesized an Fe-N/C SAzymes platform for diagnosis of AA and ACP. Because of its high OXD-like activity, the platform can be used in determination of ACP with an LOD of 0.048 U/L.[44] Sun et al. developed Co-N-C SAzymes with OXD-like catalytic ability.[50] The sensor enabled dual detection of GSH and cysteine (Cys) by exploiting thiol-induced activity inhibition, achieving impressive LODs of 0.07 and 0.06 μM, respectively. Besides, due to their regularly distributed active centers, SAzymes can be easily predicted by machine learning, which makes them suitable for creating high-performance nanozymes.[107] Li et al. developed a colorimetric sensor array assisted by machine learning (Figure 7a). Consistent with previous studies, these sensing arrays effectively discriminate between multiple foodborne pathogens based on their distinct inhibitory effects on catalytic active sites. This approach demonstrates significant potential for rapid, accurate, and high-throughput pathogen identification in food safety applications.[108] SAzymes have demonstrated remarkable potential in emulating the catalytic functions of natural enzymes, which has proved to be a capable way for applications.

4.1.2. Interfacial Modification. Interfacial modification improves nanozymes in various ways by changing the surficial characteristics of nanozymes.[109] Interfacial modification significantly enhances catalytic selectivity by adding specific ligands to the surface of nanozymes, making it attractive among many modification methods. For instance, surface modification of Prussian blue nanocubes with ATP significantly enhances nanomaterial conductivity.[110] Furthermore, functionalization of CuS nanozymes with aspartic acid introduces abundant surface negative charges, shifting the optimal catalytic pH toward neutral conditions.[111]

Variations in interfacial modification of materials significantly influence the catalytic performance of the nanozymes. The introduction of different functional groups alters the electron transfer kinetics of the nanozymes, consequently modulating their catalytic activity.[112] Therefore, diverse modification materials lead to various functional nanozymes. Application of natural enzymes in nanozyme modification can leverage its selectivity and sensitivity. This approach was successfully utilized in the detection of cholesterol, uric acid, glucose, and even tumor cells.[78−81] This advancement overcomes the traditional limitations of dispersion and selectivity in nanozymes, significantly enhancing their performance. Furthermore, macromolecular modification using proteins or antibodies offers a practical and effective strategy for nanozyme interfacial engineering. The incorporation of these macromolecules significantly enhances nanozyme detection performance, leveraging their high target affinity to achieve superior sensitivity and limit of detection.[82,83] Pan et al. developed nanozyme combined with vancomycin (Van) system, which shows high affinity with phage tail fiber protein (TFP).[113] The selective detection was applied in the diagnosis of *Listeria monocytogenes*. With a remarkable detection limit of 10 CFU/mL, this approach shows significant promise for clinical pathogen identification, meeting the sensitivity requirements for many infectious disease diagnostics. Interfacial modification provides a robust and reliable strategy for enhancing nanozyme performance, enabling precise target detection in complex matrices.

4.1.3. Aptamer Modification of Nanozymes. In recent years, aptamer nanozymes have been a popular topic in nanozyme research. This special interfacial modification uses a screening approach called systematic evolution of ligands by exponential enrichment (SELEX) to systematically evolve RNA or DNA molecules into specific sequences. It achieves target-specific recognition with high affinity via synergistic shape complementarity, electrostatic interaction, and hydrogen bonding and is known as "chemical antibody".[11,114] Although aptamer-functionalized nanozymes represent an emerging and still-developing technology, recent advances in high-precision screening methodologies are significantly enhancing their stability and applicability.[115]

Similar to many inhibition detection methods, aptamer-modified nanozyme materials can control the catalytic effect through the regulation of active sites by aptamers, thereby achieving highly selective recognition when the target appears. Cui et al. developed a colorimetric electrochemical sensing platform based on mesoporous polydopamine/MnO_2 (MPDA/MnO_2) nanozymes with specific oligonucleotide-

