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Multidimensional signal amplification architectures in electrochemical immunosensing integrate porous nanomaterials, biocatalysis, and nucleic acid circuits to achieve attomolar detection

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Electrochemical immunosensors have significantly advanced point-of-care diagnostics and environmental monitoring, owing to their high specificity, portability, and compatibility with miniaturized systems. Nevertheless, their detection sensitivity remains limited by the ultralow concentrations of target analytes (such as disease biomarkers, pathogens, or environmental contaminants), creating an urgent need for innovative signal amplification strategies to meet practical and regulatory demands. This review presents a systematic overview of emerging signal amplification strategies, placing a dedicated focus on covalent organic frameworks (COFs) and metal–organic frameworks (MOFs) as highly promising yet underexplored nanomaterials. Although traditional materials such as carbon nanotubes (CNTs), graphene, enzyme cascades, and DNA-based systems have been widely investigated in electrochemical immunosensing, COFs and MOFs have attracted comparatively less attention despite their exceptional properties. Beyond summarizing the well-established porous materials, this work delves into the distinctive roles of COF and MOF architectures in promoting electron transfer, increasing immobilization capacity, and strengthening signal amplification. A comparative analysis is provided, aligning these emerging frameworks with conventional amplification approaches, including enzymatic reactions, DNA nanotechnology, and affinity-based methods. A primary objective of this review is to highlight recent mechanistic breakthroughs and innovative applications of COFs and MOFs that remain underrepresented in existing literature. Additionally, persistent challenges such as real-sample matrix effects, multiplex detection, and sensor regeneration are discussed. We conclude with prospective research directions, incorporating advancements in microfluidics, reusable interfaces, and artificial intelligence-assisted design, to pave the way toward scalable and high-performance immunosensing platforms.

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1. Introduction

Biosensors, a specialized and rapidly evolving category of chemical sensors, are typically composed of five essential functional components, encompassing the analyte, biorecognition element, signal transducer, electronic processor, and display.^{1,2} Within this framework, the biorecognition element and the signal transducer are of paramount importance, functioning as the core units responsible for specific target detection and signal conversion, respectively.³ The fundamental operating principle relies on the incorporation of

biologically active materials, such as enzymes, microorganisms, antibodies/antigens, DNA, or tissue sections, as highly selective recognition elements.⁴ When a specific biochemical interaction occurs at the biorecognition interface (*e.g.*, antigen–antibody binding or DNA hybridization), the transducer translates this biological event into a quantifiable physical or chemical signal.⁵ This signal is subsequently amplified, processed electronically, and conveyed to the display unit for detection and analytical interpretation.⁶ As integrated analytical systems, biosensors facilitate rapid, user-friendly, and frequently label-free detection of biological or chemical substances within complex sample matrices, often obviating the need for extensive sample preparation.⁷

Immunosensors represent a rapidly advancing category of biosensors that exploit the high specificity of antigen–antibody interactions in conjunction with highly sensitive signal transduction mechanisms.⁸ These systems have emerged as powerful analytical tools, distinguished by their exceptional sensitivity

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(often achieving attomolar detection limits), outstanding molecular specificity, streamlined assay procedures, and inherent compatibility with automated platforms.^{9–11} A particularly promising subclass of these devices is electrochemical immunosensors, which effectively merge immunorecognition with electrochemical detection techniques.¹² The operational principle relies on the immobilization of antibodies on electrode surfaces to facilitate specific target capture. The formation of immunocomplexes induces measurable alterations in electrical properties, such as current, impedance, or capacitance, primarily through redox reactions occurring at the electrode–solution interface.¹³ Among the various electrochemical detection methods, voltammetry has gained widespread adoption due to its operational simplicity, rapid response kinetics, high sensitivity and selectivity, cost-effectiveness, and robust performance.¹⁴ Recent advancements in the field are focused on three key objectives, including miniaturization of sensor platforms to enhance portability, further improvements in sensitivity for detecting trace-level analytes, and the integration of full automation for user-friendly operation.¹⁵ These developments collectively contribute to the evolving applicability and reliability of immunosensors in diverse practical settings.

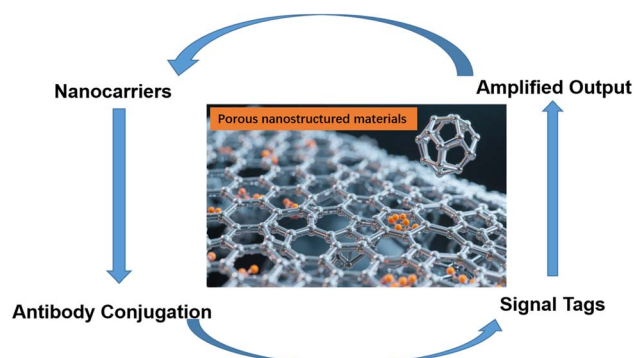
Despite their widespread adoption, conventional immunoassays are constrained by three major limitations, including insufficient sensitivity for detecting trace-level analytes, poor signal-to-noise ratios in complex matrices, and inconsistent performance when applied to real-world samples.¹⁶ To address these challenges, researchers are actively pursuing advancements along three key directions. First, efforts are being made to simplify operational protocols and introduce automated systems to improve practicality and reproducibility. Second, miniaturization is being advanced through the integration of microfluidic technologies and the development of portable sensing devices. Third, significant work is dedicated to enhancing sensitivity *via* novel signal amplification strategies and optimized sensor interface designs.¹⁷ These multidisciplinary efforts collectively aim to bridge the gap between proof-of-concept studies in controlled laboratory settings and the deployment of robust, reliable diagnostic tools capable of performing in real-world scenarios.¹⁸

Driven by these pressing needs, advanced signal amplification strategies have attracted considerable research attention, leading to significant improvements in the performance and applicability of electrochemical immunoassays, especially in clinical diagnostics and environmental monitoring.^{19,20} A highly promising approach involves the use of engineered porous nanomaterials, such as metallic nanoparticles, graphene derivatives, carbon nanotubes (CNTs), metal–organic frameworks (MOFs), covalent organic frameworks (COFs), their composites, and quantum dots, which serve as excellent carrier platforms due to their exceptional physical and electrochemical properties.^{21–23} Metallic nanoparticles (*e.g.*, gold and silver) provide high electrical conductivity, surface plasmon resonance, and large surface area-to-volume ratios, promoting efficient electron transfer and high-density antibody immobilization. Graphene derivatives (*e.g.*, graphene oxide and reduced graphene oxide) and CNTs exhibit outstanding

electrical conductivity, mechanical strength, and abundant surface functional groups, facilitating effective biomolecular conjugation. MOFs and COFs feature ultrahigh surface areas, tunable porosity, and modular functionalization, enabling high-capacity probe loading, selective molecular transport, and enhanced electrochemical reactivity. These properties directly address key challenges in immunosensing, including achieving ultra-low detection limits, improving signal-to-noise ratios, and maintaining biorecognition stability. It is essential to emphasize such structure–function relationships to establish a clear rationale for material selection in next-generation sensor design.

Composite structures combine these materials to synergize their properties, resulting in improved stability, sensitivity, and signal amplification. Quantum dots offer size-dependent electrochemiluminescence and efficient charge transfer, further boosting detection sensitivity through optoelectronic mechanisms. These materials enable high-density, oriented, and stable antibody immobilization—preserving bioactivity and enhancing immunoreaction efficiency.²⁴ In parallel, advanced signal tags such as enzyme-based catalytic systems (*e.g.*, HRP–tyramide), quantum dots with strong redox activity, and electroactive molecules (*e.g.*, ferrocene and methylene blue) further amplify detection signals.^{25–28} Integrated into modern immunoassay platforms, these strategies achieve detection limits in the attomolar to femtomolar range, markedly improving sensitivity and enabling precise quantification of trace analytes in complex samples (Scheme 1).

Building on these advances, this review critically synthesizes recent breakthroughs in highly sensitive detection methodologies for electrochemical immunosensors. We examine the implementation and mechanisms of core amplification technologies, including nanomaterial-enhanced catalysis, enzymatic cascades, avidin–biotin systems, and engineered bioreactions, integrated into electrochemical sensing platforms. The systematic use of porous nanomaterials (*e.g.*, MOFs and nanostructured carbons) as electrode modifiers, immobilization matrices, and signal tags is discussed, highlighting their roles in facilitating electron transfer, expanding surface area, and increasing biomolecular loading.²²



Scheme 1 Key components and their functional roles in achieving ultrasensitive detection.



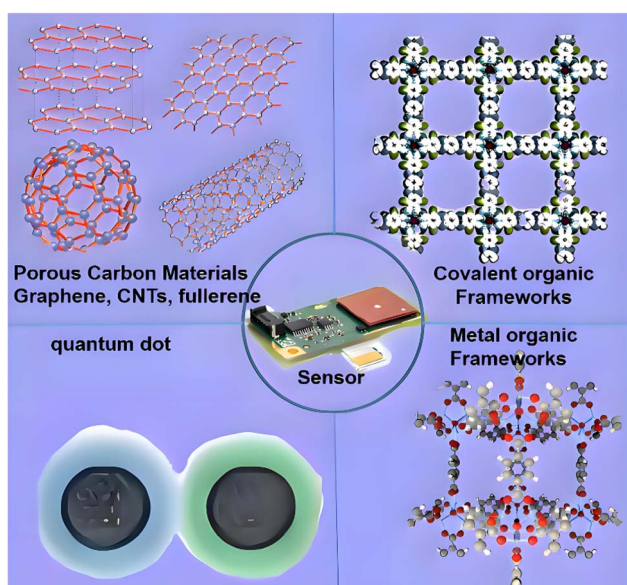
We underscore practical performance metrics, such as signal enhancement ratios, limits of detection (LOD), dynamic range, and reproducibility, supported by recent representative studies. Looking forward, we advocate for continued innovation and interdisciplinary collaboration in amplification strategy design. Future progress will depend on the seamless integration of novel signal enhancement approaches with advanced sensor architectures incorporating microfluidics, bioelectronics, and computational design. This convergence is expected to dramatically enhance analytical sensitivity, pushing detection limits to sub-femtomolar and attomolar levels, and potentially toward single-molecule resolution. These developments will support accurate, reproducible detection of low-abundance biomarkers and small molecules in complex matrices. Ultimately, such innovations are set to enable transformative applications in precision medicine, including early cancer detection and point-of-care diagnostics, as well as in environmental monitoring and food safety surveillance. By advancing reliable, miniaturized, and accessible sensing platforms, these technologies hold great potential to democratize high-sensitivity biosensing worldwide.

