



Towards engineering molecularly imprinted polymer-based sensors: linking design strategies to analytical and operational performance

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Abstract

The use of molecularly imprinted polymers (MIPs) as synthetic receptors in sensing has expanded rapidly across diagnostic, environmental, food, and pharmaceutical applications, owing to their ability to mimic biological recognition while offering chemical robustness and cost-effective synthesis. However, the analytical performance of MIP-based sensors is not determined by molecular recognition alone, but by how effectively the imprinted material is engineered and integrated within the sensing interface. Against this background, this review provides a performance-oriented analysis of strategies developed to improve MIP-based sensors by correlating specific material and device-design approaches with measurable sensing outcomes. After outlining the analytical and operational criteria used to evaluate sensor performance, the review examines how different engineering strategies address distinct performance-limiting factors, including restricted analyte accessibility, slow mass transport, binding-site heterogeneity, weak signal generation, and limited regeneration. Nanoscale and ultrathin MIP formats are discussed as approaches to improve binding-site accessibility and response dynamics, while oriented imprinting, template engineering, and solid-phase synthesis are examined as routes to enhance binding-site homogeneity and recognition reproducibility. Stimuli-responsive materials are considered for their ability to introduce active functionality into the recognition layer, enabling signal amplification, reversible target binding, and interface regeneration. By linking design strategies to sensor-level performance benefits and remaining operational limitations, this review provides a framework for evaluating how MIP engineering can contribute to more sensitive, reproducible, and functionally robust sensing platforms.

Keywords Molecularly imprinted polymers · Chemosensors · Sensors · Molecular recognition · Stimuli-responsive · Signal amplification

Introduction

Molecularly imprinted polymers (MIPs) have emerged as one of the most versatile and promising classes of synthetic recognition materials for sensor development. The concept of modern molecular imprinting originated in the early 1970s, while the application of MIP materials to sensing started only two decades later, with the first examples of MIP-based sensors in the early 1990s [1, 2]. Among these, one of the earliest examples reported was of an electrochemical MIP-based sensor, which showed that a highly

substrate-selective MIP polymeric membrane could act as a synthetic receptor capable of detecting low-molecular-weight organic substrates, including adenosine monophosphate (AMP), L-phenylalanine, atrazine, and cholesterol [3]. From that point onward, the use of MIPs as synthetic receptors in sensing has been expanding exponentially, driven by MIP's ability to mimic biological recognition processes while offering significant advantages such as chemical robustness, operational stability, and cost-effective synthesis. As a result, MIPs have provided a solid foundation for the design of sensors applied to medical diagnostics, environmental monitoring, food safety, and pharmaceutical analysis, as reported in several comprehensive reviews on the topic [4–8].

This sustained interest is further reflected in the continuous growth of scientific publications in the field of MIP-based sensors. In particular, a Scopus analysis covering the period 2015–2025 retrieved 3850 original articles (research

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“molecularly imprinted polymer* AND sensor*), highlighting a marked and steady increase in research activity over the last decade. Specifically, the annual publication output increased from below 200 articles in 2015 to more than 600 in 2025, indicating a strong upward trend in the development of MIP-based sensing technologies.

Despite this rapid growth and widespread academic interest, the translation of MIP-based sensors from laboratory-scale demonstrations to commercial and real-world applications remains limited [9]. Only a restricted number of MIP-based sensors have reached practical deployment, highlighting a persistent gap between fundamental research and technological implementation. This discrepancy between extensive scientific activity and limited commercial translation does not necessarily reflect a lack of potential of MIP-based sensors; rather, it points to a combination of material-related, device-related, and technological barriers that still hinder their broader adoption. The challenges range from the intrinsic complexity of controlling the molecular imprinting process to issues associated with sensor integration, large-scale manufacturing, and the replacement of well-established biological recognition elements, such as antibodies and enzymes, which remain the gold standard in many analytical and clinical contexts.

Understanding the barriers that currently limit the translation of MIP-based sensors from laboratory research to practical applications is, therefore, essential not to question the validity of MIPs as recognition materials, but to identify the key aspects that must be addressed to fully unlock their potential in real-world sensing applications. In this context, a more in-depth analysis of the main bottlenecks limiting practical implementation is required.

Intrinsic limitations of MIP material itself, including the quality and uniformity of binding sites, the limited control over molecular recognition processes, issues related to template removal, and the reproducibility, are among the challenges to surpass for an effective translation. At the same time, additional constraints emerge at the sensor level, such as challenges associated with the integration of MIPs within transduction platforms, suboptimal signal generation due to the limited ability of the system to convert binding events into sufficiently strong and quantifiable transduction signals, and reduced performance in complex samples, all of which ultimately affect the analytical performance of the sensor.

In response to these limitations, a wide range of strategies has been developed over the past decade to improve the analytical performance of MIP-based sensors. These approaches span multiple levels of design, encompassing both material-level engineering of the imprinted polymer and device-level optimization of the sensing architecture. Among them, particular attention has been devoted to the development of nanoscale MIPs and thin polymeric layers to enhance analyte accessibility and mass transport; the engineering of

more homogeneous and oriented binding sites to improve uniformity and reliability of molecular recognition; and the integration of signal amplification mechanisms through nanostructured and/or stimuli-responsive materials. In parallel, additional strategies have been explored to address specific challenges related to sensor implementation, including the optimization of MIP-transducer interfaces and the development of antifouling approaches to improve performance in complex sample matrices [10, 11].

In this context, the present review aims to provide a critical and performance-oriented analysis of the main strategies developed to improve MIP-based sensor performance. Rather than presenting an overview centered on the synthetic methodologies for MIP preparation, particular emphasis is placed on establishing a direct correlation between intrinsic material limitations, sensor design strategies, and their impact on analytical performance. Specifically, the review discusses how approaches such as nanoscale engineering, binding-site optimization, stimuli-responsive materials, and signal amplification mechanisms contribute to improvements in sensitivity, selectivity, response dynamics, and operational stability. Through this perspective, the review seeks to identify the most effective approaches for overcoming current limitations and to outline the key directions required to accelerate the translation of MIP-based sensors from laboratory research to practical applications.

Sensor's metrics

The strategies discussed throughout this review are ultimately intended to improve sensor performance. Their effectiveness can therefore be assessed through a set of analytical metrics that describe different aspects of sensing behavior. For clarity, these metrics are grouped here into three main categories: sensitivity-related metrics, recognition-related metrics, and operational metrics.

