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Molecularly imprinted polymer-integrated nanozymes for biosensing: advances and prospects

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Nanozymes engineered nanomaterials with enzyme-like catalytic activity—have emerged as cost-effective and stable alternatives to enzymes. However, their broad substrate range and lack of specificity limit their utility in precision biosensing. To overcome this, molecularly imprinted polymers (MIP) have been integrated with nanozymes, forming hybrid nanozyme@MIP systems that combine catalytic efficiency with molecular recognition. These materials exhibit enhanced selectivity and sensitivity, enabling their application in diverse biosensing platforms, including colorimetric, fluorescence, and electrochemical assays for the detection of drugs, pollutants, and disease biomarkers. This review critically examines recent advances in the design, synthesis, and application of nanozyme@MIP composites. This review provides a timely and comprehensive analysis of molecularly imprinted nanozymes, presenting a viable alternative to conventional enzyme-based systems. It bridges a critical gap by detailing design strategies, catalytic mechanisms, and biosensing applications. Its clarity, depth, and interdisciplinary relevance make it a valuable resource for advancing research and practical applications in this emerging field. We explore various imprinting strategies, catalytic mechanisms, and assay formats, while highlighting their advantages over conventional biosensors, such as improved stability, reusability, and cost-effectiveness. Key challenges are addressed, including the trade-off between selectivity and catalytic activity, non-specific adsorption, and the predominance of peroxidase-like mechanisms. Special attention is given to performance in complex matrices, scalability of synthesis, long-term stability, and biocompatibility. Furthermore, we discuss the need for standardized protocols to ensure reproducibility and comparability across studies and propose design principles to optimize MIP layer properties for enhanced performance. By integrating recent literature and comparative analyses, this review provides a comprehensive framework to guide future research and industrial translation of nanozyme@MIP-based biosensors for diagnostics, environmental monitoring, and point-of-care applications.

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1. Nanozyme@MIPs: tailored catalysts for enhanced detection-what are they and why do they matter?

This review is both timely and necessary as it addresses the emerging and rapidly evolving field of molecularly imprinted nanozymes, which synergistically combine the catalytic efficiency of nanozymes with the high selectivity of molecularly imprinted polymers (MIP). While several studies have reported on imprinted nanozymes, a comprehensive and focused review that systematically explores their design strategies, catalytic mechanisms, and biosensing applications remains lacking. This manuscript addresses a critical gap by providing a structured analysis of recent advancements, with emphasis on how molecular imprinting improves selectivity and catalytic performance in complex matrices. It also provides valuable insights into the mechanistic aspects of these hybrid systems, which are often underexplored. The review is particularly relevant to researchers in analytical chemistry, materials science, and diagnostics, and it contributes meaningfully to guiding future research and practical applications. Its clarity, depth, and interdisciplinary relevance make it a significant addition to the literature.

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Dr. Christopher Zaleski with a PhD in Physical Chemistry and MSc degrees in Green Chemistry and Biotechnology, boasts 20 years of experience across academia, industry, and the Environment Agency. His research expertise spans energy storage, ionic solvents, nanomaterials, biosensing, and therapeutic applications.

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Nanozymes, with their enzyme-like catalytic abilities, present a robust and cost-effective alternative to enzymes.[1–4] Their stability and ease of production make them highly attractive for various applications.[5–19] However, a significant limitation lies in their broad substrate specificity, hindering precise applications. Unlike enzymes with defined binding pockets, nanozymes often lack this selectivity, demanding innovative solutions to enhance their performance and broaden their utility.[20–24] To address the specificity challenge, MIPs are being integrated with nanozymes.[25,26] MIPs create tailored binding sites through a process where polymers form around a template molecule, leaving cavities that selectively rebind the target analyte.[27–29] This integration not only enhances specificity but also contributes to improved catalytic performance. By combining the catalytic activity of nanozymes with the selective recognition of MIPs, hybrid materials with superior sensing capabilities are being developed.[30–32] This review examines nanozyme characteristics, catalytic activity, and property tuning. It explores core–shell structures combining metal nanoparticles and MIPs, highlighting synthesis, fabrication, and sensing applications. Recent advancements in MIP-enhanced nanozyme devices, improving detection sensitivity and selectivity, are discussed. Finally, we summarize emerging developments and the future impact of these hybrid materials in diagnostics and sensing.

1.1 From enzymes to engineered catalysts: advancing nanozymes and molecularly imprinted nanozymes

Enzymes, while possessing unparalleled substrate selectivity and catalytic efficiency, are inherently limited by their sensitivity to

environmental factors, high production costs, and instability.[33] These drawbacks constrain their practical applications across diverse industries. In contrast, nanozymes offer significant advantages, including a high surface-to-volume ratio, abundant surface-active sites, customizable catalytic activity, high stability, cost-effective synthesis, and long-term stability.[34–39] Their robustness and versatility make them attractive for applications in diagnostics, environmental monitoring, and biochemical analysis.[1,40] However, nanozymes suffer from limitations, primarily their reduced substrate specificity compared to enzymes.[41] Lacking defined active sites, they often exhibit broad reactivity, hindering precise applications. Furthermore, concerns regarding potential toxicity and relatively lower catalytic activity restrict their broader adoption. Overcoming these limitations, particularly enhancing specificity, is crucial for expanding the practical utility of nanozymes. MIPs provide a viable alternative for addressing the specificity limitations of nanozymes.[42]

MIPs create tailored binding sites, mimicking the specific recognition of enzymes, using stable and cost-effective materials. By integrating nanozymes with MIPs, hybrid materials, nanozyme@MIPs, are formed. These hybrids combine the catalytic activity of nanozymes with the selective recognition of MIPs, significantly enhancing specificity. The rational design of nanozyme cores, coupled with MIP technology, allows for the creation of stimuli-responsive nanostructures, further optimizing performance. Nanozyme@MIP hybrids can function as both recognition elements and selective catalysts, broadening their applicability in assays, lateral flow devices, and biosensors (Fig. 1). This biomimetic approach, leveraging advanced nanotechnology, facilitates the development of portable, cost-effective, high stability and time-efficient diagnostic tools.[44] While challenges remain in further improving catalytic activity and specificity, the integration of MIPs with nanozymes holds immense potential for advancing biosensing applications, particularly in point-of-care diagnostics.

1.2 Engineering selectivity and catalytic activity

Engineering MIP shells on nanozymes creates substrate-specific binding pockets, a key strategy to balance selectivity and catalytic activity. These pockets selectively enrich target molecules while hindering access for non-target substrates.[45,46] Catalytic activity is further optimized by tuning nanoparticle core properties, such as employing smaller gold nanoparticles (5–20 nm) to increase surface area and reaction rates.[45] For instance, nanozyme@MIP solid phase synthesis, achieved through localized radical polymerization initiated by hydroxyl radicals from gold-catalysed H_2O_2 decomposition, ensures precise integration of recognition and catalytic sites.[4] The dual functionality of the system allows MIP shells to selectively recognize specific target molecules, such as amphetamine. This recognition event then triggers a conformational change, or actuation, in the MIP shell. The resulting change in the MIP's structure alters the surface area and catalytic activity of the inner catalytic core. This process significantly enhances reaction kinetics, as evidenced by a substantial decrease in the Michaelis–Menten constant (K_m) to the nanomolar and picomolar range.[45] Moreover, MIP coatings can synergistically boost catalytic activity by up to 100-fold compared to non-imprinted nanozymes.[47,48]

MIP coatings can enhance nanozyme activity. However, their structural design can also hinder it. Factors such as monomer composition, partition coefficient, surface area, affinity, and the balance between steric hindrance and substrate enrichment all play a role. Establishing systematic guidelines for optimizing parameters such as MIP thickness, porosity, and imprinting techniques remains challenging, especially given the diverse range of target substrates, from small drug molecules to peptides and proteins. Generally, MIP layer thickness should be proportional to analyte size, with sufficient porosity for substrate access to catalytic sites at the nanometre scale without causing fouling. For large biomolecules like proteins, surface or

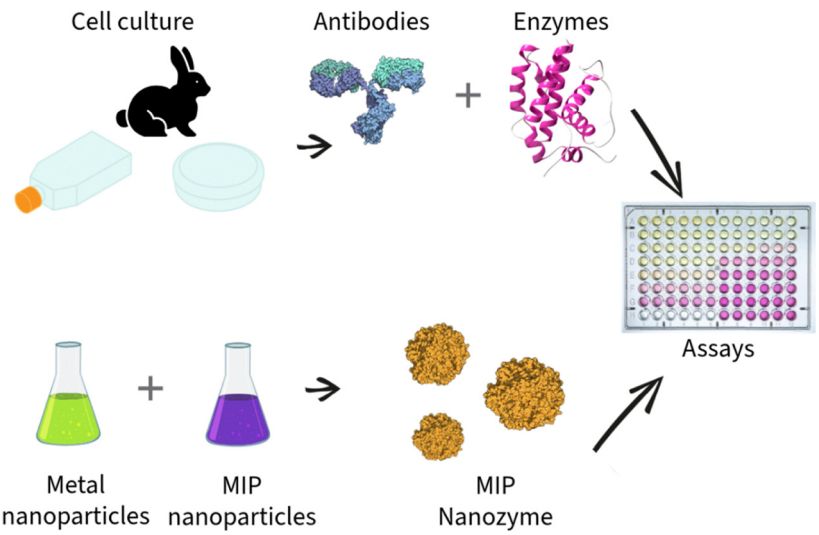

Fig. 1 Advances in analytical methods: contrasting traditional assay development with abiotic nanozyme@MIP Systems. Reproduced from ref. 43 with permission from Elsevier, copyright 2023.