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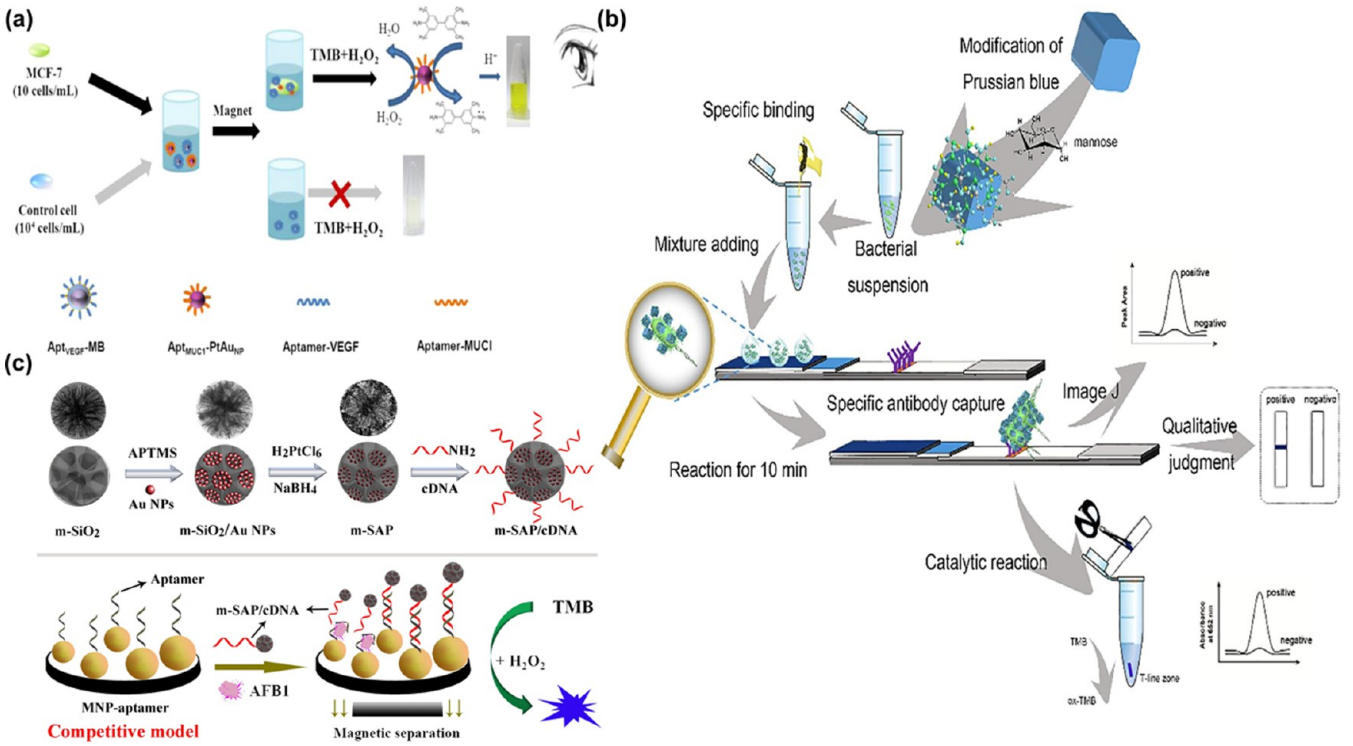

Figure 8. Enhancing selectivity of nanozymes. (a) Using an aptamer to amplify Pt@Au nanoparticle catalysis sensitivity. Reproduced with permission from ref 122. Copyright 2015 Elsevier. (b) Using AuNPs in ICA for detection of *E. coli*. Reproduced with permission from ref 56. Copyright 2020 Elsevier. (c) Using H-Co SAzyme as NLISA for determination of AFB1. Reproduced with permission from ref 126. Copyright 2020 Elsevier.