Sensitivity-related metrics describe the sensor's ability to detect and quantify analytes across a given concentration range. The broader descriptor is the dynamic range, which defines the overall concentration interval over which the sensor generates a measurable response, and it extends from the lowest concentration distinguishable from background noise to the point of saturation, where additional analyte no longer produces an increase in signal intensity. Within this interval lies the linear range, where the sensor response is directly proportional to the analyte concentration, enabling accurate and quantitative analysis [12]. The sensitivity of the sensor is then defined as the ability to generate a measurable signal in response to small changes in analyte concentration and corresponds to the slope of the calibration curve within the linear response region. At low analyte concentrations, the sensitivity becomes

particularly critical since it determines the ability to resolve small signal variations where the signal-to-noise ratio is minimal. The sensitivity at low concentration (S_{low}) can be expressed:

$$S_{low} = \frac{|y_{final} - y_{start}|}{K_{app}}$$

where y_{final} and y_{start} are the sensor responses at the final and initial analyte concentrations [13].

Consequently, the practical usefulness of a sensor is not determined solely by the magnitude of its response, but also by the ability to reliably detect and quantify trace amounts of analyte. Within this context, the limit of detection (LOD) and the limit of quantification (LOQ) represent two of the most widely used figures of merit that describe the lowest concentrations that can be reliably detected and quantitatively determined, respectively [14]. In particular, LOD is defined as the lowest analyte concentration that can be distinguished from background noise, typically calculated as three times the standard deviation of the blank divided by the sensitivity at low detection:

$$LOD = \frac{3 \cdot St.Dev_{blank}}{S_{low}}$$

LOQ represents the lowest analyte concentration that can be quantitatively determined with acceptable precision and accuracy. It is commonly estimated as ten times the standard deviation of the blank divided by the sensitivity at low detection:

$$LOQ = \frac{10 \cdot St.Dev_{blank}}{S_{low}}$$

Recognition-related metrics provide information on the quality and strength of the interactions established between the target analyte and the sensor's receptor. Among the most commonly reported descriptors, the half-maximal effective concentration (EC_{50}) corresponds to the analyte concentration that produces 50% of the maximum measurable signal. In systems operating under equilibrium conditions and linear conditions, EC_{50} typically approximates the dissociation constant (K_D), which represents the equilibrium concentration of analyte at which half of the available binding sites are occupied, thus providing a thermodynamic descriptor of the binding affinity. The K_D is often used together with its reciprocal, referred to as the apparent affinity constant (K_{aff}):

$$K_{aff} = \frac{1}{K_D}$$

Although the terms “affinity and dissociation constant” are commonly employed in the biosensing literature, they

should be regarded as a quantitative indicator of binding strength rather than a true thermodynamic constant in the strict sense [15]. Another key descriptor of molecular recognition is selectivity, which reflects the sensor's capability to discriminate the target analyte in the presence of potential interferents and can be further quantified in terms of cross-reactivity toward structurally similar compounds.

Operational metrics describe the practical usability and robustness of the sensing platform include the response time which corresponds to the time required to reach a stable analytical signal following analyte exposure; the consistency of sensor performance, which is commonly evaluated through repeatability and reproducibility and refers to the agreement between measurements performed under identical experimental conditions and to the ability to obtain comparable results across different sensors, operators, laboratories, or measurement days, respectively; long-term operation which is typically assessed based on stability, operational lifetime, and regeneration capability. In particular, stability describes the sensor's ability to maintain its analytical performance during storage and use, whereas lifetime refers to the period over which acceptable performance can be maintained, while regeneration capability represents an additional parameter of particular relevance, as it reflects the possibility of removing bound analytes and restoring recognition activity without significant deterioration of the sensor.

Strategies to enhance MIP-based sensor performance

Playing with MIP formats

The earliest examples of MIP-based sensors were predominantly based on bulk or macroscale imprinted polymers, in which the recognition sites were distributed throughout highly crosslinked polymeric matrices. Although these systems successfully demonstrated the feasibility of synthetic molecular recognition for sensing applications, their analytical performance was often limited by intrinsic structural constraints arising from the bulk imprinting process. In particular, a significant fraction of the imprinted cavities remained buried within the polymer network, resulting in restricted analyte accessibility, slow diffusion kinetics, and long equilibration times. These effects become particularly critical in sensing applications, where rapid and efficient target recognition is essential to achieve fast and reproducible analytical responses. As an example, MIPs were synthesized in bulk format to recognize the herbicide 2, 4-dichlorophenoxyacetic (2,4-D), then ground into micrometer-sized particles and employed in a competitive binding assay for selective 2,4-D binding, showing equilibrium in about 2 h of incubation. Although such equilibration time was acceptable

for batch assay applications, it highlights the mass-transfer limitations that can hinder the implementation of bulk MIPs in sensing platforms, which in contrast require rapid response and real-time analysis [16].

The transition from bulk imprinted materials to nanoscale architectures, including nanoparticles (NPs), nanolayers, and ultrathin films, therefore represented a major step forward in the evolution of MIP-based sensors. By reducing the dimensions of the imprinted matrix to the nanometer scale, recognition sites will preferentially localize at or near the surface of the material. As a consequence, nanoscale MIPs generally exhibit faster binding kinetics, reduced diffusion barriers, and significantly shorter response times, often reaching equilibrium within a few minutes.

As an example, the practical impact of reducing the dimension of the MIPs can be appreciated in a fiber-optic fluorescence sensor based on MIP microstructures photopolymerized in situ at the tip of an optical fiber. To improve mass transport and accelerate analyte recognition, the authors further engineered a core-shell configuration consisting of a core coated with a thin imprinted shell. While the original fully imprinted microstructure required approximately 45 min to reach equilibrium, reducing the thickness of the imprinted layer dramatically accelerated the binding process, enabling detectable responses within 2 min and equilibrium within approximately 10 min, with nearly 80% of the total signal developed during the first 4 min (Fig. 1) [17].