epitope imprinting is recommended to maintain accessibility and recognition.[49,50]

Developing generalizable design principles necessitates comparative studies examining the relationship between MIP layer characteristics and catalytic activity. Typically, catalyst conditions are optimized first, followed by systematic evaluation of MIP parameters. Future strategies may involve computational modelling of MIP–target interactions and mechanistic studies of surface behaviour to guide rational design. A major challenge in MIP design is selecting an optimal polymerization protocol due to the need to choose and optimize numerous variable parameters, such as the types of monomers for polymer synthesis.[51] Advanced approaches for selecting appropriate functional monomers include combinatorial and computational methods.[51]

Combinatorial approaches involve simultaneous synthesis and testing of many small-scale imprinted polymers to identify the best composition. Computational approaches perform virtual monomer screening. These two methods allow the creation of MIPs with affinities and selectivities comparable to antibodies.[51] Popular synthetic strategies for nanozyme@MIPs include free radical polymerization, precipitation polymerization, mini- and micro-emulsion polymerization, core–shell approaches, and living radical polymerization processes like atom transfer radical polymerization (ATRP) and reversible addition–fragmentation chain transfer polymerization (RAFT).[52] In MIP synthesis, the choice of chemical reagents is crucial for obtaining efficient functional MIPs. Each procedure has its own advantages and disadvantages, and tuning the polymerization method can lead to homogenous particles with customisable properties.[52] Designing a new MIP system suitable for a specific template molecule often requires significant time and effort for synthesis, washing, and testing, with many attempts needed to optimize experimental parameters.[53] Solid phase synthesis of nanozyme@MIP is considered a new and advantageous technology for creating artificial antibodies and enzyme mimics.[45] Given the increasing importance of Nanozymes@MIP as substitutes for antibodies and enzymes, automation of their manufacture warrants investigation. An automated system should ensure the fabrication and purification of nanozyme@MIP nanoparticles with a uniform size distribution and be adaptable for imprinting various template types. Thus, solid phase synthesis of Nanozymes@MIP allows for large-scale production similar to peptide and DNA synthesizers.

1.3 Impact of molecular imprinting on the nanozyme catalytic activity

The integration of MIPs with nanozymes introduces a nuanced interplay between target recognition and catalytic activity. While MIPs enhance selectivity, their impact on catalytic activity can vary, leading to both decreased and increased performance depending on the specific system and design. Several studies have reported a decrease in catalytic activity upon MIP coating.[54–60] Nanozymes, nanomaterials with enzyme-like catalytic activity, offer unique advantages over enzymes. Their catalytic mechanisms, however, often differ significantly. While enzymes rely on specific, well-defined active sites and intricate protein structures, nanozymes utilize surface-active sites and exhibit more diverse catalytic pathways. The catalytic process unfolds in four key steps: selective substrate recognition and adsorption at molecularly imprinted sites, followed by the enrichment of the substrate near the catalytic core, which can increase its local concentration by up to eight times. This concentration increase, along with a reduction in activation energy, significantly enhances catalytic efficiency. The final step is the facilitated product release, which ensures high turnover rates. In certain cases, the initial recognition step can even cause a structural change, or actuation, that directly alters the nanozyme's catalytic activity.[21,48,61,62]

One common mechanism involves the generation of reactive oxygen species (ROS).[63] For example, iron oxide nanozymes can catalyse the decomposition of hydrogen peroxide, producing hydroxyl radicals.[64] These radicals can then oxidize substrates, leading to colorimetric or fluorescent changes. The catalytic performance of nanozymes is governed by a range of physicochemical parameters, such as size, shape, surface composition, and the local environment. Size is critical, as smaller nanoparticles offer a larger surface-to-volume ratio, thereby exposing a greater number of active sites. Additionally, surface engineering through coatings or the introduction of functional groups offers a powerful strategy for modulating catalytic activity and selectivity. Unlike enzymes, nanozymes can exhibit multiple enzyme-like activities, known as multi-enzyme mimicry. This versatility allows them to catalyse a wider range of reactions. The integration of MIPs can further enhance their selectivity and catalytic efficiency by creating tailored binding sites. This combination enables the development of highly sensitive and specific biosensors and catalytic systems.

This reduction is primarily attributed to the physical barrier created by the MIP layer, which can obstruct access to the nanozyme's active sites. For instance, the creation of Fe_3O_4@MIP nanozymes by coating Fe_3O_4–COOH with an MIP layer resulted in a notable decrease in the chromogenic reaction of 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of hydrogen peroxide.[54] This observation indicates that the MIP layer partially inhibited the peroxidase (POD)-like activity of Fe_3O_4–COOH, likely by physically covering the active sites and hindering substrate access. This steric hindrance is a common consequence of MIP coating, especially when the MIP layer is dense or thick, directly limiting the interaction between the substrate and the catalytic surface. Conversely, numerous studies have demonstrated that MIPs can significantly enhance the catalytic activity of nanozymes.[21,25,58,65–73] This enhancement is often attributed to the MIP's ability to selectively enrich substrate molecules in the vicinity of the nanozyme's active sites, effectively increasing the local substrate concentration and thereby accelerating the catalytic reaction.[21,65] For example, Wu *et al.* showed that molecular imprinting significantly boosted the catalytic performance and specificity of graphitic carbon nitride (g-C_3N_4) in the TMB oxidation colour reaction.[68]

Similarly, Fan *et al.* developed a gold nanoparticle (AuNP)-based mimic glucose oxidase (GOD) with enhanced glucose selectivity and catalytic activity.[66] By incorporating aminophenylboronic

acid (APBA) and heptadecafluoro-*n*-octyl bromide (PFOB), they facilitated glucose affinity and oxygen supply, respectively, while the porous MIP structure enriched the substrate near active sites, leading to a 270-fold increase in catalytic efficiency. Furthermore, Liu's group demonstrated that molecularly imprinted polymer DNAzyme (MIP-DNAzyme) exhibited significantly higher catalytic activity towards Amplex Red (AR) compared to free DNAzyme.[25] The MIP synthesized using AR as a template, not only enhanced substrate specificity but also facilitated efficient substrate binding and subsequent catalytic turnover. This selective enrichment and facilitated substrate access are key mechanisms through which MIPs can augment nanozyme catalytic activity, overcoming the inherent limitations of the nanozyme itself.

2. Application of nanozyme@MIP in sensing platforms and diagnostics

Researchers have continuously sought to improve the selectivity of nanozymes, addressing their inherent limitation of poor catalytic specificity.[74] Two primary strategies have been identified for achieving selective bioanalysis using nanozyme catalysis: integrating nanozymes with biological recognition components or designing nanozymes with intrinsic catalytic specificity.[41,48,75]

Molecular imprinting offers a simpler and more adaptable method for creating substrate recognition sites on nanozymes, overcoming the instability and cost challenges associated with biological components and the complexity of structure-mimetic designs. Nanozyme@MIP-based colorimetric assays typically rely on the catalytic oxidation of substrates like ABTS and TMB in the presence of a target analyte. In 2014, Piletsky and colleagues pioneered the molecularly imprinted nanoparticle-based assay (MINA), using Fe_3O_4 nanoMIPs to detect vancomycin (Fig. 2).[76,77] This approach, eliminating the need for antibodies, demonstrated excellent sensitivity and specificity in detecting vancomycin, leukotrienes, and insulin.[76] Liu's group further improved Fe_3O_4-NPs' catalytic activity by developing a molecularly imprinted layer, achieving a 100-fold increase in specificity.[26] These Fe_3O_4@MIP nanoparticles, exhibiting POD-like activity, have also been employed for tetracycline (TC) detection. The assay is based on TMB oxidation, which is inhibited when tetracycline binds to the imprinted cavities, resulting in a "turn-off" detection mechanism (Fig. 3).