modulated OXD-like activity for the detection of *Staphylococcus aureus* (Figure 7b).[10] In their work, the SA31 aptamer was used to take advantage of the competitive coordination effect to influence the catalytic activity of the MnO_2 nanozymes. In the presence of *S. aureus*, the aptamer recognizes and dissociates from the nanozymes, restoring their catalytic activity and thus generating a colorimetric effect. This sensing platform exhibits an extensive linear range ($5-10^{-7}$ CFU/mL) and a low detection threshold (3 CFU/mL). Esmaelpourfarkhani et al. designed an aptamer sensor that selectively detects ampicillin (AMP) by taking advantage of the unique properties of MnO_2 nanoparticles and Au NPs.[116] In the absence of AMP, the aptamer immobilized on Au NPs bridges to the modified complementary strand (mCS). After centrifugation, the supernatant contains no mCS, leaving the catalytic activity of nanoflowers unaffected, which results in a high TMB absorbance. Conversely, AMP addition inhibits aptamer−mCS hybridization, allowing free mCS in the supernatant to reduce the nanoflower activity. This sensor achieves a detection range of 70 pM to 10 nM with an LOD of 21.7 pM. Zheng et al. designed an aptamer-functionalized Ti_3C_2-based nanoplatform for detection of exosomes (Figure 7c).[117] Combination with Fe_3O_4 enables exceptional magnetic ability, making it convenient and cost-effective in target separation. These results highlight the ability of aptamers in precise regulation of nanomaterial enzymatic activity and their excellent selectivity.

Aptamers can achieve fluorescence detection with high selectivity, demonstrating the same effect in visual detection. Rahmatian et al. prepared carbon dots (CDs) by the hydrothermal method and cross-linked them with a mixture of alginate through Ca^{2+} to fabricate alginate−CD nanocomposites.[118] Besides, they designed an aptamer capable of selectively targeting (1→3)-β-D-glucan on the cell wall surface of *Candida albicans*. The FRET effect between the aptamer and nanocomposite decreases the nanocomposite's fluorescence, allowing the creation of a turn-on probe. Upon exposure to *C. albicans*, the aptamer selectively binds to the (1,3)-β-D-glucan surface RNA chains via hydrogen bonds, displacing it from the nanocomposite and restoring the fluorescence intensity proportionally to fungal concentration. The system exhibits dual linear ranges (50−200 and 1000−6000 cells/mL) with a 40 cells/mL detection limit, realizing rapid, direct, and robust *C. albicans* detection.

Meanwhile, aptamers are able to combine inherent properties of the material itself (such as magnetism) to give full play to its role in screening and enrichment. Yan et al. developed a dual-recognition fluorescence method for quantifying *Bacillus cereus* and yeast samples in food, integrating broad-spectrum nano-MOF fluorescent probes with aptamer-conjugated magnetic nanoparticles (Figure 7d).[119] The biosensing probe was synthesized using fragment crystalline mannose-binding lectin (FcMBL)-modified nano MIR-53(Al)-NH_2, where FcMBL selectively binds terminal mannose residues concentrated on pathogenic bacterial surfaces. Apt-MBs enabled efficient *B. cereus* enrichment, leveraging aptamer advantages over antibodies, including higher specificity, nonimmunogenicity, compact size, and superior chemical stability. The system achieved exceptional quantitative performance, with a broad detection range ($20-2 \times 10^8$ CFU/mL) and low LOD (4 CFU/mL), demonstrating strong potential for applications in food safety, public health, infectious disease diagnostics, and the beverage industry.

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4.2. Selective Detection Applications of Nanozymes.

4.2.1. Constructing Dual Identification. To date, most of the applications of nanozymes are concentrated in single-signal sensing.[120] Single recognition mechanisms have limited recognition sites for target molecules, leading to poor specific binding capacity in complicated settings and a disability to mediate interactions among several molecules. Utilizing the collaborative potential of nanozymes and other signal-sensing molecules allows optimal exploitation of each recognition molecule's advantages, enhances design flexibility, and effectively addresses the limitations of single-molecule recognition strategies, thereby improving sensitivity and selectivity while aiding in the regulation of biological processes.