2. Nanomaterial-enabled signal amplification strategies

Porous nanomaterials, characterized by over 50% of their structural composition consisting of particles in the 1–100 nm range across three dimensions, have significantly advanced electrochemical immunosensors (Scheme 2).²⁹ Their distinctive properties, such as quantum confinement effects, ultrahigh surface-to-volume ratios, and macroscopic quantum tunneling, endow them with exceptional electrocatalytic activity, tunable electronic band structures, and superior mechanical and

thermal stability, setting them fundamentally apart from bulk materials.^{30–32} These advantages have spurred widespread adoption across diverse fields including catalysis, nanomedicine, and defense technologies. Within electrochemical immunosensing, effective nanomaterials must demonstrate high electrocatalytic activity, efficient electron transfer capability, and biocompatibility to interface with biomolecules such as antibodies.³³ Their extensive surface area facilitates increased biomolecular loading, thereby enhancing both bioanalytical performance and electrical conductivity.³⁴

The synthesis of porous and low-dimensional nanomaterials is essential for tailoring their structural and electrochemical properties to meet advanced application requirements. These synthetic strategies are broadly classified into bottom-up and top-down approaches, each offering specific benefits in precision, scalability, and functional customization. For example, COFs are built through reversible covalent bonds between organic linkers, which allow structural error-correction and result in highly ordered crystalline frameworks. Conversely, MOFs form *via* coordination-driven self-assembly, wherein metal ions or clusters connect with multitopic organic ligands to generate porous architectures. Both COFs and MOFs are commonly synthesized under solvothermal or microwave-assisted conditions, enabling controlled crystallization and yielding materials with ultrahigh surface areas, uniform pore size distributions, and precisely tunable functionalities, all critical for efficient biomolecular immobilization and mass transport. Graphene-based nanomaterials are typically synthesized *via* chemical vapor deposition (CVD) to produce high-quality continuous films ideal for electronic applications, or through chemical exfoliation and reduction of graphite oxide (a scalable and cost-effective route) that yields chemically functionalized graphene with tailored surface properties. Metallic nanoparticles, such as gold and silver, are often prepared by chemical reduction methods, permitting meticulous control over size, morphology, and surface characteristics, which directly modulate their plasmonic and electrocatalytic behaviors. Similarly, quantum dots are synthesized *via* colloidal methods, providing exceptional control over dimensions and resulting optoelectronic properties, making them particularly suitable for signal amplification in sensing platforms. Furthermore, composite materials, such as graphene–MOF hybrids or polymer–nanoparticle assemblies, are fabricated through *in situ* growth, self-assembly, or layer-by-layer deposition. These strategies exploit synergistic effects, leading to enhanced electrical conductivity, mechanical robustness, and tailored surface reactivity, which are indispensable for constructing high-performance and durable biosensors. Each synthesis method offers unique advantages in scalability, reproducibility, and functionalization potential, proving essential for the rational design of next-generation sensing platforms. Through deliberate selection and optimization of synthetic pathways, researchers can precisely engineer nanomaterial properties to address specific challenges in electrochemical immunosensing and broader biomedical applications.



Scheme 2 The typical porous nanomaterials used for the electrochemical signal amplification.



2.1 Carbon nanotubes

CNTs, first discovered by Iijima in 1991, have become pivotal in electrochemical immunosensing due to their exceptional electrical conductivity ($>10^4$ S cm $^{-1}$), rapid electron transfer kinetics, and robust chemical stability.³⁵ Structurally defined as needle-like tubular carbon allotropes, CNTs exhibit ultrahigh mechanical strength, extended catalytic active surfaces, and superior electrochemical activity.^{36–38} These attributes establish CNTs as ideal signal amplification platforms and electrode scaffolds. CNTs are categorized into two primary configurations, namely the single-walled CNTs (SWCNTs, ~ 1 nm diameter, exhibiting quantum confinement effects) and multi-walled CNTs (MWCNTs, 5–100 nm diameter, offering enhanced mechanical rigidity).^{39–41}

For biosensing applications, antibodies are immobilized onto CNT-modified electrodes through amine/carboxyl functionalization ($-\text{NH}_2/-\text{COOH}$ groups), enabling covalent conjugation (Fig. 1). Beyond elemental forms, CNT-based nanocomposites (e.g., Au–CNTs, graphene–CNT hybrids) demonstrate synergistic amplification.^{42–44} Multimodal functionality integrating catalytic/conductive properties, signal enhancement factors exceeding 300% compared with monocomponent systems, and diversified sensing interfaces for multiplexed detection.⁴⁵

SWNTs exhibit exceptional physicochemical properties, including large surface area-to-volume ratios, low charge density, and delocalized π -orbitals, which collectively enhance their efficacy in electrochemical sensing applications.⁴⁶ MWNTs serve as high-performance electrode scaffolds due to their exceptional conductivity and electrocatalytic activity.⁴⁷ Liu *et al.* demonstrated a novel approach for glucose oxidase (GOx) immobilization on a glassy carbon electrode (GCE) using an agarose fixation matrix.⁴⁸ Crucially, they integrated single-walled carbon nanotubes (SWCNTs) functionalized with ferrocene. This ferrocene–SWCNT hybrid exhibited exceptional stability in both aqueous and organic media, significantly enhanced electron-transfer kinetics, and provided abundant

electrochemical active sites. This design yielded two critical advantages, including the markedly improved reversibility of the ferrocene redox reaction, and effective elimination of dissolved oxygen interference. Collectively, these features enabled the development of a glucose biosensor characterized by a rapid amperometric response and enhanced sensitivity. This study effectively established an alternative pathway for efficient electrical communication, mediated by the SWCNT–ferrocene hybrid, between the GOx active center and the underlying electrode.

Yang *et al.* utilized ultrasonic dispersion to uniformly integrate SWCNTs into a chitosan matrix, forming a robust composite membrane for electrode surface modification.⁴⁹ This functionalized platform served as the foundation for constructing a highly sensitive electrochemical immunosensor targeting fumonisin B1 (FB1) in corn samples. The detection mechanism employed an indirect competitive immunoassay: free FB1 in the sample competed with FB1 conjugated to bovine serum albumin (FB1–BSA), which was immobilized on the SWCNT/chitosan-modified glassy carbon electrode, for binding to a fixed concentration of anti-FB1 antibody. Signal amplification was achieved through the subsequent binding of an alkaline phosphatase (ALP)-labeled anti-rabbit IgG secondary antibody. Catalytic hydrolysis of the substrate α -naphthyl phosphate by ALP generated the electrochemical signal. This immunosensor demonstrated exceptional performance: it achieved a broad linear detection range (0.01 to 1000 ng mL $^{-1}$) and an impressively low detection limit (2 pg mL $^{-1}$), significantly exceeding the sensitivity required by European Union legislation (2–4 mg L $^{-1}$). Furthermore, the method yielded excellent recoveries for both spiked and naturally contaminated corn samples, validating its practical utility for reliable FB1 monitoring in real-world matrices.

The growing global burden of foodborne illnesses has driven urgent demand for rapid, reliable detection technologies suitable for field deployment. Addressing this need, Joksovic group developed a cost-effective electrochemical immunosensor utilizing SWCNT-modified gold leaf electrodes (GLEs) for ultrasensitive *Escherichia coli* (*E. coli*) detection, a major foodborne pathogen. The innovative design of the sensor combines nanomaterial engineering with strategic bioconjugation. Nanostructured sensing interface was realized *via* the layer-by-layer (LbL) assembly of polyethylenimine (PEI) and carboxyl-functionalized SWCNTs. The electrostatic bonding creates a stable, conductive nanocomposite film. PEI–SWCNT film serves as an optimal substrate for antibody immobilization. The electrochemical performance validated by impedance spectroscopy (EIS). The analytical performance demonstrates a wide dynamic range of 10^1 to 10^8 CFU mL $^{-1}$ (7 orders of magnitude), with exceptional sensitivity. This platform demonstrates how nanomaterial-based interfaces can bridge the gap between laboratory sensitivity and field-deployable food safety monitoring. The SWCNT–GLE architecture offers affordability (low-cost materials and fabrication), portability (compatible with handheld readers) and operational robustness (consistent performance in real samples). By achieving pathogen detection at clinically relevant concentrations without preprocessing, this

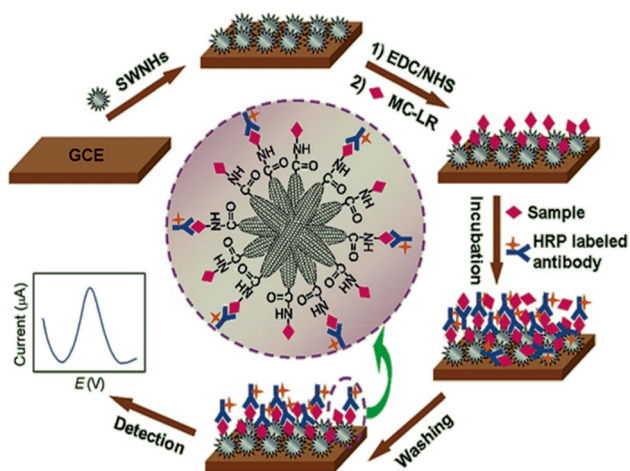


Fig. 1 Schematic representation of the preparation and detection procedure of MC-LR immunosensor.



technology represents a significant advance in preventive food safety systems (Fig. 2).⁵⁰

Researchers have engineered functional nanocomposites by integrating MWNTs with complementary materials, such as MWNT–chitosan hybrids, where chemical surface modifications enable antibody immobilization. Notably, Zhang *et al.* developed a green *in situ* synthesis of gold nanoparticles (AuNPs) on nitrogen-doped MWNTs (CNx-MWNTs) through chloroauric acid reduction, creating an effective signal amplifier for enhanced sensitivity.⁵¹

Ma *et al.* developed an ultrasensitive electrochemical immunosensor for detecting hepatitis C virus core antigen (HCVcAg) with dual-signal amplification (Fig. 3). The sensor employed a graphitized mesoporous carbon–methylene blue (GMCs–MB) nanocomposite as the electrode modifier, followed by electrodeposition of Au nanoparticles for primary antibody immobilization. The signal amplification system consisted of HRP–DNA-conjugated carboxylated multi-walled CNTs (CMWNTs) as the secondary antibody label, DNA concatemers formed *via* hybridization of biotin-tagged probes, and streptavidin–HRP for catalytic signal enhancement *via* the biotin–streptavidin system. The reduction current of MB, generated in the presence of H₂O₂, was monitored by square wave voltammetry (SWV). The immunosensor demonstrated ultralow detection limit of 0.01 pg mL⁻¹ (linear range: 0.25–300 pg mL⁻¹), high selectivity against interfering biomarkers, excellent reproducibility (RSD < 5%), and accurate recovery rates (95–102%) in human serum. With its robust performance and clinical applicability, this platform represents a significant advance in HCV diagnostics.⁵²

Acute myocardial infarction (AMI) represents a critical global health challenge, accounting for a significant proportion of mortality among non-communicable diseases due to its sudden onset, high fatality rate, and limited intervention window.⁵³ The development of rapid and accurate diagnostic technologies based on cardiac biomarkers is therefore essential for timely clinical intervention and improved patient outcomes.⁵⁴ Recent

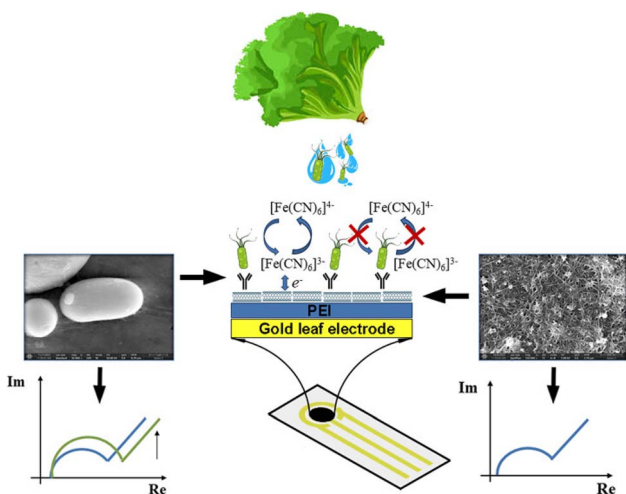


Fig. 2 Schematic layer-by-layer (LbL) procedure and functionalization steps.