AuNPs have also been explored as core catalysts. For example Zhang *et al.* developed a composite sensor, AuNPs@SiO_2-MIP, for the selective detection of glutathione (GSH) in serum (Fig. 4(a)).[78] In this system, sodium borohydride ($NaBH_4$) reduces gold ions to form gold nanoparticles (AuNPs) with peroxidase-like catalytic activity. These nanoparticles are anchored onto silica particles coated with MIPs, which are synthesized using GSH as a template to create specific recognition sites. The composite catalyses the oxidation of TMB in the presence of hydrogen peroxide, producing a visible colour change. However, GSH inhibits this reaction, enabling its quantification. The sensor exhibits high sensitivity, selectivity, and stability, making it

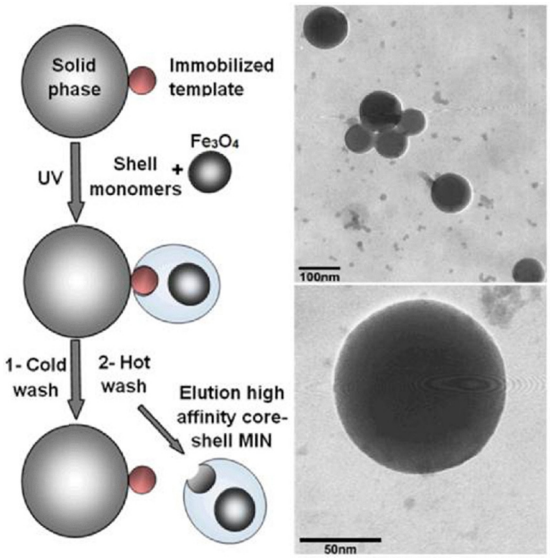

Fig. 2 Schematic representation of the solid-phase synthesis protocol integrating Fe_3O_4 for the fabrication of peroxidase-mimicking core–shell molecularly imprinted nanoparticles (MIN) (left). The TEM micrograph (right) displays the morphology and structural characteristics of the synthesized Fe_3O_4-MIN particles. Reproduced from ref. 76 with permission from The Royal Society of Chemistry, copyright 2020. Reproduced from ref. 77 with permission from Wiley-VCH, copyright 2014.

suitable for real-time clinical monitoring. Abdulsada *et al.* (2025) developed a versatile strategy for synthesizing Au@MIP nanozymes *via* localized Fenton-like polymerization (Fig. 4(b)).[45] In this approach, hydrogen peroxide decomposes on the gold nanoparticle surface to generate hydroxyl radicals, which initiate the polymerization of monomers around the gold core, forming a MIP shell. These nanozymes exhibit strong peroxidase-like activity and high substrate specificity, enabling sensitive, nanomolar-level detection of analytes such as amphetamine in complex biological samples. The method is robust, user-friendly, and well-suited for homogeneous assays and *in vitro* diagnostics.

Alternatively, Metal–organic composites like Mn-PBA-NaOH have also been used, demonstrating oxidase (OXD)-mimicking activity for TC detection (Fig. 5(a)).[79] Jing *et al.* developed an MIP/HKUST-1 composite on paper for tetrabromobisphenol A (TBBPA) detection.[25,55,57,71,73,80] This sensor couples TBBPA degradation with HKUST-1's enzyme-mimicking activity, achieving ultrasensitive detection (Fig. 5(b)).

Hsu *et al.* developed a molecularly imprinted colorimetric sensor (MICS) for morphine (MO) detection.[81] This sensor, based on MIP staining, utilizes the reduction of Fe^{3+} to Fe^{2+} by MO, forming Prussian blue. Unlike the other colorimetric methods that rely on nanozyme@MIP composites, this method utilizes the MIP component to regulate the detection mechanism, highlighting the versatility of MIPs in colorimetric sensing. Fluorescence detection, known for its higher sensitivity, offers a compelling alternative. Wang *et al.* developed a ratiometric fluorescence sensor for detecting domoic acid (DA) using NH_2-MIL-101(Fe)@MIP, an iron-based metal-organic framework (MOF) coated with a molecularly imprinted polymer (Fig. 6).[57]

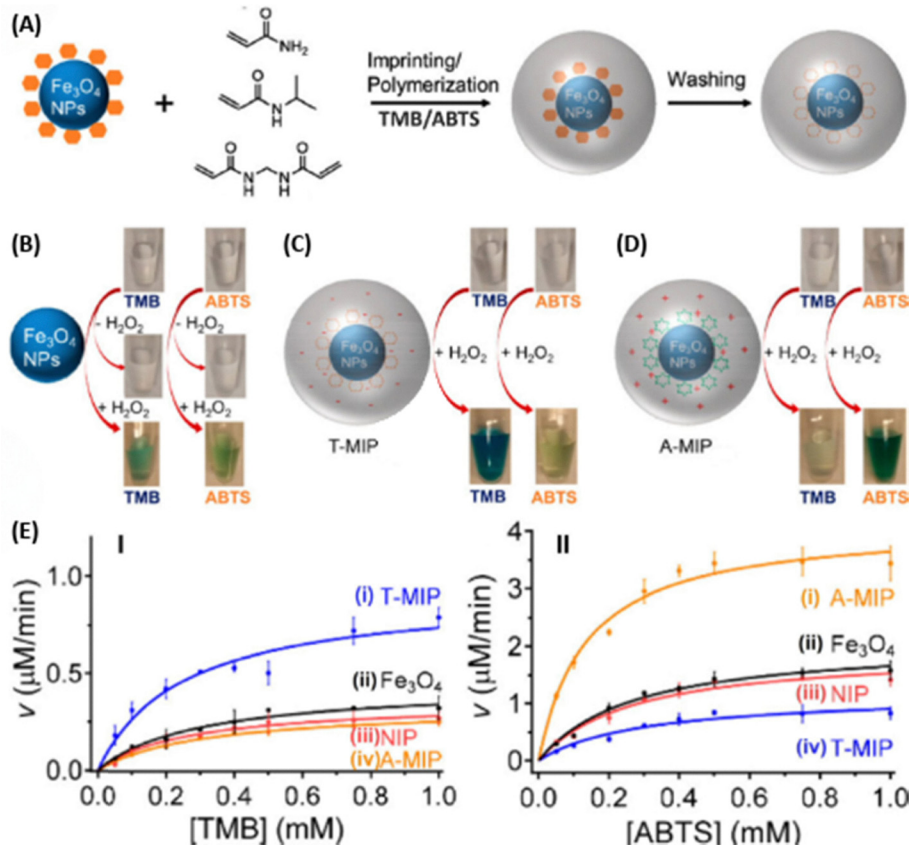

Fig. 3 (A) Schematic synthesis of MIP-polyacrylamide-coated Fe_3O_4 particles, designed for the selective and enhanced oxidation of TMB or $ABTS^{2-}$ using hydrogen peroxide (H_2O_2). Photographs illustrating the catalytic activity and specificity of (B) Fe_3O_4 nanoparticles, (C) the TMB imprinted nanogels (T-MIP), (D) the ABTS imprinted nanogels (A-MIP). Nanogels oxidize TMB and ABTS in the presence of H_2O_2. (E) This figure presents a comparative analysis of reaction rates. Panel I: shows the rate of TMB oxidation to $TMB^{+\bullet}$ by H_2O_2 using four distinct Fe_3O_4 catalysts: a TMB-imprinted polymer-coated catalyst; T-MIP (i), bare Fe_3O_4 (ii), a non-imprinted polymer-coated catalyst; NIP (iii), and an $ABTS^{2-}$-imprinted polymer-coated catalyst; A-MIP (iv). Panel II: shows the rate of $ABTS^{2-}$ oxidation to $ABTS^{-\bullet}$ by H_2O_2 using the same four catalysts, demonstrating their substrate selectivity. Reproduced from ref. 26 with permission from The American Chemical Society, copyright 2017.

The MOF exhibits peroxidase-like activity, catalyzing the oxidation of *o*-phenylenediamine (OPD) to generate a fluorescent product. This product induces an inner filter effect (IFE), which is modulated by the presence of DA. The molecular imprinting provides selective binding sites, allowing DA to influence the fluorescence intensity ratio. This dual-emission system enables sensitive, specific, and stable detection, with strong potential for applications in food safety and environmental monitoring. Quantum dots (QDs) composed of AuZnCeSeS have also been used for methamphetamine (METH) detection.[82] These QDs–MIP nanocomposites offer rapid and sensitive detection in biological samples.

Nanozyme@MIPs can also serve directly as recognition and reporting elements, leveraging LSPR. Nikhil *et al.* introduced a single nano plasmonic-based sensor platform integrated with nanoMIPs to detect multiple SARS-CoV-2 variants.[83] The study focuses on using nanostructured molecularly imprinted polymers (nanoMIPs) designed to target a specific region of the SARS-CoV-2 spike protein's receptor-binding domain (RBD), enabling the detection of Alpha, Beta, and Gamma variants. This detection method, based on LSPR associated with silver nanostructures, achieves highly sensitive detection limits. The entire assay, using blood or nasal swab samples, can be completed in under 30 min. By eliminating the need for biological binding agents, this approach circumvents a significant supply chain bottleneck in diagnostic device production.