Compared with single-signal recognition, the dual identification strategy reduces the possibility of false positives and achieves high specificity for target molecules. In terms of the visual detection of glycosylated RNA, research by Ma et al. provides a highly sensitive and selective detection scheme.[121] They utilized sialic acid aptamers and RNA in situ hybridization-mediated proximity ligation analysis (ARPLA) to visualize glycosylated RNA. Through the in situ ligation triggered by recognition, the complementary DNA undergoes further looped amplification (RCA), and a fluorescence signal is generated by binding to fluorescence-labeled oligonucleotides, thereby revealing the spatial distribution of glycosylated RNA. The advantage of this detection lies in the dual recognition of the glycan probe and the RNA-binding probe, which enables the linker hybridization and in situ ligation to be triggered only when both coexist to achieve signal output of glycosylated RNA. This strategy provides high specificity for sugar RNA and allows the imaging of naturally unlabeled sugar RNA in various types of samples without pretreatment. Therefore, it has better versatility in spatial recognition. Wang et al. developed an enzyme-free cancer cell detection platform leveraging aptamer specificity and Pt@Au nanoparticle catalysis for rapid, sensitive detection (Figure 8a).[122] Based on MUC 1 and VEGF 165, two protein markers on MCF-7 cells, the aptamer of MUC 1 modified on Pt@Au nanoparticles was used as the sensing probe, and the VEGF aptamer modified by magnetic beads was used as the capture probe. A bio-barcode configuration complex was jointly formed on the surface of cancer cells to capture them. Exerting the POD-like activity of Pt@Au, this method achieves visible sensitive detection with an LOD as low as 10 cells/mL. Compared with traditional methods, the dual-biomarker aptamer strategy enhances the detection specificity and accuracy for cancer cells. In addition, the colorimetric–electrochemical sensing platform designed by Cui et al., the FcMBL@NM5AN fluorescence platform designed by Yan et al., and the fluorescence–colorimetric dual-mode detection platform of polydopamine nanozyme fully demonstrate that the dual-recognition strategy can achieve the construction of a more efficient and accurate analysis platform.[10,119,123] It has played an unmatched role in multifunctional detection and illness treatment, hence exhibiting a superiority unattainable by the singular colorimetric mode, which addresses the increasing need for precision medicine.

4.2.2. Highly Selective Portable Test Strip. Nanozymes have emerged as highly efficient tools, enabling rapid, sensitive, and user-friendly detection. Their utility is further enhanced when combined with established techniques such as lateral flow assay (LFA) or enzyme-linked immunosorbent assay (ELISA). Colorimetric detection has grown popular as a signal readout method largely because it enables naked-eye disease detection via a simple color change, avoiding the need for expensive or complicated instruments.[124] In practical applications, LFAs demonstrated potential in biosensing during the SARS-CoV-2 epidemic. Compared with colloidal gold nanoparticles, nanozymes not only enhance color intensity through target-induced specific enrichment at the test line but also amplify the color signal by catalyzing the oxidation of colorless substrates into colored products.[123] These advantages make it a promising candidate for developing higher-performance LFAs. Fu et al. proposed a novel two-step cascade signal amplification strategy that combines in situ Au NP growth with nanozyme-mediated catalytic deposition, significantly enhancing the detection sensitivity of conventional Au NP-based immunochromatographic assays (ICAs).[72] The enhanced lateral flow strip achieves ultrasensitive detection of *E. coli* O157:H7, showing a detection limit of 1.25×10^1 CFU/mL, which is a 400-fold improvement over the conventional Au NP ICA strips. Wang et al. developed a multireadout and label-free lateral flow immunoassay based on a nanozyme, bacterium, and antibody sandwich architecture for fast detection of *E. coli* O157:H7 (Figure 8b).[56] This platform employs mannose-modified Prussian blue nanoparticles (Man-PB) as both recognition elements and signal indicators, exhibiting a broad quantitative range (10^2–10^8 CFU/mL) with a detection limit of 10^2 CFU/mL across different readout formats. Moreover, nanoparticles such as PtGNs, Fe_3O_4, Au@Pt, and Prussian blue nanoparticles are feasible to be applied as well.[57,58,70,71,73] Their intrinsic ability in biosensing extends our way of analysis and develops high-performance LFAs with the merits of high sensitivity, rapid response, simple operation, low cost, and user-friendliness.