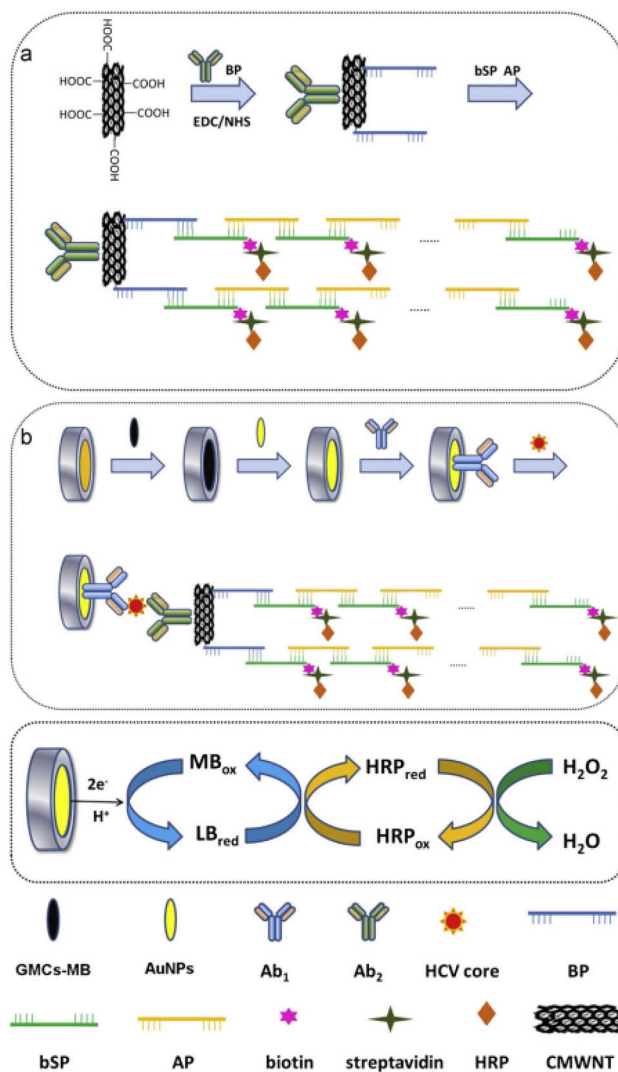


Fig. 3 (a) Preparation of the multi-HRP–DNA–CMWNTs–Ab₂ bi-conjugate, demonstrating the hierarchical assembly of horseradish peroxidase (HRP) enzymes, DNA linkers, carboxylated multi-walled carbon nanotubes (CMWNTs), and secondary antibodies (Ab₂) for signal amplification and (b) fabrication process of the hepatitis C virus (HCV) immunosensor, highlighting key steps in electrode modification, immunocomplex formation, and electrochemical signal transduction.

advances in nanomaterial-enabled electrochemical biosensors have shown particular promise for AMI biomarker detection.⁵⁵ A representative example is the HRP–Strept–Biotin–Ab–Mb/Fe₃O₄–MWCNTs–COOH/GCE immunosensor system developed Ma group for ultrasensitive myoglobin (Mb) detection, a key early diagnostic marker for AMI.⁵⁶ This innovative platform combines several strategic design elements. The base electrode incorporates Fe₃O₄ magnetic nanoparticles integrated with carboxylated multi-walled CNTs (MWCNTs–COOH), creating a hybrid nanocomposite with enhanced electrochemical activity, superior stability and dispersion properties, and excellent biocompatibility for biomolecular immobilization. This nanoplatform significantly amplifies the response signal of sensor while maintaining structural integrity. This unique



signal amplification system utilizes horseradish peroxidase (HRP)-conjugated monoclonal antibodies for specific Mb recognition, which implements a streptavidin–biotin coupling system to enhance probe immobilization efficiency and leverages enzymatic catalysis for secondary signal amplification. The optimized immunosensor demonstrates exceptional analytical performance with broad linear detection range (1.95×10^{-2} to 6.40×10^{-2} ng mL⁻¹), ultra-low detection limit: 0.1007 ng mL⁻¹ (S/N = 3), high specificity against interfering substances, excellent reproducibility (RSD < 5%) and long-term stability. This technological advance provides a rapid, sensitive point-of-care testing method for AMI biomarkers, a prototype for developing integrated AMI early warning systems, as well as a framework for multiplexed detection of cardiac biomarkers. The successful implementation of this nanomaterial-based immunosensor represents a significant step toward intelligent AMI diagnosis and demonstrates the potential of engineered nanocomposites in next-generation medical diagnostics (Fig. 4).

2.2 Nanowire

Extensive research has demonstrated the broad applicability of various nanostructures, among which nanowires represent a prominent class of one-dimensional nanomaterials.⁵⁷ Conventionally, the synthesis of such nanostructures follows two principal methodologies, including the “bottom-up” approach, involving the assembly of atomic or molecular components, and the “top-down” method, which entails the reduction of larger-scale materials to nanoscale dimensions to

achieve multifunctional structures.⁵⁸ As a representative one-dimensional nanomaterial, nanowires exhibit exceptional mechanical, electrical, thermal, and multifunctional properties. Their significant potential as alternative sensing platforms stems from key inherent attributes, including the nanoscale dimensions, a high surface-to-volume ratio, and unique electronic, optical, and magnetic characteristics.⁵⁹

Compared to CNTs, nanowires exhibit superior controllability in both material properties and surface engineering, which could be systematically analyzed from two fundamental aspects.⁶⁰ From a materials engineering perspective, the intrinsic properties of nanowires demonstrate exceptional tunability through precisely controlled synthesis parameters (including but not limited to temperature gradients, pressure conditions, and precursor stoichiometry) combined with advanced doping methodologies.⁶¹ This parameter space allows for deterministic modulation of electronic, optical, and mechanical characteristics at the nanoscale. Regarding surface chemistry, nanowire systems present unique advantages due to their inherent oxide formation tendency (particularly in semiconductor materials like silicon).⁶² This characteristic enables robust surface functionalization through well-established silane chemistry and other covalent modification protocols, and intrinsic passivation mechanisms that enhance environmental stability.⁶³ These features collectively establish nanowires as a versatile platform for applications requiring precise surface engineering.

Owing to their exceptional attributes, silicon nanowires (SiNWs) have emerged as a highly promising platform for

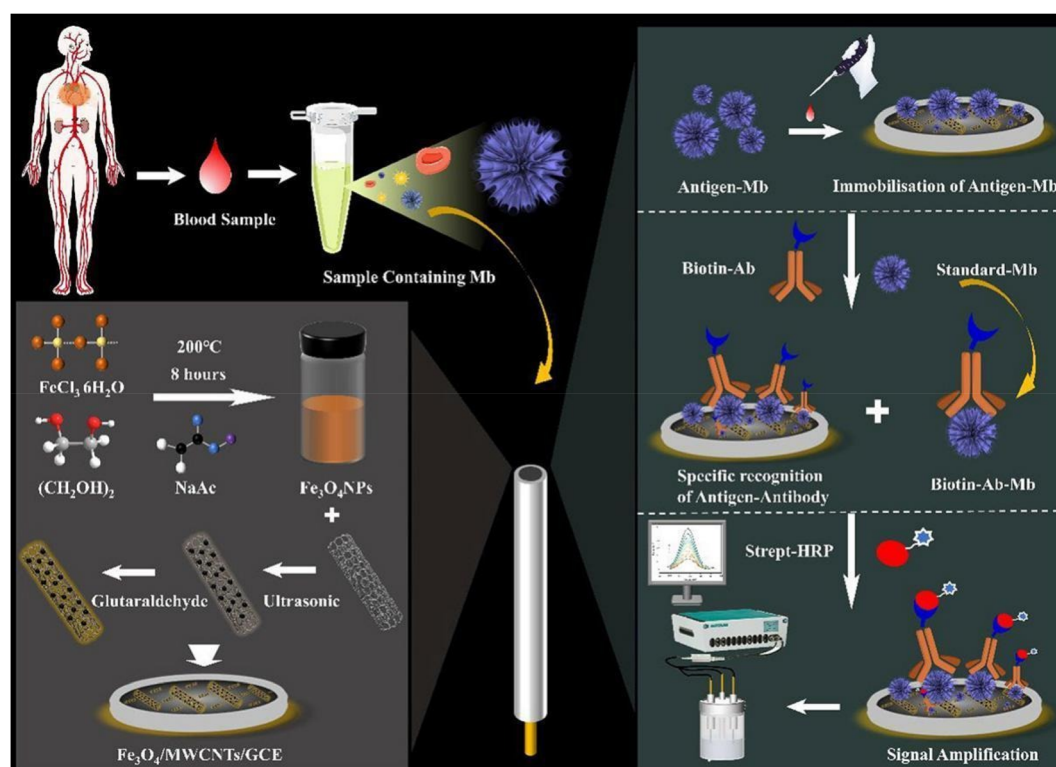


Fig. 4 Diagram of construction of HRP–Strept–Biotin–Ab–Mb/Mb/Fe₃O₄–MWCNTs–COOH/GCE electrochemical immunosensor.