2.1 Validation in complex real-world matrices

Nanozymes generally exhibit greater resistance to interference compared to enzymes, particularly in complex or harsh environments.[84] This advantage stems from their high structural stability, robustness against denaturing conditions, and lower susceptibility to inhibitors or nonspecific interactions that can affect enzymes.[84] While enzymes are highly specific and efficient, they often lose activity in the presence of interfering substances, extreme pH, or temperature changes. Conversely, nanozymes maintain catalytic activity across a broad range of conditions, making them more reliable for applications in biological fluids, environmental samples, and industrial processes.[84] Furthermore, nanozymes can be engineered with surface modifications or molecular imprinting to enhance selectivity and further reduce interference. Nanozymes@MIP

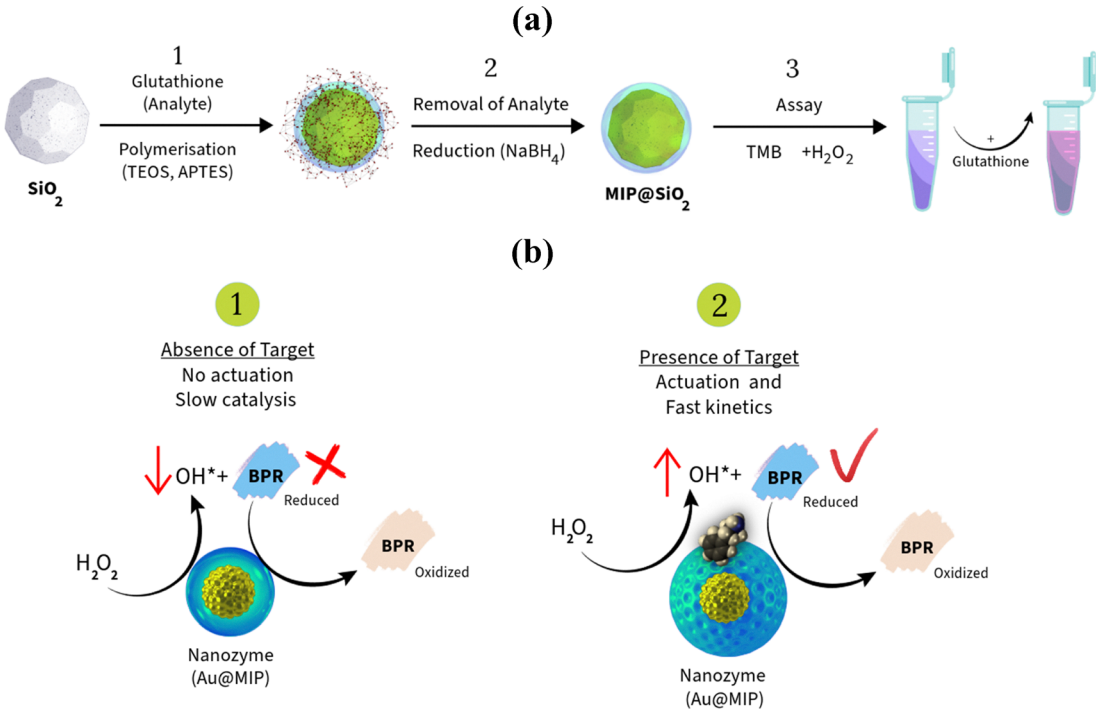

Fig. 4 (a) Illustration of the AuNPs@SiO_2-MIP composite for real-time colorimetric detection of glutathione (GSH) in serum. Reproduced from ref. 78 with permission from Springer Nature, copyright 2022. (b) Nanozyme-based colorimetric assay: (1) inactive state (no target): the nanozyme's polymeric shell inhibits radical generation, resulting in slow dye oxidation and a minimal change in absorbance. (2) Active state (target bound): target binding induces polymer swelling, exposing the nanozyme's catalytic site. This leads to rapid target oxidation, producing a detectable change in absorbance, which is used to quantify the target. Reproduced from ref. 45 with permission from The Royal Society of Chemistry, copyright 2025.

demonstrate strong performance in highly complex biological and environmental matrices, such as whole blood and wastewater, where nonspecific binding and interferents are prevalent. Integrating MIPs with nanozymes creates highly specific binding sites that significantly enhance selectivity and reduce background interference, enabling reliable detection of target analytes even at nanomolar concentrations in challenging samples.[48] Assays using Au@MIP nanozymes are robust and user-friendly, requiring minimal steps while maintaining high specificity in complex matrices.[45] As summarized in Table 1.

To further mitigate interference, additional surface modifications such as functionalizing nanozyme surfaces with specific ligands, aptamers, or other recognition elements have been employed, further improving anti-interference capability and assay robustness.[85] These strategies collectively enable Nanozymes@MIP to deliver sensitive, selective, and stable performance in real-world diagnostic and environmental applications.[86] The main challenges include enhancing specific molecular affinity in real biological fluids and complex matrices and developing customisable nanozyme@MIPs. Nevertheless, only a few examples address real biological samples, mostly in detecting small organic compounds in complex environments. Some nanozymes have demonstrated robustness in whole blood and other complex biological matrices. Magnetic nanozymes, for instance, have been used to facilitate easy sample processing, enabling efficient separation and enrichment of target analytes from whole blood, thus minimizing interference from nonspecific components.[87] Additionally, nanozymes exhibit high catalytic activity, stability, and operational robustness, making them well-suited for use in harsh environments like blood, where traditional enzymes may be less effective.[88,89] These properties, combined with surface modifications or integration with molecularly imprinted polymers, further enhance their selectivity and anti-interference capabilities, supporting reliable performance in real-world biomedical applications.[87]

Although nanozyme@MIP systems have shown promise across various analytes, most validations are limited to buffer solutions or simplified biological matrices (*e.g.*, serum). Their performance in complex environments—such as whole blood, wastewater, or food samples—remains challenging, especially with nonspecific binding and interfering substances. Demonstrating robustness under such conditions is critical for real-world applications. Incorporating antifouling strategies, such as BSA in biological samples, surface passivation, or selective coatings, could help mitigate matrix effects and improve analytical reliability. Future challenges in the field are to enhance sensitivity, selectivity, and reproducibility for industrial applications, alongside lowering costs and improving translation from laboratory to mass manufacturing.

2.2 Comparative analysis of kinetic parameters in molecularly imprinted nanozymes

The quantitative analysis of Michaelis–Menten kinetics provides critical insights into the efficacy of nanozymes@MIPs and