While conventional ELISA is limited by its relatively low sensitivity and stability, nanozyme-linked immunosorbent assay (NLISA) is a promising alternative for its robustness and cheapness.[125] Xu et al. reported an H-Co SAzyme with high oxidase-like activity.[49] Edge-hosted single-atom Co nanozymes with atomically distributed Co-N_4 moieties on nitrogen-doped hierarchical porous carbon exhibit remarkable oxidase-like activity, facilitating improved efficacy in immunoassay applications. In comparison to normal commercial ELISA kits, H-Co SAzymes offer enhanced detection sensitivity, with an LOD of 5.19 pg/mL. Wu et al. developed a nanozyme- and aptamer-based immunosorbent assay (NAISA) for detection of AFB1, the most toxic aflatoxin with potent carcinogenicity (Figure 8c).[126] The novel NAISA demonstrated enhanced analytical properties, with reduced LOD (5 pg/mL), increased accuracy (RSD = 4.2%), and improved linearity (R^2 = 0.9897) relative to traditional ELISA (t-ELISA) and enhanced ELISA (e-ELISA) techniques. Furthermore, materials such as MagPlas NZs (Fe_3O_4-Au), Fe_3O_4@COF@Os, and Au@Pt NRs are useful tools for NLISA methods.[55,74,127] Today, NLISA has transformed conventional ELISA, exhibiting greater sensitivity, diminished sample volume needs, abbreviated test durations, and improved cost-efficiency.

5. CONCLUSION AND PERSPECTIVE

The growing desire for quick, on-site microbial detection has resulted in considerable advances in nanozyme engineering. Nanozymes, as durable and cost-effective replacements for natural enzymes, have tremendous potential in visual diagnostics due to their high stability and tunable catalytic

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activity. This review has presented a systematic overview of the fundamental mechanisms of nanozyme-based detection, classified major nanozyme types, and highlighted their cutting-edge applications in microbial sensing, particularly through POD-like and OXD-like catalytic cascades that enable ultrasensitive and amplified signal detection.

Despite these advancements, significant challenges persist. While nanozymes overcome the instability and scalability constraints of natural enzymes, their low selectivity remains a restriction. Emerging techniques, such as active site advancement and interfacial modification, have shown promise for improving target specificity. However, additional optimization is required to reduce false-positive signals, particularly in complicated biological matrices. Dual-identification methodologies that incorporate several signal outputs may substantially enhance diagnostic precision through result cross-validation.

Nanozymes are still evolving; improvements in catalysis and selective performance are necessary. In the near future, certain enhancements will be imperative and efficacious, warranting exploration:

1. Expanding Nanozyme Diversity: Most reported nanozymes are limited to oxidoreductase-like activities. The discovery of hydrolase-like nanozymes or other underrepresented classes could unlock new detection paradigms, particularly for challenging targets such as polysaccharides or small molecules. This outcome requires a combination of experimental validation and rational design, a process that can be significantly streamlined with machine learning and DFT calculation assistance.
2. Enhancing Aptamer Affinity: Interfacial modification platforms, especially aptamer-based nanozyme systems, often suffer from weak binding to low-molecular-weight analytes. Innovations in SELEX techniques or hybrid binding motifs may overcome this limitation.
3. Improving Environmental Robustness: Nanozyme performance is highly dependent on pH, ionic strength, and temperature. Microenvironment engineering (e.g., hydrophobic coatings or protective matrices) could stabilize activity under physiological conditions. Encapsulation of nanozymes with chemically stable materials presents a viable solution to this challenge.
4. Next-Generation Signal Amplification: Future designs should integrate multienzyme mimics or plasmonic-enhanced catalysis to achieve exponential signal gains, pushing detection limits toward single-cell or even single-molecule levels. This can be achieved through nanozyme cascade reaction coupled with electronic signal amplification.

By addressing these challenges, nanozyme technology could revolutionize point-of-care diagnostics and enable real-time in vivo microbial monitoring. Interdisciplinary collaborations—spanning materials science, bioengineering, and data analytics—will be pivotal in translating these innovations into clinical and environmental applications. (1) Signal Amplification Focus: Explicitly links nanozyme mechanisms (POD/OXD cascades) to ultrasensitive detection. (2) Structured Solutions: Presenting challenges (e.g., selectivity and false positives) with actionable solutions (dual-mode detection and microenvironment engineering). Technical Depth: Using precise terminology while maintaining readability for broader audiences. Forward-Looking Perspective: Highlighting transformative potential (real-time diagnostics and in vivo sensing) inspires future research. In summary, nanozymes represent a promising trend in microbial detection, offering high sensitivity, rapid response, and cost-effectiveness. Continued innovation in material design, catalytic optimization, and integration with emerging technologies will further solidify their role in next-generation biosensing platforms.

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Notes

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