biosensing applications. For instance, Wang *et al.* demonstrated a SiNW field-effect transistor (FET) device achieving highly sensitive, label-free direct detection of small-molecule inhibitors targeting ATP binding sites, specifically mediated by tyrosine protein kinase (Fig. 5).⁶⁴ Beyond their electronic properties, SiNWs demonstrate exceptional multifunctionality that extends beyond their well-characterized electronic properties, establishing them as a premier material platform for next-generation sensing applications. The spontaneous formation of a native oxide layer on SiNW surfaces enables robust chemical functionalization through well-developed silane chemistry protocols, while simultaneously providing effective surface passivation.⁶⁵ Compared to other nanostructures, SiNWs benefit from relatively straightforward fabrication processes that are compatible with existing semiconductor manufacturing infrastructure.⁶⁶ The synergistic combination of intrinsic optical properties with electrical characteristics allows for ultrasensitive (sub-pM level), real-time, and label-free detection capabilities, features that have been rigorously validated across numerous studies.⁶⁷ These attributes collectively position SiNWs as a uniquely versatile material system that addresses critical requirements for practical sensor implementation, including sensitivity, stability, and manufacturability.⁶⁸

Furthermore, the strategic integration of multiple nanomaterials through co-modification presents a powerful approach to enhance sensor performance. This methodology capitalizes on the synergistic interplay between different materials, leveraging their individual strengths (*e.g.*, unique electrical, optical, or catalytic properties) to achieve complementary effects and emergent functionalities that surpass the capabilities of any single component. A compelling example is provided by Lu *et al.*, who immobilized alpha-fetoprotein (AFP) antibodies onto a composite film consisting of gold nanowires (AuNWs) and zinc oxide nanoparticles (ZnO NPs). This design effectively exploited the excellent direct electrical conductivity of AuNWs for rapid electron transfer, combined with the properties of ZnO NPs, to develop a highly sensitive current-

based AFP immunosensor. This case exemplifies how co-modification enables the creation of sensing platforms with superior properties.⁶⁹

2.3 Graphene

Graphene, a single-atom-thick, two-dimensional honeycomb lattice composed of sp^2 -bonded carbon atoms, has garnered significant attention as a revolutionary nanomaterial since its landmark isolation in 2004. Its exceptional properties, including an ultra-high specific surface area and remarkable electrical, thermal, and mechanical characteristics, underpin its widespread application in biosensing, particularly within electrochemical platforms.⁷⁰

However, a significant challenge hindering its broader utility in aqueous-based biological assays is its inherent hydrophobicity and poor aqueous dispersibility.⁷¹ To overcome this limitation, strategic surface functionalization is commonly employed. For instance, the introduction of carboxyl ($-COOH$) groups onto the graphene surface, often facilitated by the carbodiimide crosslinker EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide), serves a dual purpose. Specifically, it significantly enhances aqueous solubility and provides reactive sites for the efficient immobilization of biomolecules, such as capture antibodies, *via* stable amide bond formation.⁷²

A notable example of graphene-based biosensor functionalization was demonstrated by Narayanan *et al.*, who developed an ultrasensitive platform for botulinum neurotoxin serotype E (BoNT/E) detection. Their strategy employed a multi-step covalent modification approach involving the robust covalent modification of graphene onto a glassy carbon electrode (GCE) surface, followed by the covalent attachment of specific antibodies to the carboxylated graphene using the EDC/NHS (*N*-hydroxysuccinimide) crosslinking chemistry. Crucially, the detection of botulinum neurotoxin serotype E (BoNT/E) was achieved using a sandwich immunoassay format. Signal amplification was realized through the use of reporter antibodies conjugated to both alkaline phosphatase (RaMIG-ALP)

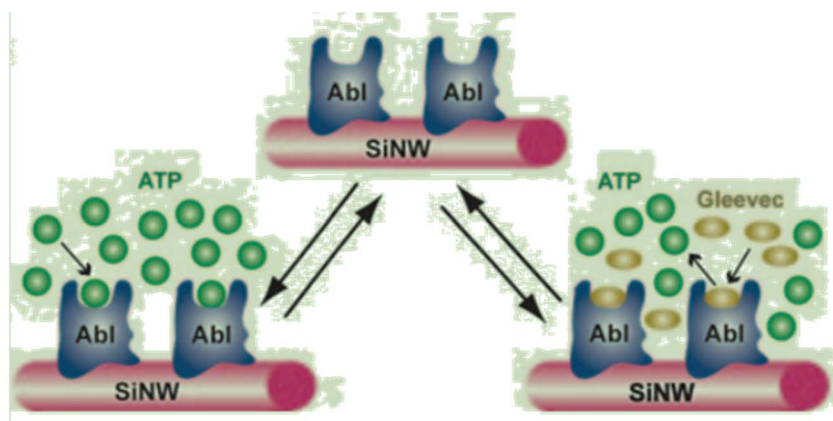


Fig. 5 Detection of ATP binding and small-molecule inhibition of binding by using a SiNW sensor device. The tyrosine kinase Abl is covalently linked to the surface of a SiNW, and then the conductance of the nanowire device is monitored to detect ATP binding and the competitive inhibition of ATP binding.



and gold nanoparticles (AuNPs), enabling highly sensitive detection.⁶³

The controlled modification of graphene through the introduction or removal of oxygenated functional groups has led to the development of key derivatives, namely graphene oxide (GO) and reduced graphene oxide (rGO). These engineered materials inherit advantageous properties from pristine graphene while exhibiting modified solubility and reactivity, significantly broadening their utility within the sensor domain.⁷³ Demonstrating the application of GO, Sharma *et al.* employed an electrochemical method to achieve *in situ* functionalization of graphene oxide directly on a screen-printed electrode (SPE).⁷⁴ Subsequently, the carboxyl groups generated on the GO surface were utilized for the covalent immobilization of specific antibodies. Leveraging the sandwich immunoassay principle, this approach successfully established an immunosensor platform for the sensitive detection of the herbicide Diuron (Fig. 6).

Beyond direct functionalization, the integration of rGO with other nanomaterials offers enhanced performance. Lai *et al.* reported an ultrasensitive immunosensor strategy based on the electrochemical detection of enzymatically synthesized polyaniline (PANI). The fabrication process involved stepwise modification (Fig. 7). First, depositing a nanocomposite of reduced graphene oxide (rGO) and gold nanoparticles (AuNPs) onto the electrode surface. This nanocomposite serves a dual purpose, in which the rGO accelerates electron transfer, while the AuNPs provide a high-surface-area substrate for the subsequent immobilization of capture antibodies. Following a sandwich immunoreaction event, captured horseradish peroxidase-conjugated gold nanoparticle (HRP-AuNP) nanoprobe catalyze the oxidation of aniline monomer in solution, leading to the *in situ* formation of electroactive polyaniline (PANI) on the sensor surface. Crucially, the electrochemical quantification of the deposited PANI film provides a novel readout strategy for HRP-based immunoassays. The synergistic combination of signal amplification *via* the HRP-AuNP nanoprobe (generating PANI) and the enhanced electron transfer facilitated by the underlying rGO/AuNP nanocomposite collectively contributes

to the greatly enhanced detection sensitivity of this immunoassay method.⁷⁵

The growing global burden of insulin resistance (IR) underscores the critical need for rapid, accurate diagnostic tools that enable early intervention. While the adiponectin-to-leptin ratio (A/L) has emerged as a promising biomarker for IR, its clinical adoption has been hindered by the lack of practical analytical platforms capable of simultaneous quantification.⁷⁶ Recent innovations in nanomaterial-based immunosensors are addressing this challenge, as exemplified by our development of a multiplexed laser-scribed graphene (LSG) biosensor developed by Rizalputri and his cooperators.⁷⁷ This integrated approach offers significant improvements over conventional methods, enabling direct A/L ratio calculation without separate assays to improve the diagnostic efficiency. Meanwhile, it realizes 80% reduction in required sample volume, while maintains ELISA-comparable accuracy. The point-of-care compatibility of this platform is enabled by a smartphone-interfaced miniaturized potentiostat, which facilitates real-time data acquisition, cloud-based result analysis, and remote monitoring capabilities, essential features for decentralized diagnostics. These advancements position multiplexed nanomaterial biosensors as powerful tools for decentralized metabolic monitoring, particularly in resource-limited settings where rapid IR assessment could significantly impact treatment outcomes. Future developments may focus on expanding the biomarker panel to include additional IR-related proteins while further simplifying the user interface for non-specialist operators (Fig. 8).

2.4 Metal nanometer

Metal nanoparticles (MNPs) serve as exceptional supporting materials in biosensor design, primarily owing to their ability to significantly enhance the effective surface area, provide excellent electrical conductivity, and facilitate efficient electron transfer.⁷⁸ Among these, gold nanoparticles (AuNPs) stand out as the most prevalent and extensively utilized nanomaterial in

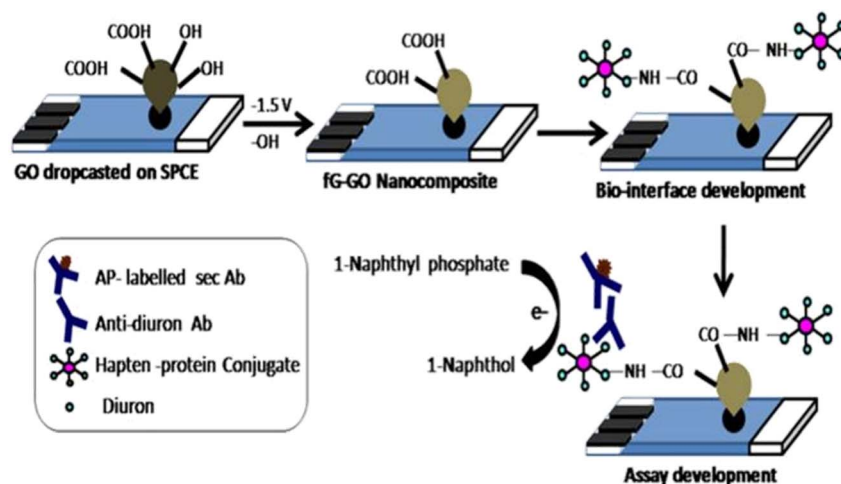


Fig. 6 *In situ* electrochemical synthesis of fG-GO nanocomposite on screen printed electrodes and subsequent immunoassay development.