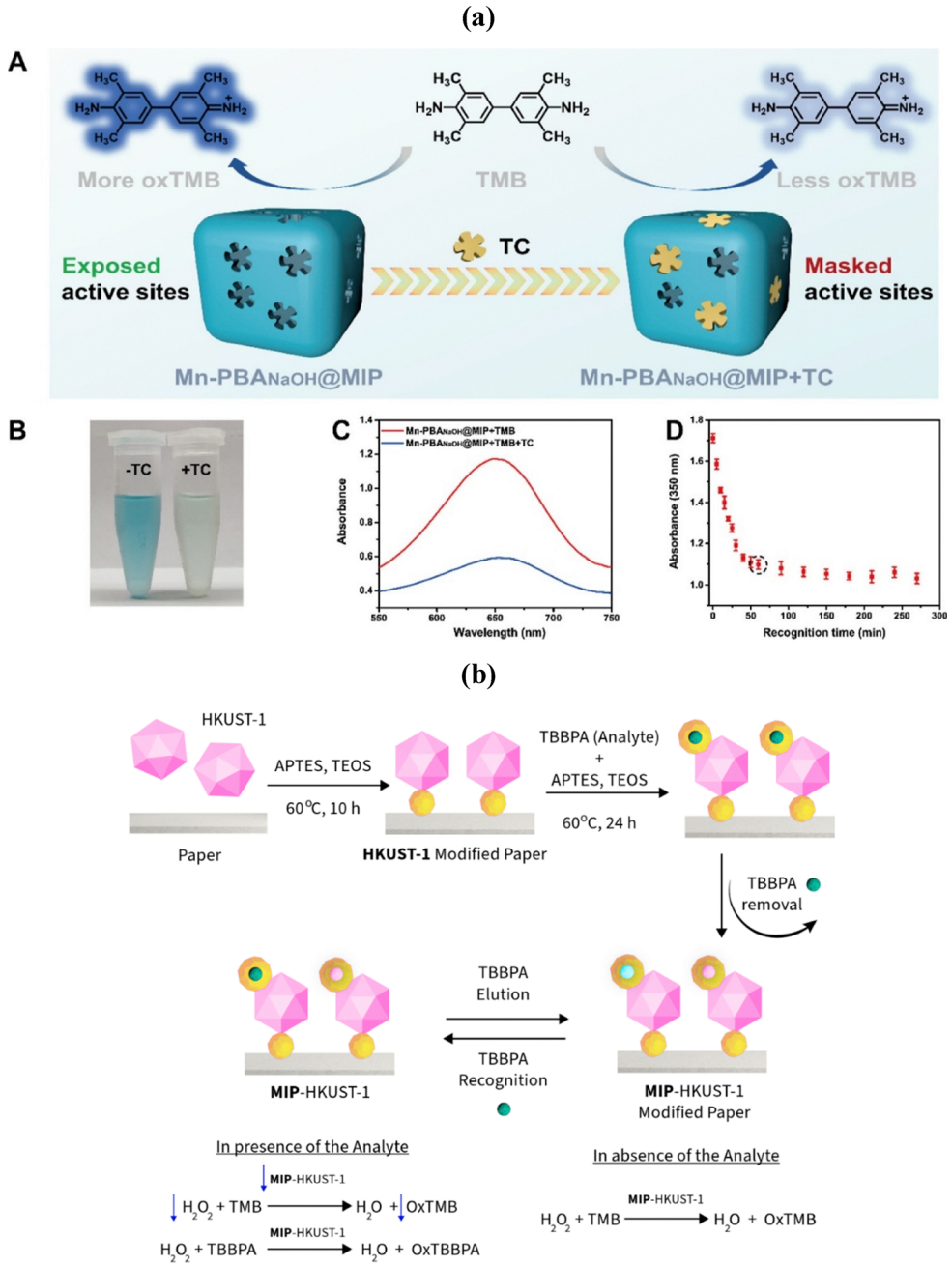

Fig. 5 (a) Colorimetric detection of tetracycline: (A) schematic depicting how TC binding to the MIP cavities hinders oxygen interaction with the Mn-PBA-NaOH core, thus inhibiting ROS generation and the subsequent TMB oxidation. Reprinted with permission from (alkali-etched imprinted Mn-based Prussian blue analogues with superior oxidase-mimetic activity and precise recognition for tetracycline colorimetric sensing), Copyright @2023, American chemical society.[79] (B) Photographs illustrating the color change of the TMB substrate in the presence and absence of TC, reflecting the inhibition of the oxidase-like activity. (C) UV-vis spectra of the reaction system under varying TC concentrations. (D) Optimization of the TC recognition time, showing the effect on the analytical signal. (b) Schematic overview of the MIP/HKUST-1 composite preparation, its mechanism of action, and its application in TBBPA detection. Reproduced from ref. 73 with permission from Springer Nature, copyright 2020.

highlights both the significant progress and the enduring challenges in the field. This discussion places nanozymes@MIPs within a broader context, comparing their performance against both conventional nanozymes and their natural counterparts in terms of catalytic efficiency and, crucially, selectivity (Table 2). A fundamental distinction between enzymes, nanozymes and nanozyme@MIP lies in their inherent selectivity. Conventional Nanozymes (*e.g.*, Fe_3O_4 NPs, CeO_2 NPs) typically possess broad and intrinsic activity. For instance, a peroxidase-mimicking nanozyme will catalyse the oxidation of a wide range of substrates (TMB, ABTS, OPD) with little discrimination. This lack of specificity is a major limitation for applications like biosensing, where cross-reactivity leads to false positives. Enzymes represent the pinnacle of selectivity, evolved to recognize

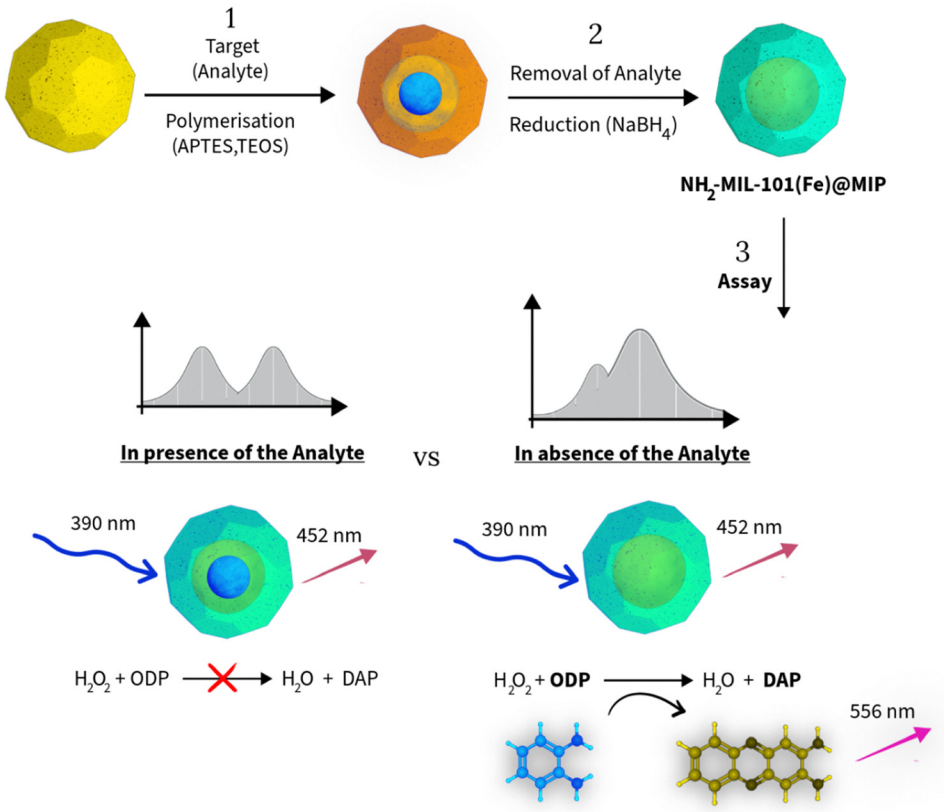

Fig. 6 Schematic illustration of NH_2-MIL-101(Fe)@MIP and the ratiometric fluorescent sensing mechanism for domoic acid (DA) detection. Reproduced from ref. 57 with permission from Elsevier, copyright 2022.

Table 1 Summary of nanozyme composites with different sensing applications

Nanozyme	Catalytic function	Analyte	Sample	LoD	Ref
AuZnCeSeS QDs-MIP	N/A	Methamphetamine	Urine	0.02 nM	82
AuNP	POD	Amphetamine	Buffer	0.17 nM	45
AuNP	Oxidase	Glutathione	Serum	1.16 μM	78
FeNP	Redox	Morphine	—	100 nM	81
Au NPs	GO_x	Glucose	Drinks, serum	—	66
PtPd-NFs	POD	H_2O_2, glucose	Human serum	5 μM	69
MIL-101 (Co, Fe) MIP	POD	Vanillin	Ice cream, candy	104 nM	90
Fe_3O_4 NPs	POD	Tetracycline	Water	400 nM	54
AgNPs@Zn-MOF	POD	Patulin	Water, juice	60 nM	56
NH_2-MIL-101(Fe) MIP	POD	Domoic acid	Lake water, shellfish	8.2 nM	57
NH_2-MIL-101(Fe) MIP	POD	Triclocarban	River water, Urine, Blood	9.8 pM	80
PtCu/PSS-Gr MIP	POD	Puerarin	Human plasma	10 μM	59
Co_3O_4 NPs	POD	Chloramphenicol	Honey, milk, chicken	0.118 pM	72
HKUST-1MIP	POD	Tetrabromobisphenol	Dust	3 pM	73
Fe_3O_4 NPs	POD	Norfloxacin	Milk	8.9 nM	91
Mn_3O_4	Oxidase	Tetracycline	Soil, water	5 nM	92
Te-CdS@Mn_3O_4	POD	2,4-Dichlorophenoxyacetic acid	Water, soil, fruit peel	0.63 nM	93
Fe_3O_4/C-dots@Ag-MOFs	POD	Parathion-methyl	Vegetables, fruits, soil	11.6 pM	94
CeO_2	POD	Glucose	Urine	1.0 μM	95
AuNPs	POD	Glutathione	Serum	1.16 μM	78
Fe_3O_4@Au NCs	POD	Glucose	Human serum	5.0 μM	96
AuNP	LSPR	Enrofloxacin	Buffer	61.1 nM	97
AgNP	LSPR	Virus		9.7 fM	83

Nanoparticles (NP), metal–organic frameworks (MOFs), graphene (Gr), peroxidase (POD), localised surface plasmon resonance (LSPR), glucose oxidase (GO_x).

a single substrate or a very narrow class of substrates with exquisite precision. Their active sites are perfectly shaped, electrostatically tuned, and often involve complex allosteric regulation. Nanozyme@MIPs are engineered to bridge this vast

Table 2 Comparison of kinetic parameters of catalytic synthetic systems and enzymes