The miniaturization of MIPs for sensing applications has been mainly achieved through two main architectures: surface-confined nanolayers or ultrathin films and molecularly imprinted nanoparticles (nanoMIPs). Although both strategies aim to reduce diffusion limitations and improve analyte accessibility, they differ substantially in their structural organization and mode of integration within sensing platforms. Ultrathin MIP films and nanolayers

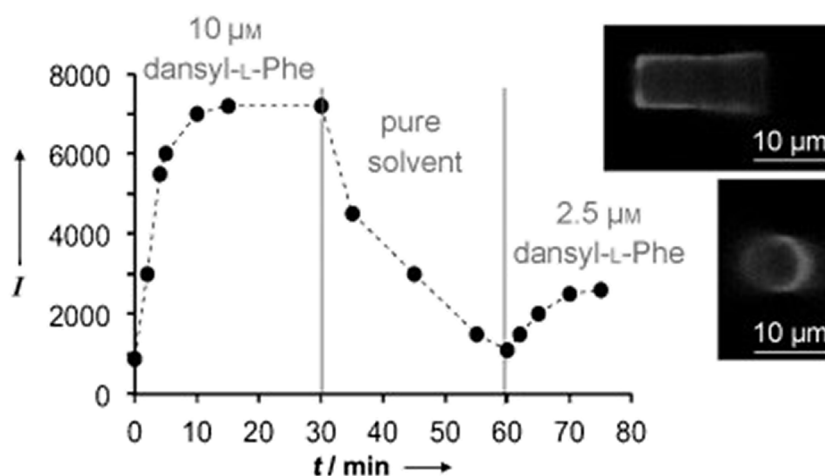
are typically prepared directly onto the transducer surface, most commonly by electropolymerization, which enables precise control over film thickness (4–20 nm) and morphology while simultaneously integrating the recognition layer with the sensing interface [18].

The advantages of this architecture are well illustrated by a tramadol-imprinted electrochemical sensor based on a thin electropolymerized polypyrrole (PPy)/sol-gel film deposited onto a functionalized multiwall carbon nanotube (f-MWCNT)-modified glassy carbon electrode. Owing to the reduced thickness and porous structure of the imprinted layer, binding equilibrium was reached within only 300 s of incubation, demonstrating how surface-confined MIP nanolayers can substantially accelerate analyte transport [19].

The alternative miniaturization strategy is represented by nanoMIPs, which are synthesized as discrete colloidal recognition elements that can subsequently be immobilized onto a transducer surface through a variety of coupling strategies, including physical adsorption, entrapment within polymeric matrices, or covalent attachment. This decouples the synthesis of the recognition element from sensor fabrication, allowing the nanoparticles to be characterized and selected prior to their integration within the sensing platform.

The impact of MIP miniaturization on recognition dynamics was demonstrated in a surface plasmon resonance (SPR) sensor developed for the detection of the peptide hormone hepcidin-25 using imprinted nanoMIPs with diameters of 20–50 nm [20]. Real-time monitoring of the binding process showed a rapid association phase, with equilibrium reached after only 3 min of analyte exposure (Fig. 2). The authors attributed this fast adsorption kinetics to the high accessibility of the binding sites within the nanoMIPs compared with conventional bulk-imprinted materials.

Fig. 1 Kinetics of the fluorescence response of the core-shell carbobenzyloxy-L-phenylalanine (Z-L-Phe) MIP tip incubated with dansyl-L-phenylalanine (dansyl-L-Phe). Reproduced and modified from ref. [17] with permission



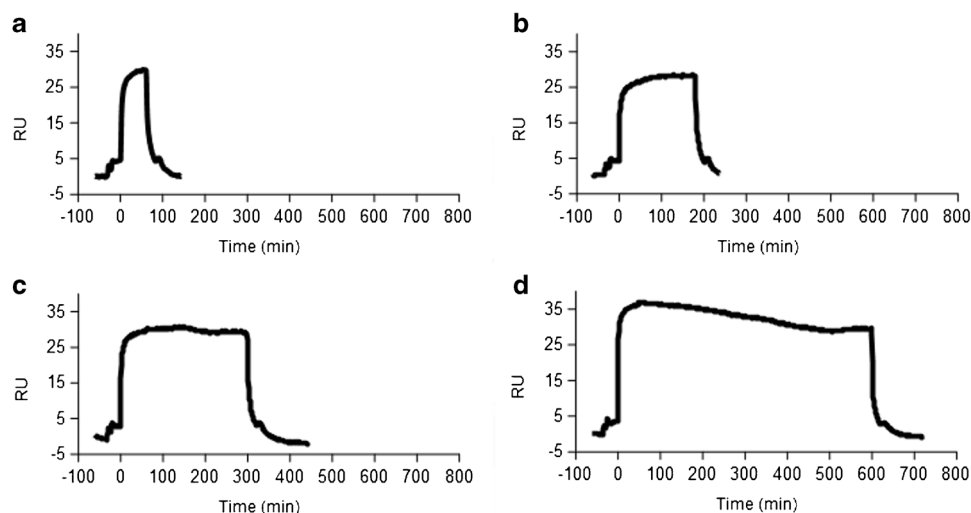


Fig. 2 The injection time of Hepcidin-25 into the MIP NP SPR sensor. Different injection volumes of Hepcidin-25 were tested (from 30 to 300 μL) at a constant flow rate of 30 $\mu\text{L}/\text{min}$ allowing the contact time between the ligand and the analyte to vary from 1 to 10 min (a

1 min, b 3 min, c 5 min, d 10 min). An injection volume of 90 μL (3 min) (b) was chosen for all the experiments. Reproduced from ref. [20] under the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>)

MIP Binding sites' homogeneity

Beyond analyte accessibility and mass-transfer limitations, the analytical performance of MIP-based sensors is strongly influenced by the quality and uniformity of the imprinted binding sites.

In ideal conditions, MIPs should provide a population of well-defined and homogeneous binding cavities capable of ensuring consistent affinity, selectivity, and reproducible molecular recognition. However, most of the MIP synthesis procedures have the drawback of forming binding cavities with intrinsic heterogeneity. This heterogeneity arises from several factors, including random template orientation within the pre-polymerization mixture, non-uniform distribution or excess of functional groups, and differences in site accessibility within the polymeric network. As a result, not all imprinted sites exhibit identical affinity or spatial arrangement toward the target, leading to a broad distribution of binding affinities. The complex distribution of binding sites typically observed in MIPs has stimulated considerable efforts toward the development of appropriate models capable of describing their recognition behavior. In this context, the Langmuir–Freundlich isotherm has been proposed as a particularly suitable model for MIPs, since it can describe systems exhibiting both homogeneous and heterogeneous binding-site distributions [21]. The Langmuir–Freundlich model can be regarded as an intermediate case between the Langmuir isotherm, which assumes a homogeneous population of equivalent recognition sites characterized by a single affinity constant, and the Freundlich isotherm, which describes heterogeneous systems

containing multiple classes of binding sites with different affinities. Within this framework, the homogeneity index (n) provides a quantitative measure of binding-site uniformity, with values approaching unity indicating increasingly homogeneous recognition environments and binding behavior closer to the ideal Langmuir model.