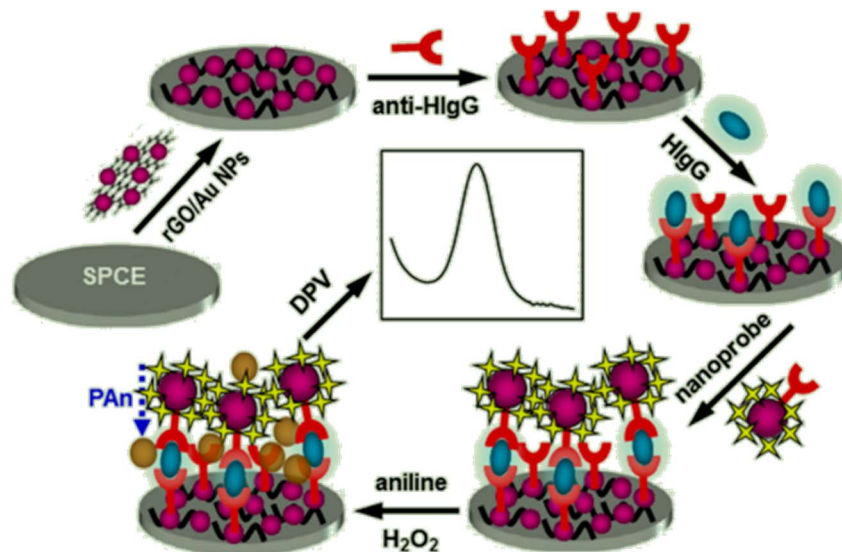


Fig. 7 Schematic representation of the preparation of immunosensor and sandwich immunoassay based on the electrochemical measurement of pAn catalytically deposited by a HRP–Au NP nanoprobe.

immunosensors. Typically ranging from 1 to 100 nm in diameter, AuNPs offer distinct advantages: relatively simple synthesis protocols, high reactivity, remarkable catalytic efficiency, excellent biocompatibility, and crucially, the ability to conjugate with biomolecules (*e.g.*, antibodies, enzymes) without compromising their inherent activity or function.⁷⁹ Beyond AuNPs, other metal nanomaterials like silver (Ag) and platinum (Pt) nanoparticles are also widely employed for electrode modification silver nanoparticles (AgNPs), in particular, offer advantages of lower preparation cost and high conductivity.⁸⁰ To further amplify performance, AgNPs are often integrated with materials like graphene oxide (GO). This synergy not only

enhances overall sensor sensitivity but also helps mitigate structural defects inherent in individual components.⁸¹

Chen's team demonstrated the utility of silver nanostructures by synthesizing functionalized silver nanowires (AgNWs) as carriers for horseradish peroxidase-conjugated anti-alpha-fetoprotein antibodies (HRP–anti-AFP).⁸² This approach enabled the development of a novel sandwich-type electrochemical immunosensor for tumor marker detection. The AgNWs served dual functions, their large surface area facilitated high antibody loading capacity, while simultaneously enhancing electrochemical signal transduction efficiency. The resulting sensor exhibited streamlined fabrication and user-

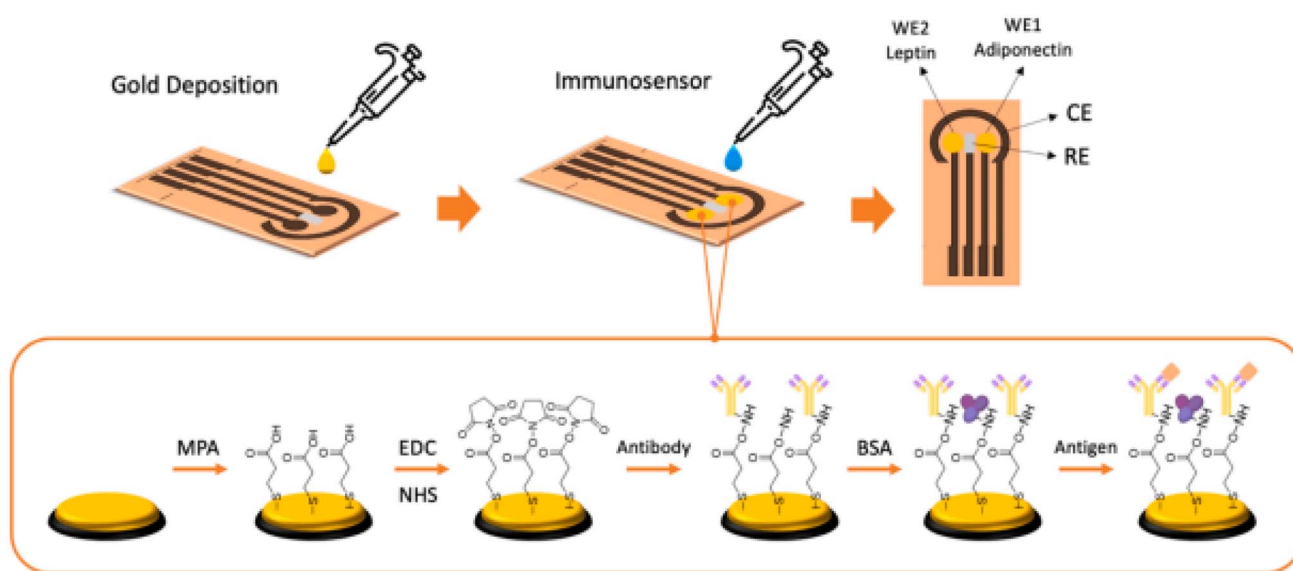


Fig. 8 Schematic illustration of the multiplexed electrochemical immunosensor fabrication process *via* EDC/NHS chemistry, enabling simultaneous detection of adiponectin and leptin biomarkers.



friendly operation with wide linear range ($0.05\text{--}400\text{ ng mL}^{-1}$), low detection limit ($\text{LOD} = 5\text{ pg mL}^{-1}$, $\text{S/N} = 3$), ultrahigh precision, as well as acceptable selectivity and stability, demonstrating promising applicability for AFP detection in clinical serum samples (Fig. 9).

In addition to the metal nanomaterials discussed above, a diverse array of other nanostructures, including QDs, and various metal oxide nanoparticles, have also been actively investigated and applied in immunosensor development.^{83–85} Researchers frequently modify electrode surfaces with these nanomaterials to achieve substantial enhancements in analytical sensitivity. Concurrent efforts are continuously focused on improving critical performance metrics such as stability and reproducibility, collectively driving the advancement and broader adoption of nanomaterial-based sensing platforms.⁸⁶

2.5 Covalent organic frameworks

As an emerging class of crystalline organic polymers, COFs exhibit highly ordered porous structures, large specific surface areas, tunable pore microenvironments, and exceptional chemical stability.⁸⁷ Since their inaugural synthesis *via* boroxine/boronate ester linkages in 2005, COFs have rapidly evolved as versatile materials with significant potential in sensing, catalysis, separation, and energy storage. Among these applications, COF-based passive sensing platforms, operating without external energy input, have garnered particular interest due to their intrinsic molecular recognition and signal transduction capabilities.⁸⁸ Tunable pore sizes ($0.5\text{--}10\text{ nm}$) and functional groups (*e.g.*, amino, sulfonic acid) enable precise adsorption of target analytes.⁸⁹ Analyte binding directly alters the electronic structure of COFs, triggering fluorescence quenching/enhancement or conductivity changes (*e.g.*, pyrene-based COFs for nitroaromatics).⁹⁰

Recent innovations highlight COFs as versatile carriers for signal probes. For instance, antibody-modified COF membranes detect C-reactive protein with a linear range of $1\text{--}100\text{ ng mL}^{-1}$. DNA-conjugated COFs exhibit enhanced ATP affinity, surpassing the performance of free aptamers. In enzymatic amplification-based detection of cardiac troponin I

(cTnI), $\text{COF}_{\text{TAPB-DMTP}}$ loaded with AuNPs, antibodies, and horseradish peroxidase (HRP) achieved a low detection limit (LOD) of 1.7 pg mL^{-1} by catalyzing the hydroquinone-to-benzoquinone conversion followed by electrochemical reduction.⁹¹ Similarly, COF-LZU1 co-doped with AuNPs, antibodies, and the toluidine blue (TB) electron mediator, combined with polypyrrole-modified TiO_2 NPs, enabled cTnI detection from 0.5 pg mL^{-1} to 10.0 ng mL^{-1} .⁹² Furthermore, magnetic COFBD-Tp incorporating methylene blue (MB) *via* supramolecular hosts, integrated with a black phosphorene conductive matrix, quantified prostate-specific antigen (PSA) down to 30 fg mL^{-1} .

COFs also excel as conductive electrode matrices.⁹³ Their layered π -cloud arrays enhance charge transport, exemplified by Pt/Ru/C nanoparticles-decorated COF-LZU1 platforms detecting C-reactive protein.⁹⁴ A highly sensitive biosensing platform was developed based on AuNPs/COF substrates combined with $\text{NiCo}_2\text{S}_4@\text{CeO}_2$ tags for the detection of kidney injury molecule-1 (KIM-1). This system achieved an exceptional detection limit (LOD) of 2.00 fg mL^{-1} for KIM-1 in plasma samples, demonstrating its strong potential as an effective tool for monitoring acute kidney injury.⁹⁵ A novel electrochemical COF-based immunosensor employing multi-stage signal amplification was developed by Chen *et al.*, for detecting Apolipoprotein A4 (Apo-A4), a promising biomarker for depression diagnosis.⁹⁶ The sensor architecture utilized a composite electrode material (NG-PEI-COF) engineered through integration of bipyridine-functionalized covalent organic frameworks (COF) with polyethyleneimine-modified nitrogen-doped graphene (NG-PEI). This hybrid substrate provided enhanced surface area and facilitated electron transfer, establishing the primary amplification stage for electrical signal conduction. Subsequently, electrodeposited gold nanoparticles (AuNPs) introduced biocompatibility and abundant antigen-binding sites, enabling secondary amplification in target recognition. To address the redox-inert nature of Apo-A4, a tracer probe was fabricated by sequential immobilization of AuNPs, anti-Apo-A4 antibodies, and toluidine blue (TB) onto COF carriers, achieving tertiary signal conversion amplification *via* TB's redox activity. The optimized immunosensor (TB/Ab/AuNPs/COF-Apo-A4/AuNPs/NG-PEI-COF/GCE) demonstrated exceptional analytical

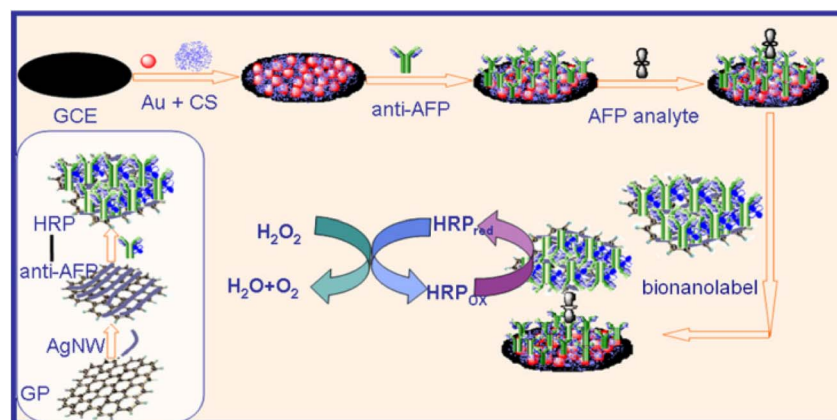


Fig. 9 Fabrication process of the electrochemical immunosensor and the sandwich-type electrochemical measurement protocol.



performance with linear detection range (0.01–300 ng mL⁻¹), ultralow detection limit of 2.16 pg mL⁻¹ (S/N = 3), reproducibility (RSD = 2.31%) and specificity (minimal cross-reactivity with co-existing depression biomarkers). Validation studies confirmed successful quantification of Apo-A4 in human serum samples, highlighting its clinical translation potential for depression monitoring (Fig. 10). This multi-amplification strategy further establishes a versatile template for developing COF-based sensing platforms.