Nanozyme@MIP systems	Enzyme-mimicking type	Substrate	K_m (μM)	V_{max} (M s^{-1})	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})	Ref.
HRP	POD	TMB (H_2O_2)	434	N.R.	N.R.	N.R.	98
Fe_3O_4 NPs	POD	TMB (H_2O_2)	154	N.R.	N.R.	N.R.	99
Fe_3O_4@MIP (control)	POD	TMB (H_2O_2)	193.3	N.R.	N.R.	N.R.	99
Fe_3O_4@SiO_2-MIP	POD	TMB (H_2O_2)	68.9	N.R.	N.R.	N.R.	26
Catalase	CAT	H_2O_2	1 100 000	N.R.	4.0×10^7	3.6×10^7	98
CeO_2 NPs	CAT	H_2O_2	39 200	N.R.	N.R.	N.R.	100
CeO_2@MIP	Oxidase	TMB (H_2O_2)	220	8.62×10^{-8}	N.R.	N.R.	48
CeO_2@NIP (Control)	Oxidase	TMB (H_2O_2)	480	6.78×10^{-8}	N.R.	N.R.	48
GO_x	GO_x	Glucose	33 000	190 U mg^{-1}	700	2.1×10^4	101
AuNP@MIP	GO_x	Glucose	17 000	0.678 U mg^{-1}	N.R.	N.R.	66
AuNP@NIP (Control)	GO_x	Glucose	23 000	0.281 U mg^{-1}	N.R.	N.R.	66

Nanoparticles (NPs), Horseradish peroxidase (HRP), peroxidase (POD), catalase (CAT), glucose oxidase (GO_x), 3,3′,5,5′-tetramethylbenzidine (TMB); units: 1 unit (U) of GO_x activity is often defined as 1 μmol of product formed per minute, V_{max} depends on assay conditions, kinetic parameters are highly dependent on experimental conditions (pH, temperature, buffer). N.R. – not reported in the source.

selectivity gap. The molecular imprinting process creates artificial binding cavities tailored to a specific target molecule (template). This is evidenced by the consistent trend of nanozyme@MIP showing significantly lower K_m values than their NIP controls, indicating higher binding affinity and specificity for the imprinted template (Table 2).

For example, the Fe_3O_4@SiO_2-MIP's affinity for TMB (K_m, 68.9 μM) is nearly three times greater than that of its non-imprinted counterpart (K_m, 193.3 μM). This demonstrates that imprinting successfully introduces a level of biomimetic recognition that is absent in conventional nanozymes, moving them closer to the selective nature of true enzymes (Table 2). Building on this, nanozyme@MIP systems have demonstrated significant progress in mimicking key enzyme activities, most notably peroxidase- and glucose oxidase-like functions, highlighting their growing potential in advanced biosensing applications. Peroxidase-like nanozyme@MIPs, such as Fe_3O_4@MIP, catalyse the oxidation of chromogenic substrates like TMB in the presence of hydrogen peroxide, directly mirroring the function of horseradish peroxidase (HRP). Here, molecular imprinting enhances substrate specificity by creating selective binding pockets, which is reflected in improved catalytic efficiency (k_{cat}/K_m) and affinity (lower K_m) compared to their non-imprinted analogues. Similarly, glucose oxidase (GO_x)-like nanozyme@MIPs mimic oxidase activity by catalysing glucose oxidation using molecular oxygen, producing gluconic acid and hydrogen peroxide without the need for exogenous H_2O_2, thereby enabling self-contained sensing platforms. The K_m for natural GO_x is often high as it operates under physiological conditions where blood glucose levels (~5.6 mM) are well below its K_m (33 mM), allowing the reaction rate to be sensitive to substrate concentration changes. Conversely, Nanozymes are often optimized for low K_m for high-sensitivity detection applications. Furthermore, k_{cat} is typically not reported for MIP-based systems because the exact number of "active sites" is difficult to quantify, unlike in nanoparticles with defined surfaces or enzymes with known molecular weights.

A comparative evaluation of kinetic parameters for these systems reveals key trends in their catalytic performance. Although direct comparisons across studies are complicated by variations in substrates, assay formats, and experimental conditions, the available data provide meaningful insights (Table 2). Nanozyme@MIPs generally exhibit enhanced affinity, often reflected in lower K_m values, due to the imprinting-induced pre-concentration of target molecules at the catalytic interface. However, a common trade-off is observed: this increased selectivity can sometimes reduce turnover rates if the imprinted cavities restrict substrate access or alter the nanozyme's surface environment. Consequently, while their k_{cat} values tend to be lower than those of enzymes such as horseradish peroxidase (HRP), their overall catalytic efficiency (k_{cat}/K_m) can be significantly improved through selective molecular recognition. This enhancement is particularly valuable in complex biological matrices, where robustness and specificity are essential. This analysis underscores a persistent performance gap when benchmarking catalytic efficiency (k_{cat}/K_m) against enzymes. This gap is the most telling metric of the difference between synthetic mimics and biological marvels.

Enzymes exhibit phenomenal catalytic efficiencies, often in the range of 10^6–10^8 M^{-1} s^{-1}, due to their perfectly evolved active sites that maximize both binding (low K_m) and rapid turnover (high k_{cat}). Nanozyme@MIPs and conventional nanozymes overwhelmingly lack reported k_{cat} and k_{cat}/K_m values (Table 2). This absence stems from a fundamental methodological challenge: the difficulty in quantifying the exact number of catalytically active sites on a heterogeneous nanoparticle surface. Without this, a true comparison of intrinsic catalytic prowess is impossible. In the literature, enzymatic activity is frequently reported as V_{max} or U mg^{-1}, with k_{cat} estimated by normalizing V_{max} to the molar concentration of the catalyst. However, V_{max} values vary widely depending on nanozyme composition and assay conditions, further complicating cross-study comparisons. These findings highlight the need for standardized assay protocols and rational design strategies to optimize both recognition and catalytic performance. The provided comparative table thus serves as both a reference and a foundation for refining assay conditions and guiding the development of next-generation biosensors.

The discussion of catalase-mimicking nanozymes illustrates that the goal of biomimicry isn't always to copy nature exactly.

The reported CeO_2@MIP system has a much higher affinity for H_2O_2 (K_m of 220 μM) than natural catalase (K_m of ~1.1 M) as shown in Table 2. This difference isn't a defect; it's a reflection of different design goals. Catalase is a high-capacity, low-affinity enzyme evolved to handle large amounts of substrate efficiently. In contrast, the CeO_2@MIP system has a lower capacity but is engineered for sensing applications. For these applications, a high affinity for lower, pathological concentrations of H_2O_2 is more desirable. This example demonstrates that nanozymes can be optimized for superior performance in specific applications, even if their fundamental kinetic parameters differ from those of their natural counterparts.

To put it simply, a three-tier hierarchy emerges, reflecting increasing levels of sophistication. Conventional nanozymes offer simple, unselective catalysis. Building on this, nanozyme@MIPs introduce a crucial layer of biomimetic selectivity and enhanced affinity *via* molecular recognition. At the top of the hierarchy, enzymes remain the gold standard, possessing an unparalleled combination of specificity and catalytic efficiency. The future of the field hinges on addressing the persistent kinetic data gap. To transition from demonstrating function to understanding performance, researchers must develop standardized methods to determine the density of active sites and report k_{cat} and k_{cat}/K_m. This will enable the rational design of next-generation nanozyme@MIP that not only recognize their substrate with high specificity but also turn it over with rates approaching enzymatic efficiency, truly closing the loop between synthetic and biological catalysis.

2.3 Synthesis of strategic trade-offs and functional redefinition

The comparison elucidates that nanozyme@MIPs are not designed to be ''better enzymes'' but represent a paradigm shift towards ''functional biomimetic materials''. ''They make strategic trade-offs, exchanging the raw catalytic power and speed of enzymes for a suite of engineered advantages''. Selectivity is the paramount advantage. While enzyme selectivity is fixed by evolution, the selectivity of a nanozyme@MIP can be designed *de novo* for any molecule of interest, including antibiotics, toxins, and biomarkers for which no enzyme exists. The Cost and Production is important to consider, purifying enzymes is complex and expensive. Nanozyme@MIPs are synthesized from abundant materials using scalable chemical methods, drastically reducing cost and enabling disposable use in point-of-care diagnostics (Table 3).

In a nutshell, nanozyme@MIP systems do not outperform enzymes in the traditional metrics of catalytic kinetics. They are, by these measures, inferior catalysts. However, this analysis confirms that this is a deliberate and productive trade-off. These systems redefine the purpose of a catalyst from being a purely kinetic machine to being an integrated detection unit. Their primary application domain is not in replacing enzymes in metabolic pathways but in enabling highly specific, robust, and low-cost chemical sensing and detection outside the ideal confines of a biological cell. They are engineered for practicality and specificity in real-world environments. The kinetic parameters validate that they possess sufficient catalytic activity to generate a strong analytical signal, while their man-made specificity and robustness make them uniquely fit for purpose in the next generation of diagnostic, environmental, and industrial monitoring technologies. They are not competitors to enzymes but are complementary tools that expand the very definition of catalysis into the realm of customizable functional materials.