The applicability of the Langmuir–Freundlich model to MIP-based sensing systems has been demonstrated in several studies. For instance, adsorption data obtained for an adenosine-5'-triphosphate (ATP)-imprinted electropolymerized sensing film were more accurately fitted by the Langmuir–Freundlich isotherm than by the Freundlich model alone, particularly at high ATP concentrations where saturation effects became significant. Indeed, the Freundlich isotherm deviated from the experimental data at elevated analyte concentrations, being unable to account for the saturation behavior of the imprinted binding sites. As a result, the heterogeneity index obtained from the two models differed substantially: the Langmuir–Freundlich fit yielded $n = 1 \pm 0.06$, indicative of a relatively homogeneous population of recognition sites, whereas the Freundlich fit produced a markedly lower value ($n = 0.56 \pm 0.02$) [22].

Importantly, in the context of sensing, the information provided by the homogeneity index is not limited to the description of molecular recognition phenomena. Since analyte binding ultimately drives signal generation, variations in the distribution of binding sites also influence the analytical characteristics of the sensing device, including sensitivity. This concept was demonstrated in a comparative study involving two estradiol-imprinted electrochemical sensing interfaces characterized by different degrees of binding-site

heterogeneity. One sensor, called the Imprinted PANI layer sensor, relied on an electropolymerized imprinted polyaniline (PANI) layer, where the recognition sites were generated directly during polymer growth, whereas the second, called nanoMIP-doped PANI sensor, employed pre-synthesized nanoMIPs dispersed within a polyaniline matrix. Analysis of the binding isotherms with the Langmuir–Freundlich model revealed markedly different homogeneity indices, with $n=0.34$ for the imprinted polyaniline layer and $n=0.79$ for the nanoMIP-based architecture, indicating a substantially more homogeneous population of recognition sites in the latter case. Interestingly, this difference was accompanied by a pronounced improvement in analytical performance. In particular, the nanoMIP-doped PANI sensor exhibited an almost two-fold increase in sensitivity compared with the Imprinted PANI layer sensor, suggesting that a more uniform distribution of binding sites may promote a more efficient transduction of molecular recognition events into measurable analytical signals [23].

To improve binding site homogeneity, several strategies have been developed, ranging from controlling template orientation during the imprinting to engineering the synthesis methodology of the imprinted material itself, with the common objective of reducing the degrees of freedom of the system and limiting the formation of poorly defined binding cavities.

Oriented imprinting has emerged as a particularly effective approach, as it enables the pre-definition of the template orientation prior to polymer formation, leading to more homogeneous and structurally consistent recognition sites. In practice, oriented imprinting is typically achieved by modifying the surface or support used for polymerization with functional groups capable of selectively interacting with the target analyte. This functionalization allows the template to be anchored in a spatially defined orientation prior to polymerization, and its subsequent removal leaves directionally organized cavities that reproduce the geometry and chemical functionalities of the target molecule [24]. Various chemistries have been employed to achieve controlled template orientation, including boronate-affinity interactions, borazine (B_3N_3)-assisted interactions, cyclodextrin-mediated

interactions, or specific ligand-mediated anchoring. Representative examples are summarized in Table 1.

A representative example of the impact of oriented imprinting on binding-site homogeneity was reported by Lang et al., who combined boronate-affinity-oriented immobilization with sequential surface imprinting to fabricate synthetic glycoprotein receptors. In this approach, the glycoprotein template ovalbumin (OVA) was first anchored onto boronic acid-functionalized magnetic nanoparticles through reversible boronate-*cis*-diol interactions, thereby constraining its spatial orientation prior to polymerization. Such controlled immobilization is expected to reduce cavity-to-cavity variability and promote the formation of more structurally uniform recognition sites [25]. Evidence supporting this hypothesis was provided by the adsorption behavior of the resulting MIP, which was well described by the Langmuir isotherm model ($R^2=0.986$). Since the Langmuir model assumes a homogeneous population of energetically equivalent binding sites, the good fitting suggests that oriented immobilization contributed to the generation of a relatively uniform affinity-site distribution. More importantly, the improved structural definition of the recognition sites was reflected in the analytical performance of the sensing platform. When compared with a previously reported OVA-imprinted sensor prepared without boronate-affinity-oriented immobilization [29], the oriented system achieved a substantially lower LOD (4.5 fg/mL versus 0.82 pg/mL). Although differences in sensor architecture and transduction mechanisms prevent a strictly direct comparison, the approximately two-orders-of-magnitude improvement in detection limit is consistent with the expected benefits of generating a more homogeneous population of high-affinity binding sites.

A further demonstration of the benefits associated with oriented imprinting was provided by Longsompurana et al., who employed cyclodextrin-mediated interactions to control the spatial arrangement of galectin-3 (Gal-3) during MIP formation [28]. In this approach, hexakis-(6-mercapto-6-deoxy)- β -cyclodextrin (mCD) was immobilized onto a quartz crystal microbalance (QCM) surface through a 4-aminothiophenol (4ATP) linker and used to guide the orientation of the protein template prior to the electropolymerization

Table 1 Representative examples of oriented imprinting strategies and their analytical performance in MIP-based sensors

Orientation strategy	MIP-based sensor architecture	Target	LOD	LOQ	Linear range	Time	Ref
Boronate affinity	Double polydopamine and phenylboronic acid imprinted layer	Ovalbumin	4.5 fg/mL	16.8 fg/mL	16 fg/mL–1 μ g/mL	5 min	[25]
	Thin L-cystein MIP layer on carbon nanotubes	Horseradish peroxidase	7.4 fg/mL	/	10^{-14} – 10^{-7} g/mL	3 min	[26]
Borazide affinity	Butyl methacrylate MIP layer	Umifenovir	48.20 fM; 23.40 fM	160.51 fM; 77.92 fM	0.50–7.50 pM; 0.25–5.00 pM	7 min	[27]
Cyclodextrin affinity	PANI-3-carboxylic acid MIP layer on Au surface	Galectin-3	1.08 ng/mL	3.61 ng/mL	1–30 ng/mL	4 min	[28]

of an aniline-3-carboxylic acid (ANI3C)-based MIP layer. Molecular docking studies indicated that mCD preferentially interacted with the carbohydrate-recognition domain of Gal-3, thereby promoting a more defined template orientation during polymerization. Importantly, the authors experimentally demonstrated that the incorporation of mCD enhanced the binding affinity of the resulting imprinted layer. Rebinding experiments performed with 20 ng/mL Gal-3 revealed that the 4ATP/mCD/ANI3C MIP generated frequency shifts approximately two-fold higher than those observed for the corresponding MIP prepared without mCD. The authors attributed this improvement to the ability of mCD to constrain the spatial orientation of Gal-3 during the pre-polymerization stage, thereby promoting a more controlled cavity formation process. Although the homogeneity of the resulting binding sites was not directly quantified, the enhanced rebinding response is consistent with the formation of more structurally uniform and effective recognition cavities. Even more significantly, the use of mCD dramatically improved fabrication reproducibility. While the oriented MIP exhibited a relative standard deviation (RSD) of only 8.3% across 15 independently fabricated sensors, the corresponding non-oriented MIP showed an RSD of 71.0%. Such a substantial reduction in sensor-to-sensor variability strongly supports the notion that controlling template orientation leads to a more reproducible cavity formation process and, consequently, to a more homogeneous population of recognition sites.