2.6 Metal–organic framework

Metal–organic frameworks (MOFs) represent a class of crystalline porous materials formed by the coordination of metal nodes with organic linkers, creating well-defined three-dimensional architectures.⁹⁷ These materials exhibit exceptional characteristics including ultrahigh surface areas, precisely tunable pore environments, modular chemical functionality through linker modification, excellent biocompatibility and biodegradability.⁹⁸ Such properties stem from their unique structural design, where the metal clusters act as “molecular building blocks” and organic linkers serve as “architectural struts”. This molecular-level programmability enables unprecedented control over catalytic activity (*via* metal center selection), target specificity (through pore functionalization), and signal transduction efficiency (by energy/electron

transfer tuning).^{99,100} MOF have emerged as transformative tools for microbial biosensing, combining signal amplification with molecular recognition capabilities.

Salmonella, a leading global cause of foodborne illness, necessitates rapid and reliable detection methods.¹⁰¹ Addressing this need, recent research has developed a novel MOF-based electrochemical immunosensor for the specific and sensitive detection of *Salmonella typhimurium* (*S. typhimurium*) in milk matrices. This platform leverages the synergistic enhancement properties of a nanocomposite comprising platinum nanoparticles (PtNPs) and Co/Zn-metal–organic framework@-carboxylic multiwalled carbon nanotubes (Co/Zn-MOF@COOH-MWCNTs), significantly boosting both sensitivity and operational stability.¹⁰² The immunosensor demonstrated a wide linear dynamic range (1.3 × 10² to 1.3 × 10⁸ CFU mL⁻¹) for *S. typhimurium*, achieving a notably low detection limit (LOD) of 9.4 × 10¹ CFU mL⁻¹. Crucially, the assay exhibited excellent specificity against non-target bacteria, high reproducibility between sensor batches, robust storage stability, and promising performance in real milk samples, highlighting its practical utility. Importantly, the underlying sensing strategy possesses significant extensibility, offering potential for adaptation to detect diverse foodborne pathogens through appropriate bioreceptor modification.

While MOFs have demonstrated exceptional catalytic properties for biomedical diagnostics, their clinical translation has

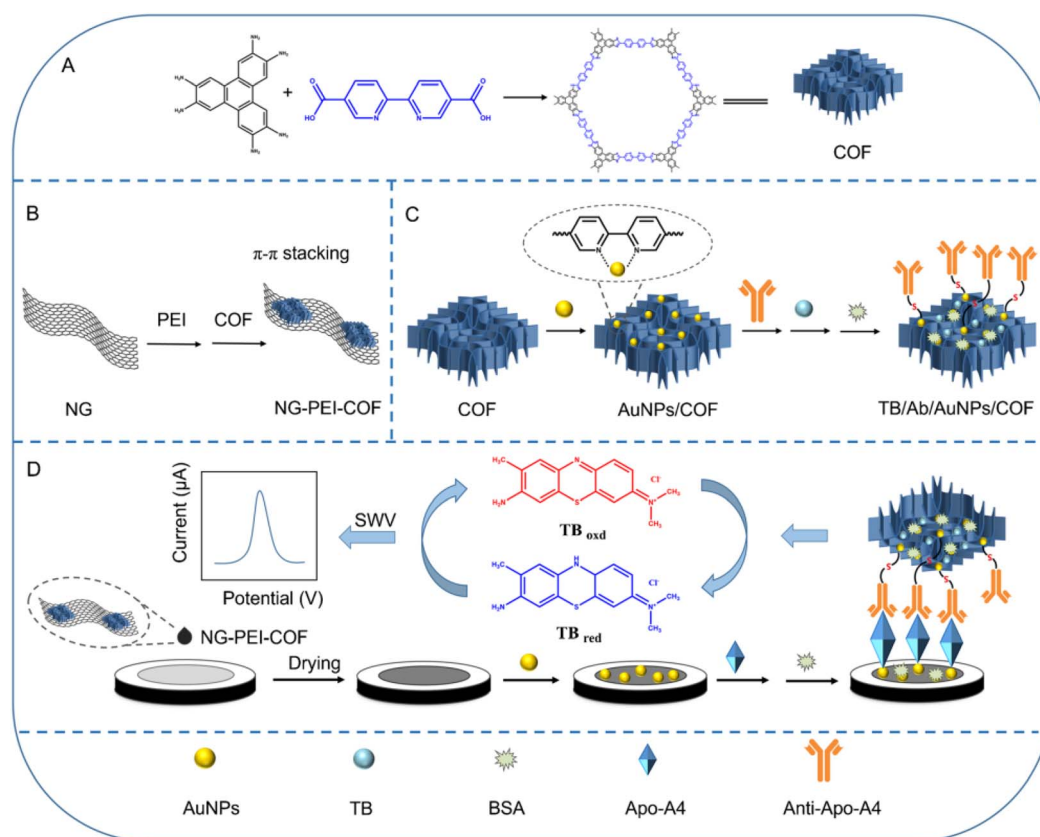


Fig. 10 Schematic diagram of the construction of a multi-signal amplification electrochemical immunosensor for Apo-A4 detection, preparation of COF (A), preparation of NG–PEI–COF composite (B), preparation of tracer probe (C), and detection process of the sensor (D).



been hindered by complex assay configurations and labor-intensive procedures.¹⁰³ To address these limitations, Xu *et al.* developed an integrated electrochemical immunosensor utilizing a Cu–Ni bimetallic MOF/carbon printed electrode (CPE) system for ultrasensitive detection of carcinoembryonic antigen (CEA), a crucial biomarker for various cancers.¹⁰⁴ The immunosensor was fabricated through a streamlined manufacturing process. High-conductivity carbon ink was screen-printed on polyethylene terephthalate (PET) substrates to create disposable CPEs. Cu–Ni MOF was synthesized *via* solvothermal method and aminated for enhanced biocompatibility. The bimetallic MOF exhibits superior peroxidase-like activity, which catalyzes HQ oxidation by H₂O₂, generating amplified electrochemical signals. Immunocomplex formation selectively inhibits catalytic activity, enabling CEA quantification. The optimized immunosensor demonstrated exceptional characteristics with wide dynamic range, ultrahigh sensitivity and recovery rates (95.2–104.6% in human serum). This work provides three key advancements in MOF-based diagnostics.

Yan *et al.* developed Ru-based MOFs with exceptional biosensing properties, using tris(4,4'-dicarboxylic acid-2,2'-bipyridyl)ruthenium(II) (Ru(dcbpy)₃²⁺) as an organic ligand.¹⁰⁵ Six carboxyl groups enable robust coordination with Zn²⁺ nodes, forming stable 2D nanosheets. Inherent ECL activity of Ru(II) complexes provides strong, stable signal output. Large surface area of 2D MOF nanosheet facilitates high-density antibody conjugation. The Ru-MOF nanosheets exhibited a concentration-dependent ECL response, with signal intensity showing a linear correlation ($R^2 = 0.998$) across five orders of magnitude of cTnI concentration (1–10⁴ pg mL⁻¹). Quantitative analysis revealed exceptional sensor performance characteristics with ultrahigh sensitivity, detection limit, wide dynamic range, and outstanding selectivity (Fig. 11).

3. Enzyme-catalyzed signal amplification strategy in electrochemical biosensing

Enzyme catalysis is a cornerstone strategy for signal amplification in electrochemical biosensing due to its exceptional catalytic efficiency, substrate specificity, and rapid reaction kinetics.^{106,107} Enzymes facilitate efficient substrate recycling, significantly enhancing signal output even at trace analyte concentrations, making them indispensable in biosensor design.

3.1 Working mechanism and device integration

The core mechanism involves enzyme-mediated generation or consumption of electroactive species near the electrode surface, inducing measurable changes in current or potential. Commonly used enzymes, including horseradish peroxidase (HRP), glucose oxidase (GOD), and alkaline phosphatase (ALP), catalyze specific redox reactions. For example, HRP reduces H₂O₂ while oxidizing electron mediators (*e.g.*, TMB), producing quantifiable amperometric or voltammetric signals.^{108–110}

Device integration benefits markedly from nanostructured materials. Mesoporous carbon, graphene oxide, and metal-organic frameworks (MOFs) provide high surface areas for efficient enzyme immobilization and enhance electron transfer rates. For instance, the hierarchical pores of COF-LZU1 facilitate HRP encapsulation while promoting direct electron communication between the enzyme's active site and the electrode (Fig. 12).⁹²

3.2 Enzyme-based amplification pathways

Several enzymatic strategies have been developed to amplify signals, such as the catalytic cycling and enzyme cascades. Enzymes such as ALP hydrolyze non-electroactive substrates (*e.g.*, *para*-aminophenyl phosphate) into electroactive products (*e.g.*, *para*-aminophenol), which undergo redox cycling at the electrode, amplifying the signal continuously. Multi-enzyme systems (*e.g.*, GOD/HRP) enable tandem reactions where the product of one enzyme serves as the substrate for the next, exponentially increasing signal output.

3.3 Synergistic nanomaterial integration

Conjugating enzymes with conductive nanomaterials (*e.g.*, AuNPs@GOx) improves enzymatic stability and enhances interfacial electron transfer. Key advantages include high surface area-to-volume ratios increasing enzyme loading capacity, intrinsic catalytic properties enhancing enzyme-substrate interactions, and superior electrical conductivity facilitating efficient electron transfer kinetics.^{111–113} This synergy significantly boosts detection sensitivity by optimizing both signal generation and transduction efficiency.