2.4 Long-term stability and reusability

The stability is weakness in enzymes which are fragile and susceptible to environmental conditions, denaturing under non-physiological conditions. Nanozyme@MIPs are incredibly robust, maintaining activity across a wide range of temperatures, pH levels, and solvents, enabling their use in harsh industrial or environmental settings. Nanozyme@MIPs exhibit markedly superior long-term stability and reusability compared to biological ligands, making them highly attractive for practical applications. Quantitative studies show that Fe_3O_4@MIP nanozymes can retain over 80% of their catalytic activity after five consecutive catalytic cycles, with some MIPs maintaining performance over 100 adsorption–regeneration cycles before significant degradation.[102] Shelf-life is also enhanced, as Nanozymes@MIP remain stable at high temperatures and across a wide pH range, conditions that typically denature enzymes.[103] In terms of shelf-life, Au@MIP nanozymes stored at 4–5 °C in

Table 3 Key trade-offs between enzymes and synthetic MIP-based nanozymes

Parameter	Enzymes	Nanozyme@MIP	Implication for nanozymes
Catalytic efficiency (k_{cat}/K_m)	Extremely high (10^3–10^8 M^{-1} s^{-1})	Moderate to low (10^0– 10^3 M^{-1} s^{-1})	Less efficient, but often ''good enough'' for many applications.
Affinity (K_m)	Very high (low μM to mM range)	Often weaker (high mM range for catalytic substrates)	Lower affinity for the reaction substrate, but high specificity for the target analyte.
Turnover (k_{cat})	Extremely fast (10^2–10^7 s^{-1})	Slower (10^{-3}–10^2 s^{-1})	Slower reaction rates, offset by using higher catalyst loads.
Selectivity	Exquisite (pre-defined by evolution)	Engineered (tuneable *via* MIP template)	Key advantage. selectivity can be designed for non-biological targets (antibiotics, toxins).
Stability	Low (denatures easily, limited pH/temp)	Very high (Robust, reusable, wide pH/temp)	Major advantage. Enables applications in harsh environments.
Cost and production	High (purification from biological sources)	Low (scalable chemical synthesis)	Major advantage. Enables mass production and disposable sensors.

buffer solution exhibited less than 7% loss in activity after 90 days and only about 13% after 180 days, after which activity stabilized, demonstrating excellent long-term stability.[45] By contrast, enzymes often require strict storage conditions and rapidly lose activity outside of optimal environments.[41] Strategies for further stabilization include lyophilization, use of preservatives, and optimizing the MIP shell composition to prevent aggregation and biological contamination. These data highlight the robust, reusable, and durable nature of nanozyme@MIPs, supporting their use in demanding analytical and sensing applications. Strategies to further improve stability and reusability include optimizing crosslinkers and functional monomers in the MIP layer, as well as employing gentle regeneration protocols to minimize irreversible structural changes.[45,48] These features enable nanozyme@MIPs to be reused multiple times with minimal loss in activity, supporting their deployment in continuous or repeated assay formats.[103] Nevertheless, most diagnostic sensing technologies used in chemical analysers for blood and urine analysis such as microplate assays, electrochemical sensors, and optical-based systems rely on disposable devices for hygiene and practical handling. Therefore, industrial requirements prioritize assays that offer high accuracy, excellent reproducibility, and long shelf-life under ambient conditions, eliminating the need for cold chain storage. Ensuring stability at room temperature is critical for widespread adoption, particularly in decentralized or resource-limited settings.

2.5 Biocompatibility and toxicity considerations

The applicability of nanozymes@MIP is highly dependent on their toxicity profile. Their characteristically low toxicity and high biocompatibility make them exceptionally suitable for *in vitro* diagnostics and sensing. Conversely, their use in *in vivo* biomedical or environmental applications demands rigorous assessment of their cytotoxic potential, especially when metal-based components are involved. Metal-based nanoparticles can induce toxicity *via* oxidative stress, inflammation, and apoptosis, with effects depending on composition, size, surface charge, and coatings.[104,105] For example, *in vivo* experiments with nanoMIPs demonstrated that, while these particles can cross biological barriers and distribute to various organs, higher doses may cause tissue-specific toxicity, such as neuronal damage or necrosis.[106] To mitigate these risks, strategies such as biocompatible polymer coatings, green synthesis methods, and the use of biodegradable materials are actively explored to reduce toxicity and enhance clearance from the body.[106] Establishing design criteria such as minimizing metal content, optimizing particle size, and employing non-immunogenic, degradable MIP shells is essential for safe *in vivo* or clinical use. Comprehensive toxicity and biodistribution studies remain necessary to fully assess long-term safety and guide the development of Nanozymes@MIP for clinical translation.[104,105]

2.6 Direct comparison with established technologies

While limited information directly compares nanozyme@MIPs with established technologies, given the extensive developments in nanoMIPs and nanozymes separately, their combination holds significant promise. Nevertheless, it has been reported that nanozyme@MIPs offer significant advantages over established technologies such as antibody-based ELISA kits, particularly in terms of stability, cost, and operational robustness. For Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) detection, nanoMIP-based sensors have demonstrated a limit of detection (LOD) as low as 3.9 fg mL^{-1} for the receptor-binding domain (RBD), which is approximately 20 times lower than the limit of detection of antibody-based sensors (85.5 fg mL^{-1}).[107] Additionally, the nanoMIP sensor achieves results in about 15 min, matching or surpassing the speed of commercial rapid antigen tests and significantly faster than traditional ELISA assays, which often require several hours.[107] In terms of specificity, nanozyme@MIP sensors show minimal cross-reactivity with common interferents in clinical samples, ensuring high diagnostic accuracy. However, direct comparisons with established diagnostic platforms remain limited. The positioning of nanozyme@MIP systems as competitive alternatives for SARS-CoV-2 detection necessitates direct, quantitative benchmarking against established commercial ELISA kits to validate claims of superior performance across critical metrics: sensitivity, specificity, and assay time.

Furthermore, nanozyme@MIPs are highly stable, retaining performance after months of storage at 4–5 °C, and are less sensitive to temperature and pH extremes than protein-based.[48] These features, combined with low production costs and ease of mass manufacturing, position nanozyme@MIPs as a compelling alternative to conventional immunoassays for sensitive, rapid, and robust diagnostics. Nanozyme@MIPs outperform traditional biological ligands (*e.g.*, antibodies) in terms of stability and cost. In contrast to biomolecules, MIPs are stable at low and high pH, pressure, and temperature (<180 °C), are less expensive than antibodies, are easier to obtain, and can be synthesized for a wide range of substances.[52] MIP nanoparticles have higher surface area-to-volume ratios, improving accessibility of imprinted cavities and binding kinetics, making them well-suited for surface imprinting strategies and *in vitro* assays.[49,108] The primary driving forces for substituting antibodies and enzymes with nanozyme@MIPs in assays are the high stability of the polymers and their low cost. Typically, the lifetime of antibodies is limited to 6–12 months, often requiring refrigerated storage, and antibody/enzyme-based devices usually cannot be regenerated for more than about 10 cycles.[51]

In contrast, nanozyme@MIPs can be stored at ambient temperature for years without noticeable loss in affinity and can be autoclaved and regenerated many times using strongly acidic or basic wash steps or organic solvents, making them very attractive for robust sensors and assays. MIPs are also two orders of magnitude cheaper than antibodies, with commercially available MIPs for various targets ranging from \$0.1–0.5 mg^{-1}, compared to antibodies typically costing \$100–\$1000 mg^{-1}. Even relatively inexpensive immunoaffinity cartridges with low antibody density are still significantly more expensive than MIPs, costing between \$10 to \$100 mg^{-1}.[51] The main challenges associated with nanozyme@MIP technology are adequate storage and test kit deployment, which often require specific pH, buffer, and

temperature conditions, and potential sensitivity to overload or poisoning with certain analytes, limiting the nanozyme lifecycle, as well as adequate calibration in biological samples.

2.7 Scalability and manufacturing challenges

Molecular imprinting technology and nanozymes have individually demonstrated strong commercial potential in fields such as environmental analysis, cell separation, and targeted theragnostic.[109] Notable commercial and academic developments highlight the practical application of molecular imprinting and nanozyme technologies. The SupelMIP SPE Cartridges product line, offered by Sigma-Aldrich (now part of Merck), was originally developed by MIP Technologies AB, these materials are designed for selective analyte extraction in complex matrices. Their design is based on early MIP research involving sol–gel and radical polymerization techniques for creating highly cross-linked polymers with specific binding sites.[110] In parallel, magnetic nanozyme reagent kits—such as those developed by Yan's team—combine magnetic nanoparticles with enzyme-mimicking catalytic activity and molecular recognition layers, including MIPs.[40] The integration of catalytic and selective binding functions makes these systems particularly suitable for biosensing and analytical applications. Foundational work by Ma *et al.* and Gao *et al.* has been instrumental in demonstrating this potential, describing the synthesis and application of such magnetic nanozyme composites.[99,111]

A few companies have begun translating this research into commercial products. Firms such as Creative Diagnostics, Abcam, Nanozyme Co., Ltd, and other specialized biotech companies offer magnetic nanozyme reagents. These products typically consist of functionalized magnetic nanoparticles with enzyme-like activity, designed for use in diagnostic assays, biosensing platforms, and biochemical analysis. However, the integration of these two technologies into a single platform molecularly imprinted nanozymes is still in its early stages, particularly in industrial and medical applications.[3] Key challenges include scalability, standardization, and competition with well-established enzymes and catalysts, which benefit from mature production processes and proven performance.[112] To enable broader adoption, it is essential to develop simple, efficient, and scalable manufacturing methods. For molecularly imprinted nanozymes to gain market traction, they must not only match but exceed the performance and cost-effectiveness of existing solutions. Despite these initial hurdles, continued scientific innovation and growing market interest are expected to drive progress and support their eventual integration into mainstream applications. Despite advances in synthesis methods such as localized Fenton-like polymerization and solid-phase synthesis, scaling up the mass production of nanozyme@MIPs remains a significant challenge.