A complementary approach to improve binding-site homogeneity relies on template engineering. In this case, the control over cavity formation is introduced directly at the template level, rather than through modifications of the polymerization substrate. By tailoring the template structure or introducing specific functional residues, the degrees of freedom available during polymerization can be reduced, facilitating the generation of more uniform recognition sites [30].

A representative example of template engineering was reported by Suda et al., who designed a cortisol motif template molecule (TM1) specifically aimed at controlling cavity formation during imprinting [31]. Unlike conventional approaches, TM1 was engineered to combine three key functions within a single structure: orientational immobilization through an adamantane moiety, polymer integration through a polymerizable group, and the generation of a specific recognition site through a reversible oxime linkage. Following polymerization and template removal, the resulting cavities retained two spatially organized interaction sites capable of recognizing cortisol through complementary hydrophobic and covalent interactions. By predetermining both the orientation of the template and the nature of the interactions encoded within the cavity, the authors generated highly homogeneous and functionally defined binding sites. This translated into a substantial improvement in analytical

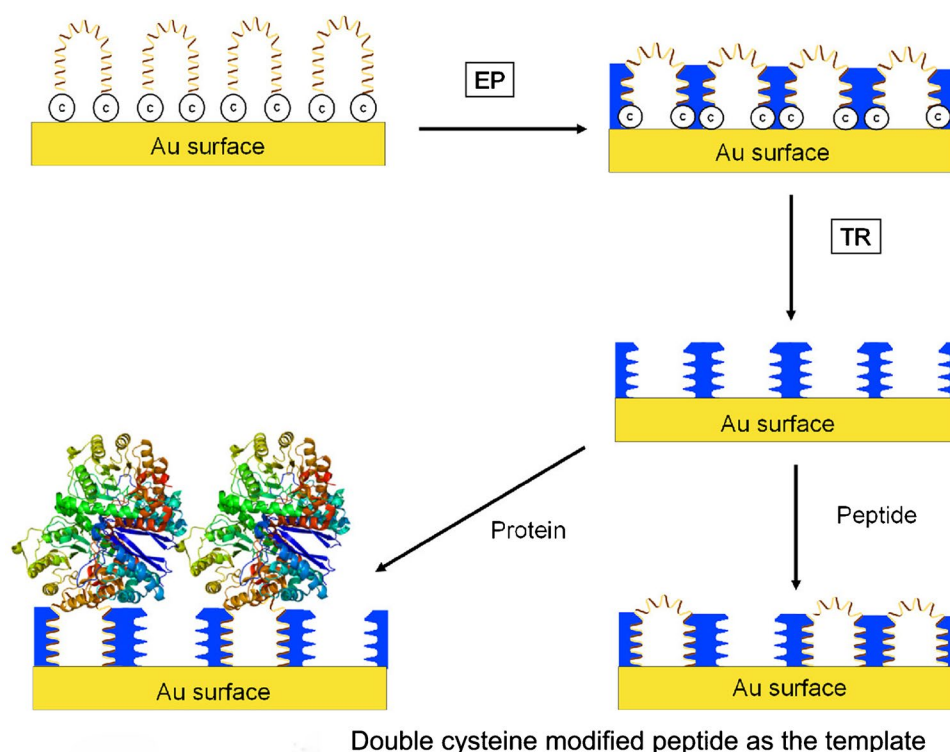
performance, reducing the LOD from approximately 80 nM in their previously reported cortisol-imprinted system to 4.8 pM in the engineered-template platform.

A particularly versatile implementation of template engineering is represented by the incorporation of specific amino acid residues, such as cysteine and histidine, into peptide or epitope templates [32]. A notable example of this approach was reported by the work of Drzazgowska et al., in which the epitope is functionalized at both N- and C-termini with cysteine residues, enabling its simultaneous anchoring to a gold surface and resulting in a bridge-like configuration (Fig. 3) [33]. In this approach, a doubly cysteine-modified peptide is immobilized onto the electrode to form a self-assembled monolayer prior to electropolymerization, forcing the template into a constrained conformation. This geometrical constraint significantly reduces the conformational flexibility of the template during polymerization, allowing a more precise transfer of structural information to the imprinted cavities. The benefits of this strategy were reflected in the recognition properties of the resulting MIP developed for neuron-specific enolase (NSE) detection. The resulting MIP-based electrochemical sensor exhibited a K_D of 2.6×10^{-11} M. Notably, this value was lower than that previously reported by the same research group for a single-cysteine epitope-imprinted system ($K_D = 5.3 \times 10^{-11}$ M), indicating an improvement in binding affinity associated with the enhanced structural control achieved through the bridge-like template configuration.

A further strategy to improve binding-site homogeneity relies on engineering the methodology for the synthesis of the imprinted material itself, whereby the main advancement comes from the solid-phase synthesis (SPS) strategy. In this approach, the template is immobilized onto a solid support prior to polymerization, so that the growing polymer nanoparticles are formed around surface-confined and spatially organized template molecules. This feature introduces an orientation constraint similar to that exploited in oriented imprinting strategies, contributing to a more controlled formation of the recognition cavities. However, the distinctive advantage of SPS lies in the subsequent affinity-based selection step. After polymerization, weakly interacting or poorly formed nanoparticles can be removed through washing, while high-affinity nanoMIPs are selectively recovered under controlled elution conditions. As a result, the final nanoparticle population is enriched in receptors displaying comparable affinity for the target, leading to a narrower distribution of binding properties and improved batch-to-batch reproducibility [34, 35].