3.4 Classification of amplification mechanisms

Enzymatic amplification strategies can be categorized into four main types based on their operational principles. Substrate cycling amplification involves the continuous regeneration of electroactive species, leading to sustained current enhancement. Enzymatic product deposition relies on the accumulation of insoluble electroactive products on the electrode surface. Enzyme cascade amplification employs multi-enzyme sequences that exponentially increase signal output. Enzyme-assisted target recycling uses enzymes to repeatedly process target molecules, enabling cyclic signal generation.^{114–118}

For instance, Bauer *et al.* developed an alkaline phosphatase (ALP) sensor utilizing a tyrosinase/glucose dehydrogenase cascade, which achieved a 35-fold signal enhancement with a detection limit as low as 3.2 fmol L⁻¹.¹¹⁷ Similarly, as shown in Fig. 13, Xu *et al.* designed a triple-enzyme cascade system incorporating glucose oxidase (GOx), Pt@Cu-MOFs, and DNAzyme for the detection of carcinoembryonic antigen, demonstrating highly sensitive detection through coordinated multi-stage signal amplification.¹¹⁹

Enzyme-catalyzed signal amplification, particularly when integrated with nanostructured materials, substantially enhances the sensitivity and specificity of electrochemical biosensors. Future efforts should focus on optimizing enzyme-



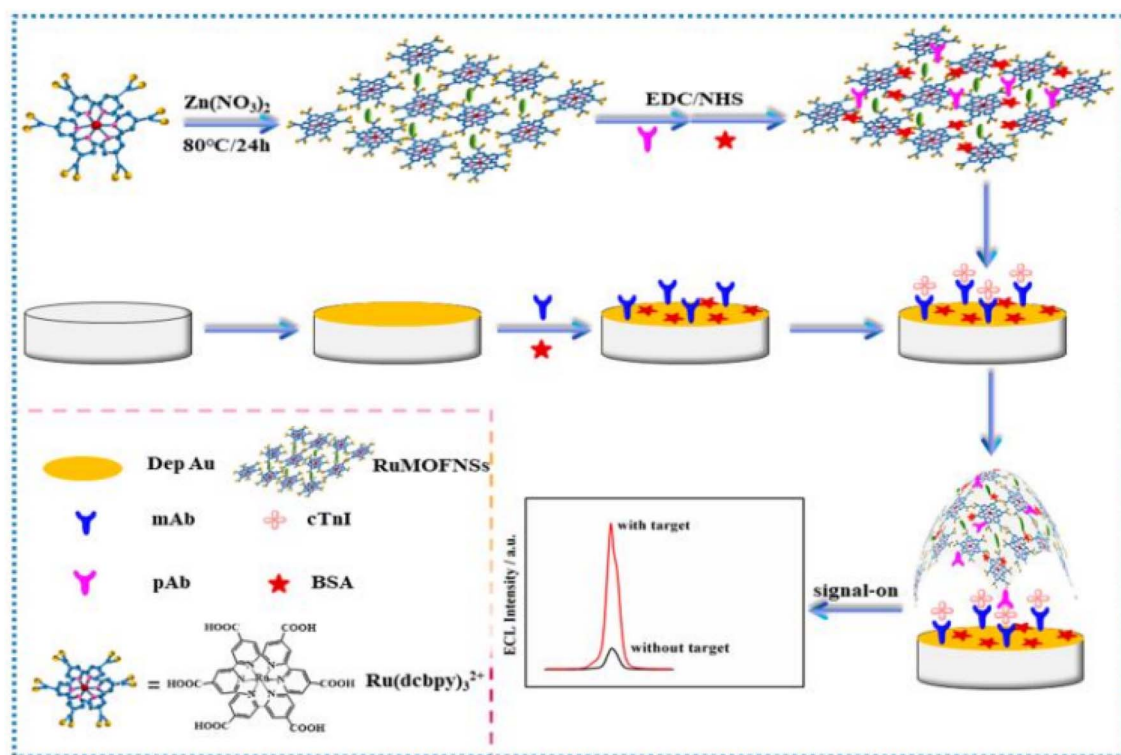


Fig. 11 Schematic fabrication process of the ECL immunosensor.

nanomaterial interfaces and developing multiplexed enzymatic platforms for complex sample analysis.

4. Signal amplification strategy based on DNA technology

Recent advances in DNA nanotechnology have enabled the development of enzyme-free signal amplification strategies that demonstrate superior environmental stability. Unlike enzyme-catalyzed techniques, these DNA-assisted approaches exhibit minimal susceptibility to fluctuations in pH, temperature, and reaction duration.¹²⁰ Contemporary DNA-assisted amplification methodologies are broadly classified into two categories encompassing the DNA amplification techniques (leveraging polymerase-driven nucleic acid replication for exponential target detection) and DNA self-assembly techniques (exploiting programmable hybridization to construct signal-enhancing nanostructures).^{121–123}

4.1 DNA amplification techniques

DNA amplification methodologies bifurcate into thermal cycling amplification and isothermal amplification. The polymerase chain reaction (PCR) represents the pioneering thermal cycling technique, a highly efficient *in vitro* nucleic acid amplification system renowned for its rapid kinetics and exceptional sensitivity.¹²⁴ PCR remains the gold standard for detecting low-abundance nucleic acid targets due to these intrinsic advantages. However, PCR implementation faces

significant constraints in requirement for precision thermocycling instrumentation and substantial operational costs. These limitations restrict the utility of PCR in point-of-care and resource-limited settings.¹²⁵ In contrast, isothermal amplification techniques provide a compelling alternative by eliminating thermal cycling requirements, operating at constant temperatures, maintaining high amplification efficiency, enabling detection of diverse analytes (proteins, cells, ions).¹²⁶ This paradigm overcomes instrumental limitations of PCR while expanding applicability to non-nucleic acid targets.

Rolling circle amplification (RCA), an isothermal nucleic acid amplification technique pioneered in the 1990s, emulates circular DNA replication mechanisms observed in viral pathogens.¹²⁷ This methodology employs three essential components (the circular DNA template, strand-displacing DNA polymerase, and isothermal reaction conditions) to generate long tandem repeats of single-stranded DNA (concatemers) complementary to the template sequence through continuous enzymatic replication.^{128,129} This templated amplification process produces extended DNA structures that serve as high-density scaffolds for signal reporters.

Loop-mediated isothermal amplification (LAMP) is a transformative nucleic acid amplification technology that offers three fundamental advantages over conventional PCR.¹³⁰ Instrument independence eliminates thermal cyclers through isothermal ($60\text{--}65^\circ C$) operation. LAMP possesses unique cost efficiency, which reduces instrumentation costs by $>90\%$ compared to qPCR system. This technique enables rapid high-throughput detection, suitable for point-of-care testing.¹³¹



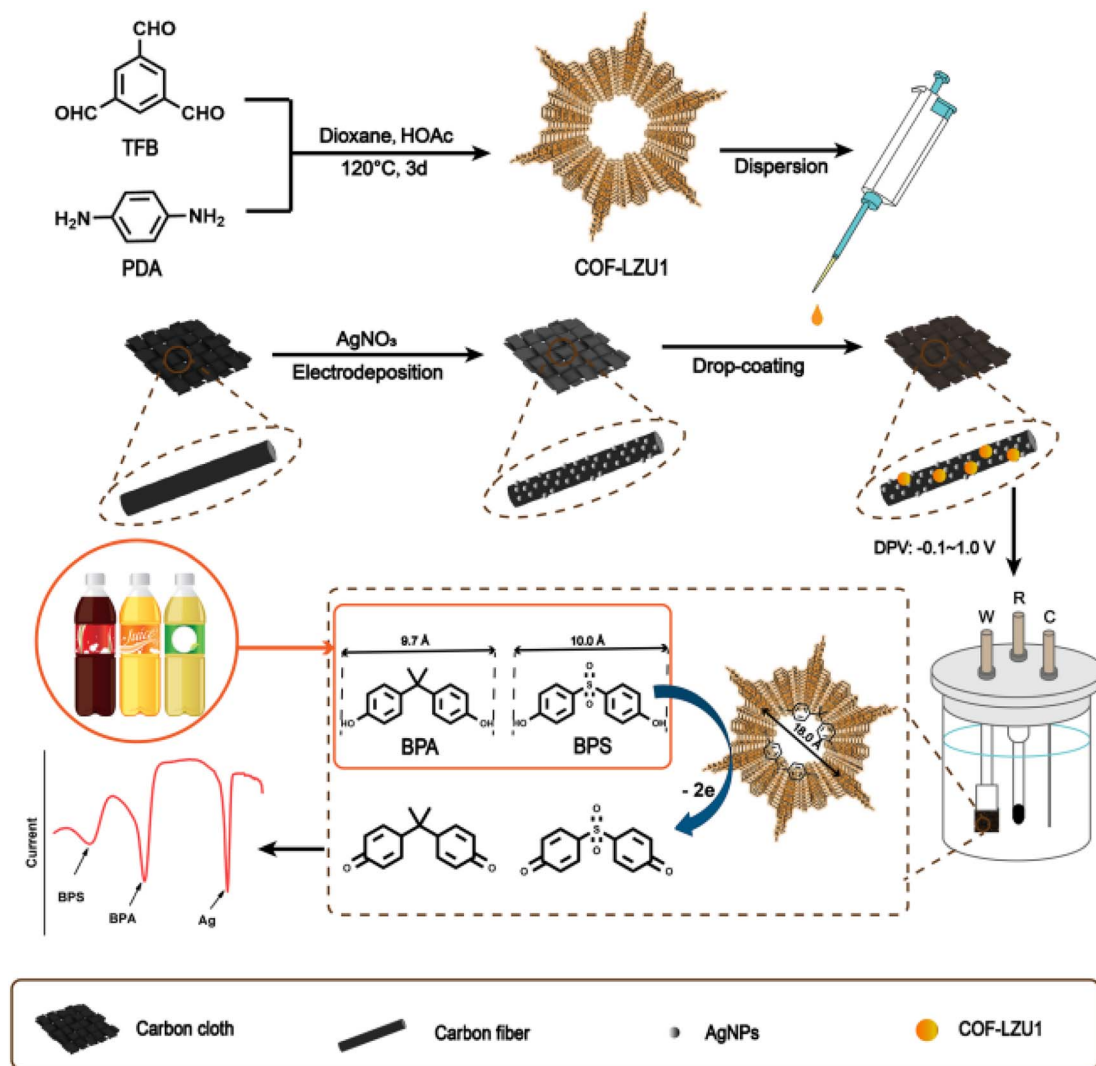


Fig. 12 Schematic illustration for preparation of COF/AgNPs/CC and the construction of ratiometric electrochemical sensor for simultaneous determination for BPA and BPS in beverage.

LAMP employs four to six primers targeting six distinct genomic regions, combined with Bst DNA polymerase (exhibiting strand displacement activity) to achieve autocycling amplification. This architecture enables exponential target amplification (10^9 – 10^{10} -fold) within 15–60 min.¹³² LAMP technology offers significant operational advantages. It has ultrahigh specificity, with dual structural recognition preventing off-target amplification.¹³³ The technology also has single-copy sensitivity with a detection limit of less than 10 copies per microliter. Additionally, it features a simplified workflow, with results interpretable through a colorimetric change. Xie *et al.* developed an innovative signal conversion strategy exploiting loop-mediated isothermal amplification (LAMP) byproducts, achieving a cascade of target amplification, ATP conversion, and detection.¹³⁴ Specifically, amplification of the target gene by LAMP releases inorganic pyrophosphate (PPi), which is subsequently converted to ATP *via* ATP sulfurylase catalysis. Subsequently, the team constructed an electrochemical sandwich aptasensor

to quantify this enzymatically generated ATP, thus achieving signal amplification through aptamer-target binding.