While these approaches can yield uniform core@shell nanoparticles with high specificity and catalytic activity, several bottlenecks impede large-scale manufacturing. Notably, batch-to-batch variability in particle size and shell thickness can affect both catalytic performance and selectivity, as evidenced by dynamic light scattering and electron microscopy analyses.[45] Efficient template removal from the MIP shell presents another critical challenge, as incomplete removal can diminish binding site accessibility and reproducibility.[48,113] Furthermore, achieving consistent polymerization around nanoparticles, particularly larger cores, necessitates precise control of reaction conditions to prevent aggregation and ensure uniformity.[45] Although rapid, green synthesis methods like microwave-assisted polymerization offer improved yields and reduced heterogeneity, further optimization is required to fully align these techniques with industrial-scale production requirements. Addressing these issues through standardized protocols and automation will be essential for the widespread commercial adoption of nanozyme@MIP.

The successful design and manufacturing of nanozyme@MIP necessitate careful consideration of materials, cost-effectiveness, and mass fabrication-compatible methods. A key objective is to enhance the large-scale synthesis of nanozymes, focusing on reproducibility, stability, and shelf-life, alongside thorough comparison or validation with existing methods such as immunoassays and microplate technology for biological sample detection. A central goal for the field is to establish robust designs and scalable production processes suitable for industrial applications, readily adaptable for detecting diverse compounds in complex matrices. Simplifying their fabrication while ensuring compatibility with mass manufacturing and green chemistry principles is crucial. However, the broader application of this technology is currently constrained by challenges related to large-scale production and synthesis optimization. Recent advancements in MIP nanoparticles may offer solutions to several performance and application-related issues.[52]

Current synthetic progress in MIPs meets the quality demands for industrial applications, with solid-phase imprinting serving as a prime example, revolutionizing nanoMIPs production.[114,115] This method enables automation and large-scale nanoMIPs fabrication, significantly enhancing the reproducibility and efficiency of nanoMIPs synthesis.[116] Despite their potential for point-of-care applications, the scalability of nanozyme@MIP fabrication is not extensively discussed. The compatibility of current synthesis methods—such as localized Fenton-like polymerization—with large-scale production remains a key question. Critical bottlenecks, including batch-to-batch variability, template removal efficiency, and reproducibility, must be addressed. Discussing strategies to overcome these challenges would pave a clearer path toward commercialization and widespread adoption.

The existence of commercial particle technology for DNA sequencing and RNA recognition contrasts with the absence of commercial nanozyme@MIP sensing devices, potentially indicating challenges in large-scale production and integrating these components into test kits. Nanozymes@MIP technology may find niche applications where biological receptors are unavailable or lack sufficient stability. Moreover, nanozyme@MIPs can serve as mimics for expensive or difficult-to-obtain bioreceptors and biocomponents. Consequently, MIP sensors can complement established conventional technologies in clinical tests and assays (*e.g.*, HPLC, spectroscopy, immunoassays),

for instance, in quality control for food and beverage screening of pesticides and antibiotics. Alternatively, nanozymes could significantly impact targeted applications where the capital investment for conventional analytical instrumentation is prohibitive, and users seek low-maintenance solutions, such as in environmental sensing of toxic compounds.

For example, nanozymes@MIP could substantially simplify laboratory protocol testing, saving time and resources, particularly in biomedicine. Another potential niche for MIPs is drug screening and clinical testing, potentially revolutionizing drug testing in pharmaceutical industries by reducing time and cost. Assuming ideal application scenarios, attractive pricing could be achieved even in cost-sensitive markets like *in vitro* diagnostics. Nanozymes@MIP can compete in markets for drug delivery, therapeutic drug monitoring, drugs-of-abuse screening, artificial recognition, and *in vivo* applications (*e.g.*, core–shell MIPs have been used as direct replacements for natural antibodies and cell imaging). Addressing these clinical and medical sensing applications requires collaboration with companies, medical professionals, and patients to meet real-world needs, and the performance and reproducibility of MIPs must be validated for mass production. Nevertheless, nanozyme@MIP technology still has a considerable way to go to meet industrial requirements for robustness, reliability, accuracy, reproducibility, long-term stability, and ease of portability. To gain a market advantage, nanozyme@MIP technology should ideally be disposable and portable, making it preferable to sensitive laboratory-based techniques.

Although MIP and nanozyme@MIP sensors hold great promise, most are not yet suitable for practical deployment. Currently, this technology remains at a low technology readiness level (TRL), typically ranging from early-stage research (TRL 1) to functional prototypes tested in laboratory settings (TRL 6). Bridging the gap between prototypes and practical applications requires substantial effort. The technology must progress to field testing (TRL 7), demonstrate compliance with end-user requirements and operational standards (TRL 8), and ultimately achieve successful implementation and commercialization (TRL 9). Advancing to higher TRLs demands that researchers tackle several key challenges. Long-term stability must be thoroughly assessed, including the effects of aging, signal drift, and shelf-life. In parallel, improvements in repeatability, accuracy, and calibration procedures are essential—alongside adherence to relevant industry standards.[3,117]

Furthermore, regulatory frameworks must be proactively integrated into the sensor design phase, as requirements are highly application specific. The successful commercialization of nanozyme@MIP technology will hinge on establishing robust standards through the direct input of stakeholders and end-users, necessitating coordinated collaboration among these groups. Although nanozyme@MIP have progressed beyond proof-of-concept, key challenges remain in identifying viable market applications, establishing standardized protocols, enhancing device robustness, and scaling up manufacturing. Translating scientific advances into practical applications is inherently complex and incremental. While the technology has matured in the laboratory, its transition into affordable, reliable diagnostic devices for medical use depends on systematically addressing these persistent technical and production hurdles. In summary, recent advancements in MIP research are positioned to enable a new generation of analytical sensing devices with the potential for transformative applications across diagnostics.

3. Concluding remarks and future perspectives

This review highlights significant advances in integrating MIPs with nanozymes to form versatile nanozyme@MIP hybrids that synergistically enhance catalytic performance and selectivity. By combining MIPs' precise molecular recognition and structural stability with the robust catalytic activity of nanozymes, these hybrids provide tailored binding sites that improve substrate specificity and, in some cases, boost catalytic efficiency. This dual functionality addresses the long-standing issue of low specificity in traditional nanozymes, enabling fine-tuned performance for targeted sensing applications. Despite impressive progress, challenges remain. Optimizing the balance between catalytic activity and selectivity requires refined fabrication methods, especially control over imprint cavity size and uniformity. Enhancing stability and biocompatibility is particularly critical for *in vivo* and point-of-care diagnostics.

Although direct comparisons of diagnostic platforms based on Nanozymes@MIP are complicated by variations in substrates, assay formats, and experimental conditions, the available data still yield meaningful insights. To translate this technology into practical applications, there is a critical need for standardized assay protocols and rational design strategies. In this context, comparative studies benchmarking Nanozymes@MIPs against gold-standard methods serve as an essential reference for refining assay conditions and guiding the development of next-generation biosensors.

Moreover, standardizing design strategies and scaling up production while maintaining batch consistency are essential for practical deployment. Nanozyme@MIPs exhibit superior durability and reusability compared to enzymes and antibodies, maintaining activity over multiple cycles and prolonged storage. Their cost-effectiveness, rapid response, and compatibility with complex matrices make them a viable alternative to conventional biosensors. Emerging green synthesis approaches and solid-phase imprinting techniques further improve their practicality. Overall, nanozyme@MIP technology stands poised to become a cornerstone of next-generation analytical platforms with broad applications in diagnostics, environmental monitoring, and drug screening. Continued innovation will drive commercialization and expand their impact on healthcare and environmental quality worldwide.

Conflicts of interest

S. P. and E.P. hold shares in Tozaro, which represents a potential financial conflict of interest. A.G.C. has a financial

relationship with Gamanity Health and Cosmetics, constituting a conflict of interest.

Data availability

This review article does not report any original experimental data. All data discussed and analyzed are derived from previously published studies, which are appropriately cited throughout the manuscript. No new datasets were generated or analyzed during the preparation of this review.

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