Direct experimental evidence supporting the affinity-selection role of SPS was recently provided by Maniglio et al., who compared gelatin-methacryloyl-based bioMIPs prepared by conventional in-solution imprinting with bioMIPs obtained through SPS for interleukin-6 (IL-6)

Fig. 3 Generation of self-assembled monolayer epitope bridges on gold surfaces using doubly cysteine-modified peptide templates for subsequent molecular imprinting via electropolymerization (EP) and target sensing (short peptide or NSE protein). TR, template removal. Reproduced with modifications from ref. [33] with permission



recognition [36]. When tested in commercial serum, in-solution-imprinted bioMIPs showed a moderate IL-6 uptake of 26 ± 3 pg/ μ g (quantity IL-6/quantity NPs), while SPS-derived bioMIPs exhibited a markedly enhanced IL-6 capture capacity, reaching 1178 ± 101 pg/ μ g, corresponding to an approximately 45-fold increase compared with the in-solution-imprinted counterpart. Although this study was not focused on sensor development, it clearly demonstrates the key advantage of SPS in enriching the final MIP population with high-affinity receptors through an affinity-based selection process.

The relevance of this strategy for sensor development has been demonstrated in several nanoMIP-based sensing platforms, where the use of SPS-MIPs was associated with

strong affinity, high selectivity, and low detection limits. Representative examples are summarized in Table 2.

A representative example is a label-free capacitive sensor based on nanoMIPs produced by SPS [41]. In this work, nanoMIPs were prepared for two targets with markedly different molecular sizes, namely tetrahydrocannabinol (THC) and trypsin, and subsequently deposited onto the sensor surface, where target rebinding was monitored through changes in capacitance. The resulting nanoMIP-modified sensor enabled the detection of both analytes at physiologically relevant concentrations, reaching a LOD of 1.0×10^{-14} M and 1.0×10^{-14} M for THC and trypsin, respectively. Importantly, the authors reported a significant improvement in selectivity and LOD, approximately one order of

Table 2 Representative examples of MIP-based sensors employing solid-phase-synthesized nanoMIPs as recognition elements and their analytical performance

MIP-based sensor	Target	Transduction method	LOD	Linear range	Ref
Electroactive nanoMIP onto Pt-SPE	Glyphosate	DPV	18.2 pM	25–500 pM	[37]
NanoMIP onto SPE or onto GCE	Norovirus	EIS Thermal	EIS: 3.4 pg/mL Thermal: 6.5 pg/mL	1–100 ng/mL	[38]
NanoMIP Au chip	Fluoxetine (FLU); carbamazepine (CBZ)	SPR	FLU: 0.39 nM; CBZ: 0.34 nM	1–1000 nM	[39]
NanoMIP onto Au chip	Glucocorticoid steroids	SPR	1.6–3.6 nM	4–64 nM*	[40]
NanoMIP onto electrode	Tetrahydrocannabinol (THC); Trypsin	Capacitive	THC: 10^{-14} M; Trypsin: 10^{-14} M	THC: 10^{-12} – 10^{-6} M; Trypsin: 10^{-13} – 10^{-10} M	[41]
NanoMIP onto carbon SPE	Cardiac troponin T	EIS	0.004 ng/mL	0.01–0.3 ng/mL	[42]

*The reported interval refers to the low analyte concentration range tested by the original authors, rather than to a broad analytical working range

magnitude compared with previously used MIP microparticles. Although this comparison does not isolate the effect of SPS alone, the enhanced performance was attributed to the nanoscale format, increased surface-to-volume ratio, and higher specificity of the solid-phase-synthesized nanoMIPs. A further example illustrating the relevance of SPS-MIPs in electrochemical sensing was reported by Gagliani et al., who developed an impedimetric sensor based on MIP nanogels (MIP-NGs) for the detection of cardiac troponin T (cTnT) [42]. The resulting sensor based on the SPS-MIP-NGs achieved a linear response in the range of 0.01–0.3 ng/mL and a LOD of 0.004 ng/mL in human serum. The authors report that this LOD was lower than that achieved by several earlier MIP-based electrochemical cTnT sensors, including o-phenylenediamine-based MIP layers on gold electrodes and other electropolymerized MIP architectures, whose LOD ranged from 6 to 17 pg/mL.

Stimuli-responsive MIPs

Beyond strategies aimed at improving analyte transport, binding-site homogeneity, and recognition-site organization, an additional route to enhance MIP-based sensor performance relies on introducing dynamic control over the recognition process itself. In many sensing platforms, target binding within the imprinted cavities produces only minimal physicochemical variations at the sensing interface,

which may limit sensitivity, reduce signal reliability, and hinder regeneration, particularly in complex analytical matrices or at ultralow analyte concentrations. To address these limitations, increasing attention has been devoted to stimuli-responsive MIPs, in which the polymer is designed to undergo controlled structural, conformational, physicochemical, or interfacial changes in response to external stimuli or to the binding event itself. In these systems, the imprinted polymer does not act merely as a passive recognition element, but actively contributes to the sensing process by modulating cavity accessibility, analyte uptake and release, interfacial properties, or signal generation. Depending on the responsive mechanism involved, such dynamic MIP platforms can improve sensitivity, enhance signal transduction, facilitate regeneration and reusability, and provide more reversible and controllable sensing interfaces. For this reason, stimuli-responsive MIPs can be more effectively discussed according to the sensor function enabled by the responsive behavior, rather than solely according to the nature of the stimulus (Table 3).

Accordingly, the following discussion focuses on three main performance-oriented roles of stimuli-responsive MIPs: amplification of weak binding-induced signals, regulation of target binding and release for improved reversibility and regeneration, and external control of recognition or transduction processes at the sensing interface. Among the different functions enabled by stimuli-responsive MIPs,

Table 3 Representative examples of stimuli-responsive MIP-based sensors

Stimuli-responsive MIP-based sensor	Stimuli-responsive mechanism	Target	Transduction methods	LOD	Performance benefit	Ref
Deformable protein-imprinted nanogels immobilized on a D-shaped POF	Analyte-induced deformation	HTR	SPR	1.2 fM	Signal amplification	[43]
Thermo-responsive poly(NIPAAm)-based imprinted memory hydrogel on GCE	Thermos-responsive	BSA	CV/EIS/DPV	0.012 μ M	Sensor regeneration	[44]
pH-responsive boronate-affinity MIP incorporated into polymeric potentiometric membrane	pH-responsive	Catechol	Potentiometric	0.12 μ M	Sensor regeneration	[45]
Xanthan–biochar–Fe ₃ O ₄ /MIPDA biopolymer hydrogel on SPE	Magnetic-responsive	L-tryptophan	SWV/EIS	0.44 μ M	Signal amplification	[46]
Hollow visible-light-responsive surface MIP	Photo-responsive	chlorpyrifos	UV–Vis	0.2 μ M	Reversible uptake/release	[47]
Photo-responsive MWCNT-MIP-modified platinum electrode	Photo-responsive	L-phenylalanine benzyl ester	CV/EIS/DPV	0.2 μ M	Reversible uptake/release	[48]
Core-shell Fe ₃ O ₄ @fluorescent MIP nanoparticles on screen-printed gold electrodes	Photo/magnetic-responsive	Interleukin-6	SWV	0.38 pM	Signal amplification	[49]