The advent of enzymatic isothermal DNA amplification techniques dates to 1992, when Walker *et al.* pioneered strand displacement amplification (SDA), a seminal methodology for *in vitro* nucleic acid amplification under constant temperature conditions.¹³⁵ Building on this foundational work, Zeng *et al.* engineered an ultrasensitive label-free electrochemical aptasensor for kanamycin (Kana) detection. This integrated platform leverages cascaded signal amplification by combining SDA with hybridization chain reaction (HCR), achieving a detection limit of 36 fM and a linear range of 0.05–200 pM in spiked samples, thereby demonstrating unprecedented analytical performance for antibiotic monitoring.¹³⁶

4.2 DNA self-assembly amplification technology

DNA self-assembly technology, pioneered by Seeman in the 1980s, represents a bottom-up molecular assembly paradigm



In a groundbreaking study, Feng and colleagues introduced an innovative electrochemical biosensing system tailored for DNA analysis.¹⁴⁵ This system leverages graded mesoporous NiO@N-doped C microspheres, which are functionalized with catalytic hairpins, to enhance detection capabilities. The electrode materials employed in this setup consist of NiO@N-doped C microspheres and multi-walled carbon nanotubes, chosen for their ability to facilitate efficient interfacial electron transfer and provide an increased number of surface-active sites for subsequent biochemical reactions. Utilizing target-assisted CHA, this platform enables a single target DNA molecule to initiate the recruitment of multiple signal probes, each labeled with ferrocene (Fc), onto the surface of the working electrode. This amplification strategy significantly enhances the sensitivity of the detection system. Notably, the developed platform exhibits a broad linear range for target DNA detection, spanning from 100 aM to 100 pM, with an impressive detection limit of 45 aM (Fig. 14). This advancement underscores the potential of CHA-based electrochemical biosensors in ultra-sensitive nucleic acid detection applications.

DNAzymes represent a unique class of DNA oligonucleotides that exhibit enzymatic activity, distinguishing them from conventional enzymes by their *in vitro* screening capabilities.¹⁴⁶ These DNA molecules are not only easily synthesized and functionalized but also possess the remarkable ability to catalyze diverse chemical reactions, including DNA cleavage, as highlighted in reference. Comprising a substrate strand and an enzyme strand, DNAzymes feature a catalytic core flanked by short binding arms on either side. The catalytic core, composed of a fixed sequence of approximately 15 nucleic acids, remains consistent, while the binding arms can be tailored to recognize specific RNA target sequences.

5. Biotin-affinity-based signal amplification

The Biotin–Avidin System (BAS) has been a cornerstone technology in biomolecular detection since its development as a cutting-edge bioreaction amplification platform in the late

1970s.¹⁴⁷ Its enduring significance stems from remarkable versatility and unparalleled sensitivity, primarily driven by the extraordinary affinity between biotin and avidin, one of the strongest known non-covalent biological interactions.¹⁴⁸ This affinity, at least 10 000 times greater than that of an antigen–antibody bond, is exceptionally stable and specific, remaining robust against variations in reagent concentration, pH, and other environmental factors.

Biotin (vitamin H/coenzyme R) features a unique structure with an imidazolone ring serving as the exclusive binding site for avidin, and a thiazole ring with a terminal carboxyl group enabling its conjugation to virtually any biological macromolecule (*e.g.*, antibodies, proteins, nucleic acids, enzymes) or solid-phase material.¹⁴⁹ Avidin, a tetrameric basic glycoprotein, possesses four identical binding sites, allowing it to simultaneously bind four biotinylated molecules.¹⁵⁰ Crucially, biotinylation typically preserves the physicochemical properties and biological activity of the conjugated molecule. This unique combination of the universal labeling capability of biotin and the multivalent, ultra-high affinity binding of avidin, facilitates powerful multi-level signal amplification. Consequently, BAS has revolutionized immunolabelling, tracer analysis, and become indispensable for the qualitative and quantitative detection of diverse biomolecules across biology, molecular biology, biochemistry, and clinical medicine.^{151,152}

Xu group developed a biotin–streptavidin amplified ELISA (BA-ELISA) using a monoclonal antibody to detect pirithramycin residues in beef muscle, milk, and honey.¹⁵³ This BAS-enhanced approach achieved a three-fold increase in sensitivity compared to conventional ELISA, demonstrating a semi-inhibitory concentration (IC_{50}) of 1.6 ng mL⁻¹ for pyrimycin in buffer.

Similarly, Du *et al.* pioneered a novel sandwich-structured electrochemical immunosensor for the quantification of the organophosphorus pesticide and nerve agent exposure biomarker, organophosphorus acetylcholinesterase (OP-AChE), leveraging the biotin–avidin system (Fig. 15). In this design, zirconia nanoparticles (ZrO₂ NPs) anchored to a screen-printed electrode (SPE) effectively capture the OP-AChE adduct *via*

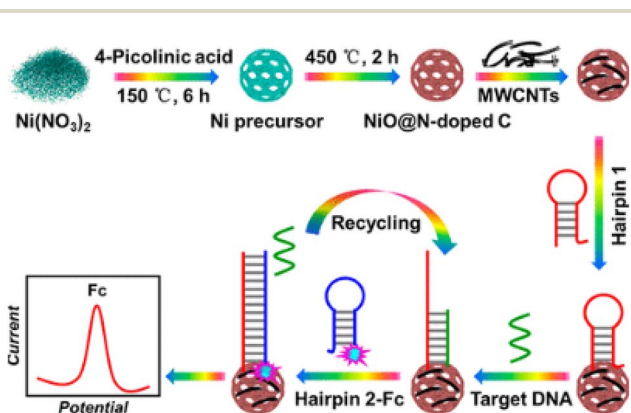


Fig. 14 Schematic route for the construction of an electrochemical biosensing platform based on hierarchical mesoporous NiO@N-doped C microspheres coupled with catalytic hairpin assembly.

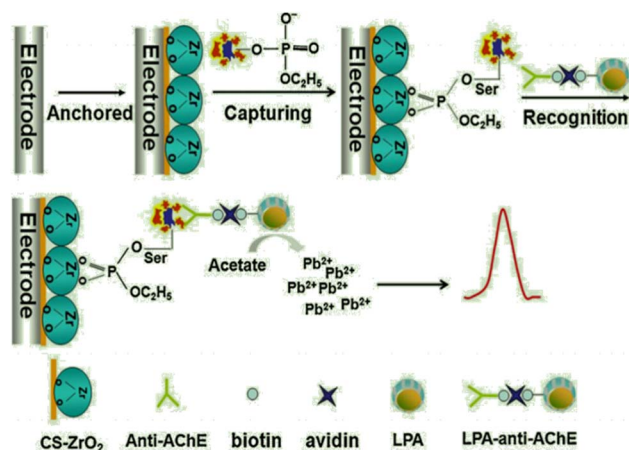


Fig. 15 Schematic illustration of sandwich-like immunoassay of OP-AChE adducts.



metal chelating phosphoryl groups, which are then selectively recognized by a lead phosphate-apatite-labelled anti-AChE antibody (LPA-anti-AChE). Through biotin-affinity amplification, a substantial amount of lead phosphate-depsiphyrin can be modified onto the electrode surface, facilitating the release of large quantities of lead ions. This, in turn, exponentially enhances the electrochemical signal, enabling the immunosensor to exhibit a linear response current with sufficient sensitivity across the OP-AChE concentration range of 0.05–10 nM.¹⁵⁴ These advancements underscore the immense potential of BAS in enhancing the sensitivity and specificity of biosensing technologies for various applications.

6. Conclusion, challenges and outlook

This review highlights pivotal advances in signal amplification strategies for electrochemical immunosensors, with a particular emphasis on the transformative role of porous nanostructured materials. We have critically examined techniques such as nanomaterial engineering, enzyme catalysis, and nucleic acid-based amplification, all of which collectively enhance detection sensitivity and specificity. These approaches have enabled unprecedented LOD, making trace-level analyte quantification feasible even within complex sample matrices. Emerging paradigms, including entropy-driven amplification, illustrate innovative pathways to improve biorecognition efficiency during immunobinding events, thereby significantly augmenting signal generation. Together, these strategies contribute to biosensors with broad dynamic ranges, reduced cost, and operational simplicity, facilitating the transition of trace analyte detection from a technical challenge to practical reality.

Despite these advances, several interdisciplinary challenges hinder the translation of these technologies into real-world applications. A major limitation lies in the poor reusability of sensing interfaces, particularly those functionalized with biological recognition elements. Issues such as heterogeneity in biomolecular immobilization and signal probe degradation often restrict commercial immunosensors to single-use formats, necessitating repetitive and costly electrode renewal. Furthermore, achieving reliable multiplexed detection within complex matrices (*e.g.*, blood or tissue lysates) remains challenging. This requires either sophisticated spatial functionalization of electrodes with different capture probes or the development of cross-reactive antibodies capable of recognizing multiple antigens with high specificity. Essential to addressing these issues are improvements in biointerface stability, coupling efficiency, and the ultrasensitive discrimination of coexisting biomarkers at low abundances.

6.1 Strategies to overcome challenges and future opportunities

To tackle the issue of sensor regeneration, the integration of stimuli-responsive materials (*e.g.*, pH-, temperature-, or light-switchable probes) offers promising avenues for reversible antigen binding and release. Porous framework materials such

as COFs, and MOFs, known for their structural tailorability and high surface area, are particularly suited for designing renewable sensing interfaces. Their functional versatility allows multiple usage cycles without significant loss of activity. Similarly, reusable electrode configurations, often embedded within microfluidic architectures, enable *in situ* regeneration through chemical or electrochemical treatments, substantially extending operational lifetimes.

For enhancing multiplexing capability, spatially encoded platforms, including electrode arrays and microfluidic chips, permit simultaneous detection of multiple analytes by immobilizing distinct capture agents at predefined locations. Nanomaterials such as COFs and MOFs can be engineered to exhibit unique electrocatalytic or redox signatures at different sites, improving signal discrimination and encoding capacity. Additional strategies involving spectral and electronic tagging further enhance multiplexing performance.

The integration of EC and ECL modalities within microfluidic systems represents a highly promising direction toward automated, miniaturized, and multiplexed immunoassays. Such platforms enable precise fluid manipulation, reduce reagent consumption, and facilitate operational standardization, critical attributes for the next generation of point-of-care diagnostic devices.

In conclusion, while challenges related to sensor reusability and multiplex detection persist, converging advances in smart materials, reversible chemistry, and integrated lab-on-a-chip systems are steadily narrowing the gap between laboratory innovation and clinical utility. Interdisciplinary collaboration across materials science, engineering, and biology will be essential to develop robust, renewable, and multiplex-capable electrochemical immunosensors. These innovations hold great potential to enable decentralized, high-sensitivity diagnostic applications across diverse fields such as healthcare, environmental monitoring, and food safety.

Conflicts of interest

There are no conflicts to declare.

Data availability

Data will be made available on request.

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