signal amplification via analyte-induced structural changes was effectively demonstrated by us [43]. In this work, protein-imprinted nanogels selective for human serum transferrin (HTR) were integrated into a D-shaped plastic optical fiber surface plasmon resonance (POF-SPR) platform through covalent immobilization onto the plasmonic surface. The imprinted nanogels were synthesized by precipitation polymerization and were characterized by a soft, hydrogel-like structure, which conferred a marked deformability to the recognition layer. This analyte-induced deformation amplified the plasmonic response, enabling femtomolar detection with a linear range from 1.2 fM to 1.8 pM. Therefore, the sensitivity gain was attributed to the MIP nanogel, which not only provides molecular selectivity toward the target protein but also acts as a deformable transducing layer, as demonstrated by the force distance curves, capable of converting analyte binding into a larger plasmonic response.

Beyond signal amplification, stimuli-responsive MIPs can also improve the operational performance of sensors by enabling controlled target binding and release. This aspect becomes particularly important in sensing platforms where target rebinding is not easily reversible, leading to incomplete regeneration of the imprinted interface and limiting sensor reusability. A representative example of this strategy was reported by Wei et al., who developed a self-cleaned electrochemical protein-imprinting biosensor based on a thermo-responsive poly(*N*-isopropylacrylamide)-based memory hydrogel for bovine serum albumin (BSA) detection [44]. The imprinted hydrogel was constructed on a glassy carbon electrode (GCE) by free radical polymerization and acted as a temperature-controlled gate, regulating BSA adsorption and desorption through reversible hydrophilic/hydrophobic conformational changes of the polymer network. The regeneration capability of the sensor was experimentally evaluated through controlled self-cleaning tests. The authors compared different temperatures and electrochemical cycling conditions, identifying 37 °C combined with potential cycling between -0.8 and $+0.8$ V as the most effective regeneration protocol, demonstrating that under these conditions, BSA could be efficiently removed in phosphate-buffered saline (PBS) without the use of organic solvents or harsh washing treatments. Importantly, the reversible operation of the sensing interface was further confirmed by alternating BSA rebinding at 20 °C and release at 37 °C. After three uptake/release cycles, the thermo-responsive MIPs/GCE retained more than 90% of its affinity toward BSA, indicating that the responsive hydrogel preserved its recognition capability after repeated regeneration. The main relevance of this example, therefore, lies in the operational functionality introduced by the thermo-responsive MIP layer, which, rather than providing a major gain in detection limit, improved sensor reusability by enabling solvent-free regeneration and repeated use of the imprinted interface.

A similar regeneration-oriented strategy was reported by Qi et al., who developed a pH-responsive MIP-based potentiometric sensor for the reversible detection of neutral phenols [45]. As a proof of concept, catechol, a *cis*-diol-containing phenol, was selected as the template, while 4-vinylphenylboronic acid was used as the functional monomer to generate boronate-affinity binding sites. Under weakly alkaline conditions, the boronic acid groups within the imprinted polymer formed reversible cyclic esters with catechol, promoting selective extraction of the analyte into the sensing membrane through covalent recognition interactions. Under acidic conditions, these complexes dissociated, allowing catechol release and regeneration of the imprinted receptor sites. The effectiveness of this pH-controlled regeneration mechanism was confirmed by repeated potentiometric measurements. After each detection cycle, the sensor was regenerated with 10 mM HCl and reconditioned in alkaline PBS, without showing an evident decrease in response over successive cycles (Fig. 4). Therefore, this example further supports the role of stimuli-responsive MIPs in improving operational reversibility and sensor lifetime by enabling mild, aqueous regeneration of the recognition interface.

Beyond chemically triggered regeneration, external fields can also be exploited to improve the operational performance of stimuli-responsive MIP-based sensors. A recent example was reported by Hosseini et al., who developed a remote magnetically stimulated electrochemical sensor for the enantioselective detection of L-tryptophan. The sensing platform consisted of a xanthan hydrogel incorporating loofah-derived biochar-stabilized Fe_3O_4 nanoparticles coated with molecularly imprinted polydopamine (MIPDA), and was deposited onto a screen printed electrode (SPE) [46]. In this architecture, the different components played

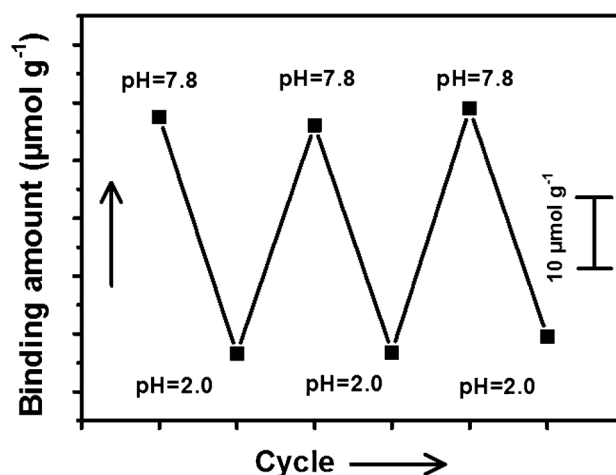


Fig. 4 Changes in the catechol binding amount of the pH-responsive MIP under alkaline (10 mM PBS buffer of pH 7.8) and acid conditions (0.01 M HCl). Reprinted from ref. [45] with permission

complementary roles: the MIPDA layer provided enantioselective recognition sites for L-tryptophan; the xanthan hydrogel created a three-dimensional adhesive microenvironment for analyte diffusion and redox-probe transport; the biochar-supported Fe_3O_4 nanoparticles introduced magnetic responsiveness and improved the electrochemical interface. The role of magnetic stimulation was specifically investigated by comparing the magneto-electrochemical behavior of the XG-LBC- Fe_3O_4 /MIPDA-modified electrode in the presence and absence of an external magnetic field. Cyclic voltammetry (CV) and squarewave voltammetry (SWV) measurements showed an enhanced electrochemical signal under magnetic stimulation, while electrochemical impedance spectroscopy (EIS) analysis revealed a smaller Nyquist semicircle, indicating a decrease in charge-transfer resistance. The authors attributed this behavior to the magnetic-field-induced attraction of the active composite toward the electrode surface, which promotes closer interfacial contact, expands the effective working area, and accelerates both mass transfer and charge transfer. Therefore, in this system, the magnetic field acts as an external stimulus capable of improving the electrochemical transduction efficiency of the MIP-based sensing interface.

Conclusions and outlooks

MIP-based sensors have undergone substantial development over the past decade, evolving from simple synthetic receptor-based devices towards increasingly engineered sensing platforms in which the imprinted material is designed to actively address specific analytical and operational limitations. The examples discussed in this review show that sensor performance cannot be fully interpreted by considering analytical figures of merit alone, such as sensitivity, selectivity, or LOD. Rather, these parameters should be correlated with the structural and functional properties of the imprinted material, including binding-site accessibility and homogeneity, affinity-site distribution, analyte transport, interfacial organization, signal transduction efficiency, and operational aspects such as reproducibility, regeneration, reusability, and long-term stability. From this perspective, the advancement of MIP-based sensors depends not only on the development of new imprinting formulations but also on the rational design of MIP architectures capable of translating molecular recognition into robust, reproducible, and practically usable analytical responses.

From a translational perspective, the imprinting strategies discussed in this review should be viewed as modular design tools that can be selected according to the requirements of the intended sensing application. NanoMIPs and nanostructured architectures are particularly valuable when rapid mass transport and surface accessibility are the dominant

limitations, whereas oriented imprinting and solid-phase synthesis provide solutions when reproducibility and binding-site homogeneity become the critical determinants of analytical reliability. Likewise, stimuli-responsive polymers introduce functionalities that extend well beyond molecular recognition, enabling active participation of the polymer matrix in signal transduction, reversible target capture, and sensor regeneration. Such dynamic behavior represents a significant conceptual evolution of MIPs, transforming them from passive recognition elements into adaptive materials capable of improving both analytical sensitivity and operational robustness. Their incorporation into sensing platforms may therefore become increasingly important as applications shift toward continuous monitoring, wearable devices, and reusable point-of-care technologies.

From a commercialization perspective, however, these strategies differ substantially in their balance between performance gain, cost, and scalability. Among the approaches discussed in this review, miniaturized and surface-confined MIP formats currently appear to present the lowest barrier to practical implementation, particularly when the imprinted layer is directly generated on planar or screen-printed transducers by controlled thin-film deposition or electropolymerization. These formats require small amounts of monomers and template, avoid separate nanoparticle purification steps, and are compatible with disposable electrode formats and scalable sensor fabrication. Their main limitation is that improved mass transport and faster response do not necessarily guarantee higher recognition reproducibility unless film thickness, morphology, and template removal are tightly controlled. Oriented imprinting and solid-phase synthesis address a different bottleneck, namely, binding-site quality and receptor reproducibility, but they introduce different levels of manufacturing complexity. Oriented surface imprinting can be commercially attractive when template anchoring relies on simple and reproducible surface chemistry, because the recognition layer can be generated directly on the transducer. This reduces the number of fabrication steps compared with SPS, which requires separate nanoparticle production, affinity-based selection, purification, and subsequent immobilization onto the sensor surface. In contrast, SPS provides affinity-enriched nanoMIP populations with improved binding-site quality and batch-to-batch consistency, but its scalability is more demanding because the same selection process that improves receptor quality can reduce the recoverable yield, increase production time, and cost. Therefore, SPS may be commercially justified for high-value targets requiring highly reproducible synthetic receptors, whereas direct surface-confined imprinting currently presents a lower process-complexity barrier for disposable or large-scale sensor formats. Stimuli-responsive MIPs offer the greatest functional added value, particularly for signal amplification, reversible binding, and regeneration,

but also the highest translational risk because they require additional material design, stimulus control, cycling stability, and device integration. Their commercial viability will therefore depend on applications in which the added complexity is compensated by clear operational advantages, such as reusable point-of-care devices, wearable sensors, or continuous-monitoring platforms.

Although antifouling and matrix-tolerance strategies are not the primary focus of this review, they represent an important boundary condition for translating performance improvements into real-sample analysis. The design strategies discussed here may influence nonspecific binding in different ways, but they do not inherently guarantee resistance to biofouling or matrix effects. Miniaturized and ultrathin MIP layers can reduce diffusion distances and limit nonspecific uptake into the polymer bulk, but their highly exposed interfaces may remain susceptible to site blocking or nonspecific adsorption. Oriented imprinting and SPS can improve recognition-site definition and selectivity, yet they do not by themselves prevent fouling of the surrounding non-imprinted surface. Stimuli-responsive hydrogels and dynamic polymer networks may facilitate mild regeneration after matrix exposure, but changes in hydration, charge, or hydrophobicity may also affect nonspecific adsorption under application-relevant conditions. Therefore, matrix resistance should be evaluated as a distinct performance requirement through matrix-matched calibration, recovery experiments, appropriate negative controls, repeated-use tests, and direct comparison between buffer and real-sample responses.

Despite these significant advances, the translation of MIP-based sensors into products and practical applications remains more limited than the volume of academic research would suggest. Although numerous studies report excellent analytical performance under controlled laboratory conditions, challenges related to reproducibility, large-scale fabrication, long-term stability, regeneration, and validation in real-world samples are still insufficiently addressed. In addition, the current literature frequently compares systems employing different analytes, transduction principles, sample matrices, and experimental protocols, making it difficult to distinguish genuine advances in imprinting technology from improvements arising from the sensing platform itself.

A key priority for the community is therefore to establish harmonized benchmarking approaches capable of linking specific design choices to measurable sensor-level benefits. Statistical analyses and emerging AI-assisted tools for structure–performance correlation may support this effort by improving the comparability of published studies and helping to identify the most effective combinations of imprinting methodologies and transduction technologies for specific analytical scenarios.

Future progress in MIP-based sensing will likely depend less on the continuous introduction of new imprinting

chemistries and more on the rational integration of existing strategies into robust, scalable, and application-relevant sensor architectures. Rather than further increasing the complexity of individual material designs, future studies should prioritize controlled comparisons, reproducible fabrication, compatibility with miniaturized and portable transducers, and validation under realistic operating conditions. In this way, complementary functionalities such as enhanced site accessibility, improved binding-site homogeneity, signal amplification, matrix tolerance, and regeneration could be combined within unified sensing platforms capable of addressing multiple performance-limiting factors simultaneously. This shift from isolated performance enhancement toward integrated and validated sensor design will be essential for supporting the transition of MIP-based sensing technologies from academic proof-of-concept studies toward broader practical and commercial implementation.

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Declarations

Conflict of interest The authors declare no competing interests.

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